0031-6997/91/4302-0143$03.00/0
PHARMACOLOGICAL REVIEWS

Vol. 43, No. 2
Printed in U.S.A.

Capsaicin: Cellular Targets, Mechanisms of Action, and Selectivity for Thin Sensory Neurons*

PETER HOLZER†

Department of Experimental and Clinical Pharmacology, University of Graz, Graz, Austria

* Work in the author's laboratory was supported by the Austrian Scientific Research Funds (grants 5552 and 7845).
† Correspondence: Department of Experimental and Clinical Pharmacology, University of Graz, Universitätsplatz 4, A-8010 Graz, Austria.

I. Introduction

THE function of primary afferent neurons is to receive and transmit information from the internal and external environment and thereby contribute to the organism's ability to maintain homeostasis. The cell bodies of primary afferent neurons are located in the spinal (dorsal root) or cranial sensory ganglia and send fibers in both the central and peripheral directions. The endings of the

peripheral fibers may be either receptors themselves or are connected to special sensory structures. Much of the current information concerning the histochemistry, physiology, and pathophysiology of primary afferent neurons has been obtained in the last 15 years. This resulted not only from the advent of new experimental techniques but also from the availability of capsaicin which, during this time, proved to be an important pharmacological tool in sensory neuroscience.

Capsaicin is the pungent ingredient in a wide variety of red peppers of the genus *Capsicum*. Chemically, it is a derivative of vanillyl amide, 8-methyl-N-vanillyl-6-nonenamide (fig. 1) and has a molecular weight of 305.42. Hot peppers have been eaten and used by humans since prehistorical times (Lembeck, 1987b). A. Högyes (1878) was the first to state that the pungent and irritant action of capsicol, an extract of *Capsicum*, is mediated mainly by sensory nerves. It was not before the middle of this century, however, that another Hungarian investigator, N. Jancsó, realized that capsaicin, the pure substance, also exerts a long-term sensory receptor-blocking action which can be made use of in the functional investigation of afferent neurons (Jancsó, 1960, 1968; Szolcsányi, 1982, 1984a). A large number of studies have since corroborated this discovery and established capsaicin as an important probe for sensory neuron mechanisms. The last few years have witnessed important insights into the mechanism of action of capsaicin, and with these advances it has become possible to put forward a framework hypothesis that explains the selectivity of capsaicin's action on a molecular and cellular basis.

With respect to potency, target, and mechanism of action, two effects of capsaicin can be differentiated: (*a*) One action is grossly selective for thin afferent neurons of mammalian species and expresses itself in an initial short-lasting stimulation that can be followed by desensitization to capsaicin and other stimuli of sensory neurons. With clearly suprathreshold doses of capsaicin a long-term functional or even morphological ablation of thin sensory neurons is achieved. Both the stimulant and long-term inhibitory effects of capsaicin appear to arise from a common mechanism of action, i.e., activation of a cation channel in the cell membrane of mammalian sensory neurons. (*b*) The other action of capsaicin is cell nonselective and is seen throughout the animal kingdom. In many, but not all, cases, it manifests itself as a transient depression of excitability with no long-lasting consequences for the cell. The concentrations of capsaicin needed to elicit these cell-nonselective effects are usually orders of magnitude higher than those sufficient to stimulate sensory neurons.

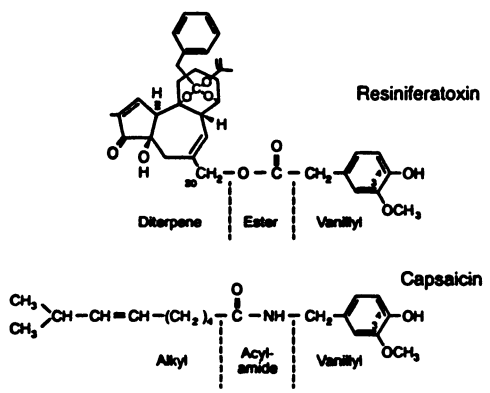

FIG. 1. Chemical structures of capsaicin and resiniferatoxin.

The scope of the present article is to describe and discuss the phenomenology, cellular targets, and mechanisms of action of capsaicin and to present an integrated summary of the current neuropharmacology of this drug. My review concentrates on papers in which the cellular target and mechanism of the actions of capsaicin were explored and attempts to address the practical value of capsaicin as a pharmacological research tool and to point out the limitations of its usefulness. Many other advances arising from the use of capsaicin have been summarized in various reviews (Nagy, 1982; Szolcsányi, 1982, 1984a,b, 1990; Fitzgerald, 1983; Coleridge and Coleridge, 1984; Russell and Burchiel, 1984; Marley and Livett, 1985; Buck and Burks, 1986; Lembeck, 1987a, 1988; Chahl, 1988; Holzer, 1988; Maggi and Meli, 1988; Donnerer et al., 1990; Lynn, 1990; Maggi, 1991) and are considered here only marginally. The disposition of the article follows the time course of capsaicin's action on sensory neurons, proceeding from the acute to the intermediate and long-term effects of the drug. This order is adhered to in the description of both its cellular targets and mechanisms of action.

II. Phenomenology and Selectivity of the Actions of Capsaicin on Primary Afferent Neurons

A. Types of Primary Afferent Neurons

The classification of primary afferent neurons according to morphological, functional, and neurochemical criteria is dealt with here only to the extent that the target of action of capsaicin can be appreciated. *Morphologically*, primary afferent neurons are divided into those having large light somata (A-type neurons) and those having small dark somata (B-type neurons), although there is no sharp separation with respect to cell body diameter (for example, see Lawson and Harper, 1984). The size of the cell bodies is grossly, but not strictly, related to the diameter of the fibers that arise from them (Harper and Lawson, 1985; Hoheisel and Mense, 1986). Three groups of afferent nerve fibers can be separated: (*a*) thick myelinated, (*b*) thin myelinated, and (*c*) thin unmyelinated. This morphological heterogeneity of afferent nerve fibers is closely paralleled by a functional heterogeneity with regard to projection into different layers of the spinal cord or medulla, conduction velocity, and sensory modality (Salt and Hill, 1983).

Physiologically, the thick myelinated fibers have the highest conduction velocities (A$\alpha\beta$-fibers) and carry non-nociceptive mechanical information from the skin and

muscle. The thin unmyelinated fibers have the slowest conduction velocities (C-fibers) and are primarily nociceptors (polymodal nociceptors, chemonociceptors) which respond to noxious mechanical, thermal, and/or chemical stimuli. In addition, they also comprise some specific thermonociceptors as well as some nonnociceptive mechanical, warmth, and cold receptors. The small myelinated fibers conduct at intermediate velocities (Aδ-fibers) and carry both nociceptive (mechanonociceptors and polymodal nociceptors) and nonnociceptive (mechanoreceptors, cold receptors) information. The relative proportions of these sensory receptors among the respective afferent fiber classes show marked species differences.

Primary afferent neurons can be further differentiated by their ultrastructural and by their *biochemical* and *histochemical* properties. A large number of bio- and histochemical markers has been found to be associated with primary afferent neurons. The A-type neurons in the rat can be labeled selectively with RT97, a monoclonal antibody to a neurofilament protein that is absent from B-type neurons (Lawson and Harper, 1984; Lawson et al., 1984; Kai-Kai et al., 1986; Winter, 1987). Some biochemical and histochemical markers that have been found primarily in B-type neurons or thin afferent nerve fibers, respectively, are listed in table 1. These markers include a number of peptides such as substance P, neurokinin A, calcitonin gene-related peptide, galanin, vasoactive intestinal polypeptide, and somatostatin which play a role in the communication of primary sensory neurons with other neuronal and nonneuronal cells (Salt and Hill, 1983; Weihe, 1990). The peptide markers are by no means exclusive for afferent neurons but also label many neurons of the central, motor, autonomic, and enteric nervous systems.

B. Acute Excitatory Effects of Capsaicin on Mammalian Sensory Neurons

1. Excitatory threshold doses or concentrations. On first contact with capsaicin, afferent neurons are invariably stimulated, and there seems to be no gross difference whether the drug is applied to the peripheral or central endings or to the cell bodies of sensory neurons. Administration of capsaicin to the peripheral nerve endings results in depolarization and discharge of action potentials, which in turn evokes burning pain (Pórszász and Jancsó, 1959; Jancsó et al., 1968; Bernstein et al., 1981; Carpenter and Lynn, 1981; Geppetti et al., 1988b; Stjärne et al., 1989). This painful sensation is produced by a threshold concentration of 30 nM capsaicin on the rat eye (Szolcsányi and Jancsó-Gábor, 1975) or blister base in human skin (Szolcsányi, 1977), whereas the threshold concentration in the oral cavity and on the tongue of

TABLE 1
Some markers of capsaicin-sensitive primary afferent neurons

Marker	Selected references
Adenosine deaminase	Nagy and Daddona, 1985
Arginine vasopressin	Kai-Kai et al., 1986
Bombesin/gastrin-releasing peptide	Decker et al., 1985
Calbindin D28k	Kashiba et al., 1990b
Calcitonin gene-related peptide	Gibbins et al., 1985; Lundberg et al., 1985; Skofitsch and Jacobowitz, 1985b; Franco-Cereceda et al., 1987b; Gibbins et al., 1987; Carr et al., 1990; Kashiba et al., 1990a
Cholecystokinin*	Jancsó et al., 1981; Gibbins et al., 1987
Cholecystokinin receptor binding	Ladenheim et al., 1986
Corticotropin-releasing factor†	Skofitsch et al., 1985
Dynorphin	Gibbins et al., 1987; Weihe, 1990
Fluoride-resistant acid phosphatase	Jancsó and Knyihár, 1975; Jessel et al., 1978; Ainsworth et al., 1981; Nagy et al., 1981a,b; Gamse et al., 1982; McDougal et al., 1983, 1985
GABA receptor binding	Singer and Placheta, 1980
Galanin	Skofitsch and Jacobowitz, 1985a
β-Glycerophosphatase	Bucsics et al., 1988
5-Hydroxytryptamine receptor binding	Hamon et al., 1989
Lactoseries carbohydrate antigens	Kirchgessner et al., 1988
Leucine enkephalin	Weihe, 1990
Neurokinin A	Maggio and Hunter, 1984; Hua et al., 1985
Opiate receptor binding	Gamse et al., 1979a; Nagy et al., 1980; Laduron, 1984
Peptide histidine methionine	Chéry-Croze et al., 1989
Peripherin	Ferri et al., 1990
Somatostatin	Gamse et al., 1981b; Jancsó et al., 1981; Nagy et al., 1981a,b
Substance P	Jessell et al., 1978; Gamse et al., 1980, 1981b; Hayes and Tyers, 1980; Nagy et al., 1980, 1981a,b; Jancsó et al., 1981
Thiamine monophosphatase	Inomata and Nasu, 1984; Bucsics et al., 1988
Vasoactive intestinal polypeptide	Jancsó et al., 1981; Skofitsch et al., 1985

* Cholecystokinin-like immunoreactivity in rat sensory neurons (Jancsó et al., 1981) may represent calcitonin gene-related peptide (Ju et al., 1986).

† Corticotropin-releasing factor-like immunoreactivity in rat sensory neurons (Skofitsch et al., 1985) may represent substance P (Berkenbosch et al., 1986).

humans is about 0.7 μM (Szolcsányi and Jancsó-Gábor, 1975; Szolcsányi, 1977; Rozin et al., 1981; Sizer and Harris, 1985). When given as an aerosol by nebulising solutions of capsaicin, concentrations of ≥2 μM produce coughing in humans (Collier and Fuller, 1984). In the rat and monkey, intradermal doses of 30 nmol capsaicin are sufficient to activate polymodal nociceptors and to elicit pain (Martin et al., 1987; LaMotte et al., 1988). Injected close arterially to exteroceptive fields, doses of 0.07 nmol capsaicin in the cat (Szolcsányi, 1977) and guinea pig (Szolcsányi et al., 1986), 0.3 nmol in the rat (Szolcsányi et al., 1988), and 7 nmol in the rabbit (Szolcsányi, 1987) are suprathreshold in activating polymodal nociceptors or producing pain.

This high potency of capsaicin is also seen in vitro (Dray et al., 1989a, 1990a,b,d) and when capsaicin is administered to the axons or somata of afferent neurons. Periaxonal concentrations as low as 30 to 100 nM capsaicin are capable of depolarizing afferent nerve fibers in the rat isolated vagus, sciatic and sural nerves, or lumbar dorsal roots (Ault and Evans, 1980; Yanagisawa et al., 1980; Hayes et al., 1984a; Bevan et al., 1987; Marsh et al., 1987). Similar concentrations of capsaicin depolarize dorsal root and nodose ganglion cells of the rat in vitro or in culture (Williams and Zieglgänsberger, 1982; Baccaglini and Hogan, 1983; Heyman and Rang, 1985; Bevan et al., 1987; Marsh et al., 1987; Winter et al., 1990). Behavioural evidence indicates that intrathecal, intracisternal, or intracerebroventricular injection of 33 to 330 nmol capsaicin activate the central endings of nociceptive afferent nerve fibers (Yaksh et al., 1979; Gamse et al., 1981b; Jancsó, 1981).

2. Targets and selectivity of the excitatory action. The conjecture that pain receptors, but not mechanoreceptors, are activated by capsaicin (Pórszász and Jancsó, 1959; Jancsó, 1968) was later confirmed by single-unit recordings from cutaneous nerves. It is now evident that the primary targets of the excitatory action of capsaicin are thin primary afferent neurons that are connected to distinct sensory receptors (table 2). When capsaicin is administered to the peripheral endings of cutaneous sensory neurons of humans, cat, rabbit, and rat, many, but not all, C-fiber polymodal nociceptors (Szolcsányi, 1977, 1987; Foster and Ramage, 1981; Kenins, 1982; Konietzny and Hensel, 1983; Martin et al., 1987; Szolcsányi et al., 1988; Lang et al., 1990), some C-fiber warmth receptors (Szolcsányi, 1977, 1983a; Foster and Ramage, 1981; Kenins, 1982), and some Aδ-fiber polymodal nociceptors (Matsumiya et al., 1983; Szolcsányi et al., 1988; Hartung et al., 1989) are stimulated. Accordingly, perineural application of capsaicin to the cat saphenous nerve depolarizes C-fibers and some Aδ-fibers (Such and Jancsó, 1986). Other types of cutaneous C-fiber afferents such as low- and high-threshold mechanoreceptors and cold receptors are not affected by capsaicin (Kenins, 1982; Szolcsányi, 1987, 1990; Szolcsányi et al., 1988). With the exception of some Aδ-fiber polymodal nociceptors, none of the cutaneous Aδ- and Aβ-fiber afferents appear to respond to capsaicin (Foster and Ramage, 1981; Szolcsányi, 1987, 1990).

In the dog skeletal muscle, capsaicin has been found to stimulate the majority of group IV (C-fiber) and some group III (Aδ-fiber) skeletal muscle afferents (Kaufman et al., 1982). Exactly the same spectrum of action is seen in the knee joint of the cat in which most of the group IV and some of the group III articular afferents are stimulated by the drug (He et al., 1988, 1990). Capsaicin appears to have an equivalent target of action on visceral afferent neurons as it does with somatic afferents; capsaicin preferentially stimulates C- but also some chemosensitive Aδ-fibers (Coleridge and Coleridge, 1977, 1984; Longhurst et al., 1984; Szolcsányi, 1984b). This is in keeping with the observation that perineural application of capsaicin to the rat vagus nerve depolarizes only C-fibers (Bevan et al., 1987).

Polymodal nociceptors are usually identified by their sensitivity to mechanical and thermal noxious stimuli. Only recently has it been recognized that a considerable proportion of C-fiber afferents in somatic and visceral nerves do not respond to excessive mechanical and thermal stimulation ("silent nociceptors") but are sensitive to algesic chemicals such as bradykinin and histamine (LaMotte et al., 1988; Schaible and Schmidt, 1988; Häbler et al., 1990; McMahon and Koltzenburg, 1990). These chemonociceptors acquire sensitivity to mechanical stimuli during inflammation. All afferent C-fibers in the rabbit great auricular nerve that respond to bradykinin are also sensitive to capsaicin injected intraarterially into the ear (Szolcsányi, 1987). Similarly, those afferent C-fibers in the skin of the monkey (LaMotte et al., 1988) and the rat (Lang et al., 1990) which are stimulated by capsaicin are, in addition, responsive to histamine or bradykinin. Sensitivity to capsaicin and other algesic chemicals is shared in an analogous manner by visceral afferent C-fibers in, for example, the vagal and renal nerves (Coleridge and Coleridge, 1977, 1984; Longhurst et al., 1984; Szolcsányi, 1984b). In contrast, there is much less overlap between cutaneous units sensitive to capsaicin and units responsive to mechanical noxious stimulation (LaMotte et al., 1988; Lang et al., 1990). Thus, it appears as if responsiveness to capsaicin is a particular property of C-fiber chemonociceptors, which might be of great practical value in probing the chemical sensitivity of polymodal nociceptors and in identifying silent nociceptors as suggested by Szolcsányi in 1984 (Szolcsányi, 1984b). However, the precise relationship between capsaicin-sensitive afferents and silent nociceptors remains to be determined.

When administered to the cell bodies of afferent neurons, capsaicin stimulates only somata that are connected to C-fibers as observed both in vitro (Heyman and Rang, 1985; Bevan et al., 1987; Marsh et al., 1987)

TABLE 2

*Targets and selectivity of capsaicin's actions on excitable cells in mammals**

A. Acute effects

1. *Excitation of primary afferent neurons (high potency)*
   a. Application of capsaicin to peripheral endings of sensory neurons:
      Excitation of most, if not all, unmyelinated afferent axons (C-fibers) connected to chemonociceptors
      Excitation of many unmyelinated afferent axons (C-fibers, group IV afferents) connected to polymodal nociceptors
      Excitation of some afferent C-fibers connected to warmth receptors
      Excitation of a minority of thinly myelinated afferent axons (Aδ-fibers, group III afferents) connected to polymodal nociceptors
   b. Application of capsaicin to cell bodies in sensory ganglia:
      Excitation of somata connected only to C-fibers
2. *Excitation of thermosensitive neurons in the preoptic region of the hypothalamus*
3. *Contraction of vascular smooth muscle (low potency)*
4. *Inhibition of the activity of cardiac and visceral smooth muscle (low potency)*
5. *Variable effects on a variety of excitable cells* (effects on the cell membrane and on cytoplasmic systems, typically produced by very high concentrations of capsaicin)

B. Long-term neurotoxic effects

2. *Ablation of primary afferent neurons*
   a. Ablation of majority of afferent neurons with small-diameter somata (small dark B-type somata, neurofilament protein-negative, typically containing the markers listed in table 1)
   b. Ablation of minority of afferent neurons with somata of intermediate diameter (light A-type somata, neurofilament protein positive)
   c. Ablation of majority of afferent neurons with unmyelinated axons (C-fibers) connected to chemonociceptors, polymodal nociceptors, chemoceptors, or warmth receptors
   d. Ablation of minority of afferent neurons with thinly myelinated axons (Aδ-fibers) connected to polymodal nociceptors
2. *Secondary effects on systems related to capsaicin-sensitive afferent neurons*
   a. Reorganization of capsaicin-insensitive primary afferent neurons
   b. Alterations in second and higher-order afferent pathways in the central nervous system
   c. Alterations in the sympathetic nervous system
3. *Ablation of some neurons in the preoptic region of the hypothalamus*
4. *Ablation of some neurons in certain forebrain nuclei, in the retina, and in the enteric nervous system* (to be corroborated)

* Potency and effectiveness of capsaicin depend on route of administration and age, strain, and species of the mammal under study.

and in tissue culture (Bevan et al., 1987; Wood et al., 1988), albeit one report holds that the drug activates somata connected to both C- and A-fibers (Williams and Zieglgänsberger, 1982).

Taken together, capsaicin is selective in *stimulating* primary afferent C- and Aδ-fibers, although a few other A-fibers also might respond to the drug (Williams and Zieglgänsberger, 1982; Longhurst et al., 1984). However, not all C- and Aδ-fibers and not all sensory neuron somata connected to these fibers are sensitive to capsaicin. In terms of sensory receptors, it is nociceptors (polymodal nociceptors, silent nociceptors) and some warmth receptors that are stimulated by the drug. The capsaicin-sensitive nociceptors seem to be characterized by their particular responsiveness to a variety of algesic chemicals and may, therefore, be designated as chemonociceptors. Because there is indirect evidence that capsaicin-sensitive afferents might, in addition, be activated by innocuous chemical stimuli (for example, see MacLean, 1985; Amann and Lembeck, 1986; Raybould and Taché, 1988; South and Ritter, 1988; Forster et al., 1990), it is conceivable that sensitivity to capsaicin is a distinct trait of chemoceptive afferent neurons in general. This conjecture, however, requires direct experimental proof.

The selectivity of the excitatory action of capsaicin toward sensory neurons is best exemplified by the lack of a direct excitatory action on neurons other than primary afferents, the only exception to date being a group of thermosensitive neurons in the hypothalamus (Szolcsányi, 1982; Hori, 1984). Ventral roots of the spinal cord (Ault and Evans, 1980; Marsh et al., 1987), fibers of the optic nerve (Marsh et al., 1987), pre- and postganglionic sympathetic nerve fibers and sympathetic ganglion cells (Ault and Evans, 1980; Baccaglini and Hogan, 1983; Such and Jancsó, 1986; Bevan et al., 1987; Marsh et al., 1987; Wood et al., 1988), and cerebellar neurons (Salt and Hill, 1980) are not stimulated by capsaicin.

There is indirect evidence that neurons of the enteric nervous system are not activated either (Barthó and Szolcsányi, 1978; Barthó et al., 1982a; Holzer, 1984; Takaki and Nakayama, 1989). The excitatory actions of capsaicin seen, for example, in neurons of the enteric nervous system (Barthó and Szolcsányi, 1978; Takaki and Nakayama, 1989), in the spinal cord (Yanagisawa et al., 1980; Chung et al., 1985a; Urbán et al., 1985), in the medulla (Salt and Hill, 1980), and in certain areas of the brain (Rabe et al., 1980; Andoh et al., 1982; Braga et al., 1987; Zagami and Lambert, 1991) are considered to be secondary consequences of sensory neuron stimulation. This argument, however, is not conclusive in all instances and one cannot rule out that capsaicin is capable of activating certain nonsensory neurons. This uncertainty also applies to the finding that systemic capsaicin produces acute changes in the synthesis rate and levels of monoamines in substantia nigra, striatum, and hypothalamus (Hajós et al., 1986b) and in the rate of glucose

utilization in certain forebrain nuclei (Szikszay and London, 1988).

The acute actions of capsaicin, however, are not restricted to neurons (table 2), and there are a number of reports of capsaicin influencing nonneural systems. These *cell-nonselective* effects of capsaicin and capsaicin congeners include inhibition of cardiac muscle excitability (Zernig et al., 1984; Franco-Cereceda and Lundberg, 1988), inhibition of visceral smooth muscle activity (Szolcsányi and Barthó, 1978; Holzer and Lembeck, 1979; Barthó et al., 1982b, 1987; Maggi et al., 1987c, 1989b; Takaki et al., 1990), and contraction of vascular smooth muscle (Donnerer and Lembeck, 1982; Duckles, 1986; Saito et al., 1988; Bény et al., 1989; Edvinsson et al., 1990; Holzer et al., 1990b). Contradictory data suggest that capsaicin and related substances might either enhance (Juan et al., 1980; Moritoki et al., 1990) or inhibit (Flynn et al., 1986) the formation of prostanoids in vascular tissue of rabbits and humans, respectively, whereas no effect on prostanoid formation is observed in the rat gastric mucosa (Holzer et al., 1990a) and other tissues (Brand et al., 1990). In addition, capsaicin and its congeners have been reported to inhibit platelet aggregation (Wang et al., 1984, 1985; Brand et al., 1990) and to influence a variety of enzymatic activities (Chudapongse and Janthasoot, 1981; Ki et al., 1982; Miller et al., 1983; Negulesco et al., 1983; Modly et al., 1986; De and Ghosh, 1989; Srinivasan and Satyanarayana, 1989; Savitha et al., 1990; Yagi 1990) and other cell and tissue functions (Kenins et al., 1984; Nagabhushan and Bhide, 1985; Agarwal and Bhide, 1988; Gannett et al., 1988; Muralidhara and Narasimhamurthy, 1988; Lawson and Gannett, 1989; Knyazev et al., 1990; Matucci-Cerinic et al., 1990).

Importantly, many of the cell-nonselective effects of capsaicin are produced by, or were studied with, doses of the drug far in excess of those necessary to stimulate thin afferent neurons. For example, the sensory neuron-selective and cell-nonselective effects of the drug in the guinea pig ileum are separated by a dose ratio of 300 to 3000 (Szolcsányi and Barthó, 1978; Barthó et al., 1987). In addition, capsaicin's nonselective actions differ profoundly from its stimulant action on sensory neurons in that they are sustained, do not undergo desensitization, and are easily reproducible on reapplication of capsaicin (Szolcsányi and Barthó, 1978; Holzer and Lembeck, 1979; Barthó et al., 1982b, 1987; Maggi et al., 1987c, 1989b; Edvinsson et al., 1990).

3. Consequences of excitation: afferent and local effector roles of sensory neurons. It is beyond the scope of this article to discuss all the functional consequences of the capsaicin-induced stimulation of afferent neurons. Given the selectivity with which capsaicin stimulates thin afferent neurons, however, this drug was instrumental in providing evidence (Jancsó, 1960, 1981) for a "dual sensory-efferent function" (Szolcsányi, 1984b) of afferent neurons. Thus, excitation of nociceptive nerve fibers by either capsaicin or other stimuli is followed not only by conduction of nerve activity to the central nervous system but also by the release of transmitter substances from the activated peripheral nerve endings themselves. In addition, nerve activity may travel among the peripheral branches of sensory nerve fibers, and this process is thought to account for local "axon reflexes" such as the spreading flare (vasodilatation) around a focal injury of the skin (for reviews of this concept, see Szolcsányi, 1984b, 1988; Holzer, 1988; Maggi and Meli, 1988).

The *afferent function* of sensory neurons enables information to be transmitted to the central nervous system. Stimulation of afferent neurons by capsaicin gives rise to a painful sensation and activates protective reflexes including avoidance or escape reactions (Gamse, 1982; Fitzgerald, 1983; Russell and Burchiel, 1984; Buck and Burks, 1986) or sneezing, coughing, and bronchoconstriction in response to airway irritation (Lundberg and Saria, 1987). Other reflexes arising from capsaicin-induced stimulation of sensory neurons involve thermoregulatory (Rabe et al., 1980; Szikszay et al., 1982; Szolcsányi, 1982; Donnerer and Lembeck, 1983; Hayes et al., 1984b; Hori, 1984; de Vries and Blumberg, 1989; Szallasi and Blumberg, 1989), cardiovascular (Crayton et al., 1981; Donnerer and Lembeck, 1982; Jancsó and Such, 1983; Ordway and Longhurst, 1983; Longhurst et al., 1984; Szolcsányi et al., 1986; Amann et al., 1989a), and neuroendocrine (Mueller, 1981; Watanabe et al., 1988b) control mechanisms.

The local release of peptide mediators from peripheral sensory nerve endings enables these neurons to exert a *local effector function* (Holzer, 1988), because the released peptides influence a variety of local tissue functions (for reviews, see Szolcsányi, 1984b; Lundberg and Saria, 1987; Chahl, 1988; Holzer, 1988; Maggi and Meli, 1988; Nilsson, 1989; Donnerer et al., 1990). These include changes in local blood flow, vascular permeability, cardiac and smooth muscle activity, tissue growth and repair, immunological processes, and regulation of activity in postganglionic sympathetic efferents. Substance P, neurokinin A, calcitonin gene-related peptide, and vasoactive intestinal polypeptide are among the identified peptides released by capsaicin from peripheral endings of afferent neurons (Holzer, 1988; Maggi and Meli, 1988; Saria et al., 1988; Maggi et al., 1989d). However, capsaicin also releases substance P, somatostatin, and calcitonin gene-related peptide from their central endings in the spinal cord in vitro and in vivo (Gamse et al., 1979b, 1981a; Thériault et al., 1979; Yaksh et al., 1980; Helke et al., 1981b; Jhamandas et al., 1984; Saria et al., 1986). Substance P in neurons of the central (Gamse et al., 1979b; Helke et al., 1981b) or enteric (Holzer, 1984) nervous system is not released by capsaicin. Likewise, the release of neurotransmitters such as glutamic acid (Akagi et al., 1980; Singer et al., 1982), γ-aminobutyric acid, glycine

(Akagi et al., 1980), 5-hydroxytryptamine (Bergstrom et al., 1983), vasoactive intestinal polypeptide, cholecystokinin (Yaksh et al., 1982; Jhamandas et al., 1984), or bombesin (Moody et al., 1981) in the spinal cord is not affected by capsaicin.

C. Intermediate Effects of Capsaicin on Mammalian Sensory Neurons

1. Sensitization. Desensitization and blockade of nerve conduction are very common but not exclusive sequelae of the excitatory action of capsaicin on sensory neurons. Under certain conditions the opposite can be seen; excitation is followed by sensitization to thermal, mechanical, and chemical stimuli, particularly if low doses of capsaicin are administered repeatedly. There is only one report indicating that neuronal sensitivity to capsaicin can increase with repeated applications of the drug to the human tongue; paradoxically, a pause in the stimulation cycle gives rise to desensitization (Green, 1989). The capsaicin-induced sensitization of C-fiber polymodal nociceptors to thermal (Kenins, 1982; Konietzny and Hensel, 1983) and mechanical (Kenins, 1982) noxious stimuli is a more frequent observation, and sensitization appears to precede desensitization (Kenins, 1982). Sensitization could have a bearing on the capsaicin-induced hyperalgesia to thermal (Jancsó, 1960; Szolcsányi, 1977, 1990; Carpenter and Lynn, 1981; Green, 1986; Simone et al., 1987) and mechanical (Szolcsányi, 1977; Culp et al., 1989; Simone et al., 1989) stimuli, but it is not clear whether hyperalgesia is mediated by those nerve fibers that are desensitized to capsaicin. However, it is worth mentioning in this context that the mechanical hyperalgesia resulting from chronic paw inflammation in the rat is mediated by afferent neurons sensitive to capsaicin (Barthó et al., 1990).

2. Desensitization. A more typical feature of capsaicin-induced stimulation of primary afferent neurons is that excitation soon subsides and the neurons become unresponsive to further applications of the drug. Capsaicin desensitization has been observed both by recording the activity of sensory neurons and by examining the consequences of sensory neuron activation, e.g., neuropeptide release from peripheral and central terminals of afferent neurons, depolarization of spinal dorsal horn and ventral root neurons (Dickenson et al., 1990a,b), and behavioural (pain) reactions. The rapidity and extent with which desensitization to capsaicin develops is related to the dose of, and the time of exposure to, capsaicin and the time interval between consecutive dosings (Szolcsányi et al., 1975; Szolcsányi and Jancsó-Gábor, 1976; Szolcsányi, 1977, 1987; Barthó and Szolcsányi, 1978; Foster and Ramage, 1981; Kaufman et al., 1982; Zernig et al., 1984; He et al., 1988, 1990; Maggi et al., 1988f, 1990a; Dray et al., 1989a,b, 1990b; Amann, 1990; Winter et al., 1990).

With low suprathreshold doses of capsaicin given at appropriate time intervals, desensitization does not necessarily take place so that excitation of thin sensory neurons (Kaufman et al., 1982; Kenins, 1982; Longhurst et al., 1984; Szolcsányi, 1987; Szolcsányi et al., 1988), pain reactions (Szolcsányi et al., 1975; Szolcsányi and Jancsó-Gábor, 1976; Szolcsányi, 1977; Green, 1989; Dray et al., 1989a, 1990b), cardiovascular responses (Longhurst et al., 1980; Donnerer and Lembeck, 1982; Szolcsányi et al., 1986; Amann et al., 1989a), and peptide release from peripheral sensory nerve endings (Dray et al., 1989b; Amann, 1990) can be reproduced with each application of the drug. With higher doses of capsaicin or prolonged exposure to the drug, however, desensitization ensues and consecutive applications of capsaicin become less effective or fail to produce any effect.

Whereas desensitization to comparatively low doses of capsaicin may be specific for this drug and its congeners (Szolcsányi, 1977; Bernstein et al., 1981; Dray et al., 1989a,b, 1990b,c; Winter et al., 1990), desensitization to higher doses of the drug also is associated with a loss of responsiveness to other chemical (Jancsó, 1960; Szolcsányi, 1977, 1987; Foster and Ramage, 1981; Kenins, 1982; Jancsó and Such, 1983; Ueda et al., 1984; Geppetti et al., 1988c; Dray et al., 1989a, 1990b; He et al., 1990; Lang et al., 1990), warmth (Szolcsányi, 1977, 1983a, 1987; Foster and Ramage, 1981; Kenins, 1982; Williams and Zieglgänsberger, 1982), and noxious (high-threshold) mechanical stimuli (Kenins, 1982; Szolcsányi, 1987; He et al., 1990; Lang et al., 1990) and to potassium depolarization (Saria et al., 1983a; Dray et al., 1989b; Amann, 1990; Donnerer and Amann, 1990; Maggi et al., 1990b). Specific taste chemoreceptors, cold receptors, and low-threshold mechanoreceptors are not inhibited by desensitization to capsaicin (Szolcsányi, 1977, 1990; Dray et al., 1989b, 1990b). Thus, only thin sensory neurons appear to be rendered insensitive to capsaicin and their other sensory modalities.

Nonspecific desensitization to capsaicin is probably the first manifestation of the long-term neurotoxic action of the drug on sensory neurons, the adjective long-term referring to a time scale of several weeks to months. The frequent use of the term desensitization to denote chronic defunctionalization of sensory neurons has created some confusion because it is at variance with the meaning of desensitization in pharmacology. The problem relates to the fact that most, if not all, long-term neurotoxic effects of capsaicin involve morphological changes, whereas the pharmacological term desensitization implies a transient functional refractoriness in the absence of long-lasting morphological or other toxic changes. Although in the present article I attempt to use the term desensitization in this latter sense only, it is not possible in many cases to clearly distinguish between desensitization and neurotoxicity because the reversibility of defunctionalization and a possible association with morphological alterations have not been examined.

The duration of the desensitization to capsaicin ap-

pears to be a matter of a few hours to a few days. It is not known whether there is any temporal relationship between desensitization and the presence of capsaicin in the tissue. In the rat, topical administration of approximately 100 μM capsaicin is necessary to make the cornea insensitive to chemical noxious stimuli for at least 2 h (Szolcsányi et al., 1975; Szolcsányi and Jancsó-Gábor, 1976). Capsaicin-induced desensitization of a C-fiber warmth receptor also was noted to last for at least 2 h (Szolcsányi, 1983a). When given systemically, 1 to 400 mg/kg capsaicin are able to reduce cutaneous sensitivity to chemical (Szolcsányi et al., 1975; Hayes et al., 1981b), mechanical (Hayes et al., 1981b, 1984b), and thermal noxious stimuli (Hayes et al., 1981b; Gamse, 1982; Bittner and LaHann, 1985; Szolcsányi, 1990) for a few hours up to several days. Likewise, desensitization of the human tongue to low concentrations of capsaicin lasts several hours but disappears within a day (Szolcsányi, 1977; Green, 1989). It is not possible, however, to deduce from these behavioural data whether the time course of the antinociceptive effects of capsaicin reflects that of nociceptor desensitization. Antinociceptive doses of capsaicin and the related compound, olvanil, produce a selective reduction in the responses of dorsal horn neurons to peripheral C- and Aδ-fiber stimulation, and there is circumstantial evidence that capsaicin/olvanil-induced antinociception may be due to inhibition of transmission from afferent nerve terminals in the spinal cord rather than defunctionalization of the peripheral axons (Dickenson et al., 1990a,b).

3. Blockade of nerve conduction. Within a few minutes from the application of capsaicin to axons of sensory neurons, nerve conduction through the treated segment is blocked (Petsche et al., 1983; Pini, 1983; Handwerker et al., 1984; Lynn et al., 1984; Baranowski et al., 1986; Such and Jancsó, 1986; Marsh et al., 1987; Waddell and Lawson, 1989; Brugger et al., 1990). Capsaicin inhibits conduction in most, but not all, afferent C-fibers of the rat coccygeal, saphenous, sciatic, sural, and vagus nerve and of the monkey sural nerve, polymodal C-fiber nociceptors being most often affected (Petsche et al., 1983; Welk et al., 1983; Lynn et al., 1984; Chung et al., 1985a; Waddell and Lawson, 1989). Nerve conduction in the dorsal roots of the rat is blocked as well (Brugger et al., 1990). Except in the ferret in which perineural capsaicin blocks C-fibers only (Baranowski et al., 1986), the conduction in afferent A-fibers of the rat, rabbit, guinea pig, and monkey also is reduced to a minor degree (Pini, 1983; Lynn et al., 1984; Chung et al., 1985a; Baranowski et al., 1986; Such and Jancsó, 1986; Marsh et al., 1987).

The conduction block in the A-fibers is primarily due to a block of Aδ-fibers, although some fast-conducting Aαβ-fibers can be affected as well. The potency of capsaicin in blocking C- and Aδ-fibers, however, is considerably higher than that for fast-conducting A-fibers (Baranowski et al., 1986; Marsh et al., 1987; Waddell and Lawson, 1989). Although the block of Aαβ-fibers is fully reversible within 1 h, the C-fibers show only partial, if any, recovery during the first 2 h after capsaicin application. The duration of the C-fiber block at the site of capsaicin application appears to last up to 3 days (Jancsó and Such, 1983; Welk et al., 1983; Lynn et al., 1984). In the rat vagus nerve the C-fiber conduction block is composed of two components, one that is reversible within 90 min and another one that is produced by perineural concentrations of capsaicin of >1 μM that is not reversible within this time frame (Waddell and Lawson, 1989). There are also species differences in that C-fibers in the saphenous nerve of the guinea pig and rabbit are less sensitive to capsaicin than those in the rat, and full recovery from the conduction block produced by as much as 33 mM capsaicin takes place within 1 h (Baranowski et al., 1986).

Nerve conduction in sympathetic efferent fibers, ventral roots of the spinal cord, or fibers of the optic nerve is not altered (Handwerker et al., 1984; Marsh et al., 1987) or only temporarily reduced (Such and Jancsó, 1986). The latter observation could either represent an example of the cell-nonselective effects of capsaicin or partly be due to the use of 10% ethanol in the vehicle which, by itself, can block nerve conduction (Wall and Fitzgerald, 1981). The use of paraffin or olive oil (Petsche et al., 1983; Pini, 1983) avoids this element of vehicle interaction.

D. Long-term Neurotoxic Effects of Capsaicin on Mammalian Sensory Neurons

1. Effects of systemic capsaicin in newborn mammals.

a. RAT. **i. Morphological changes.** It had long been assumed that the persistent inhibition of sensory neuron functions produced by systemic administration of relatively large doses of capsaicin to adult rats merely reflected a sustained defunctionalization of these neurons. In 1977, however, G. Jancsó and his associates (1977) reported that a subcutaneous dose of 50 mg/kg capsaicin given to newborn rats caused a lifelong degeneration of B-type primary afferent neurons. This degeneration takes place within 30 min, is permanent, and involves most B-type neurons and their peripheral and central nerve processes (Jancsó et al., 1977; Jancsó and Király, 1980, 1981; Nagy et al., 1980, 1983; Nagy and Hunt, 1983; Holje et al., 1983; Dinh and Ritter, 1987).

The neurotoxic effect of neonatal capsaicin is dose dependent; the threshold dose of the drug in inducing degeneration of unmyelinated dorsal root fibers and axon terminals in the spinal cord lies between 5 and 15 mg/kg (Jancsó and Király, 1981; Nagy et al. 1983; Jancsó, 1984). The dose of 50 mg/kg capsaicin, which now is most widely used, is thought to cause maximal degeneration of unmyelinated afferent neurons. The loss of unmyelinated fibers from the rat saphenous, inferior alveolar, and mental nerves ranges from 40% (Lynn, 1984), 50% (Welk et al., 1984), 58% (Holje et al., 1983),

and 64% (Scadding, 1980) to 67% (Jancsó et al., 1977, 1980a). This variability in nerve fiber loss is likely to be due to regional differences in the proportion of unmyelinated afferent/efferent nerve fibers in mixed nerves. Furthermore, the proportion of B-/A-type cell bodies in the dorsal root ganglia shows some regional variation (Lawson et al., 1984), which implies a regional difference in the relative proportion of afferent neurons that are sensitive to the neurodegenerative action of capsaicin (Doucette et al., 1987). In addition, the number of degenerated axons also can differ within different branches of a nerve. Thus, although the number of unmyelinated fibers in the inferior alveolar and mental nerves of the rat is decreased by approximately 58% (Holje et al., 1983), no changes are found in the pulpal nerves (Holje et al., 1983; Fried et al., 1988).

The loss of thin afferent nerve fibers is associated with the degeneration of cell bodies from the spinal and cranial sensory ganglia (Jancsó et al., 1977; Lawson and Nickels, 1980; Otten et al., 1983; Lawson and Harper, 1984; McDougal et al., 1985; Arvidsson and Ygge, 1986). The loss of somata from the ganglia ranges between 28% (McDougal et al., 1985), 43% (Arvidsson and Ygge, 1986), and 44% (Otten et al., 1983). Although it is primarily the small dark somata that are destroyed, there also is some degeneration of large light A-type somata, especially if doses of capsaicin >50 mg/kg are used (Lawson and Harper, 1984). A major loss of afferent neurons also is demonstrated by a marked reduction in the number of dorsal root ganglion cells that are labeled by retrograde transport of horseradish peroxidase injected into the urinary bladder wall (Jancsó and Maggi, 1987). In the spinal cord, transganglionic labeling is totally absent in the central termination areas of primary afferent neurons supplying the urinary bladder (Jancsó and Maggi, 1987). Similarly, the number of trigeminal ganglion cells, which are labeled retrogradely by a fluorescent dye applied to the cornea, is diminished by 87% (Ogilvy et al., 1991).

In the dorsal roots 72% (Arvidsson and Ygge, 1986) to 95% (Lawson and Nickels, 1980; Nagy et al., 1981b, 1983; Holje et al., 1983) of all unmyelinated fibers are lost. The number of myelinated Aδ-fibers is either unchanged (Scadding, 1980; Nagy et al., 1981b; Arvidsson and Ygge, 1986) or reduced by 10% (Jancsó et al., 1980a) to 40% (Lawson and Nickels, 1980; Nagy and Hunt, 1983; Nagy et al., 1983) particularly if doses >50 mg/kg capsaicin are used. Conversely, doses of only 10 to 25 mg/kg lead to a selective loss of up to 95% of all unmyelinated afferent nerve fibers without affecting myelinated fibers (Nagy et al., 1983). Consistent with these figures, a 93% loss of type I synaptic glomeruli, thought to be terminations of unmyelinated afferent fibers, is observed in the dorsal horn of the rat spinal cord (Ribeiro-da-Silva and Coimbra, 1984). These glomeruli could be related to low-density synaptosomes containing substance P (and probably other neuropeptides), which also are absent from the dorsal spinal cord following neonatal capsaicin treatment (Bucsics et al., 1984). Axon terminal degeneration is seen in all areas of the spinal cord and brainstem which are known to receive primary afferent C-fiber input (Jancsó et al., 1977; Jancsó and Király, 1980, 1981; Nagy et al., 1980; Dinh and Ritter, 1987).

ii. Neurochemical and histochemical changes. The morphological ablation of sensory neurons produced by capsaicin in the newborn rat is reflected by neuro- and histochemical as well as functional deficits. Neuro- and histochemically, capsaicin leads to a depletion of markers associated with thin primary afferent neurons. This depletion of markers is seen in sensory ganglia, in nerves containing afferent fibers, and in tissues innervated by these fibers in the periphery and in the terminal regions of the spinal cord and brainstem. Substance P was the first marker found to be depleted by capsaicin treatment of newborn rats (Gamse et al., 1980; Nagy et al., 1980; Holzer et al., 1982), the depletion being permanent because no recovery is seen within 9 months (Gamse et al. 1981b). Accordingly, the synthesis of substance P, as measured by the incorporation of [^{35}S] methionine, is reduced by 80 to 90% in tissue cultures of dorsal root ganglia taken from rats treated with capsaicin while neonates (Harmar et al., 1981), indicating that the majority of substance P-containing afferent neurons are ablated by neonatal capsaicin.

Calcitonin gene-related peptide also is depleted from the dorsal horn of the spinal cord 10 days after capsaicin treatment of newborn rats (Hammond and Ruda, 1989). There is some controversy, however, relating to the persistence of depletion in the spinal cord. Two reports hold that calcitonin gene-related peptide, in contrast to substance P, can be replenished over a posttreatment period of 6 to 16 weeks to near-normal levels in the spinal cord (Diez Guerra et al., 1988; Hammond and Ruda, 1989) although the cellular source of peptide replenishment is unknown. This observation may be explained by considering that in the spinal cord chronic deafferentation can lead to sprouting and synaptogenesis of surviving primary afferent nerve fibers containing calcitonin gene-related peptide (McNeill et al., 1990). Other reports, however, show that the depletion of calcitonin gene-related peptide in spinal cord (Skofitsch and Jacobowitz, 1985b; Carr et al., 1990; Marlier et al., 1990; Pohl et al., 1990), sensory ganglia, and peripheral targets of sensory neurons (Skofitsch and Jacobowitz, 1985b; Franco-Cereceda et al., 1987b; Sternini et al., 1987; Su et al., 1987; Diez Guerra et al., 1988; Geppetti et al., 1988a; Varro et al., 1988; Carr et al., 1990; Pohl et al., 1990) persists. It has also been found that neonatal capsaicin seems to deplete more calcitonin gene-related peptide from visceral than from somatic sensory pathways (Kashiba et al., 1990a), but it is not known whether this factor contributes to the variability in the findings with calcitonin gene-related peptide.

A list of markers of afferent neurons, including the substance P-related peptide neurokinin A, dynorphin, leucine-enkephalin, galanin, somatostatin, and vasoactive intestinal polypeptide, all of which can be depleted by neonatal capsaicin to varying degrees, is given in table 1. The chemical coding of afferent neurons is heterogeneous in that the markers listed in table 1 are not present in all afferent neurons, and there are differences in the combinations of coexisting markers both within and across different species (Costa et al., 1986; Gibbins et al., 1987; Weihe, 1990). As a further limitation, many of the neurochemical markers of capsaicin-sensitive afferent neurons also occur in afferent and nonafferent neurons that are not sensitive to capsaicin.

The capsaicin-induced depletion of peptides and other markers from sensory neurons (table 1) and the classification of capsaicin-sensitive afferent neurons according to different patterns of chemical coding have been summarized in other reviews (Marley and Livett, 1985; Buck and Burks, 1986). In addition to the markers referred to in table 1, large light A-type sensory neurons are characterized by the selective presence of a neurofilament protein that is absent in the small dark B-type neurons (Lawson and Harper, 1984; Kai-Kai et al., 1986). Although the majority of capsaicin-sensitive afferent neurons fall within the group of neurofilament-negative B-type neurons (Lawson and Harper, 1984; Kai-Kai et al., 1986), some of the neurofilament-containing A-type neurons also are destroyed by neonatal capsaicin, at least at doses >50 mg/kg (Lawson and Harper, 1984). Furthermore, capsaicin treatment of newborn rats leads to inhibition of the axoplasmic transport of organelle-specific enzymes and the retrograde transport of nerve growth factor in sensory, but not sympathetic, nerves of adult animals (McDougal et al., 1983). These changes can be explained by a deficit of thin primary afferent nerve fibers.

iii. Functional changes. As might be expected from the morphological ablation of afferent neurons, capsaicin treatment of newborn rats is associated with permanent sensory and functional deficits which involve both the afferent and local effector functions of sensory neurons (Nagy, 1982; Szolcsányi, 1982, 1990; Jancsó, 1984; Russell and Burchiel, 1984; Buck and Burks, 1986; Maggi and Meli, 1986, 1988; Lembeck, 1987b, 1988; Lundberg and Saria, 1987; Chahl, 1988; Holzer, 1988; Donnerer et al., 1990; Maggi, 1990, 1991). The activity of the drug to induce neuronal degeneration correlates well with its ability to inhibit the function of fine afferent neurons (Jancsó, 1984). The many functional changes brought about by neonatal capsaicin are not detailed here except that some salient features are discussed.

Capsaicin treatment of newborn rats has been used widely to explore the functional implications of capsaicin-sensitive afferent neurons, this approach being based on the assumption that capsaicin affects thin sensory neurons only. A number of local effector functions including vasodilatation, increase in vascular permeability, changes in the activity of cardiac, bronchial, and visceral muscle, and changes in the activity of the immune system (Lembeck and Holzer, 1979; Morton and Chahl, 1980; Saria, 1984; Lundberg and Saria, 1987; Ahlstedt et al., 1986; Helme et al., 1987; Chahl, 1988; Holzer, 1988; Maggi and Meli, 1988; Nilsson, 1989; Donnerer et al., 1990; Maggi, 1990) are inhibited or abolished by neonatal capsaicin. The vulnerability of the gastric mucosa is enhanced (Holzer and Sametz, 1986), and the occurrence and persistence of lesions in skin and cornea of capsaicin-treated animals are seen to reflect a trophic role of sensory nerve endings in these tissues (Gamse et al., 1981b; Shimizu et al., 1984; Kjartansson et al., 1987; Maggi et al., 1987a; Ogilvy et al., 1991).

Afferent functions that are impaired by neonatal capsaicin comprise warmth reception and thermoregulation (Hori and Tsuzuki, 1981; Donnerer and Lembeck, 1983; Dib, 1983; Hajós et al., 1983, 1986a; Obál et al., 1983; Jancsó, 1984), cardiovascular reflexes (Bond et al., 1982; Lembeck and Skofitsch, 1982; Lorez et al., 1983; Bennett and Gardiner, 1984; Donnerer et al., 1989), visceral reflexes (Cervero and McRitchie, 1982; Sharkey et al., 1983; Santicioli et al., 1985; Holzer et al., 1986), neuroendocrine reflexes (Traurig et al., 1984a,b; Amann and Lembeck, 1986, 1987; Bennett and Gardiner, 1986; Donnerer et al., 1989; Donnerer and Lembeck, 1990), and satiety (MacLean, 1985). These changes are likely to be related to ablation of unmyelinated afferent neurons and to inhibition of slow synaptic transmission in the dorsal spinal cord (Urbán et al., 1985).

In keeping with the sensory modalities of capsaicin-sensitive afferent neurons there also are changes in nociception and nociception-associated avoidance and escape reactions. Perception of chemical noxious stimuli is permanently inhibited by neonatal capsaicin (Jancsó et al., 1977, 1980a; Faulkner and Growcott, 1980; Gamse et al., 1980; Hayes et al., 1981a; Gamse, 1982; Saumet and Duclaux, 1982; Jancsó, 1984), but there is disagreement as to whether mechano- and thermonociception also are impaired (Holzer et al., 1979; Faulkner and Growcott, 1980; Jancsó and Jancsó-Gábor, 1980; Nagy et al., 1980; Cervero and McRitchie, 1981; Hayes et al., 1981a; Gamse, 1982; Saumet and Duclaux, 1982; Jancsó, 1984; Doucette et al., 1987; Hammond and Ruda, 1989; Ogilvy et al., 1991). The discrepant results are probably due to several factors including the use of different strains of rats, age-related changes in capsaicin-induced antinociception (Hammond and Ruda, 1989), intrastrain variability in the sensitivity to capsaicin (Nagy and van der Koy, 1983), and differences in the experimental protocols and procedures. Thus, tests of nociception that use suprathreshold or even supramaximal strengths of stimuli are likely to give different results than tests that use threshold stimuli (Szolcsányi, 1985). In addition, regional dif-

ferences in the proportion of afferent nerve fibers sensitive to capsaicin (Holje et al., 1983; Doucette et al., 1987; Fried et al., 1988) are likely to account for differences in capsaicin-induced antinociception in different skin areas (Doucette et al., 1987).

iv. Secondary changes in sensory pathways. The lack of a consistent effect of neonatal capsaicin on mechanical and thermal nociception is a paradox in view of the permanent loss of unmyelinated afferent neurons caused by this treatment. This paradox probably reflects one of the many aspects of secondary changes in sensory pathways that occur in response to ablation of primary afferent neurons by neonatal capsaicin. Hence, functional alterations seen in adult rats treated with capsaicin as neonates cannot unequivocally be used to draw straightforward conclusions as to specific functional implications of capsaicin-sensitive afferent neurons. Given the plasticity of the nervous system in the newborn rat, degeneration of the majority of unmyelinated primary afferent neurons is likely to have a significant impact on afferent nerves themselves, on second- and higher-order afferent neurons and related systems in the central nervous system, and on systems associated with the peripheral endings of sensory neurons.

Reorganization of the primary afferent system is indicated by the findings that the loss of small sensory ganglion cells and unmyelinated afferent nerve fibers is associated with a numerical increase of dorsal root ganglion cells which are of intermediate size and stain for both peripherin and neurofilament triplet (Ferri et al., 1990) and by changes in the diameter of the remaining populations of sensory ganglion cells (McDougal et al., 1985; Ferri et al., 1990). Although the number of unmyelinated fibers is diminished by about 40 to 50% in afferent nerves of adult rats treated with capsaicin as neonates (Lynn, 1984; Welk et al., 1984), there is no change in the proportions of the C-fiber receptor types (Lynn, 1984; Welk et al., 1984; Cervero and Sharkey, 1988). Accordingly, application of capsaicin to afferent nerves of capsaicin-treated rats produces excitation of (Szolcsányi, 1990), and a conduction block in, (Welk et al., 1984) C-fiber polymodal nociceptors, the magnitude of these effects being similar to that found in control rats. These data indicate that adult rats treated with capsaicin as neonates possess capsaicin-sensitive afferent neurons; yet it is not known whether these neurons escaped or survived the neonatal capsaicin treatment or evolved at a later stage. In the cornea, sprouting of the surviving sensory fibers occurs to the extent that the density of nerve fibers in the epithelium is considerably higher than in control animals, although the overall innervation of the cornea by trigeminal afferent fibers is greatly diminished (Ogilvy et al., 1991). Sprouting of calcitonin gene-related peptide-containing afferent neurons may also take place in the spinal cord of rats treated with capsaicin at birth (Diez Guerra et al., 1988; Hammond and Ruda, 1989; McNeill et al., 1990). This may explain why capsaicin is able to release some calcitonin gene-related peptide and neurokinin A into the blood stream of adult rats treated with capsaicin as neonates (Diez Guerra et al., 1988).

In the central nervous system, the organization of sensory pathways in the spinal cord and nucleus gracilis (Nagy and Hunt, 1983; Réthelyi et al., 1986; Shortland et al., 1990), of the spinothalamic tract (Saporta, 1986), and of the somatotopic maps in the cerebral cortex (Wall et al., 1982a) is profoundly altered by neonatal capsaicin treatment. Reorganization of 5-hydroxytryptamine-containing nerve fibers also occurs in the spinal cord (Marlier et al., 1990). As a consequence, the processing of sensory information in the spinal cord (Wall, 1982; Wall et al., 1982b; Cervero et al., 1984; Cervero and Plenderleith, 1987) and brainstem (Salt et al., 1982) shows marked differences when compared with vehicle-treated controls.

In the periphery, neonatal capsaicin treatment can lead to permanent changes in the morphology of the cornea (Shimizu et al., 1984; Ogilvy et al., 1991; but see also Knyazev et al., 1990) and lung (Ahlstedt et al., 1986) and to increased tissue concentrations of histamine and 5-hydroxytryptamine in the skin and lung (Holzer et al., 1981). The responsiveness of blood vessels (Jancsó et al., 1980a; Jancsó, 1984) and mast cells (Skofitsch et al., 1983) to substance P, bradykinin, 5-hydroxytryptamine, and histamine may be altered permanently. A direct relationship of these changes to sensory neuron ablation is not clear. This also holds true for the transformation of fast muscle fibers to slow muscle fibers in the sternohyoid superior muscle of the rat (Müntener, 1985) and to some ultrastructural alterations in the gastrointestinal mucosa (Pfeiffer and Evangelista, 1991), although biochemical indices of the intestinal endocrine mucosa and brush border remain unchanged (McGregor and Conlon, 1991).

An intriguing observation is the existence of an apparent reciprocal relationship between the peripheral endings of sympathetic and sensory neurons. Capsaicin-induced long-term elimination of afferent neurons results in an increase in the transmitter content and innervation density of sympathetic nerve endings, whereas long-term ablation of sympathetic neurons is followed by an increase in the sensory innervation (Terenghi et al., 1986; Nielsch and Keen, 1987; Luthman et al., 1989; Aberdeen et al., 1990). One group (Aberdeen et al., 1990) holds that following sympathectomy in the neonate with guanethidine only calcitonin gene-related peptide-containing sensory neurons react with increased transmitter levels and innervation density, whereas no such changes are seen with sensory neurons containing substance P or vasoactive intestinal polypeptide. Whether these reciprocal changes arise from a competition of sensory and sympathetic neurons for nerve growth factor, in which

case elimination of one nerve population might increase the availability of nerve growth factor for the other nerve population (Terenghi et al., 1986; Nielsch and Keen, 1987; Luthman et al., 1989; Aberdeen et al., 1990), is in need of experimental verification.

v. Selectivity of the action of capsaicin. There is no doubt that the primary target of the neurodegenerative action of capsaicin in the newborn rat is a group of fine primary afferent neurons. This inference is corroborated by the findings that, with the exception of some myelinated Aδ-fibers, other primary afferent as well as efferent motor and autonomic neurons are not affected by capsaicin. Thus, myelinated afferent neurons such as muscle stretch receptors (Soukup and Jancsó, 1987) and the unmyelinated efferent fibers of the sympathetic (Jancsó et al., 1980a; Cervero and McRitchie, 1982; Takano et al., 1988) and parasympathetic (Sharkey et al., 1983) nervous systems are not damaged by neonatal capsaicin treatment. Whether all capsaicin-induced alterations of sensory pathway-related systems are secondary to the degeneration of primary afferent neurons is not yet clear. The possibility, therefore, remains that capsaicin also has some direct effects on neuronal and nonneuronal systems other than primary afferent neurons. However, most neurons of the central nervous system are not susceptible to the neurotoxic effect of neonatal capsaicin, although this is not true for all central neurons.

The capsaicin-induced degeneration of axon terminals in the dorsal horn of the spinal cord and in the brain stem (Jancsó et al., 1977; Jancsó, 1978; Jancsó and Király, 1980, 1981; Dinh and Ritter, 1987) is considered to reflect degeneration of the central endings of primary afferent neurons only, because markers of thick afferent as well as of spinal and brainstem neurons are not depleted by capsaicin. Thus, the tissue levels of glutamic acid, glutamic acid decarboxylase, γ-aminobutyric acid, glycine, glycine receptors, aspartic acid, taurine, choline acetyltransferase, noradrenaline, 5-hydroxytryptamine, and histamine are not decreased in the dorsal horn of the spinal cord of adult rats treated with capsaicin as neonates (Nagy et al., 1980; Singer and Placheta, 1980; Holzer et al., 1981; Jancsó et al., 1981; Singer et al., 1982; Hajós et al., 1986b; Holzer-Petsche et al., 1986). In one study even an increase in the 5-hydroxytryptamine and histamine content of the dorsal spinal cord was found (Holzer et al., 1981), whereas these changes were not seen in another study (Hajós et al., 1986b). Furthermore, the levels of substance P, somatostatin, and other neuropeptides found both in primary afferent and central neurons are not altered in the ventral spinal cord and in regions of the brain above the brainstem (Gamse et al., 1980, 1981b; Nagy et al., 1980; Helke et al., 1981a; Jancsó et al., 1981; Priestley et al., 1982; Panerai et al., 1983). Likewise, neuropeptides not associated with sensory neurons such as neurotensin and methionine-enkephalin remain unaffected throughout the central nervous system (Gamse et al., 1981b; Jancsó et al., 1981; Priestley et al., 1982; Singer et al., 1982; Panerai et al., 1983). There is, however, evidence that some neurons in the forebrain and retina degenerate following pre- or neonatal capsaicin treatment (Dinh and Ritter, 1987; Ritter and Dinh, 1990) and that β-endorphin is permanently depleted from the hypothalamus but not from other regions of the brain (Panerai et al., 1983). Although these observations are in need of further confirmation, they do throw some doubt on the exclusive selectivity of capsaicin for primary afferent neurons.

The unmyelinated neurons of the enteric nervous system seem to be insensitive to the neurotoxic effect of neonatal capsaicin insofar as gastrointestinal tissue levels of peptides associated with the enteric nervous system such as substance P and calcitonin gene-related peptide do not change (Holzer et al., 1980; Geppetti et al., 1988a), except in the upper gastrointestinal tract where sensory nerve endings contribute significantly to the tissue content of these peptides (Sharkey et al., 1984; Sternini et al., 1987; Su et al., 1987; Green and Dockray, 1988; Geppetti et al., 1988a). The tissue levels of vasoactive intestinal polypeptide in the gut remain unchanged after neonatal capsaicin treatment (McGregor and Conlon, 1991), but an immunohistochemical study has shown that, although this peptide is depleted from fibers, but not somata, of submucosal neurons, a lactoseries carbohydrate antigen colocalized with the peptide is lost from both fibers and somata (Kirchgessner et al., 1988). This finding suggests that capsaicin might have some neurotoxic effect on certain enteric nerve fibers, but further proof of such a target of the drug is needed.

b. OTHER MAMMALS. The neuropharmacological effects of neonatal capsaicin treatment have been studied most extensively in the rat, but there is some information that treatment of newborn mice (Scadding, 1980; Jancsó et al., 1985a; Hiura and Sakamoto, 1987b; Hiura and Ishizuka, 1989; Hiura et al., 1990b) and dogs (Jancsó et al., 1985a) also causes degeneration of small- to medium-sized afferent neurons.

In the lumbar dorsal root ganglia of the newborn mouse, 50 mg/kg capsaicin leads to rapid degeneration of a proportion of B-type somata sending unmyelinated fibers into the dorsal roots (Hiura and Ishizuka, 1989). Later, ultrastructural and degenerative changes also take place in A-type somata with myelinated fibers. This sequence of events is interpreted to indicate that degeneration of A-type somata is not due to a direct action of capsaicin on these neurons, however this contention needs to be proven. Doses of 50 to 150 mg/kg capsaicin cause a 41 to 75% reduction of unmyelinated fibers and a 6 to 12% loss of myelinated fibers in the dorsal roots; the number of small dorsal root ganglion cell bodies decreases by 51 to 77% and that of large somata by 14 to 52% (Hiura and Sakamoto, 1987b). These degenera-

tive changes are accompanied by a 50% loss of unmyelinated fibers from the sural nerve, whereas the number of myelinated fibers stays the same in response to 50 mg/kg capsaicin given to newborn mice (Scadding, 1980). A loss of nerve axons also is seen in the cornea (Fujita et al., 1984) and in the superficial dorsal horn (Hiura et al., 1990b) of the mouse. The neurotoxic action of capsaicin in the newborn mouse is associated with depletion of substance P from the dorsal spinal cord and cornea and with deficits in chemo- but not thermonociception (Hayes et al., 1981a; Gamse, 1982; Keen et al., 1982). Vascularization of, and lesions in, the cornea (Keen et al., 1982; Shimizu et al., 1984, 1987; Fujita et al., 1984) and changes of the somatotopic maps in the cerebral cortex (Wall et al., 1982a) are considered to represent changes secondary to the removal of sensory neurons. The gustatory afferent and parasympathetic efferent nerve fibers in the chorda tympani are not affected by 50 mg/kg capsaicin given to newborn mice (Hiura et al., 1990a).

Administration of 70 mg/kg capsaicin to newborn rabbits fails to cause any long-term depletion of substance P from the spinal cord and eye, although the miotic and hyperaemic effects of acute intracameral injection of capsaicin are inhibited (Tervo, 1981). No morphological study of the neurotoxic effect of capsaicin in the newborn rabbit has yet been carried out. In contrast, administration of 200 mg/kg capsaicin to newborn cats has been found to induce neuronal degeneration in the small intestine when examined 24 to 72 h later (Fehér and Vajda, 1982). Degenerating axons were seen in the myenteric and submucosal plexuses, and a few cell bodies in the two plexuses also were severely damaged. Whether this observation points to degeneration of enteric neurons awaits further verification, particularly because no further analysis of the effect of capsaicin on primary afferent neurons of the newborn cat has been made.

2. Effects of systemic capsaicin in adult mammals. a. RAT. **i. Morphological changes compared with those produced by neonatal capsaicin.** In the rat, unmyelinated primary afferent neurons are particularly sensitive to the neurotoxic action of capsaicin at the age of 1 to 12 days, whereas when capsaicin is given at the age of 14 days or more no major degeneration of nerve fibers is noticeable (Jancsó and Király, 1981). Accordingly, capsaicin treatment of 2- to 10-day-old rats leads to a permanent depletion of substance P from primary afferent pathways, a permanent deficit in chemonociception, and a permanent absence of sensory nerve-mediated increases in vascular permeability (Jancsó et al., 1977, 1980a; Gamse et al., 1980; Nagy et al., 1980). In contrast, treatment of 20-day-old or adult rats reversibly reduces chemonociception (Jancsó, 1960), sensory nerve-mediated increases in vascular permeability (Jancsó et al., 1977), and substance P levels in sensory pathways (Gamse et al., 1980). Thus, there is a critical period during the ontogeny of unmyelinated primary afferent neurons during which they are especially vulnerable to capsaicin. However, capsaicin can have a long-lasting sensory neuron-blocking effect also when it is given to adult rats (Jancsó et al., 1967), and some functional deficits produced by capsaicin in the adult rat are virtually irreversible (Jancsó-Gábor et al., 1970; Szolcsányi et al., 1975). Adult rats given capsaicin show recovery which may take several weeks to several months (Szolcsányi and Jancsó-Gábor, 1976; Jancsó et al., 1977; Gamse et al., 1980, 1981b; Gamse, 1982; Bittner and LaHann, 1985; Maggi et al., 1987d; South and Ritter, 1988; Gardiner et al., 1989; Szallasi et al., 1989).

Ultrastructurally, subcutaneous administration of 35 to 300 mg/kg capsaicin to adult rats causes swelling of mitochondria in B-type sensory neurons, but not in A-type afferent or sympathetic efferent neurons, when examined 1 to 60 days posttreatment (Joó et al., 1969; Szolcsányi et al., 1975; Chiba et al., 1986). Although destruction of cell bodies was not identified in these studies, G. Jancsó and his associates found that within a few hours after systemic administration of similar capsaicin doses a proportion of the B-type somata exhibit severe ultrastructural changes thought to reflect degeneration (Jancsó et al., 1985b). The effect of capsaicin is dose dependent; the dose of 100 mg/kg capsaicin is maximally effective and damages 17% of the B-type cell bodies in the dorsal root ganglia. In addition, degenerating axon terminals are seen in those areas of the spinal cord and brainstem that are known to be the central projection areas of thin primary afferent neurons (Jancsó et al., 1985b; Jancsó and Maggi, 1987; Ritter and Dinh, 1988). Six days after capsaicin treatment, the number of unmyelinated fibers in the rat saphenous nerve is diminished by 45%, whereas the number of myelinated fibers is reduced by only 16% (Jancsó et al., 1985b).

Extensive degeneration of axons and axon terminals is also observed in the ureter, duodenum (Hoyes and Barber, 1981), and trachea (Hoyes et al., 1981) 24 h after subcutaneous injection of 50 mg/kg capsaicin to adult rats. Degeneration is confined to axons with terminals containing mainly large dense-cored vesicles. In the ureter as many as 90% of all axons degenerate, whereas no appreciable degeneration in the dorsal roots is noted (Chung et al., 1985b). Likewise, 49% of the axons in the subepidermal nerve plexus are lost 1 to 112 days after treatment with 50 mg/kg capsaicin, whereas the number of axons in the sural nerve is not significantly altered (Chung et al., 1990).

Thus, capsaicin treatment of adult rats can lead to severe morphological changes in some small sensory neurons and predominantly unmyelinated afferent nerve fibers. A very important point is that, although somata and nerve fibers show no or only moderate degeneration, axon terminals in the periphery are destroyed to a much larger extent (Hoyes and Barber, 1981; Chung et al.,

1985b, 1990). Therefore, the extent of degeneration of somata and axons in the adult rat is clearly less pronounced than when capsaicin is given to the newborn animal (Jancsó et al., 1977, 1980a, 1985b, 1987a), and only a subpopulation of capsaicin-sensitive afferent neurons appears to undergo degeneration (Jancsó et al., 1985b).

ii. Neurochemical and histochemical changes compared with those produced by neonatal capsaicin. The neurochemical consequences of systemic capsaicin treatment of adult rats are qualitatively similar to those in newborn rats, whereas the extent and reversibility of the neurotoxic effect of capsaicin may differ. Depletion of substance P from sensory nerve pathways is produced by 50 to 125 mg/kg capsaicin given subcutaneously or intraperitoneally to adult rats (Gamse et al., 1981b; Gamse, 1982), the intraperitoneal route being more effective than the subcutaneous one (Gamse et al., 1981b). Subcutaneously, the dose of 125 mg/kg capsaicin seems to be maximally effective (Gamse et al., 1981b), making it unnecessary to use doses as high as 950 mg/kg (Jessell et al., 1978) which may give rise to cell-nonselective neurotoxic effects (Harti, 1988). The depletion of substance P and somatostatin from sensory pathways produced by 125 mg/kg capsaicin in adult Sprague-Dawley rats is less than that caused by 50 mg/kg capsaicin in newborn animals (Gamse et al., 1981b; Gamse, 1982; Priestley et al., 1982). Likewise, neonatal capsaicin treatment is more effective in depleting vasoactive intestinal polypeptide from the dorsal spinal cord than adult capsaicin treatment (Skofitsch et al., 1985). In MRC Porton and Wistar rats, however, the depletion of substance P and calcitonin gene-related peptide is the same when capsaicin is given to newborn or adult rats (Salt et al., 1982; Geppetti et al., 1988a), and 125 mg/kg capsaicin given to adult animals is no more effective than 50 mg/kg (Geppetti et al., 1988a). Opiate receptor binding in the dorsal horn of the spinal cord remains unaltered after capsaicin treatment of adult rats (Jessell et al., 1978) but is decreased by neonatal capsaicin (Gamse et al., 1979a; Nagy et al., 1980).

After capsaicin treatment of newborn rats, peptide markers (table 1) are permanently depleted from sensory pathways, but partial or total recovery of the marker levels takes place following capsaicin treatment of adult rats, although the recovery rates differ with tissue and peptide. After administration of 125 mg/kg capsaicin to adult rats, substance P recovers completely within 4 months in the saphenous nerve, dorsal root ganglia, and dorsal roots, whereas in the cornea, vagus nerve, dorsal spinal cord, and brainstem recovery is not complete even after 9 months (Gamse et al., 1981b). In other studies, however, a complete replenishment of substance P was found in the spinal cord and urinary bladder 39 to 60 days after administration of 50 to 100 mg/kg capsaicin to adult rats (Bittner and LaHann, 1985; Maggi et al., 1987d). The depletion of somatostatin is completely reversible within 4 months in all tissues investigated (Gamse et al., 1981b). A relationship, if any, between replenishment of neurochemical markers and morphological recovery of sensory neurons is not known.

iii. Functional changes compared with those produced by neonatal capsaicin. There are some very important age-dependent differences in the functional consequences of systemic capsaicin treatment. Whereas capsaicin treatment of newborn rats does not change the proportion of relative receptor types in afferent nerves (Lynn, 1984; Welk et al., 1984; Cervero and Sharkey, 1988), there is a significant reduction in the proportion of C-fiber polymodal nociceptors in the rat saphenous nerve following capsaicin treatment of adult rats (Lynn et al., 1984; Szolcsányi et al., 1988). The responsiveness of the remaining C-fiber polymodal nociceptors to close arterial injection of capsaicin is diminished but not abolished (Szolcsányi et al., 1988). In contrast, thermonociception seems to be impaired only temporarily by systemic capsaicin treatment of adult rats (Hayes et al., 1981b; Buck et al., 1982; Gamse, 1982; Bittner and LaHann, 1985; Szolcsányi, 1990), whereas permanent changes may occur after neonatal treatment (Holzer et al., 1979; Nagy et al., 1980; Gamse, 1982). Chemonociception, however, is reduced for a long time after capsaicin treatment of adult rats (Szolcsányi et al., 1975; Szolcsányi and Jancsó-Gábor, 1976; Hayes and Tyers, 1980; Gamse et al., 1981b; Gamse, 1982). In contrast, mechanonociception is either unchanged (Barthó et al., 1990), facilitated (Szolcsányi, 1985), or inhibited (Hayes and Tyers, 1980; Gamse, 1982), this variability being probably due to different nociception tests (Barthó et al., 1990). Mechanical hyperalgesia associated with chronic paw inflammation, however, is markedly reduced by systemic administration of capsaicin to adult rats (Barthó et al., 1990).

The differences between the effects of neonatal and adult capsaicin treatment can be explained in at least two different ways. On the one hand, they could mirror different degrees of morphological and functional ablation of sensory neurons. Such a difference, for instance, is reflected by the finding that capsaicin-induced release of substance P from the dorsal spinal cord in vitro is inhibited to a greater extent by neonatal capsaicin pretreatment than by pretreatment of adult animals (Gamse et al., 1981a; Gamse, 1982). A further example relates to the catecholamine secretion from the adrenal gland induced by an acute intravenous infusion of capsaicin. This effect, which is due to a neural reflex (Watanabe et al., 1988b), is reduced by capsaicin pretreatment of newborn rats to a larger extent than by pretreatment of adult animals (Watanabe et al., 1988a). On the other hand, certain differences such as that found with thermonociception may be accounted for by changes at the level of second-order neurons in the spinal cord and medulla

(Salt et al., 1982). Thus, neonatal capsaicin treatment greatly reduces the number of brainstem cells responding to noxious pressure and heat, whereas after treatment of adult rats no major change is observed (Salt et al., 1982). An analogous explanation could hold true for the observation that analgesia induced by mechanical stimulation of the rat vagina is blocked by neonatal, but not adult, capsaicin treatment (Rodriguez-Sierra et al., 1988).

Very pronounced differences in the long-term effects of capsaicin when given to newborn or adult rats are evident with the micturition reflex. Capsaicin treatment at any age impairs this reflex, probably by interfering with its afferent arc only (Sharkey et al., 1983; Holzer-Petsche and Lembeck, 1984; Maggi et al., 1984, 1987d, 1989b; Santicioli et al., 1985; Maggi and Meli, 1986). Although the depletion of substance P from the urinary bladder is similar (Holzer et al., 1982; Maggi et al., 1987d; Geppetti et al., 1988a), neonatal capsaicin treatment leads to a permanent abolition of distension-induced micturition and to pronounced hypertrophy of the bladder (Sharkey et al., 1983; Santicioli et al., 1985; Maggi et al., 1989b), whereas treatment of adults with 25 to 350 mg/kg capsaicin causes only a reversible increase in the pressure threshold of micturition (Maggi et al., 1984, 1987d, 1989b). This has been taken to suggest that there are two populations of capsaicin-sensitive afferent neurons in the rat urinary bladder, one population being sensitive to the neurotoxic action of capsaicin at all ages of life and the other vulnerable to capsaicin only in the newborn rat (Maggi et al., 1987d, 1989b; Maggi and Meli, 1988; Szolcsányi, 1990). Because morphological evidence for this proposition is not available, however, it ought to be considered that there is only one population of afferent neurons whose sensitivity to the neurotoxic effect of capsaicin diminishes as they mature.

Differences also exist with respect to inhibition of local effector functions of sensory nerve endings in peripheral tissues. The exudation of plasma proteins induced by cutaneous application of mustard oil can be inhibited for a much longer time when capsaicin is administered to newborn rather than to adults rats (Jancsó et al., 1967, 1977). Similarly, capsaicin treatment of newborn rats results in a decreased basal blood flow in the superior mesenteric artery of adult rats, whereas treatment of adults is without effect (Hottenstein et al., 1991). In the perfused hindquarter from rats treated with capsaicin as adults, substance P is able to release histamine as it does in untreated rats, whereas in rats treated with capsaicin as neonates the peptide is inactive (Skofitsch et al., 1983). An appraisal of these differences has to take into account that some of the functional alterations caused by neonatal capsaicin treatment probably reflect changes not only in sensory neurons but also in systems closely related to these neurons. It needs to be stressed, however, that capsaicin treatment of newborn and adult rats does not necessarily result in different degrees of functional ablation of sensory neurons. Thus, the vulnerability of the rat gastric mucosa by acid, ethanol, or indomethacin seems to be aggravated to the same degree in rats treated with capsaicin either as neonates (Holzer and Sametz, 1986) or as adults (Szolcsányi and Barthó, 1981; Evangelista et al., 1986; Esplugues et al., 1989; Holzer et al., 1991).

iv. Comparison of the onset of morphological, neurochemical, and functional changes. Degeneration of B-type sensory neurons takes place within 1 h after subcutaneous administration of capsaicin to adult rats (Jancsó et al., 1985b), and inhibition of the afferent and local effector functions of sensory neurons is observed within a few minutes to 1 h after capsaicin treatment (Hayes et al., 1981b; Lembeck and Donnerer, 1981; Bittner and LaHann, 1985; Maggi et al., 1987d; Dickenson et al., 1990a,b). In contrast, depletion of substance P from peripheral targets of sensory neurons is not appreciable until 3 h posttreatment (Bittner and LaHann, 1985; Maggi et al., 1987d,e) or later (Lembeck and Donnerer, 1981). Peptide depletion from the sensory ganglia of adult rats can be preceded by an increase in the ganglionic levels of substance P (Lembeck and Donnerer, 1981; Gamse et al., 1981b; Geppetti et al., 1988a) and calcitonin gene-related peptide (Geppetti et al., 1988a) during the first 4 days after capsaicin treatment.

v. Selectivity of the action of capsaicin. The capsaicin-induced depletion of substance P appears to be restricted to areas containing primary afferent neurons because the peptide concentrations in the ventral spinal cord and in the brain above the brainstem remain unaffected (Hayes and Tyers, 1980; Gamse et al., 1981b; Gamse, 1982; Virus et al., 1982). Similarly, levels of glutamic acid decarboxylase and choline acetyltransferase in the dorsal spinal cord (Jessell et al., 1978; Holzer-Petsche et al., 1986) and of neurotensin and methionine-enkephalin in the central nervous system (Gamse et al., 1981b; Priestley et al., 1982) are not altered by capsaicin treatment of adult rats. The tissue concentrations of noradrenaline, dopamine, and 5-hydroxytryptamine in the spinal cord and brain either stay unchanged (Hajós et al., 1986b) or may even increase (Virus et al., 1983) in response to systemic capsaicin application. This observation indicates that monoamine systems in the central nervous system are not damaged by the drug.

A synopsis of the literature supports the assumption that the neurotoxic effect of capsaicin given to adult rats is more selective for fine sensory neurons than when given to newborn rats. This contention derives from the relative inability of the drug to induce secondary changes in sensory neuron-related systems of the adult rat, a finding that could be due to the weak effect of capsaicin in producing morphological ablation of primary afferent neurons and/or to the lack of plasticity of the nervous system in the adult animal. However, a systematic comparison of this issue in newborn and adult rats has not

yet been made, and there is some indirect evidence that capsaicin treatment of adult rats can lead to functional changes in both the central and autonomic nervous systems (Jancsó-Gábor, 1980; Miller et al., 1983; Holzer, 1986) as well as nonneural tissues (Cui et al., 1990).

The absence of any capsaicin-induced depletion of neurochemical markers from central neurons is at variance with the finding that intraperitoneal administration of 50 to 90 mg/kg capsaicin to adult rats can induce nerve terminal degeneration not only in areas of the spinal cord and brainstem known to be innervated by primary sensory neurons but also in certain discrete fore- and hindbrain areas not yet associated with terminations of primary afferents (Ritter and Dinh, 1988). The observation of degenerating cell bodies in the ventromedial midbrain tegmentum, supramammillary nucleus, and posterior hypothalamus points to a permanent destruction of some central neurons by capsaicin (Ritter and Dinh, 1988). Because capsaicin-induced degeneration of central neurons has been reported by one research group only (Dinh and Ritter, 1987; Ritter and Dinh, 1988, 1990), it will be important to see this finding confirmed by an independent laboratory.

One group of central neurons that have long been recognized as being sensitive to capsaicin lie in the preoptic region of the hypothalamus. Administration of 30 to 70 mg/kg capsaicin to adult rats produces ultrastructural changes in these neurons including swelling of mitochondria (Szolcsányi et al., 1971). These neurons are thought to be thermosensitive neurons involved in central thermoregulation (Szolcsányi, 1982). Investigation of the effect of capsaicin on thermoregulation, therefore, is complicated by the fact that both peripheral C-fiber warmth receptors and central thermosensitive neurons are affected by the drug (for reviews see Szolcsányi, 1982; Hori, 1984). Whereas warmth sensitivity mediated by primary afferents is impaired by capsaicin treatment of both newborn and adult rats (Hajós et al., 1986a), only treatment of adult rats produces a long-term defunctionalization of central thermosensitive neurons (Hori, 1981; Dib, 1983; Hajós et al., 1983; Obál et al., 1983). Likewise, only treatment of adult but not newborn rats with systemic capsaicin abolishes the increases in the synthesis rate and tissue level of monoamines produced by acute capsaicin application in the area of the preoptic region and hypothalamus (Hajós et al., 1986b).

The enteric nervous system seems to be spared by treatment of adult rats with capsaicin unless excessively high doses (950 mg/kg) are used. Such doses can cause the disappearance of some nerve fibers containing substance P from the myenteric plexus of the small intestine (Harti, 1988). There is no direct evidence that enteric neurons are damaged by 50 mg/kg capsaicin because the degeneration of axon terminals seen in the enteric plexuses probably reflects destruction of primary afferent nerve endings only (Hoyes and Barber, 1981); the levels of substance P and calcitonin gene-related peptide in the enteric nervous system remain unaltered (Holzer et al., 1980; Geppetti et al., 1988a). Furthermore, morphological (Szolcsányi et al., 1975) and functional (Stein et al., 1986) observations indicate that neurons of the sympathetic nervous system are not susceptible to the neurotoxic action of capsaicin in the adult rat.

b. GUINEA PIG. **i. Morphological, neurochemical, and histochemical changes.** Systemic administration of capsaicin to adult guinea pigs produces extensive axon terminal degeneration in the dorsal spinal cord, brainstem (Jancsó et al., 1987a), and periphery (Papka et al. 1984). Perivascular nerve endings containing substance P deteriorate within 5 min after the subcutaneous injection of 50 mg/kg capsaicin, and by 4 h posttreatment the number of substance P-containing nerve fibers is visibly diminished (Papka et al., 1984). Depletion of substance P and ultrastructural signs of axon degeneration are appreciable for at least 1 year (Papka et al., 1984), which is in keeping with virtually irreversible functional deficits produced by capsaicin in the adult guinea pig (Jancsó, 1968; Jancsó-Gábor et al., 1970). Quantitatively, a dose of 2.5 mg/kg capsaicin given subcutaneously appears to be suprathreshold, whereas 10 mg/kg capsaicin is capable of producing maximal depletion of substance P from afferent neurons (Miller et al., 1982b; Buck et al., 1983). With low doses of capsaicin (≤5 mg/kg) depletion does not become apparent before 2 days posttreatment, but following administration of 50 mg/kg capsaicin the substance P content of dorsal root ganglia and dorsal spinal cord is significantly diminished within 12 h (Buck et al., 1983). Depletion of substance P proceeds with time to reach a maximum of 10 days after capsaicin treatment. The loss of substance P from the dorsal root ganglia induced by 10 mg/kg capsaicin can be approximately 80 to 90%; this degree of depletion is not surpassed even by doses up to 1250 mg/kg capsaicin (Gamse et al., 1981c; Miller et al., 1982a,b; Buck et al., 1983).

The tissue levels of vasoactive intestinal polypeptide (Buck et al., 1983; Della et al., 1983) and somatostatin (Buck et al., 1983) and those of substance P in the ventral spinal cord and brain above the brainstem are not affected (Buck et al., 1983). However, calcitonin gene-related peptide (Gibbins et al., 1985, 1987; Lundberg et al., 1985; Franco-Cereceda et al., 1987b), cholecystokinin (Buck et al., 1983; Gibbins et al., 1987), and dynorphin (Gibbins et al., 1987) are depleted from sensory neurons. The loss of substance P and calcitonin gene-related peptide is seen in all areas containing primary afferent pathways including medulla oblongata, dorsal spinal cord, dorsal roots, sensory ganglia, afferent nerves, and peripheral targets of sensory neurons such as skin, cardiovascular system, respiratory tract, urogenital system, and parasympathetic and sympathetic ganglia (Gamse et al., 1981c; Furness et al., 1982; Matthews and Cuello, 1982; Miller et al., 1982a,b; Buck et al., 1983; Papka et

al., 1984; Gibbins et al., 1985, 1987; Lundberg et al., 1985; Franco-Cereceda et al., 1987b).

ii. Functional changes. The sensitivity of the cornea to chemical noxious stimuli is abolished by the same doses of capsaicin that reduce the substance P content of sensory neurons (Buck et al., 1983). Chemonociception may be impaired lifelong (Jancsó, 1960). Unlike in the rat, capsaicin treatment of adult guinea pigs also is followed by cutaneous insensitivity to nonnoxious and noxious heat, but these changes require doses of at least 10 mg/kg capsaicin (Miller et al., 1982a; Buck et al., 1983). In contrast, the cutaneous sensitivity to nonnoxious and noxious cold and to nonnoxious and noxious mechanical stimuli is not changed by doses of capsaicin as high as 950 mg/kg (Buck et al., 1983). The loss of sensitivity to thermal noxious stimuli induced by 50 mg/kg capsaicin precedes the depletion of substance P from the dorsal root ganglia (Miller et al., 1982a). Local effector functions of sensory nerve endings are also inhibited by systemic pretreatment of adult guinea pigs with capsaicin, defunctionalization being best documented for sensory nerve-mediated neurogenic inflammatory and constrictor processes in the respiratory tract (Szolcsányi and Barthó, 1982; Lundberg and Saria, 1983, 1987; Saria et al., 1983b; Manzini et al., 1987).

iii. Selectivity of the action of capsaicin. A selective action of capsaicin on primary afferent neurons is supported by the absence of effects of capsaicin on nonsensory central neurons (Buck et al., 1983), autonomic efferent neurons (Matthews and Cuello, 1982; Della et al., 1983), and enteric neurons (Gamse et al., 1981c; Furness et al., 1982; Matthews and Cuello, 1982; Gibbins et al., 1985; Harti, 1988). Neurotransmission processes in autonomic efferent and enteric pathways also are spared by capsaicin (Szolcsányi and Barthó, 1978; Barthó et al., 1982b; Donnerer et al., 1984; Szolcsányi, 1984b). It is not known, however, whether the corneal and cutaneous lesions produced by 950 mg/kg capsaicin (Buck et al., 1983) reflect a trophic role of sensory neurons in these tissues or other effects of the drug. Capsaicin-induced changes in the number of substance P-binding sites in the vas deferens are considered to arise from the elimination of presynaptic substance P receptors on sensory nerve endings and the upregulation of postsynaptic receptors in response to sensory denervation (Mussap et al., 1989).

c. OTHER MAMMALS. Systemic treatment of mice at the age of 2, 4, and 7 days and of adult mice with 50 mg/kg capsaicin results in inhibition of thermo- and chemonociception, the magnitude of which increases with the age at the time of the treatment (Gamse, 1982). Chemonociception is almost completely abolished by capsaicin treatment of 10-day-old or older mice (Gamse, 1982). Although the capsaicin-induced release of substance P from the isolated spinal cord also is inhibited to a larger degree in mice pretreated with capsaicin as adults than those pretreated as neonates, the degree of substance P depletion from the spinal cord and sciatic nerve is the same. A lower dose of capsaicin, 10 mg/kg, given to adult mice reduces the substance P content of the spinal cord, but not the sciatic nerve, and fails to cause long-term changes in thermonociception (Gamse, 1982). These observations suggest that primary afferent neurons in the newborn mouse are not as susceptible to the neurotoxic action of capsaicin as they are later in life. However, morphological data concerning this issue are not yet available, except that a marked loss of axons is seen in the cornea, this effect being associated with corneal lesions (Fujita et al., 1984). Subcutaneous treatment of adult cats with 50 mg/kg capsaicin is followed by extensive axon terminal degeneration in areas of the brainstem and spinal cord known to be the central projection areas of primary sensory neurons (Jancsó et al., 1987a). Injection of the same dose of capsaicin to adult rabbits does not alter the heat thresholds of primary afferent neurons in the saphenous nerve but abolishes the acute excitatory effects of capsaicin application to the skin (Lynn et al., 1984). Subcutaneous administration of up to 280 mg/kg capsaicin to adult rabbits does not alter the substance P content of the spinal cord and eye (Tervo, 1981) but reduces the miotic and hyperemic effects of acute intracameral injection of capsaicin (Tervo, 1981) and the contractile effect of capsaicin on isolated bronchi (Manzini et al., 1990). Administration of a cumulative dose of 50 mg/kg capsaicin to pigs results in a 50 to 90% depletion of calcitonin gene-related peptide and neurokinin A from the skin, respiratory tract, and skeletal muscle and blocks the vasodilator effect of an acute intradermal injection of capsaicin (Franco-Cereceda and Lundberg, 1989; Alving et al., 1991) or exposure to a capsaicin aerosol (Matran et al., 1990; Alving et al., 1991). Data from other mammalian species are not available.

3. Effects of periaxonal administration of capsaicin. In this article, periaxonal or perineural administration of capsaicin denotes application of the drug to nerve trunks as opposed to application to cell bodies or endings of sensory neurons.

a. RAT. **i. Morphological changes.** Application of capsaicin to axons of afferent neurons not only results in acute excitation and reversible inhibition of nerve conduction in C- and some A-fibers but also is followed by long-term ablation of primary afferent C-fiber neurons. One to several days after periaxonal application of 1% (33 mM) capsaicin to the saphenous and sciatic nerves swelling of numerous unmyelinated fibers is observed both distal and proximal to the application site, and 2 to 3 weeks posttreatment the structural organization of the nerves is changed (Handwerker et al., 1984; Jancsó et al., 1985a). This damage is confined to unmyelinated fibers (Handwerker et al., 1984) and involves the whole sensory neuron (Lynn et al., 1987; Jancsó and

Lawson, 1990; Pini et al., 1990). Quantitatively, periaxonal administration of capsaicin to the rat saphenous and sciatic nerves leads to a 34% reduction of cell bodies in the corresponding dorsal root ganglia (Jancsó and Lawson, 1990) and a 32 to 40% reduction of unmyelinated fibers in the treated nerve (Lynn et al., 1987; Jancsó and Lawson, 1990), whereas the number of myelinated fibers remains unaltered. The degeneration extends transganglionically to the central terminals of sensory neurons in the dorsal spinal cord (Jancsó and Lawson, 1990). Thus, a considerable proportion of fine afferent neurons is eliminated in response to periaxonal capsaicin application in the rat.

ii. Neurochemical and histochemical changes. In keeping with the degeneration of thin afferent neurons, the neurochemical changes caused by periaxonal capsaicin persist for a long time (Jancsó and Lawson, 1988). Three months after application of capsaicin to the rat sciatic nerves, the proportions of small-sized cell bodies immunoreactive for substance P, calcitonin gene-related peptide, somatostatin, carbohydrate epitopes, or sensory neuron-specific acid phosphatase in the fifth lumbar dorsal root ganglia are decreased by 24 to 61%. To the contrary, the percentage of large-sized somata immunoreactive for neurofilament protein is enhanced (Jancsó and Lawson, 1988). Peptide depletion from the axons and endings of sensory neurons is most probably related to the fact that application of capsaicin to nerve trunks blocks the ortho- and retrograde transport of peptides and macromolecules in afferent fibers in a dose-related manner (for a review see Jancsó et al., 1987b). Thus, when capsaicin is applied to the rat sciatic nerve, the axoplasmic flow of substance P and somatostatin is inhibited (Gamse et al., 1982; Handwerker et al., 1984). This effect is seen 1 day posttreatment and depends on the dose of capsaicin (0.33 to 33 mM) (Gamse et al., 1982). The retrograde transport of horseradish peroxidase toward the dorsal root ganglia also is significantly reduced by perineural capsaicin (Jancsó et al., 1985a, 1987b). In contrast, the axoplasmic flow of acetylcholinesterase and noradrenaline is not interrupted, indicating that efferent motor and sympathetic nerve fibers are not affected (Gamse et al., 1982). During the 1 to 13 days after periaxonal capsaicin application to the sciatic nerve, substance P is progressively lost from all parts of afferent neurons, although distal to the treatment site the loss is maximal already by 4 days posttreatment (Gamse et al., 1982). Proximal to the treatment site, peptide depletion becomes maximal 2 weeks posttreatment and shows various degrees of recovery during the following 4 to 6 months (Gamse et al., 1982; Gibson et al., 1982). In the spinal cord substance P and somatostatin, cholecystokinin-like immunoreactivity which in fact may represent calcitonin gene-related peptide (Ju et al., 1986) and fluoride-resistant acid phosphatase, are depleted (Ainsworth et al., 1981; Gamse et al., 1982; Gibson et al., 1982) in a concentration-dependent manner (0.1 to 49 mM). In contrast, vasoactive intestinal polypeptide levels in the sciatic nerve and dorsal horn of the spinal cord are increased following periaxonal application of capsaicin (Anand et al., 1990). Substance P in spinal cord regions not receiving primary afferent input, as well as peptides primarily present in intrinsic neurons of the spinal cord (neurotensin, neurophysin, methionine-enkephalin, bombesin), remains unchanged by perineural capsaicin (Ainsworth et al., 1981; Gibson et al., 1982). Likewise, noradrenergic sympathetic efferent neurons are spared by perineural capsaicin treatment (Gazelius et al., 1983; Jancsó et al., 1987b).

iii. Functional changes. Perineural capsaicin inhibits chemo- and thermonociception in the areas supplied by the treated nerve (Jancsó et al., 1980b, 1987b; Fitzgerald and Woolf, 1982; Gamse et al., 1982; Gibson et al., 1982; Coderre et al., 1984; McMahon et al., 1984; Szolcsányi, 1990); this effect is seen as early as 5 h posttreatment, i.e., before substance P is depleted from afferent nerve fibers (Gamse et al., 1982). The sensitivity to noxious cold and noxious and nonnoxious mechanical stimuli is not affected by periaxonal capsaicin (Fitzgerald and Woolf, 1982; Coderre et al., 1984). The early onset of functional inhibition is in keeping with the prompt blockade of nerve conduction caused by periaxonal capsaicin (Petsche et al., 1983; Lynn et al., 1984; Chung et al., 1985a; Marsh et al., 1987; Waddell and Lawson, 1989). Initially, nerve conduction in polymodal C- and Aδ-fiber nociceptors is inhibited at the treatment site only (Petsche et al., 1983; Lynn et al., 1984; Chung et al., 1985a). Whereas conduction in the A-fibers is soon resumed, the polymodal C-fiber nociceptors remain unresponsive to their natural sensory modalities (Welk et al., 1983; Handwerker et al., 1984; Lynn et al., 1984). The proportion of C-fibers responding only to noxious heat can be increased 1 day after perineural capsaicin treatment (Welk et al., 1983), which may reflect a transient stage in the gradual defunctionalization of polymodal C-fiber nociceptors (Handwerker et al., 1984). Thirteen to 21 days posttreatment nerve conduction in the surviving C-fibers appears to be rather normal, although the conduction velocity may still be slowed (Wall and Fitzgerald, 1981; Lynn et al., 1984).

As is expected from the degeneration of sensory neurons (Lynn et al., 1987; Jancsó and Lawson, 1990), defunctionalization of afferent C-fibers persists for several months (Lynn and Pini, 1985; Pini et al., 1990). Both the afferent functions of sensory neurons (Jancsó et al., 1980b; Gamse et al., 1982; Gibson et al., 1982; Raybould and Taché, 1988, 1989; South and Ritter, 1988; Esplugues et al., 1990) and the local effector functions of peripheral sensory nerve endings are inhibited. Thus, vasodilatation and increase in vascular permeability induced by electrical or chemical stimulation of sensory nerve endings are strongly reduced by periaxonal capsai-

cin treatment (Jancsó et al., 1980b, 1987b; Gamse et al., 1982; Coderre et al., 1984; Pertovaara, 1988; Raybould et al., 1991). The onset of this effect of perineural capsaicin parallels that of substance P depletion from afferent nerve fibers distal to the treatment site (Gamse et al., 1982). The reduction of thermonociception does not reverse within 3 months (Szolcsányi, 1990), and cholecystokinin-induced satiety, which is attenuated by perivagal capsaicin, takes as many as 5 months for full recovery (South and Ritter, 1988). Functional recovery may not take place even within 1 year from perineural capsaicin treatment (Jancsó and Király, 1984; Jancsó et al., 1987b).

The processing of sensory information in the spinal cord also is altered by perineural capsaicin. The number of cells in the dorsal horn of the spinal cord that respond to noxious heating of the skin is significantly reduced 1 day after capsaicin application to the rat sciatic nerve (Fitzgerald and Woolf, 1982). In contrast, the number of dorsal horn cells responding to electrical stimulation of afferent C-fibers does not decrease until day 3 posttreatment, this effect reaching a maximum on day 7 (Fitzgerald and Woolf, 1982) and remaining constant up to 3 weeks (Wall and Fitzgerald, 1981; Fitzgerald, 1982; McMahon et al., 1984). The number of dorsal horn cells responding to noxious mechanical stimulation of the skin (Fitzgerald, 1982; Fitzgerald and Woolf, 1982) or electrical stimulation of afferent A-fibers (Wall and Fitzgerald, 1981) is not altered. In addition, the size of cutaneous receptive fields is increased for many dorsal horn cells ipsilateral to the sciatic nerve treated with periaxonal capsaicin (Fitzgerald, 1982; Wall et al., 1982b), whereas other dorsal horn cells may have an ill-defined receptive field (McMahon et al., 1984). Furthermore, excitatory receptive fields are seen not only in the ipsilateral but also in the contralateral dorsal horn of the spinal cord, whereas in control rats these contralateral inputs are invariably inhibitory (Fitzgerald, 1982; Wall et al., 1982b).

iv. Topical selectivity of the action of periaxonal capsaicin. Periaxonal capsaicin produces a selective long-term ablation of primary afferent C-fibers with polymodal nociceptors, whereas other afferent or nonsensory (efferent motor and autonomic) nerve fibers are not directly affected. The question arises, however, as to whether the actions of periaxonal capsaicin are restricted to afferent fibers of the treated nerve or whether afferent fibers in other nerves are affected as well. The latter possibility is not unlikely because systemic parenteral administration of capsaicin to the rat results in rapid distribution of the drug throughout the body (Saria et al., 1982). Indeed, 30 min after application of a 33 mM solution of capsaicin (maximal estimated dose: 1.6 μmol) to the rat sciatic nerve, the drug is found not only in the treated nerve segment and the adjacent distal and proximal segments but also in the blood (Gamse et al., 1982; Petsche et al., 1983), indicating absorption and delivery of capsaicin to other tissues. However, the amounts of capsaicin delivered to other tissues are too small to exert a long-term neurotoxic effect on nerves other than the treated one (Jancsó et al., 1980b, 1987b; Gamse et al., 1982; South and Ritter, 1988).

A particularly valuable study in this respect was one in which the effects of capsaicin were compared when given intraperitoneally (225 mg/kg), perivagally (165 mM), peripylorically (165 mM), intracerebroventricularly (330 nmol), or intrathecally (330 nmol) on cholecystokinin-induced satiety, chemosensitivity of the eye, and substance P tissue levels in the spinal cord and brainstem (South and Ritter, 1988). Consistent with the conclusion that cholecystokinin-induced inhibition of food intake is mediated by vagal afferent neurons, satiety is prevented by perivagal, intracerebroventricular, and intraperitoneal capsaicin and peripyloric and intrathecal capsaicin are without effect. The sensitivity of the cornea to chemical noxious stimuli, on the other hand, is abolished by intraperitoneal and intracerebroventricular capsaicin only, which is in keeping with trigeminal afferents mediating corneal nociception (South and Ritter, 1988). Finally, substance P in the brainstem is depleted by intraperitoneal and intracerebroventricular administration of capsaicin, whereas the substance P content of the spinal cord is reduced by intraperitoneal and intrathecal capsaicin only. The perivagal and peripyloric administration of capsaicin has no effect on spinal or medullar substance P levels as measured by immunohistochemistry (South and Ritter, 1988). Thus, perineural capsaicin ablates afferent nerve fibers in the treated nerve only and, hence, represents an important tool for functional neuroanatomy.

b. OTHER MAMMALS. As in the rat, periaxonal application of capsaicin (33 mM) to the sciatic nerves of guinea pig, cat, and rabbit inhibits the axoplasmic transport of substance P (Gamse et al., 1982). However, whereas in the rat, guinea pig, and cat the capsaicin-induced inhibition of substance P transport is of the same degree as that produced by nerve ligation, the effect of capsaicin in the rabbit is less than one-half of that caused by nerve ligation (Gamse et al., 1982). In addition, capsaicin has to be injected subepineurally in the rabbit to inhibit the axonal transport of substance P, whereas perineural application with a cuff is ineffective (Gamse et al., 1982). This observation is consistent with the absence of morphological or functional signs of axon degeneration 10 days after application of capsaicin to the rabbit saphenous nerve, although the substance P content of the skin is diminished and sensory nerve-mediated increases in vascular permeability are reduced (Lynn and Shakhanbeh, 1988). Periaxonal application of capsaicin to somatic nerves of the guinea pig is followed by a long-lasting inhibition of thermonociception (Szolcsányi, 1990). In the cat, perivagal capsaicin treatment impairs vagal cardiovascular and respiratory chemoreflexes

which are blocked 3 to 5 days posttreatment (Jancsó and Such, 1983).

4. Effects of capsaicin administered to the central endings of afferent neurons. a. INTRATHECAL, INTRACISTERNAL, OR EPIDURAL APPLICATION OF CAPSAICIN. Local administration of 100 to 130 nmol capsaicin into the rat cisterna magna (Jancsó, 1981; Gamse et al., 1984) or lumbar/cervical subarachnoid space (Palermo et al., 1981; Ribeiro-da-Silva and Coimbra, 1984) leads to destruction of axon terminals in the primary afferent terminal regions of the brainstem and spinal cord. Degeneration is confined to the glomerular C-type nerve terminals (Palermo et al., 1981) or glomerular type I nerve terminals (Ribeiro-da-Silva and Coimbra, 1984) which are considered to be terminations of unmyelinated afferents. One to 2 days posttreatment 53 to 56% of the type I nerve terminals have degenerated, whereas the number of type II nerve terminals thought to arise from myelinated afferents are reduced by only 2 to 5% (Ribeiro-da-Silva and Coimbra, 1984). Importantly, the neurotoxic effect of capsaicin administered into the subarachnoid space is restricted to the central endings of primary afferent neurons, because no signs of degeneration are noted in the trigeminal roots, trigeminal ganglion, and maxillary nerve (Jancsó, 1981; Gamse et al., 1984).

The topical selectivity of intracisternal capsaicin is underlined by a selective depletion of substance P and neurokinin A from the sensory nerve terminal areas in the medulla but not from the trigeminal roots, trigeminal ganglion, and maxillary nerve of the rat and guinea pig (Gamse et al., 1984, 1986). Intrathecal administration of capsaicin (50 to 1000 nmol) at the lumbar level causes a long-term depletion of substance P and somatostatin from the dorsal spinal cord of rat and mouse (Yaksh et al., 1979; Nagy et al., 1981a; Gamse, 1982; Micevych et al., 1983; Jhamandas et al., 1984; South and Ritter, 1988). Substance P also is depleted from the dorsal roots of the rat but rather increased in the dorsal root ganglia and sciatic nerve when measured 6 days posttreatment (Gamse, 1982). Substance P levels in the ventral half of the spinal cord and in areas remote from the injection site, i.e., cervical spinal cord, brainstem, and forebrain, are not changed by intrathecal capsaicin (Yaksh et al., 1979; Nagy et al., 1981a; South and Ritter, 1988). Other markers of sensory and nonsensory neurons such as vasoactive intestinal polypeptide and cholecystokinin (Jhamandas et al., 1984), glutamic acid decarboxylase (Yaksh et al., 1979; Nagy et al., 1981a), neurotensin (Gamse, 1982), noradrenaline (Yaksh et al., 1979), 5-hydroxytryptamine, and methionine-enkephalin (Micevych et al., 1983) remain grossly unaltered after intrathecal capsaicin application.

Although the nociceptive functions of sensory neurons are inhibited by intracisternal capsaicin for several months, the local effector functions of peripheral sensory nerve endings remain unaffected (Jancsó, 1981; Gamse et al., 1984). Thus, sensory nerve-mediated increases in vascular permeability in the skin of the rat cheek and nose are virtually unchanged, whereas the sensitivity to chemical noxious stimuli in the ears, eyes, and forepaws of the rat and guinea pig is greatly diminished as early as 1 h posttreatment (Jancsó, 1981; Gamse et al., 1984, 1986). Whereas chemonociception in the corresponding ecteroceptive areas also is lost after intrathecal or epidural application of capsaicin (Yaksh et al., 1979; Jancsó, 1981; Gamse, 1982; Eimerl and Papir-Kricheli, 1987), thermonociception is either reduced for up to 5 months after intrathecal or epidural injection of capsaicin in the rat and mouse (Yaksh et al., 1979; Palermo et al., 1981; Gamse, 1982; Micevych et al., 1983; Jhamandas et al., 1984; Eimerl and Papir-Kricheli, 1987) or not consistently changed (Hayes et al., 1981b; Nagy et al., 1981a). These discrepancies may be related, in part, to variability between different experimental animals (Yaksh et al., 1979; Nagy et al., 1981a; Palermo et al., 1981), different nociception tests (Gamse, 1982), nonspecific interactions between capsaicin and its vehicle (Jancsó, 1981), or the catheterization procedure (Nagy et al., 1981a). Mechanonociception is only temporarily diminished by intrathecal capsaicin (Hayes et al., 1981b) but unchanged in the long term (Yaksh et al., 1979).

Taken together, only the central terminals of unmyelinated afferent neurons in the vicinity of the injection site are ablated after intrathecal or intracisternal application of capsaicin, whereas the cell bodies and peripheral processes of these neurons are left intact. When doses of capsaicin shown to be effective intrathecally or intracisternally are administered intravenously or intraperitoneally, no long-term neurotoxic effects are noted (Yaksh et al., 1979; Gamse et al., 1986).

b. INTRACEREBROVENTRICULAR APPLICATION OF CAPSAICIN. Morphological changes that might occur in response to intracerebroventricular application of capsaicin have not yet been examined. Intracerebroventricular injection of 650 to 1000 nmol capsaicin reduces the content of substance P, but not somatostatin and neurotensin, in the medulla oblongata, with no changes in other brain areas, spinal cord, and trigeminal ganglion (Gamse et al., 1981b; Bodnar et al., 1982; South and Ritter, 1988). The reduction (30%) of the substance P content in the medulla is rather small (Gamse et al., 1981b), making it difficult to be picked up by immunohistochemistry (Bodnar et al., 1982). In the hypothalamus, however, β-endorphin is depleted by intracerebroventricular injection of capsaicin for 2 weeks, whereas the β-endorphin content of other brain areas and the hypothalamic content of substance P, somatostatin, and methionine-enkephalin are left unaltered (Panerai et al. 1983). Functionally, chemonociception in the cornea (Gamse et al., 1981b; South and Ritter, 1988), but not in skin areas supplied by the lumbar spinal cord (Yaksh et al., 1979), is greatly impaired. Intracerebroventricular

capsaicin also attenuates cholecystokinin-induced satiety; this functional deficit takes as many as 5 months for full recovery (South and Ritter, 1988).

c. TOPICAL ADMINISTRATION OF CAPSAICIN TO SPECIFIC BRAIN REGIONS. Microinjection of 2 to 83 nmol capsaicin into the preoptic region of the rat hypothalamus causes hypothermia; this response is absent after systemic or intrahypothalamic treatment of the animals with this drug (see Szolcsányi, 1982). Furthermore, intrahypothalamic injection of nanomole to micromole doses of capsaicin results in a long-lasting impairment of thermoregulation against overheating in rats and cats (see Szolcsányi, 1982). Topical administration of a capsaicin solution (320 μM) to the brainstem of the cat causes degeneration of nerve terminals in a specific region of the ventral medulla oblongata, these structures being involved in the regulation of the cardiovascular and respiratory systems (Jancsó and Such, 1985). Injection of 80 nmol capsaicin into the area postrema and adjacent nucleus tractus solitarii of the rat brainstem results in overconsumption of preferred food (South and Ritter, 1983). No other long-term effects of capsaicin injected topically into the central nervous system have been reported. The behavioural and biochemical alterations involving 5-hydroxytryptamine and dopamine neurons in the substantia nigra and striatum, observed after injection of 100 nmol capsaicin into the rat substantia nigra, are only short lasting and have abated by day 6 posttreatment (Dawbarn et al., 1981).

5. *Effects of capsaicin administered to peripheral endings of sensory neurons.* Repeated administration of capsaicin (66 nM to 33 mM) to the cornea of rats and guinea pigs causes insensitivity to further instillation of capsaicin-related drugs (Jancsó, 1968; Szolcsányi et al., 1975; Szolcsányi and Jancsó-Gábor, 1976; Gamse et al., 1981b). Inhibition of corneal chemonociception may last for days or weeks, depending on the dose applied, but is of shorter duration than that produced by systemic administration of 50 mg/kg or higher doses of capsaicin (Szolcsányi et al., 1975; Gamse et al., 1981b). Although capsaicin does not induce any gross or ultrastructural damage of the corneal epithelium, it produces swelling of mitochondria, disorganization of mitochondrial cristae, and a 90% reduction of the number of microvesicles in unmyelinated nerve endings of the cornea (Szolcsányi et al., 1975). These ultrastructural changes, which are observed 5 h after repeated instillation of 33 mM capsaicin, are strictly confined to nerve endings which in the cornea are all unmyelinated (Szolcsányi et al., 1975). No direct sign of axonal degeneration was seen by Szolcsányi et al. (1975), but this issue calls for reinvestigation and confirmation. Repeated topical application of 33 mM capsaicin to the rat eye also is followed, within 4 h, by an 80% depletion of substance P from the cornea, which reverses over the following 3 weeks as does the corneal sensitivity to chemogenic pain (Gamse et al., 1981b).

Local application of capsaicin to the skin of primates and rodents is followed by a long-term defunctionalization of sensory neurons supplying the treated area. Repeated administration of 33 mM capsaicin to the monkey skin results in a significant reduction of the cutaneous substance P content and in inhibition of neurally mediated increases in vascular permeability (Alber et al., 1989). Likewise, application of several doses of 3.3 to 33 mM capsaicin to the human skin inhibits the axon reflex vasodilatation induced by histamine, substance P, somatostatin, vasoactive intestinal polypeptide (Bernstein et al., 1981; Anand et al., 1983; Tóth-Kása et al., 1983; Szolcsányi, 1988), allergen (Lundblad et al., 1987; McCusker et al., 1989), heat injury, or mechanical trauma (Carpenter and Lynn, 1981). In addition, chemo- and thermonociception (Jancsó, 1960, 1968; Bernstein et al., 1981; Carpenter and Lynn, 1981; Anand et al., 1983; Tóth-Kása et al. 1983, 1986; Szolcsányi, 1990) are impaired for several days to weeks in the skin area treated with capsaicin, whereas mechanonociception and perception of touch and temperature remain unaltered in the long term (Jancsó, 1960, 1968; Bernstein et al., 1981; Tóth-Kása et al., 1983; Szolcsányi, 1990). Repeated topical application of capsaicin (33 mM) to exposed blister bases on the human forearm blocks the algesic effects of capsaicin, bradykinin, and acetylcholine (Szolcsányi, 1977).

Topical application of 3 to 30 mM capsaicin to the nasal mucosa of the guinea pig results in long-lasting depletion of substance P from the nasal mucosa, inhibition of protective reflexes in response to nasal irritation, and block of sensory nerve-induced extravasation of plasma proteins (Lundblad, 1984). These changes are of the same magnitude as those produced by systemic capsaicin treatment; they are evident for at least 2 months after the topical application of capsaicin and are restricted to the treated organ as no neurochemical or functional alterations are seen, for example, in the ureter (Lundblad, 1984). Similarly, repetitive administration of 250 nmol capsaicin to the human nasal mucosa leads to abolition of the irritating and secretory effects of the drug, which lasts for several weeks and is confined to the treated nostril (Geppetti et al. 1988b). When applied 10 times within 40 min to the human tongue, capsaicin (33 mM) abolishes the burning sensation evoked by capsaicin or mustard oil; responsiveness to these chemicals recovers during the following 2 days. However, taste, sensitivity to tactile stimuli, and the cold sensation evoked by menthol remain unaltered (Szolcsányi, 1977). Thus, consumption of hot food rich in capsaicin is unlikely to be toxic for the tongue, and there is, in fact, no evidence that ingested capsaicin exerts any neurotoxic effect in humans.

The selective and reversible effects of capsaicin applied locally to the skin or nasal mucosa are beginning to be used as a treatment for certain neurological disorders

involving sensory neurons. Thus, several applications of capsaicin into the nasal mucosa have been reported to cure vasomotor rhinitis (Marabini et al., 1988; Saria and Wolf, 1988) and to be beneficial in cluster headache (Sicuteri et al., 1989). In some, but not all, patients topical administration of 0.8 mM capsaicin to the skin is able to relieve pain associated with postherpetic neuralgia (Bernstein et al., 1987; Don, 1988; Watson et al., 1988; Bjerring et al., 1990), pain associated with diabetic neuropathy (Ross and Varipapa, 1989), pain associated with psoriasis (Bernstein et al., 1986), postmastectomy pain (Watson et al., 1989), and local stump pain (Rayner et al., 1989). Treatment for several days may suppress pain for several weeks.

Local injection of capsaicin into tissues is another route by which peripheral sensory nerve endings can be defunctionalized, although in this case an axonal site of action may also play a role. Injection of 25 nmol dihydrocapsaicin, a congener of capsaicin, into the footpad skin of guinea pigs causes a prompt and long-lasting inhibition of thermonociception in the area surrounding the site of injection but does not deplete substance P from dorsal root ganglia or inhibit the retrograde transport of nerve growth factor (Miller et al., 1982a). However, injection of a much higher dose of capsaicin (8 μmol) into the skin of the rat scrotum inhibits the retrograde transport of horseradish peroxidase in the pudendal nerve toward the sixth lumbar and first sacral dorsal root ganglia (Taylor et al., 1984, 1985). This effect, which involves predominantly small afferent neurons (Taylor et al., 1985), seems to be permanent because it is seen even 1 year posttreatment, although no sign of cell body degeneration in the dorsal root ganglia is observed (Taylor et al., 1984). Retrobulbar injection of capsaicin (16 μmol) into the rabbit eye inhibits ocular inflammation in response to various irritant stimuli for several weeks, this effect being confined to the treated eye (Camras and Bito, 1980; Bynke, 1983). Likewise, topical application of 33 mM capsaicin to the exposed urinary bladder of the adult rat depletes substance P from the urinary bladder, but not from the adjacent ureter, and leaves chemonociceptive and local effector functions of sensory neurons unaltered in organs distant from the urinary bladder such as the skin and eye (Maggi et al., 1989b). The neurochemical and functional changes produced by local application of capsaicin to the rat urinary bladder are of the same magnitude as those produced by systemic capsaicin treatment (Maggi et al., 1989b).

6. Effects of capsaicin on sensory neurons in vitro. After only a 5-min exposure to 640 μM capsaicin, nerve fibers in the epithelium of the isolated rat trachea exhibit ultrastructural changes that are indicative of degeneration (Hoyes et al., 1981). A significant increase in axon diameter, a reduction in axoplasmic density, a loss of microtubules, and a disruption of axonal membranes are the most prominent morphological features. These degenerative alterations are confined to axons with terminals containing a high proportion of large dense-cored vesicles and only scattered small vesicles, whereas axons with terminals containing large numbers of closely packed small vesicles are not affected (Hoyes et al., 1981). Exactly similar changes are seen in the isolated guinea pig ureter (Sikri et al., 1981) in which concentrations of 1 to 10 μM capsaicin are able to produce degeneration of up to 70% of all axonal profiles when examined 1 h after a 6-min exposure to the drug (Király et al., 1991). Although the origin of the degenerating axons has not been identified, it is likely that they are of primary afferent origin, because the same concentrations of capsaicin (1 to 10 μM) induce ultrastructural damage of most small cell bodies in the isolated nodose ganglion of the rat (Marsh et al., 1987). Within 5 min of exposure to the drug, ultrastructural changes including swelling of mitochondria, disruption of neurofilament organization, and fiber enlargement take place (Marsh et al., 1987). All of these observations indicate rapid in vitro degeneration of sensory neurons exposed to capsaicin.

The rapidity with which morphological changes take place after in vitro exposure to capsaicin is paralleled by the quick onset of functional changes. Isolated muscle tissues including cardiac muscle (Zernig et al., 1984; Franco-Cereceda and Lundberg, 1988), visceral smooth muscle (Barthó and Szolcsányi, 1978; Barthó et al., 1982a, 1987; Chahl, 1982; Hua and Lundberg, 1986; Lundberg and Saria, 1987; Maggi et al., 1987c), vascular smooth muscle (Toda et al., 1972; Duckles, 1986; Saito et al., 1988; Edvinsson et al., 1990), and the iris sphincter muscle (Ueda et al., 1984) promptly respond to capsaicin with neurogenic contraction or relaxation. In the isolated inferior mesenteric ganglion of the guinea pig capsaicin elicits a slow depolarization of the principal ganglion cells (Tsunoo et al., 1982; Dun and Kiraly, 1983). All of these acute effects of capsaicin are short lasting and, within a few minutes, the tissues become unresponsive to both capsaicin and other means of sensory nerve stimulation. It can be assumed that this state of "desensitization" reflects not only defunctionalization but also degeneration of sensory nerve fibers because desensitization to capsaicin is achieved by in vitro concentrations of capsaicin in the range of 0.3 to 10 μM which are of the same magnitude as those producing degenerative changes in the isolated ureter (Király et al., 1991) and nodose ganglion of the rat (Marsh et al., 1987) and in dorsal root ganglia grown in tissue culture (Wood et al., 1988). Furthermore, a 5-min exposure of the isolated rat urinary bladder to a solution containing 1 to 10 μM capsaicin leads to substantial depletion of substance P, which does not become appreciable, however, until 3 h posttreatment (Maggi et al., 1987e).

7. Effects of capsaicin on sensory neurons in culture. The sensitivity to the acute and neurotoxic effects of

capsaicin is maintained when dorsal root ganglia are grown in tissue culture. Like in vivo, two populations of neurons, large light and small dark cell bodies, can be distinguished in cultures of dorsal root ganglia from newborn or adult rats (Winter, 1987; Wood et al., 1988; Winter et al., 1990). The large light neurons are selectively labeled with an antibody to neurofilament protein, whereas the small dark cell bodies are neurofilament negative. Capsaicin stimulates a certain proportion of the cultured dorsal root ganglion cells, excitation being visualized by a cobalt uptake stain (Winter, 1987; Wood et al., 1988; Winter et al., 1990). Those cells that are sensitive to capsaicin are usually neurofilament negative and fall totally within the population of small dark cells. Approximately 50% of the neuronal cell bodies derived from neonatal dorsal root ganglia are sensitive to capsaicin, and overnight treatment of cultures with 2 μM capsaicin results in the loss of most cell bodies which show cobalt staining in response to acute capsaicin or the related resiniferatoxin (Winter, 1987; Wood et al., 1988; Winter et al., 1990). Because prolonged exposure to capsaicin also leads to a 37% reduction in the total number of cell bodies in the culture, it follows that most of the capsaicin-sensitive cells are killed (Wood et al., 1988). Thus, degeneration of sensory neurons also takes place in tissue cultures of dorsal root ganglia and the extent of cell loss is similar to that seen after systemic capsaicin treatment of newborn rats (Otten et al., 1983; McDougal et al., 1985; Arvidsson and Ygge, 1986). The cellular specificity of capsaicin's and resiniferatoxin's effects on cultured dorsal root ganglia is underlined by the finding that cultures of large light neurons and nonneuronal cells from rat dorsal root ganglia, nonneuronal cells from the rat sciatic nerve, rat sympathetic neurons, neural crest-derived neuroblastoma cells, and hybrid cells derived from embryonal carcinoma cells are not affected by the drugs (Bevan et al., 1987; Winter et al., 1988, 1990; Wood et al., 1988).

E. Differences in the Sensitivity to Capsaicin among Mammalian Species

Although capsaicin acts as a stimulant of thin sensory neurons in all mammalian species that have been investigated so far including humans, rat, mouse, guinea pig, hamster, rabbit, cat, dog, goat (Glinsukon et al. 1980; Szolcsányi 1990), pig (Franco-Cereceda et al. 1987c; Franco-Cereceda and Lundberg, 1989; Matran et al., 1989, 1990; Pierau and Szolcsányi, 1989; Alving et al., 1991), and bear (Rogers, 1984), there are considerable species differences in the sensitivity of afferent neurons to capsaicin which have not yet been examined systematically. While sensory neurons in the cornea of guinea pig and rat are similarly sensitive to the irritant effect of capsaicin (Szolcsányi et al., 1986), there are some differences in capsaicin's neurotoxic effect. As judged from dose-response relationships, thin afferent neurons of the adult guinea pig (Buck et al., 1983) appear to be slightly more sensitive to the neurotoxic action of systemic capsaicin than are those of newborn rats (Nagy et al., 1983). Inhibition of the micturition reflex is more pronounced in guinea pigs treated as adults than in rats treated at the same age (Maggi et al., 1987b), and the reduction of thermonociception is definitely more marked in adult guinea pigs (Buck et al., 1983) than in rats and mice treated as neonates or as adults (Gamse, 1982). However, when capsaicin is administered perineurally, afferent C-fibers of the guinea pig are significantly less sensitive to a conduction block than are C-fibers in the rat and ferret (Baranowski et al., 1986), although axoplasmic transport of substance P is inhibited to the same degree in rat and guinea pig (Gamse et al. 1982) and thermonociception in the guinea pig is even more inhibited than in the rat (Szolcsányi, 1990).

Afferent C-fibers of the rabbit also are less sensitive to a conduction block than those in the rat (Baranowski et al., 1986), but this observation is consistent with a weaker inhibition of the axoplasmic transport of substance P in the rabbit when compared with the rat (Gamse et al., 1982). Furthermore, no degeneration of axons is seen after application of capsaicin to afferent nerve axons of the rabbit (Lynn and Shakhanbeh, 1988), whereas a considerable proportion of C-fibers degenerate in the rat (Lynn et al., 1987; Jancsó and Lawson, 1990; Pini et al., 1990). Thus, afferent neurons in the rabbit are in general resistant to a long-term ablation by capsaicin, although this drug is able to produce acute effects indicative of sensory neuron stimulation, desensitization, and defunctionalization (Barthó and Szolcsányi, 1980; Camras and Bito, 1980; Tervo, 1981; Bynke, 1983; Baranowski et al., 1986; Lynn and Shakhanbeh, 1988; Moritoki et al., 1990; Manzini et al., 1990; Trad et al., 1990). However, the rabbit is about 20 times less sensitive to the irritant effect of capsaicin than is the rat (Szolcsányi, 1987). There also are species differences in the sensitivity of afferent A-fibers to capsaicin. Whereas perineural capsaicin induces a reversible conduction block primarily in Aδ-fibers of the rat, guinea pig, rabbit, cat, and monkey (Lynn et al., 1984; Chung et al., 1985a; Baranowski et al., 1986; Such and Jancsó, 1986; Marsh et al., 1987), only C-fibers are blocked in the ferret (Baranowski et al., 1986).

Other species differences in the excitatory and long-term effects of capsaicin are not necessarily due to differences in sensory neuron sensitivity to capsaicin but rather to different distributions of capsaicin-sensitive afferent neurons, different neurotransmitter contents, different actions and functions of these afferent neurons, or species differences in the metabolic effects (Miller et al., 1983) and pharmacokinetic fate of capsaicin. One example refers to the indirect contractile effects of capsaicin on isolated cardiac muscle, which is thought to reflect a local effector function of sensory neurons in this organ. Although in the rat (Sigrist et al., 1986) and guinea

pig (Zernig et al., 1984; Franco-Cereceda and Lundberg, 1988) capsaicin causes a positive ino- and chronotropic effect, no such effect is apparent in the cardiac muscle of rabbit, dog (Toda et al., 1972), and man (Franco-Cereceda et al., 1987a). A similar situation applies to the motor effects of capsaicin on tracheobronchial (Lundberg and Saria, 1987) and gastrointestinal (Barthó et al., 1987; Maggi et al., 1986, 1987c, 1989d) smooth muscle which varies among species.

Other examples include differences in the ability of capsaicin to release substance P or calcitonin gene-related peptide in the ureter of rat and guinea pig, the tissue levels of these peptides being profoundly dissimilar in the two species (Amann et al., 1988). Because the two peptides exert either relaxant (calcitonin gene-related peptide) or contractile (substance P) effects on the ureter (Hua and Lundberg, 1986), it is not unexpected that the contractile effects of capsaicin also differ in the ureter of the rat and guinea pig (Amann et al., 1988). Unlike in the rat skin in which intravenous capsaicin induces leakage of plasma proteins (Saria et al., 1983b), intradermal capsaicin fails to produce extravasation in the skin of humans (Lundblad et al., 1987) and pigs (Alving et al., 1991). However, capsaicin gives rise to chemosis (conjunctival edema) when it comes into contact with the human eye (J. Szolcsányi, personal communication). Furthermore, capsaicin increases vascular permeability in the urinary bladder of rat, mouse, and guinea pig but fails to do so in the hamster bladder (Maggi et al., 1987b). Similarly, capsaicin elicits sensory nerve-mediated contractions of urinary bladders from rat, mouse, and guinea pig but not from rabbit and hamster (Maggi et al., 1987b). Substance P is considered to be the main mediator of sensory neurons for increasing vascular permeability and causing smooth muscle contraction (Lundberg and Saria, 1987; Chahl, 1988; Holzer, 1988; Maggi and Meli, 1988), and it has been speculated that the inactivity of capsaicin in the rabbit and hamster bladder is related to the low substance P content of the rabbit bladder and the absence of this peptide from the hamster bladder (Maggi et al., 1987b). Altogether, the hamster seems to be a mammalian species that is comparatively insensitive to capsaicin because even systemic treatment with the drug is relatively well tolerated (Glinsukon et al., 1980) and does not result in, for example, changes of the micturition reflex (Maggi et al., 1987b).

Finally, the overall toxicity of capsaicin, when administered systemically to mammals (Glinsukon et al., 1980), does not necessarily correlate with the species sensitivity of afferent neurons to the drug. Capsaicin is an extremely irritating substance that, by stimulating sensory neurons, gives rise not only to nociception and nociception-associated reactions but also to peripheral release of neuropeptides that cause generalized vasodilatation, extravasation of plasma protein, and bronchoconstriction. The guinea pig is particularly susceptible to these immediate consequences of sensory neuron stimulation, and even low doses of systemic capsaicin (<1 mg/kg) may be fatal. Another limiting factor in the use of capsaicin in the rat is the induction of the Bezold-Jarisch reflex which arises from activation of pulmonary chemoreceptors and leads to apnoea, hypotension, and bradycardia (Szolcsányi et al., 1990). As a consequence, capsaicin has to be administered to animals under general anaesthesia to avoid undue pain. Especially in the guinea pig, capsaicin-induced bronchoconstriction, hypersecretion in the airways, and leakage of plasma protein ought to be limited by pretreatment of the experimental animals with drugs such as theophylline, β_2-adrenoceptor agonists, and atropine (Jancsó, 1960; Gamse et al., 1981c; Buck et al., 1983). In addition, it is necessary to split the total dose into a schedule of gradually increasing doses to keep the adverse effects of the initial doses tolerable. The "ultrapotent" analogue of capsaicin, resiniferatoxin, seems to be comparatively less toxic because it does not elicit the full Bezold-Jarisch reflex in the rat (Szolcsányi et al., 1990).

F. Age and Strain Differences in the Capsaicin Sensitivity of Mammalian Species

Age-dependent differences in the effects of capsaicin on mammalian species are best documented in the rat, with some information also available for the mouse. In the newborn rat, thin primary afferent neurons are considerably more susceptible to the neurotoxic action of capsaicin than they are in the adult animal. This difference certainly is true for the cell bodies and nerve axons, whereas degeneration of the peripheral terminals of thin sensory neurons in the adult rat may be as extensive as in the newborn animal (Chung et al., 1985b, 1990). Conversely, in the mouse it appears as if some functional and neurochemical consequences of the neurotoxic action of capsaicin are more pronounced in adults than in neonates (Gamse, 1982), although morphological evidence for such a difference is not yet available. The ontogenetic shift in the sensitivity of sensory neurons to capsaicin also poses problems for the classification of capsaicin-sensitive sensory neurons because the neurons that are vulnerable to the drug in the newborn animal are not completely identical with those affected in the adult animal (Jancsó et al., 1987a; Maggi and Meli, 1988; Szolcsányi, 1990). Hence, the existence of more than one population of capsaicin-sensitive afferent neurons has been proposed, the groups differing in their age-dependent sensitivity to capsaicin (Maggi and Meli, 1988; Szolcsányi, 1990).

The sensitivity to, and the effectiveness of, capsaicin in the rat depends to some degree on the *strain* of the animals and, in addition, may be influenced by experimental conditions such as the anaesthetic used (J. Szolcsányi, personal communication). Following intravenous administration of capsaicin, Wistar and Sprague-Dawley rats respond with similar blood pressure changes,

but, whereas Wistar rats also exhibit bradycardia and apnoea, these effects are absent in Sprague-Dawley rats (Donnerer and Lembeck, 1982). Differences between these two strains of rats also are noted after systemic treatment of adult animals with a high dose of the capsaicin congener, nonanoyl vanillylamide. The long-term alterations include different weight losses posttreatment, different degrees of tachycardia in response to captopril, and different changes in the sensitivity to the anaesthetic methohexital (Gardiner et al., 1989).

The extent of long-term peptide depletion from sensory neurons of Wistar rats may be independent of the age of the animal treated with a high dose of capsaicin (Geppetti et al., 1988a), whereas in Sprague-Dawley rats treatment of newborn animals seems to be more effective than is treatment of adults (Gamse et al., 1981b). In addition, following capsaicin treatment as neonates, Charles River and Wistar-Morini rats develop persistent skin wounds, whereas Sprague-Dawley rats do not (Gamse et al., 1981b; Maggi et al., 1987a). Differences are also seen after capsaicin treatment of adult Wistar-Kyoto and spontaneously hypertensive rats. Thermonociception is impaired only in the spontaneously hypertensive rats (Virus et al., 1981); additionally these animals show a more pronounced substance P depletion and 5-hydroxytryptamine/noradrenaline accumulation in the spinal cord than do Wistar-Kyoto rats (Virus et al., 1983).

G. Acute and Long-term Effects of Capsaicin in Nonmammalian Species

In contrast to mammals, which in general are sensitive to the irritant and long-term inhibitory effects of capsaicin on sensory neurons, nonmammalian species appear to be only poorly sensitive to the drug. In a marsupial species, the North American opossum, acute exposure to capsaicin has been found to release substance P from the muscularis mucosae of the esophagus, whereas systemic pretreatment does not change the tissue level of the peptide (Daniel et al., 1987).

Acute local or systemic administration of capsaicin in pigeons and other birds, at concentrations of 33 mM or doses up to 600 mg/kg, either fails to evoke pain or reactions indicative of pain or is only weakly active (Mason and Maruniak, 1983; Szolcsányi et al., 1986; Sann et al., 1987). Similar to the findings made in mammalian species, both substance P and calcitonin gene-related peptide are markers of primary afferent neurons in the pigeon (Pierau et al., 1987; Harti et al., 1987, 1989). Capsaicin (10 μM), however, is unable to evoke a detectable release of substance P from the central endings of these neurons in pigeon spinal cord slices (Pierau et al., 1987). No changes in chemonociception have been noted after systemic capsaicin treatment of pigeons (Szolcsányi et al., 1986). Thermoregulation has been examined in some avian species and was shown not to be affected by the drug (Mason and Maruniak, 1983; Geisthövel et al., 1986; Sann et al., 1987).

In spite of the absence of persistent functional alterations, systemic treatment of pigeons with 950 mg/kg capsaicin gives rise to a partial depletion of substance P and calcitonin gene-related peptide from nerve fibers in the myenteric plexus of the small intestine (Harti, 1988), ureter, and cornea (Harti et al., 1989). A reduction of peptide levels in the pigeon cornea also is seen after topical application of 33 mM capsaicin to this tissue (Harti et al., 1989). Likewise, application of 330 nmol capsaicin to the sciatic nerve of the pigeon gives rise to a 40% depletion of substance P and calcitonin gene-related peptide from the nerve axons, whereas these peptides accumulate in the dorsal horn of the spinal cord (Harti et al., 1987). Taken collectively, these neurochemical changes have been taken to suggest that systemic capsaicin acts primarily on axons but not on nerve terminals of sensory neurons (Harti et al., 1989). It is not known, however, whether peptide depletion in the pigeon is associated at all with degenerative alterations in sensory neurons.

Morphological changes are unlikely to occur because systemic application of 500 mg/kg capsaicin to the newborn chick fails to cause degeneration of dorsal root ganglion cells (Jancsó et al., 1985a). This lack of effect is in keeping with the finding that cultured dorsal root ganglia from the newborn chick do not respond to capsaicin or resiniferatoxin in the cobalt stain assay (Bevan et al., 1987; Wood et al., 1988; Winter et al., 1990). In contrast, exposure (30 min to 28 h) of cultured chick sensory neurons to 32 to 160 μM capsaicin has been reported to cause a concentration-dependent retardation of neurite outgrowth (Hiura and Sakamoto, 1987a). This retardation is associated with a disappearance of the neurite tips due to degeneration but does not seem to be permanent because quick neurite regeneration may occur even in the continued presence of capsaicin (Hiura and Sakamoto, 1987a). Because degeneration of nerve cell bodies has not been noted, it would seem that the somata of cultured chick sensory neurons are resistant to the degenerative action of capsaicin, whereas neurites may temporarily be damaged by the drug. This inference is in line with the suggestion that in the pigeon capsaicin receptors are present only on the axons but not on the other parts of sensory neurons (Harti et al., 1989).

Unlike birds, reptiles such as the African crocodile seem to be extremely sensitive to the irritant effect of capsaicin. When instilled into the crocodile's eye, concentrations of capsaicin as low as 3.3 nM cause reactions indicative of pain (Kanui et al., 1990). This reaction, however, does not show any desensitization, because repeated instillation of the drug produces consistent pain reactions (Kanui et al., 1990). In contrast, amphibian species appear to be insensitive to the irritant (Szolcsányi et al., 1986) and neurotoxic effects of capsaicin. Follow-

ing systemic treatment with up to 100 mg/kg of capsaicin, frogs do not exhibit any long-term peptide depletion from, or defunctionalization of, sensory neurons (Chéry-Croze et al., 1985). However, overnight exposure of the isolated ileum of the toad to 300 μM capsaicin is followed by the disappearance of most substance P-containing nerve fibers from the intestinal wall and by inhibition of contractions due to extrinsic nerve stimulation (Osborne and Campbell, 1986). The cellular specificity of capsaicin's effects in the toad has not yet been characterized.

H. Summary: Targets of Action

1. Capsaicin-sensitive neurons. It has become common usage to refer to those afferent neurons which, in the rat, are excited *and subsequently* inhibited by nanomolar concentrations of capsaicin as "capsaicin-sensitive sensory neurons" (Szolcsányi and Barthó, 1978; Szolcsányi, 1982, 1984b). Their sensitivity to these actions of capsaicin appears to be mediated by a specific membrane recognition site for the drug (see below). The main classification traits of the neurons that are the target of the sensory neuron-selective effects of capsaicin are summarized in table 2. Apart from the trait of capsaicin sensitivity, however, capsaicin-sensitive afferent neurons are difficult to define because they do not completely overlap with any population of afferent neurons that have been classified according to morphological, neurochemical, or functional criteria.

First, the majority of capsaicin-sensitive afferent neurons have small- to medium-sized cell bodies that are connected to unmyelinated (C) or thinly myelinated (Aδ) nerve fibers (Lawson and Harper, 1984). However, not all unmyelinated afferent neurons conducting in the C- and Aδ-fiber range are sensitive to capsaicin.

Second, capsaicin-sensitive afferent neurons contain a variety of peptides that are thought to play a transmitter or mediator role. The best known among these peptides are substance P and calcitonin gene-related peptide, but there is a long list of peptide and other markers of the neurons under consideration (table 1). However, none of these markers is an exclusive constituent of capsaicin-sensitive sensory neurons because not all afferent neurons containing, for example, substance P or calcitonin gene-related peptide are sensitive to capsaicin, and these peptides also are contained in nonsensory neurons. There is, in fact, no specific histochemical marker for this group of neurons (Lawson and Harper, 1984; Kirchgessner et al., 1988).

Third, capsaicin-sensitive afferent neurons are heterogeneous in terms of their sensory modality and the functions they subserve. The most consistent functional change associated with the neurotoxic effect of capsaicin is a long-term inhibition of chemonociception, which underlines the concept that capsaicin-sensitive afferents are chemoceptive neurons (Szolcsányi, 1984b). However, the somatic capsaicin-sensitive afferents include not only chemonociceptors and polymodal nociceptors but also some warmth receptors. Visceral afferent neurons sensitive to capsaicin have been found to respond to noxious or potentially noxious (Coleridge and Coleridge, 1977, 1984; Cervero and McRitchie, 1982; Longhurst et al., 1984; Szolcsányi, 1984b; Stein et al., 1986; Martling and Lundberg, 1988; Geppetti et al., 1990, 1991; Forster et al., 1990; Holzer et al., 1991) as well as nonnoxious "physiological" stimuli (MacLean, 1985; McCann et al., 1988; Raybould and Taché, 1988, 1989; South and Ritter, 1988; Yox and Ritter, 1988; Rózsa and Jacobson, 1989; Forster et al., 1990).

Fourth, age, strain, and species differences in the sensory neuron-selective effects of capsaicin are other factors that complicate the classification of capsaicin-sensitive neurons.

2. Neurons not sensitive to the neurotoxic action of capsaicin. Although some afferent neurons with thickly myelinated axons conducting in the Aα and Aβ range are first stimulated and then blocked transiently by acute administration of capsaicin, there is consistent evidence that this population of afferent neurons is not sensitive to the long-term neurotoxic action of the drug. Likewise, efferent motor neurons and efferent neurons of the autonomic nervous system are not directly sensitive to the excitatory or neurotoxic action of capsaicin (Szolcsányi et al., 1975; Ault and Evans, 1980; Jancsó et al., 1980a; Cervero and McRitchie, 1982; Gamse et al., 1982; Matthews and Cuello, 1982; Della et al., 1983; Sharkey et al., 1983; Handwerker et al., 1984; Stein et al., 1986; Such and Jancsó, 1986; Bevan et al., 1987; Marsh et al., 1987; Takano et al., 1988; Wood et al., 1988). The enteric nervous system also is believed to be grossly insensitive to capsaicin (Barthó and Holzer, 1985), and the reports that a minority of enteric neurons is susceptible to the neurotoxic action of the drug (Fehér and Vajda, 1982; Harti, 1988; Kirchgessner et al., 1988) need to be confirmed. A similar restriction applies to neurons in the central nervous system, the majority of which are insensitive to the acute and long-term effects of capsaicin. However, the situation is not yet clear and calls for further examination. It needs to be emphasized, therefore, that the selectivity with which capsaicin acts on a group of unmyelinated and thinly myelinated primary afferent neurons is exceptional but certainly not absolute.

3. Cell-nonselective effects of capsaicin. Apart from its sensory neuron-selective effects, capsaicin exerts a number of "nonselective" effects which can be differentiated into three categories. (*a*) Capsaicin can, both acutely and in the long term, affect neural and nonneural systems that are functionally related to primary afferent neurons. Capsaicin-induced changes in these systems are interpreted to be secondary to the action of capsaicin on primary afferent neurons. (*b*) Certain long-term neurotoxic actions of capsaicin on neurons in the central and enteric nervous systems cannot be assessed in terms of

a secondary sequel of sensory denervation but appear to reflect a direct effect of the drug on these neurons. Thus, a group of thermosensitive neurons in the preoptic region of the hypothalamus is defunctionalized by systemic capsaicin given to adult rats, and some neurons in other nuclei of the brain appear to degenerate in response to the drug (Dinh and Ritter, 1987; Ritter and Dinh, 1988). (*c*) Capsaicin exerts a transient effect on many neural as well as nonneural systems in both vertebrate and invertebrate species, which seem to be unrelated to its excitatory action on thin primary afferent neurons. Although the potency of capsaicin in producing these cell-nonselective effects is, in most cases, orders of magnitude lower than that in exciting sensory neurons, it follows that the acute effects of capsaicin are less selective for thin afferent neurons than are its long-term effects.

III. Mechanisms of Action of Capsaicin

A. Evidence for the Presence of a Specific Recognition Site for Capsaicin

1. Structure-activity relationship for the excitatory effect of capsaicinoids on sensory neurons. Ever since the acutely stimulant and long-term inhibitory actions of capsaicin on afferent neurons were disclosed, investigators were intrigued by the remarkable selectivity of these actions for thin sensory neurons. The finding of Szolcsányi and Jancsó-Gábor (1975, 1976) that the effects of capsaicin are shared by structurally related analogues of the drug first suggested that certain afferent neurons are sensitive to capsaicin because they possess a selective recognition site for capsaicinoids, a pharmacological receptor that mediates the cellular responses to the drug. As shown in fig. 1, capsaicin consists of a vanillyl moiety, an acylamide group, and an alkyl chain, certain features of which are required for capsaicin-like activity.

The acutely stimulant activity of capsaicin is determined by the presence of a 4-hydroxy-3-methoxy benzyl ring, the polar nature of the acylamide bond, and the apolar nature of the alkyl chain. The substituted aromatic ring is essential for the excitatory activity of capsaicin congeners (Szolcsányi and Jancsó-Gábor, 1975; Hayes et al., 1984b; Dray et al., 1990c; Szallasi and Blumberg, 1990b), and this structural requirement has been confirmed by studying the activity of capsaicin-like photoaffinity probes on neurons cultured from rat dorsal root ganglia (James et al., 1988). Thus, aryl azido analogues of capsaicin produce a long-lasting stimulation of calcium uptake after ultraviolet irradiation only if these analogues contain a 4-hydroxy-3-methoxy benzyl ring (James et al., 1988).

There is some flexibility in the polar bond because the acylamide group can be replaced by an ester group (Szolcsányi and Jancsó-Gábor, 1975), particularly in resiniferatoxin (Szallasi and Blumberg, 1989, 1990a,b). In capsaicin congeners of the homovanillyl alkylamide type, the position of the amide (NH) and carbonyl (CO) functions is reversed, but excitatory activity is retained (Szolcsányi and Jancsó-Gábor, 1975). However, a change of the secondary to a tertiary amide function (replacement of the H in the NH group by, for example, an alkyl group) strongly reduces noxious activity of capsaicinoids on the rat cornea (Szolcsányi and Jancsó-Gábor, 1975). Most variability is permitted in the apolar residue which, in capsaicin, is an alkyl chain. It appears as if the optimal chain length for activity on the rat cornea is approximately 8 to 10 carbon atoms because either an increase or decrease in the chain length reduces activity, whereas the introduction of a double bond in the alkyl chain has no effect (Szolcsányi and Jancsó-Gábor, 1975). The presence of an alkyl chain as such is not essential because its substitution by a cycloalkyl ring results in a compound that retains much of its activity (Szolcsányi and Jancsó-Gábor, 1975); the exchange of the alkyl chain by a diterpene, as occurs in the resiniferatoxin molecule, leads to a markedly increased activity in a number of assays (de Vries and Blumberg, 1989; Szallasi and Blumberg, 1989, 1990a,b; Szallasi et al., 1989; Dray et al., 1990a; Maggi et al., 1990c; Winter et al., 1990).

Some exceptions to the described structural requirements for capsaicin-like activity need to be mentioned. Removal of the 3-methoxy substituent in the resiniferatoxin molecule, such as is found in tinyatoxin, results in only a minor decrease in activity as compared with resiniferatoxin (Szallasi and Blumberg, 1990b). Mustard oil (allyl isothiocyanate, CH_2 = CH-CH_2-NCS) stimulates capsaicin-sensitive afferent nerve fibers (Jancsó et al., 1967, 1977, 1980a; Lembeck and Holzer, 1979; Gamse et al., 1980; Patacchini et al., 1990) of both the C and Aδ type (Harris and Ryall, 1988), although its chemical constitution is very different from that of capsaicinoids. Furthermore, piperine which lacks a polar moiety in the place of capsaicin's acylamide retains considerable capsaicin-like activity in certain assays (Micevych et al., 1983; Jhamandas et al., 1984; Miyauchi et al., 1989; Takaki et al., 1990). It is not totally clear, however, whether the cellular site of action of these compounds is identical with that of capsaicin. This shortcoming curtails the validity of all structure-activity studies that do not directly examine the stimulant effect of capsaicinoids on sensory neurons but utilize effects subsequent to this primary event, e.g., nociceptive reflexes or tissue responses to the local release of sensory neurotransmitters.

Despite these uncertainties, there is a good correlation between the noxious activity of capsaicin congeners on the rat cornea (Szolcsányi and Jancsó-Gábor, 1975) with their potency in eliciting the Bezold-Jarisch reflex (Szolcsányi, 1982), producing hypothermia (Szolcsányi, 1982; Hayes et al., 1984b; de Vries and Blumberg, 1989; Szallasi and Blumberg, 1990b), causing contraction of the trachea (Szolcsányi, 1983b), and facilitating the release of substance P from the spinal cord (Bucsics and

Lembeck, 1981; Jhamandas et al., 1984). The situation is different, however, when capsaicin and resiniferatoxin are compared with each other. In some assays of capsaicin-like activity (Szallasi and Blumberg, 1989; Maggi et al., 1990c), resiniferatoxin is not or is only marginally more active than capsaicin, whereas in other tests (de Vries and Blumberg, 1989; Szallasi and Blumberg, 1989, 1990a,b; Szallasi et al., 1989; Dray et al., 1990a; Maggi et al., 1990c; Winter et al., 1990), resiniferatoxin is 100 to 10,000 times more potent.

On the basis of the described structural requirements for a capsaicin congener to stimulate sensory neurons, Szolcsányi and Jancsó-Gábor (1975) proposed a hypothetical model of a capsaicin receptor in which multiple interactions between the capsaicin molecule and the recognition site determine optimal activity. In this model, the 4-hydroxy substitutent on the phenyl ring and the carbonyl and NH functions in the acylamide moiety (or its polar equivalents) interact with polar constituents of the receptor, whereas the phenyl ring, as well as the alkyl chain or its apolar substitution, provide for apolar ligand-receptor interactions (Szolcsányi and Jancsó-Gábor, 1975). However, the capsaicin-like activities of irritant compounds with partial, little, or no structural similarity to capsaicin such as xylene or mustard oil are not accounted for by this model.

2. Structure-activity relationships for the desensitizing and neurotoxic effects of capsaicinoids on sensory neurons. The activities of capsaicin analogues in inducing desensitization to their stimulant effect on sensory neurons are still poorly defined but, as it appears, do not completely overlap with their excitatory activities (Szolcsányi and Jancsó-Gábor, 1976; Hayes et al., 1984b; Dickenson et al., 1990b; Dray et al., 1990c). These differences are best illustrated by the fact that there are capsaicin analogues such as zingerone and homovanillic octylester that exhibit noxious activity but are devoid of desensitizing activity on the rat cornea (Szolcsányi and Jancsó-Gábor, 1976) and that, in contrast, other analogues such as olvanil are able to desensitize against capsaicin in the absence of stimulant activity (Dickenson et al., 1990b; Dray et al., 1990c). Furthermore, in some assays of capsaicin-like activity, such as corneal nociception (Szallasi and Blumberg, 1989) and contraction of the rat urinary bladder (Maggi et al., 1990c), resiniferatoxin is orders of magnitude more potent in desensitizing than in exciting sensory nerve fibers.

It is not yet clear how the divergence in the stimulant and desensitizing activities of some capsaicin derivatives is to be explained, but two possibilities are especially worth considering. One line of evidence suggests that excitation, desensitization, and neurotoxicity of capsaicinoids are mediated by a common site of action and that mismatches in the activities of some congeners arise from differences in their pharmacokinetic behaviour and/or metabolic stability or from differences in the ligand-receptor interactions with respect to binding forces and binding reversibility (Szolcsányi and Jancsó-Gábor, 1976). Support for this contention comes from the findings that the potencies of many capsaicin congeners to stimulate and desensitize sensory neurons are grossly correlated with each other (Szolcsányi and Jancsó-Gábor, 1975, 1976; Szolcsányi, 1982; Hayes et al., 1984b; James et al., 1988) and with their potency to induce afferent neuron degeneration in the newborn rat (Jancsó and Király, 1981). Furthermore, the structure-activity relationships of intrathecal capsaicinoids for the acute release of substance P in the spinal cord, desensitization to this effect, long-lasting antinociception, and long-term depletion of substance P from afferent nerve terminals in the spinal cord are comparable (Jhamandas et al., 1984). The potency of a number of capsaicin congeners in preventing labeling of cultured sensory neurons with a photoaffinity probe also correlates well with their potency as agonists in the calcium uptake assay (James et al., 1988).

Other data prompt speculation that capsaicin-induced excitation and desensitization of sensory neurons involve two different sites of action. In line with this contention, differences in the structural requirements for capsaicin analogues to cause stimulation and desensitization of sensory neurons are apparent. The most important difference seems to relate to the apolar moiety, because changes in this group (e.g., olvanil) lead to a loss of excitatory activity, whereas desensitizing potency is maintained (Dickenson et al., 1990b; Dray et al., 1990c). Furthermore, the optimal length of capsaicin's alkyl chain for desensitizing potency (10 to 12 carbon atoms) is somewhat longer than that required for excitatory activity (8 to 10 carbon atoms) (Szolcsányi and Jancsó-Gábor, 1976), and the especially high desensitizing activity of resiniferatoxin with its diterpene substituent underlines the importance of the apolar moiety for desensitization (Szallasi and Blumberg, 1989, 1990b). Another point concerns the acylamide linkage, the presence of which was previously believed to be required for desensitizing activity (Jancsó, 1968; Szolcsányi and Jancsó-Gábor, 1976). This bond is not essential, however, because resiniferatoxin, which has an ester group instead of the acylamide, displays a particularly high desensitizing potency (Szallasi and Blumberg, 1989).

3. Resiniferatoxin as a very potent capsaicin agonist. Apart from capsaicin, there are a number of other natural and synthetic pungent compounds, including zingerone, shogaol, chavicine, piperine, guajacol, isoeugenol, eugenol, xylene, curcumin, and mustard oil, that show variable degrees of structural and/or pharmacological overlap with capsaicin (Jancsó, 1968; Szolcsányi and Jancsó-Gábor, 1975, 1976; Szolcsányi, 1982; Abelli et al., 1988; Miyauchi et al., 1989; Patacchini et al., 1990; Takaki et al., 1990). Among these, capsaicin is by far the most potent compound in terms of nociceptor stimula-

tion and desensitization and sensory neuron ablation. Another compound, resiniferatoxin, has been known for some time to be a potent irritant whose mechanism of action is not understood. It is a substance that occurs naturally in the latex of certain species of *Euphorbia*. Structurally, it combines characteristics of the phorbol esters and of capsaicin (fig. 1). Like capsaicin, it contains a 4-hydroxy-3-methoxy benzyl (vanillyl) moiety connected to an ester group which, in the capsaicin molecule, can replace the acylamide group in reversed position without loss of activity (Szolcsányi and Jancsó-Gábor, 1975). This ester group connects the vanillyl moiety to the 20-hydroxyl substituent of the complex diterpene structure. Thus, unlike active phorbol esters, resiniferatoxin lacks a free 20-hydroxyl group which is essential for phorbol ester activity and thus fails to induce the typical phorbol ester effects including tumor promotion, binding to, and stimulation of, protein kinase C (Szallasi and Blumberg, 1989, 1990b; Dray et al., 1990a; Winter et al., 1990).

In keeping with the structural similarity between resiniferatoxin and capsaicin, resiniferatoxin appears to share most, if not all, of the effects of capsaicin on sensory neurons. Thus, resiniferatoxin depolarizes cultured neurons from rat dorsal root ganglia and increases cation fluxes across the cell membrane but is approximately two orders of magnitude more potent than capsaicin (Winter et al., 1990). A similar activity of resiniferatoxin relative to capsaicin is seen with resiniferatoxin's stimulant effect on cutaneous nociceptors in an in vitro preparation of the neonatal rat tail-spinal cord (Dray et al., 1990a; Winter et al., 1990) and on the release of substance P and calcitonin gene-related peptide from the rat urinary bladder, rat spinal cord, and rabbit ear (Maggi et al., 1990c). In other tests, such as induction of hypothermia (de Vries and Blumberg, 1989; Szallasi and Blumberg, 1989), induction of plasma protein extravasation (Szallasi and Blumberg, 1989) and inhibition of electrically induced twitch contractions of the rat vas deferens (Maggi et al., 1990c), resiniferatoxin is even three to four orders of magnitude more potent than capsaicin. To complicate the situation further, resiniferatoxin is not or only marginally more active than capsaicin in inducing corneal nociception (Szallasi and Blumberg, 1989), reflex hypotension, and urinary bladder contraction (Maggi et al., 1990c) and, unlike capsaicin, fails to evoke the full Bezold-Jarisch reflex in the rat (Szolcsányi et al., 1990). In the cat, however, the full Bezold-Jarisch reflex is evoked by resiniferatoxin (Szolcsányi et al., 1990). Thus, although the excitatory effects of capsaicin and resiniferatoxin on sensory neurons are qualitatively similar, there are some important quantitative differences between the potencies of the two drugs.

There also are similarities and differences between the intermediate (desensitizing) and long-term neurotoxic effects of the two drugs. In contrast to its stimulant effect, resiniferatoxin is generally more active in producing desensitization to itself and capsaicin, the desensitizing potency of resiniferatoxin exceeding that of capsaicin by three to four orders of magnitude (de Vries and Blumberg, 1989; Szallasi and Blumberg, 1989, 1990b; Dray et al. 1990a; Maggi et al., 1990c; Winter et al., 1990; Szolcsányi et al., 1991). In the rat, intravenous doses of resiniferatoxin that are too low to evoke the Bezold-Jarisch reflex are able to desensitize the pulmonary chemoreceptors, which are responsible for this reflex, against the excitatory action of capsaicin (Szolcsányi et al., 1991). At low doses, desensitization involves only the excitatory responses to resiniferatoxin and capsaicin (Dray et al., 1990a; Winter et al., 1990), whereas at higher doses insensitivity to all noxious stimuli of sensory neurons is produced (Szallasi and Blumberg, 1989; Dray et al., 1990a; Winter et al., 1990). Unlike capsaicin, resiniferatoxin is also able to desensitize pulmonary chemoreceptors to the excitatory effect of phenyldiguanide (Szolcsányi et al., 1990).

Systemic treatment of adult rats with resiniferatoxin (100 to 400 μg/kg) causes defunctionalization of sensory neurons as shown by the abolition of capsaicin- or resiniferatoxin-evoked hypothermia, leakage of plasma protein in the skin and trachea, and chemonociception in the cornea (Szallasi and Blumberg, 1989; Szallasi et al., 1989; Szolcsányi et al., 1990). In contrast, cardiovascular responses to stimulation of parasympathetic efferent neurons are not affected by systemic resiniferatoxin pretreatment (Szolcsányi et al., 1990).

The defunctionalization of sensory neurons is due to resiniferatoxin's neurotoxic action as shown by morphological alterations in their cell bodies. Like capsaicin, resiniferatoxin given to adult rats induces ultrastructural changes in small dark neurons of the dorsal root ganglia, the alterations being characterized by swelling and disorganization of mitochondria, but resiniferatoxin is approximately three orders of magnitude more potent than capsaicin (Szallasi et al., 1989; Szolcsányi et al., 1990). A-type neurons in dorsal root ganglia or sympathetic ganglion cells in the superior cervical ganglion are not affected by resiniferatoxin (Szolcsányi et al., 1990). In addition, systemic resiniferatoxin leads to the accumulation of ionic calcium in some small sensory neurons, an effect that is more marked than that occurring with capsaicin (Szallasi et al., 1989) and which, according to Jancsó et al. (1978), could indicate that in the adult rat resiniferatoxin causes more sensory neurons to degenerate than does capsaicin. Neurochemically, resiniferatoxin has been found to deplete calcitonin gene-related peptide from the spinal and cranial sensory ganglia and the dorsal horn of the spinal cord (Szolcsányi et al., 1990).

Administration of resiniferatoxin (300 μg/kg) to newborn rats leads to approximately a 50% degeneration of

cell bodies in the dorsal root ganglia, which is associated with an almost complete depletion of calcitonin gene-related peptide from the spinal and cranial sensory ganglia and the dorsal horn of the spinal cord (Szallasi et al., 1990). These morphological and neurochemical alterations induced by administration of resiniferatoxin to newborn rats are paralleled by a complete loss of chemonociception in the cornea and by inhibition of neurogenic edema formation in the skin (Szallasi et al., 1990). The range and magnitude of the neurotoxic effects produced by neonatal resiniferatoxin are similar to those produced by neonatal capsaicin, but resiniferatoxin is at least two orders of magnitude more potent than capsaicin (Szallasi et al., 1990). Similarly, a 50-min exposure of cultured sensory neurons from newborn rats to 10 to 100 nM resiniferatoxin causes the same extent of neuritic damage as exposure to 1 to 10 μM capsaicin (Winter et al., 1990).

Further differences between capsaicin and resiniferatoxin concern the time course of their acutely excitatory and long-term neurotoxic effects. Thus, resiniferatoxin's effects in depolarizing cultured sensory neurons (Winter et al., 1990), in producing corneal nociception (Szallasi and Blumberg, 1989), in inducing plasma protein extravasation in the skin (Szallasi and Blumberg, 1989), and in depressing electrically evoked contractions of the rat isolated vas deferens (Maggi et al., 1990c) are slower in onset but longer lasting than those of capsaicin. Accordingly, the inhibitory effects of systemic resiniferatoxin on sensory nerve-mediated chemical pain, hypothermia, and plasma protein leakage also take longer to recover than do the equivalent effects of systemic capsaicin (Szallasi et al., 1989). There is evidence that the slower onset of resiniferatoxin's acute effects is, at least in part, due to a slower tissue penetration rate of this drug as compared with that of capsaicin (Maggi et al., 1990c). This difference in the pharmacokinetic behaviour of capsaicin and resiniferatoxin could explain why the relative potencies of the two drugs in acutely stimulating sensory neurons vary so much in different assay systems, whereas their relative activities in causing desensitization and neurotoxicity are less variable. Whether, in addition, a heterogeneity of receptors accounts for some of the differences in the actions of capsaicin and resiniferatoxin (Szallasi and Blumberg, 1990b) has not yet been established. The two drugs have been found to bind to a cellular recognition site, the kinetics of which are best described by a one-site model (Szallasi and Blumberg, 1990a). However, following resiniferatoxin treatment of newborn rats there is not only a permanent reduction in the number of [^{3}H]resiniferatoxin-binding sites in sensory ganglia but also a 4- to 6-fold decrease in the affinity of the remaining binding sites, a finding that is consistent with a heterogeneity of resiniferatoxin/capsaicin receptors (Szallasi et al., 1990).

Cross-desensitization between the stimulant effects of capsaicin and resiniferatoxin on sensory neurons (Szallasi and Blumberg, 1989; Szallasi et al., 1989; Dray et al., 1990a; Maggi et al., 1990c; Winter et al., 1990) is another finding that may indicate a common site of action of the two drugs. However, this argument is only valid for the specific desensitization and cross-desensitization to capsaicin/resiniferatoxin (Amann, 1990; Winter et al., 1990). In the case of nonspecific desensitization, responsiveness to the drugs seems to be lost because of cell damage. Consequently, no inference as to a common site of action can be made, and all that can be deduced is that the cross-desensitizing drugs act on the same group of cells (Maggi et al., 1990c; Winter et al., 1990).

4. Capsazepine as a competitive antagonist of capsaicin. An important step in proving the existence of a specific recognition site for capsaicin was the development of a competitive antagonist, capsazepine. This compound (2-[2-(4-chlorophenyl)ethylamino-thiocarbonyl]-7,8-dihydroxy-2,3,4,5-tetrahydro-1H-2-benzazepine) is structurally related to capsaicin and potently antagonizes all sensory neuron-selective effects of capsaicin but lacks agonistic activity (Bevan et al., 1991; Dray et al., 1991). In cultured dorsal root ganglion cells from newborn rats, capsazepine reversibly antagonizes the excitatory action of capsaicin. The concentration of the antagonist needed to reduce capsaicin-evoked uptake and accumulation of $^{45}Ca^{2+}$ in these neurons is 0.5 μM (Bevan et al., 1991). Analysis of the inhibition of capsaicin-induced $^{86}Rb^+$ efflux from cultured dorsal root ganglion neurons and [^{14}C]guanidinium efflux from isolated rat vagus nerve segments indicates that the antagonism exerted by capsazepine is competitive. Thus, log concentration-effect curves are shifted to the right in a parallel manner with increasing concentrations of the antagonist. Schild plots are linear, display a slope very close to 1, and give K_d estimates of 0.1 to 0.7 μM (Bevan et al., 1991).

In keeping with these pharmacological properties, capsazepine has been found to antagonize the effects of capsaicin in various test preparations (Dray et al., 1991). Pain evoked by intradermal injection of capsaicin into human skin is inhibited by 50% after intradermal administration of 10 to 100 pmol of the antagonist. The concentration of capsazepine that is required to reduce by one-half the capsaicin-evoked activation of nociceptive C-fiber units in an isolated preparation of the rat saphenous nerve and hindpaw skin is approximately 1 μM (Dray et al., 1991). This potency of capsazepine compares well with its activity in antagonizing capsaicin-evoked stimulation of nociceptors in the spinal cord-tail preparation from the neonatal rat (IC_{50} 0.2 μM) and capsaicin-induced activation of afferent C-fiber terminals in the isolated mouse spinal cord (IC_{50} 1 to 5 μM). Furthermore, the antagonist blocks the capsaicin-induced reflex decrease in rat blood pressure, 50% reduction being seen with doses of 10 nmol given intraarterially, and reduces sensory nerve-mediated contractions of

the guinea pig isolated ileum with an IC_{50} of 0.01 to 0.05 μM (Dray et al., 1991). Importantly, in all of these test preparations only responses to capsaicin, but not to other stimuli, are blocked by capsazepine.

The discovery of capsazepine as a competitive antagonist of capsaicin has added significant support to the hypothesis that capsaicin's actions on sensory neurons are mediated by a specific recognition site with the pharmacological properties of a receptor. The availability of such an antagonist is a significant asset in the further exploration of the sensory neuron-selective actions of capsaicin, their mechanisms, and functional significance.

5. Biochemical characterization of the recognition site for capsaicin. Biochemical characterization of the cellular recognition site for capsaicin through a study of its specific binding has not been possible because of technical problems arising from the lipophilic nature of this ligand (James et al., 1988). This problem was overcome when [^{3}H]resiniferatoxin was used as the ligand (Szallasi and Blumberg, 1990a). Binding studies with this labeled compound have indicated the presence of a single class of specific saturable binding sites for [^{3}H]resiniferatoxin on membranes from dorsal root and trigeminal ganglia (Szallasi and Blumberg, 1990a). This neuronal binding site for resiniferatoxin, characterized by a K_d of 0.13 to 0.27 nM, is distinct from protein kinase C which seems to recognize a different aspect of the diterpene moiety (Szallasi and Blumberg, 1990a,b). Subcellularly, most of the binding sites for resiniferatoxin are located in the microsomal/plasma membrane fraction of pig dorsal root ganglia (Szallasi and Blumberg, 1990a). The specific binding of [^{3}H]resiniferatoxin to sensory ganglion membranes is displaced by resiniferatoxin, capsaicin, and other capsaicinoids, their relative potencies being similar to those in producing hypothermia and plasma protein leakage in vivo (Szallasi and Blumberg, 1989, 1990a). Inorganic calcium channel blockers (nickel and cadmium) and ruthenium red, which can block certain phases of capsaicin's and resiniferatoxin's stimulant effects on sensory neurons (Wood et al., 1988; Amann et al., 1989a; Maggi et al., 1988d; Dray et al., 1990d) apparently do not inhibit [^{3}H]resiniferatoxin binding (Szallasi and Blumberg, 1990a,b). These compounds, therefore, do not seem to interact with the recognition site for resiniferatoxin per se. Sodium deoxycholate, which has been reported to facilitate capsaicin's acute effects (Jin and Nakayama, 1990), is likewise without effect on the resiniferatoxin-binding site (Szallasi and Blumberg, 1990b).

Specific recognition sites for [^{3}H]resiniferatoxin have been found in dorsal root and trigeminal ganglia of rat, pig, cow, and sheep but not in those of the chicken, a species that is not responsive to the pungent action of capsaicin (Szallasi and Blumberg, 1990a). No specific binding of resiniferatoxin has been detected in the preoptic region of the brain, striatum, substantia nigra, cerebellum, and whole spinal cord of the rat, but one must consider that minor binding components could be obscured by a high level of nonspecific binding of resiniferatoxin (Szallasi and Blumberg, 1990a,b). Nevertheless, the available findings indicate that specific binding of [^{3}H]resiniferatoxin reflects the existence of a specific cellular recognition site for resiniferatoxin/capsaicin that is exclusively present on membranes of sensory ganglia. This contention is underlined by the finding that resiniferatoxin treatment of newborn rats leads to a 41 to 53% loss of cell bodies and to a 79 to 91% reduction of specific [^{3}H]resiniferatoxin-binding sites in the dorsal root ganglia of adult rats; the binding sites in the trigeminal ganglion are reduced by 30 to 56% (Szallasi et al., 1990). It can be anticipated that the [^{3}H] resiniferatoxin-binding assay will have great potential in further unraveling the molecular structure, biochemical processing, and properties of the resiniferatoxin/capsaicin receptor.

The ability of photoaffinity probes to irreversibly label the capsaicin recognition site (James et al., 1988) has been utilized in an attempt to isolate protein components of the putative capsaicin receptor (Wood et al., 1990). Thus, a major protein with an apparent molecular weight of 58 kD and a minor protein with an apparent molecular weight of 42 kD can be isolated from rat and chick dorsal root ganglia but are absent from rat liver and cerebellum (Wood et al., 1990). However, the inability of capsaicin to protect these proteins from labeling with the photoaffinity probe does not allow them to be designated as part of the capsaicin recognition site (Wood et al., 1990). This inference is also supported by the finding that the labeled proteins are present in both rat and chick dorsal root ganglion neurons, although chick sensory neurons are insensitive to capsaicin in the calcium uptake assay (Wood et al., 1988, 1990). Consequently, a possible relationship of the labeled proteins to the capsaicin receptor/cation channel complex remains unknown.

B. Mechanisms of the Sensory Neuron-selective Effects

1. Acute excitation of sensory neurons. a. PRIMARY EFFECT: ACTIVATION OF A CATION CONDUCTANCE. As potent stimulants of sensory neurons, capsaicin and resiniferatoxin depolarize both axons and somata of rat primary afferent neurons. The depolarization derives from an inward current which sometimes is followed by an outward current (Ault and Evans, 1980; Yanagisawa et al., 1980; Williams and Zieglgänsberger, 1982; Baccaglini and Hogan, 1983; Hayes et al., 1984a; Heyman and Rang, 1985; Bevan et al., 1987; Marsh et al., 1987; Winter et al., 1988; Bevan and Szolcsányi, 1990; Bleakman et al., 1990; Dray et al., 1990d; Docherty et al., 1991). Because the inward current is associated with a conductance increase, it would follow that ion channels are opened by the drugs. Indeed, ion flux (Wood et al., 1988, 1989; Winter et al., 1990) and patch clamp (Bevan and Forbes, 1988; Forbes and Bevan, 1988; Bevan and Szolcsányi, 1990) studies of cultured sensory neurons

indicate that capsaicin and resiniferatoxin open a membrane ion channel that is relatively nonselective for cations and which allows the passage of both mono- and divalent cations including Na^+, K^+, Cs^+, Rb^+, Ca^{2+}, Mg^{2+}, and guanidium ions. The reversal potential for the inward current evoked by capsaicin and resiniferatoxin is approximately 0 mV, which is also consistent with the opening of a nonselective cation channel (Bevan and Forbes, 1988; Winter et al., 1990). In contrast, the efflux of Cl^- is left unaltered by capsaicin (Wood et al., 1988), and it seems that Cl^- and other anions do not participate directly in the conductance increase (Bevan and Szolcsányi, 1990; Winter et al., 1990), although there could be a secondary increase in the conductance for Cl^- ions (Marsh et al., 1987).

The permeability sequence of the capsaicin-operated cation channel is $Ca^{2+} > Mg^{2+} > K^+ > Na^+$ (Bevan and Szolcsányi, 1990). Thus, under physiological conditions both Ca^{2+} and Na^+ move into the cell and carry the capsaicin/resiniferatoxin-induced inward current while K^+ ions leave the cell (Wood et al., 1988; Bleakman et al., 1990; Dray et al., 1990d; Winter et al., 1990). Removal of either Ca^{2+} or Na^+ from the extracellular fluid does not abolish the inward current because in this instance the inward current will be carried, at least in part, by the other cation (Bevan and Forbes, 1988). Thus, capsaicin-induced depolarization is reduced but not prevented by removal of extracellular Na^+ (Bevan et al., 1987; Marsh et al., 1987). In contrast, omission of extracellular Ca^{2+} completely fails to inhibit the excitatory action of capsaicin but, if anything, enhances it (Yanagisawa et al., 1980; Baccaglini and Hogan, 1983; Marsh et al., 1987; Bettaney et al., 1988; Amann et al., 1989a; Dray et al., 1990b; Docherty et al., 1991).

Further analysis of the capsaicin/resiniferatoxin-induced membrane current on outside-out membrane patches has shown that capsaicin evokes single-channel currents which display the same cation permeability as the currents obtained from whole cell recordings (Forbes and Bevan, 1988; Bevan and Szolcsányi, 1990; Dray et al., 1990d). The conductance of single channels opened by capsaicin and resiniferatoxin is approximately 100 pS at +60 mV and 20 to 30 pS at −60 mV (Forbes and Bevan, 1988; Bevan and Szolcsányi, 1990). The data obtained from isolated membrane patches of sensory neurons indicate that capsaicin-like drugs act directly on the plasma membrane without any intervention of a second-messenger system, a conclusion for which there is also biochemical evidence (Wood et al. 1989; Bevan and Szolcsányi, 1990; Dray et al., 1990a,b; Winter et al., 1990). Accordingly, inhibition or activation of protein kinase C, cyclic AMP‡-dependent kinase, cyclic GMP-dependent kinase, calmodulin-dependent kinase, phospholipase A_2, and cyclooxygenase does not affect the sensory neuron responses to capsaicin and resiniferatoxin (Dray et al., 1990a,b; Winter et al., 1990). The capsaicin/resiniferatoxin-evoked increase in the levels of cyclic GMP in sensory neurons does not have any bearing on the capsaicin/resiniferatoxin-evoked ion fluxes through the cell membrane (Wood et al., 1989; Winter et al., 1990).

There are several lines of evidence indicating that the capsaicin-operated cation channel is distinct from voltage-dependent cation channels in the cell membrane. First, the effect of capsaicin to release peptides is not prevented by K^+ depolarization of sensory nerve terminals (Donnerer and Amann, 1990). Second, blockers of voltage-dependent Ca^{2+} channels of the T-, N- and L-type (such as nickel ions, omega-conotoxin, and nifedipine or verapamil, respectively) fail, in general, to inhibit the stimulant effect of capsaicin (Zernig et al., 1984; Maggi et al., 1988a,c,e, 1989c,e, 1990b; Wood et al., 1988; Dray et al., 1990b). The effect of low capsaicin concentrations, however, may be reduced by omega-conotoxin (Lou et al., 1991). Third, local anaesthetics and particularly tetrodotoxin are without effect on capsaicin's local depolarizing and excitatory actions on sensory nerve endings and axons (Jancsó et al., 1968; Gamse et al., 1979b; Saria et al., 1983a; Szolcsányi, 1983b, 1984b; Hayes et al., 1984a; Hua et al., 1986; Marsh et al., 1987; Santicioli et al., 1987; Maggi et al., 1989c, 1990b). This latter finding indicates that fast voltage-dependent Na^+ channels are not involved in the action of capsaicin on the cell membrane.

The capsaicin/resiniferatoxin-induced activation of a nonselective cation conductance leads to a sustained increase in the intracellular concentration of free Ca^{2+} (Bleakman et al., 1990; Dray et al., 1990d) and to a net uptake of $^{45}Ca^{2+}$ into the cell (Wood et al., 1988, 1989; Winter et al., 1990). This Ca^{2+} uptake is a specific response of B-type sensory neurons of the rat and mouse (and probably other mammalian species) which is absent in the vast majority of rat A-type sensory neurons, in rat sympathetic neurons, Schwann cells and fibroblasts, in neuronal cell lines, and in chick sensory neurons (Wood et al., 1988; Winter et al., 1990). The activity of capsaicin in causing an influx of calcium (EC_{50} 100 to 300 nM) agrees very well with estimates of its potency in depolarizing sensory neurons (Marsh et al., 1987), activating nociceptors (Dray et al., 1990b), and evoking peptide release from sensory nerve terminals (Amann, 1990).

Divalent cations (order of potency: cadmium ≫ nickel ≫ cobalt > barium > strontium > manganese > magnesium) can inhibit the capsaicin-induced uptake of $^{45}Ca^{2+}$ into sensory neurons (Wood et al., 1988; Bleakman et al., 1990). These data, however, do not necessarily indicate that the capsaicin-operated cation channel is blocked as well. Thus, cadmium fails to prevent the capsaicin-induced increase in free intracellular Ca^{2+}, and it seems as if the inhibitory effect of this ion on the

‡Abbreviations: AMP, adenosine monophosphate; GMP, guanosine monophosphate; NGF, nerve growth factor.

capsaicin-induced Ca^{2+} uptake arises from inhibition of intracellular Ca^{2+} sequestration or cellular Ca^{2+} extrusion (Dray et al., 1990d). Inhibition of cation influx also is ruled out by the finding that capsaicin-induced depolarization and activation of sensory nerve endings is not blocked by either cadmium (Marsh et al., 1987; Dray et al., 1990b,d) or nickel (Maggi et al., 1988e). It may well be that some of the divalent ions that block the capsaicin-induced Ca^{2+} uptake do so by entering the cells themselves instead of Ca^{2+}. Thus, cobalt appears to be taken up via the capsaicin-operated cation channel, which explains why cobalt staining is selective for capsaicin-sensitive B-type sensory neurons (Winter, 1987; Winter et al., 1988, 1990; Wood et al., 1988). In addition, cadmium itself has been reported to display some capsaicin-like stimulant activity on capsaicin-sensitive sensory neurons (Patacchini et al., 1988).

The stimulant effect of capsaicin on sensory neurons in vivo displays a high degree of temperature dependency. The capsaicin-induced burning sensation on the human tongue and skin is inhibited or even abolished by cooling of the respective tissue to 24°C (Green, 1986) and 20°C (Szolcsányi, 1977), whereas warming augments burning. Capsaicin-induced action potential activity in afferent nerve fibers is likewise inhibited by cooling of the skin (Szolcsányi, 1977). Thus, the temperature dependency of capsaicin's effect seems to be related to a peripheral process occurring at, or close to, the sensory receptors (Szolcsányi, 1977, 1990). However, the capsaicin-evoked uptake of Ca^{2+} into rat cultured dorsal root ganglion cells is not much different at 37° and 20°C (Wood et al., 1988), although peptide release from sensory nerve terminals is reduced at 18°C (Amann, 1990). It would seem, therefore, that the opening of capsaicin-operated cation channels is not blocked by cooling but that in some way the initiation of propagated action potentials is prevented and peptide release is reduced. This poorly understood aspect of capsaicin's action on sensory neurons needs to be investigated further.

Taken together, the available evidence indicates that capsaicin stimulates sensory neurons by an interaction with a specific recognition site on their cell membrane, which in turn leads to activation of a conductance that is nonselective for cations and different from voltage-dependent ion channels. Capsaicin-induced depolarization and Ca^{2+} influx through this mechanism, however, exerts secondary effects on voltage-dependent and Ca^{2+}-activated membrane currents of sensory neurons. The increase in the intracellular concentration of Ca^{2+} also leads to activation of intracellular enzymes and to transmitter (peptide) release.

b. SECONDARY EFFECTS. **i. Intracellular ion accumulation.** The capsaicin/resiniferatoxin-induced opening of a nonselective cation channel leads to the net uptake of Ca^{2+} and Na^{+} (Wood et al., 1988; Winter et al., 1990). The magnitude of the intracellular $^{45}Ca^{2+}$ accumulation in sensory neurons exposed to capsaicin is unique in that no other stimuli have yet been identified to cause a comparable accumulation. Quantitatively, it is estimated that intracellular concentrations of up to 12 mM calcium can be reached in response to 1 μM capsaicin (Wood et al., 1988). Calcium is thus concentrated in the cell but, because the intracellular concentration of free Ca^{2+} does not exceed 0.6 to 0.9 μM (Bleakman et al., 1990; Dray et al., 1990d), it follows that influxing Ca^{2+} is sequestered by intracellular organelles. There are some data to indicate that the major part of Ca^{2+} entering the cell is taken up by mitochondria rather than by the endoplasmic reticulum and that this process requires an oxidative energy supply (Wood et al., 1988). Thus, drugs known to interfere with the calcium binding of the endoplasmic reticulum (e.g., caffeine, isobutylmethylxanthine) do not inhibit capsaicin-induced uptake of calcium (Wood et al., 1988). In contrast, uncoupling of oxidative phosphorylation in the mitochondria by cyanide or dinitrophenol inhibits the capsaicin-induced uptake of calcium (Wood et al., 1988) and release of calcitonin gene-related peptide from sensory nerve endings (Amann et al., 1990c). It is not clear from these findings, however, whether the process of calcium accumulation is inhibited because of energy deprivation of the cell or because of blockade of calcium sequestration in the mitochondria (R. Amann, personal communication). Nevertheless, a mitochondrial site of calcium accumulation is strongly suggested by electron microscopical observations of swollen mitochondria (Joó et al., 1969; Szolcsányi et al., 1975, 1990; Chiba et al., 1986; Marsh et al., 1987; Szallasi et al., 1989) and by mitochondrial Ca^{2+} deposits (Jancsó et al., 1978, 1984) in sensory neurons exposed to capsaicin or resiniferatoxin. The inhibitory effect of ruthenium red on capsaicin-induced uptake of calcium, previously thought to occur by interference at a mitochondrial level (Wood et al., 1988), is now thought to result from an action on the cell membrane (Chahl, 1989; Bleakman et al., 1990; Dray et al. 1990d). The inhibitory effects of cinnarizine and trifluoperazine on the capsaicin-induced accumulation of calcium are not understood in terms of site and mechanism of action (Wood et al., 1988). Trifluoperazine does not block capsaicin-induced stimulation of nociceptors (Dray et al., 1990b).

Capsaicin also causes an influx of Na^{+} in cultured sensory neurons (Wood et al., 1988). There is indirect evidence that this ion, as it accumulates intracellularly, is followed passively by Cl^{-}, which results in a net uptake of NaCl (Hogan, 1983; Winter et al., 1990).

ii. Effects on voltage-dependent and calcium-activated membrane currents. Capsaicin administration can lead to activation of voltage-dependent Ca^{2+} channels in sensory neurons (Petersen et al., 1989), but from ion flux studies it appears as if this occurs secondarily to the capsaicin-induced depolarization through opening of a nonselective cation-permeable channel

(Wood et al., 1988, 1989). Those somata in the dorsal root ganglia of the guinea pig which respond to capsaicin (about 50% of all somata) exhibit both fast- and slow-inactivating Ca^{2+} channels, whereas the capsaicin-insensitive neurons display slow-inactivating currents only (Petersen et al., 1989). As recorded under voltage clamp and intracellular perfusion conditions, capsaicin (30 μM) activates selectively the fast-inactivating channel, which may correspond to a T-type Ca^{2+} channel because it is blocked by nickel ions (Petersen et al., 1989). The action of capsaicin manifests itself in acceleration of channel activation and inactivation and in an increase in the Ca^{2+} current. In cultured rat dorsal root ganglion neurons, however, capsaicin was found to be unable to activate voltage-dependent Ca^{2+} channels (Docherty et al., 1991).

The capsaicin/resiniferatoxin-induced influx of Ca^{2+} appears to have at least two secondary effects on Ca^{2+}-dependent ion channels in the cell membrane. First, the increase in the intracellular Ca^{2+} concentration seems to activate a Ca^{2+}-dependent K^+ conductance, a contention that is supported by both electrophysiological (Marsh et al., 1987; Bleakman et al., 1990) and ion flux (Wood et al., 1988; Winter et al., 1990) studies. Although the capsaicin-operated cation channel per se will allow K^+ to leave the cell (Bevan and Szolcsányi, 1990), it appears as if most of the capsaicin/resiniferatoxin-evoked increase in the efflux of K^+ (measured by the efflux of $^{86}Rb^+$) reflects a Ca^{2+}-activated K^+ outward current, because it is inhibited in the absence of extracellular Ca^{2+} (Wood et al., 1988; Winter et al., 1990). Blockade of this K^+ outward current by removal of extracellular Ca^{2+} has been proposed to account for the facilitatory effect of Ca^{2+} removal on capsaicin's excitatory effect on sensory neurons (Amann et al., 1989a). The argument goes that in the absence of extracellular Ca^{2+} capsaicin can still activate an inward current carried by Na^+ but, because of the absence of Ca^{2+} entry, the K^+ outward current will be inhibited. Consequently, the inward current is enhanced because it is not cut short by the K^+ outward current and depolarization of sensory neurons is augmented (Amann et al., 1989a). An alternative explanation considers that Ca^{2+} ions normally impede the capsaicin-evoked passage of Na^+ ions (Forbes and Bevan, 1988). If so, removal of external Ca^{2+} will facilitate the entry of Na^+ and thereby amplify the capsaicin-induced depolarization of sensory nerve endings (Dray et al., 1990b).

Second, capsaicin-evoked influx of Ca^{2+} leads to inhibition of voltage-dependent Ca^{2+} channels (Bleakman et al., 1990; Docherty et al., 1991). This effect of capsaicin on cultured dorsal root ganglion cells, which is extremely long lasting (>1 h), is seen only in neurons that are depolarized by the drug (Docherty et al., 1991). Replacement of Ca^{2+} by Mg^{2+} or Ba^{2+} either strongly reduces (Bleakman et al., 1990) or abolishes (Docherty et al., 1991) the capsaicin-induced inhibition of voltage-gated Ca^{2+} currents, which indicates that this effect of capsaicin depends on a substantial increase in the intracellular Ca^{2+} concentration (Bleakman et al., 1990; Docherty et al., 1991). In addition, voltage-gated Ca^{2+} inward currents may be inhibited to a minor degree by the capsaicin-induced activation of an outward current and/or by a small direct blocking effect of the drug (Bleakman et al., 1990).

iii. Peptide release. Another consequence of the capsaicin-induced entry of Ca^{2+} is the release of peptides from sensory nerve endings. Exocytotic release of neurotransmitter substances is a process that depends on an influx of extracellular Ca^{2+}. Accordingly, capsaicin-induced release of neuropeptides from the central and peripheral endings of sensory neurons is inhibited by removal of extracellular Ca^{2+} (Gamse et al., 1979b; Thériault et al., 1979; Yanagisawa et al., 1980; Helke et al., 1981b; Hua et al., 1986; Maggi et al., 1988d, 1989e; Amann et al., 1989a; Amann, 1990). However, whereas the peptide release that occurs in response to electrical field stimulation is prevented by blockers of voltage-dependent Ca^{2+} channels (Holz et al., 1988; Maggi et al., 1989c, 1990b), peptide release induced by capsaicin is not blocked by Ca^{2+} channel inhibitors (Maggi et al., 1988c,e, 1989e) or by K^+ depolarization of the nerve terminals (Donnerer and Amann, 1990). This observation indicates that Ca^{2+} entry through the capsaicin-operated nonselective cation channel provides sufficient intracellular Ca^{2+} to induce peptide release. The massive uptake of Ca^{2+} induced by capsaicin (Wood et al., 1988) probably also explains why the requirements of extracellular Ca^{2+} for capsaicin-induced release of substance P from sensory nerve endings are substantially lower than those necessary to maintain release induced by other depolarizing stimuli (Gamse et al., 1979b; Maggi et al., 1988e, 1989e).

iv. Enzyme activation. The buildup of Ca^{2+} in mammalian sensory neurons also is likely to account for the activation of Ca^{2+}-dependent enzymes. Thus, capsaicin and resiniferatoxin increase cyclic GMP levels in those sensory neurons that also show uptake of calcium, but this biochemical change is unrelated to the drugs' primary effects on the cell membrane (Wood et al., 1989; Winter et al., 1990). The levels of cyclic AMP are not changed in cultured sensory neurons (Wood et al., 1989; Winter et al., 1990) which contrasts with reports that capsaicin can increase the levels of this phosphate in the rat brain (Horváth et al., 1979) and spinal cord (Northam and Jones, 1984). However, this effect of capsaicin in vivo may conceivably be an indirect action caused by transmitter release (Wood et al., 1989).

2. Desensitization. a. SPECIFIC DESENSITIZATION. Although the phenomenon of desensitization to capsaicin and structurally related substances has been well documented, its mechanism of action is still poorly understood. Desensitization to capsaicin can be differentiated

into a process that is specific for this drug and related compounds (Szolcsányi, 1977, 1987; Bernstein et al., 1981; Dray et al., 1989a,b, 1990a,b; Amann, 1990; Winter et al., 1990) and a process that is drug nonspecific and, hence, is better described as a defunctionalization of sensory neurons. Specific desensitization, as studied on capsaicin-evoked release of neuropeptides from peripheral sensory nerve terminals, is restricted to low, near-threshold concentrations of the drug (Dray et al., 1989b; Amann, 1990). However, the range of concentrations of capsaicin producing specific desensitization seems to be extended by lowering the experimental temperature (Dray et al., 1989b) so that, in the neonatal rat spinal cord-tail preparation kept in vitro at 24°C, specific desensitization can be demonstrated with capsaicin concentrations up to 2 μM, a concentration that is supramaximal with regard to nociceptor stimulation (Dray et al., 1989a, 1990a,b,c; Winter et al., 1990).

As its definition implies, specific desensitization will only affect a signaling mechanism that is selectively operated by capsaicin-like drugs and does not impair the function of sensory neurons. Consequently, specific desensitization may arise from either desensitization of the capsaicin receptor itself (Szolcsányi and Jancsó-Gábor 1976; Dray et al., 1989a,b, 1990b; Amann 1990; Bleakman et al., 1990) or desensitization of the specific cell membrane response to capsaicin-like drugs (Amann, 1990; Bleakman et al., 1990). Like the temperature-dependent desensitization of muscarinic acetylcholine receptors (El-Fakahany and Richelson, 1980), desensitization to the capsaicin-induced peptide release from guinea pig ureter and rat urinary bladder is attenuated by lowering the temperature from 37° to 18° to 20°C, even when concentrations of capsaicin are used that release as much peptide as at 37°C (Dray et al., 1989b; Amann, 1990; Maggi et al., 1990a). Although this finding is compatible with the idea of receptor desensitization (Amann, 1990), this possibility is difficult to reconcile with the observation that the capsaicin-induced opening of nonselective cation channels in isolated cell membrane patches from sensory neurons is sustained as long as the drug is present (Forbes and Bevan, 1988; Dray et al., 1990d). Thus, receptor desensitization and rapid inactivation of the capsaicin-operated cation channel are unlikely to account for desensitization provided that the temporal characteristics of this channel are identical in membrane patches and intact cells. Moreover, there is no loss of resiniferatoxin-binding sites in dorsal root ganglia 12 h after systemic treatment of rats with resiniferatoxin (Szallasi and Blumberg, 1990a). The available data, therefore, suggest that specific desensitization to capsaicin arises from a mechanism beyond the capsaicin receptor and the capsaicin-operated cation channel.

The time and dose relationships between capsaicin-induced excitation and desensitization suggest that these phenomena are consecutive manifestations of one common effect of capsaicin on sensory neurons. Like excitation, specific desensitization does not involve any second-messenger system such as protein kinase C, cyclic AMP-dependent kinase, cyclic GMP-dependent kinase, calmodulin-dependent kinase, phospholipase A_2, or cyclooxygenase (Dray et al., 1990a,b; Winter et al., 1990). Removal of external Na^+ fails to influence specific desensitization, and neither excitation nor specific desensitization of nociceptors requires the presence of extracellular Ca^{2+} in the neonatal rat tail/spinal cord preparation (Dray et al., 1990b). This observation, however, is at variance with the finding that specific desensitization to the capsaicin-evoked peptide release from peripheral sensory nerve terminals requires the presence of external Ca^{2+} (Amann, 1990). This discrepancy might be resolved by speculating (R. Amann, personal communication) that desensitization to the stimulant effect of capsaicin in the neonatal rat tail/spinal cord preparation may, at least in part, be due to blockade of nerve conduction in afferent nerve fibers, a process that is independent of Ca^{2+} (Waddell and Lawson, 1989).

There are some data to suggest that capsaicin-induced excitation and specific desensitization are not related to each other but involve two different sites and/or mechanisms of action. Thus, specific desensitization can occur independently of capsaicin-induced stimulation of nociceptors (Dray et al., 1990b). Furthermore, the potencies of structurally related analogues of capsaicin in exciting and desensitizing sensory neurons do not always correlate with each other (Szolcsányi and Jancsó-Gábor, 1976; Hayes et al., 1984b; Szallasi and Blumberg, 1989, 1990b; Dray et al., 1990c; Maggi et al., 1990c). These studies, however, have rarely considered that differences in the activities may in part be determined by differences in the rates with which the different compounds can gain access to their sites of action in the tissue (Maggi et al., 1990c). It is not known to what extent such pharmacokinetic factors contribute to the activity profile of olvanil which is a capsaicin congener that can produce specific desensitization in the absence of excitation (Dickenson et al., 1990b; Dray et al., 1990c). The mechanism of olvanil-induced desensitization to capsaicin requires further exploration, particularly in view of the reports that in the tail of the neonatal rat 50 μM olvanil produces specific desensitization (Dray et al., 1990c), whereas in the spinal cord of the adult rat olvanil concentrations as low as 2 μM give rise to a nonspecific depression of stimulus-induced peptide release from sensory nerve terminals (Dickenson et al., 1990b).

b. NONSPECIFIC DESENSITIZATION. The mechanism of nonspecific desensitization to capsaicin implies a process that takes place beyond the level of a specific response to capsaicin because it is associated with the loss of excitability and physiological function of sensory neurons. The details of this mechanism are still not understood, but there are a number of possibilities to be

considered. Many reports concerning capsaicin desensitization, however, are difficult to assess because neither the reversibility of the process nor any relationship to morphological changes in sensory neurons has been examined. Consequently, it is often not possible to differentiate between temporary defunctionalization or longer-lasting neurotoxicity. Given the fact that in vitro 0.5 to 2 μM capsaicin or 1 to 10 nM resiniferatoxin can lead to damage of sensory neurons (Bevan et al., 1987; Marsh et al., 1987; Winter, 1987; Wood et al., 1988; Király et al., 1991; Winter et al., 1990) within 5 min (Marsh et al., 1987), it seems that nonspecific desensitization to these concentrations of the drugs reflects neurotoxicity. However, because nonspecific desensitization to low doses of capsaicin can be reversible within a couple of hours (He et al., 1990; Szolcsányi, 1990), there might be a range of concentrations that produce nonspecific desensitization in the absence of neurotoxicity. The width of the transition zone between concentrations of capsaicin that cause specific desensitization only and those that give rise to nonspecific desensitization as well appears to depend on the experimental temperature. At 37°C it is difficult to demonstrate specific desensitization at capsaicin concentrations >0.3 μM (Amann, 1990), whereas at 20° to 24°C desensitization is specific for the drug at concentrations up to 2 μM (Dray et al., 1989a,b, 1990a,b,c; Winter et al., 1990). However, a direct comparison of specific and nonspecific desensitization at different temperatures has not yet been made.

Fatigue and functional exhaustion of the neurons due to excessive stimulation are generally dismissed as causes of desensitization because the depolarizing effect of capsaicin abates within a few minutes (Hayes et al., 1984a; Bevan et al., 1987; Marsh et al., 1987; Winter et al., 1990; Docherty et al., 1991). This also rules out that a sustained depolarization of the neurons accounts for their prolonged inexcitability. Unlike specific desensitization, which can occur in the absence of an excitatory effect of capsaicin-like drugs (Dray et al., 1990b,c), nonspecific desensitization seems to be related to an initial excitatory effect (Dray et al., 1990c; He et al., 1990). Thus, olvanil causes nonspecific desensitization of nociceptors in the tail of newborn rats only at a concentration (500 μM) that is sufficient to produce initial excitation (Dray et al., 1990c). In contrast, 2 μM olvanil, which is only weakly active in stimulating peptide release from sensory nerve terminals in the spinal cord of adult rats, suppresses stimulus-evoked peptide release in a nonspecific manner (Dickenson et al., 1990b). Although it remains to be determined whether this peculiarity of olvanil's action is compatible with a common mechanism of excitation and nonspecific desensitization, it may at present be postulated that a component of the initial excitatory action of capsaicin on sensory neurons is a priming event that triggers a functional change that outlasts the initial effect. Unlike excitation and specific desensitization (Dray et al., 1990b), nonspecific desensitization depends critically on the availability of extracellular Ca^{2+} because removal of external Ca^{2+} blocks (Santicioli et al., 1987; Wood, 1987; James et al., 1988; Jin et al., 1989; Maggi et al., 1989e; Amann, 1990; Bleakman et al., 1990), and an increase of the extracellular Ca^{2+} concentration augments (Dray et al., 1990b), nonspecific desensitization. Furthermore, ruthenium red is considerably more potent in preventing desensitization than in blocking excitation, and protection from desensitization lasts much longer than does antagonism of excitation (Maggi et al., 1988b,d, 1989a; Chahl, 1989; Amann et al., 1990b).

Although the precise desensitization mechanism that is controlled by Ca^{2+} has not yet been elucidated, there are at least three possibilities to consider, all of which depend critically on the influx of Ca^{2+}. First, it has been speculated (Amann, 1990; Bevan and Szolcsányi, 1990) that nonspecific desensitization to capsaicin-evoked peptide release is caused by the sustained inactivation of voltage-gated Ca^{2+} channels which results from the capsaicin-induced influx of Ca^{2+} (Bleakman et al., 1990; Docherty et al., 1991). This effect is indeed very likely to contribute to defunctionalization of sensory neurons, and the long duration of the block of voltage-gated Ca^{2+} channels has been suggested to reflect a structural alteration of the channels induced by proteolytic enzymes (Docherty et al., 1991). Second, nonspecific desensitization may be a direct functional correlate of a mild and, therefore, reversible form of capsaicin's neurotoxic action on sensory neurons, a contention that receives strong support from the similarity in the ionic requirements of nonspecific desensitization (Dray et al., 1990b) and neurotoxicity (Winter et al., 1990). Thus, expression of nonspecific desensitization depends on the presence of extracellular Ca^{2+} and Na^{+} and may, like the neurotoxic action of capsaicin, arise from intracellular accumulation of calcium and NaCl (Dray et al., 1990b). The capsaicin/resiniferatoxin-evoked increase in cyclic GMP levels, another consequence of Ca^{2+} entry, is without influence on desensitization to these drugs (Wood et al., 1989; Winter et al., 1990).

A third aspect arises when desensitization to capsaicin is studied during the release of peptides from sensory neurons (Dray et al., 1989b; Amann, 1990; Amann and Lembeck, 1991). The characteristics of the capsaicin-induced release of substance P from, and the capsaicin-induced contraction of, the rabbit iris sphincter suggest that desensitization to capsaicin in this tissue could be accounted for by depletion of the releasable peptide pool (Ueda et al., 1984; Håkanson et al., 1987; Amann and Lembeck, 1991). In agreement with this contention, specific desensitization to capsaicin-induced peptide release does not occur in the rabbit iris sphincter (Amann and Lembeck, 1991). As neuropeptide release depends on Ca^{2+} influx, depletion of the releasable peptide pool will be prevented in the absence of extracellular Ca^{2+} and,

consequently, desensitization to capsaicin will not take place. In contrast, desensitization to capsaicin-induced peptide release in the guinea pig ureter (Dray et al., 1989b) and rat urinary bladder (Amann, 1990; Amann and Lembeck, 1991; Maggi et al., 1990a) does not seem to result exclusively from depletion of the releasable peptide pool because, with low concentrations of capsaicin, it is possible to demonstrate specific desensitization to capsaicin (Dray et al., 1989b; Amann, 1990; Amann and Lembeck, 1991). Furthermore, the total amount of peptide released by capsaicin is in the range of that released by other nondesensitizing stimuli and has been considered to be too small to significantly change the peptide content of sensory nerve endings in the rat and guinea pig (Gamse et al., 1979b; Hua et al. 1986; Dray et al. 1989b; Amann, 1990; Maggi et al., 1990a). In addition, the release of peptides induced by capsaicin is not sustained but abates within a matter of 10 to 20 min, whereas it takes hours until a depletion of peptides from rat sensory neurons becomes appreciable (Lembeck and Donnerer, 1981; Bittner and LaHann, 1985; Maggi et al., 1987e; Donnerer and Amann, 1990). These latter data certainly do not rule out that some exhaustion of the releasable peptide pool, the size of which may be small compared to the total peptide content, could occur (Amann, 1990). It is unlikely, however, that such a mechanism could have any bearing on desensitization to the depolarizing effect of capsaicin, peptide release being a secondary consequence of capsaicin's excitatory action on sensory neurons. Peptide release depends on Ca^{2+} entry through voltage-dependent membrane channels, and at present it is most reasonable to assume that the long-lasting inhibition of voltage-dependent Ca^{2+} channels (Bleakman et al., 1990; Docherty et al., 1991) underlies the capsaicin-induced nonspecific inhibition of peptide release.

3. Conduction block. Periaxonal application of capsaicin blocks conduction in afferent nerve fibers (Petsche et al., 1983; Lynn et al., 1984), an effect that phenomenologically resembles nonspecific desensitization. The mechanisms underlying these two effects, however, do not seem to be identical. Conduction block ensues within a few minutes and, in analogy to observations made on dorsal root ganglia (Williams and Zieglgänsberger, 1982), it could be argued that the initial phase of nerve block is due to a still prevailing depolarization of the axons (Handwerker et al., 1984; Baranowski et al., 1986). However, the block of nerve conduction lasts much longer than depolarization (Ault and Evans, 1980; Hayes et al., 1984a; Bevan et al., 1987; Marsh et al., 1987) and, with respect to duration, it has actually been possible to distinguish two components of nerve block in the isolated vagus nerve of the rat (Waddell and Lawson, 1989). Concentrations of up to 1 μM capsaicin cause a C-fiber block that is reversible within 90 min, whereas higher concentrations of the drug give rise to a block that is irreversible within the time of the experiment (Waddell and Lawson, 1989). The dose dependency of the reversible conduction block in vagal C-fibers (Waddell and Lawson, 1989) is similar to that found for the capsaicin-induced depolarization of the vagus (Marsh et al., 1987) and sciatic (Hayes et al. 1984a) nerves. Thus, although the transient depolarization does not explain the prolonged block of C-fiber conduction, it appears conceivable that a common factor triggers both effects of capsaicin or that depolarization triggers some functional change in the axons that outlasts the initial depolarization (Waddell and Lawson, 1989).

The long-lasting inhibition of voltage-gated Ca^{2+} channels that capsaicin induces in sensory ganglia (Bleakman et al., 1990; Docherty et al., 1991) could be a major factor in the conduction block if a similar effect were to be evoked in afferent axons. However, whereas the inhibition of voltage-gated Ca^{2+} channels depends on the presence of extracellular Ca^{2+} (Bleakman et al., 1990; Docherty et al., 1991), the block of nerve conduction in the vagus nerve is either not changed (Waddell and Lawson, 1989) or even enhanced (Marsh et al., 1987) by removal of extracellular Ca^{2+}. The presence of Ca^{2+}, however, is required for the recovery of the reversible component of the conduction block (Waddell and Lawson, 1989). Because the ionic dependency of the capsaicin-induced C-fiber block is similar to that of excitation (Yanagisawa et al., 1980; Baccaglini and Hogan, 1983; Marsh et al., 1987; Bettaney et al., 1988; Amann et al., 1989a), it could be proposed that opening of the capsaicin-operated nonselective cation channel leads to a temporary change in the axons which precludes the conduction of action potentials.

The dose dependency of the persistent ("irreversible") C-fiber block in the vagus nerve (Waddell and Lawson, 1989) is similar to that of the A-fiber block (Baranowski et al., 1986; Marsh et al., 1987). The blockade of afferent A-fibers, however, is readily reversible and most probably a reflection of the cell-nonselective actions which capsaicin exerts on a variety of neurons, as is discussed in a separate section, whereas the irreversible block of C-fiber conduction is a sensory neuron-selective effect of the drug. Because perineural application of millimolar solutions of capsaicin leads to a permanent degeneration of unmyelinated (probably afferent) nerve fibers (Lynn et al., 1987; Jancsó and Lawson, 1990; Pini et al., 1990), it would be logical to infer that the irreversible block of C-fiber conduction induced by concentrations of 3 to 50 μM (Waddell and Lawson, 1989) reflects neurotoxicity of the drug. However, although the neurotoxic effects of capsaicin can be inhibited by the removal of extracellular Ca^{2+} (Marsh et al., 1987; Winter et al., 1990), the irreversible block of C-fiber conduction is not blocked by this maneuver (Waddell and Lawson, 1989). It follows that the persistent C-fiber block, at least in its initial phase, does not depend on intracellular calcium accu-

mulation as is the case for the neurotoxic action of capsaicin. In conclusion, the mechanisms of capsaicin-induced blockade of C-fiber conduction remain to be elucidated.

4. Neurotoxicity. a. INTRACELLULAR ACCUMULATION OF CALCIUM AND NaCl. From the evidence available it seems that the stimulant and initial neurotoxic effects of capsaicin-related compounds arise from similar mechanisms of action. Two pathways leading to neurotoxicity can be differentiated, one pathway depending on the influx of Ca^{2+} and the other involving intracellular accumulation of NaCl. The critical involvement of Ca^{2+} in the expression of neurotoxicity is demonstrated by the findings that the neuronal damage caused by capsaicin and resiniferatoxin is markedly reduced by removal of extracellular Ca^{2+} (Marsh et al., 1987; Winter et al., 1990) but not by blockade of voltage-dependent calcium channels (Marsh et al., 1987). These observations have been taken to infer that entry of Ca^{2+} into the cell is a priming event for the neurotoxic action of capsaicin (Marsh et al., 1987). Entry of Ca^{2+} is an early response to capsaicin and related compounds and, in line with this, exposure of isolated nodose ganglion cells to 1 to 10 μM capsaicin leads to disruption of the microtubular and neurofilament organization within 5 to 10 min (Marsh et al., 1987). Cell damage in cultured sensory neurons develops at a similar speed (Winter et al., 1990), and the ultrastructural alterations are paralleled by a quick defunctionalization of sensory neurons (Marsh et al., 1987; Winter et al., 1990). The way in which Ca^{2+} influx leads to damage has not yet been studied, but it is likely that the massive accumulation of intracellular calcium that capsaicin and resiniferatoxin bring about in cultured sensory neurons (Wood et al., 1988; Winter et al., 1990) is a key event. This inference is supported by the finding that 2 μM capsaicin, which induces maximal calcium accumulation, causes a similar proportion of cultured sensory neurons to degenerate (Wood et al., 1988) as does systemic treatment of newborn rats with 50 mg/kg capsaicin. Furthermore, exposure of B-type sensory neurons to capsaicin or resiniferatoxin in vivo is followed by the histochemically demonstrable appearance of calcium in the cell bodies of these neurons (Szallasi et al., 1989), an effect that is particularly pronounced when capsaicin is given to newborn rats (Jancsó et al., 1978, 1984).

Excessive accumulation of calcium within cells is extremely toxic and appears to be a common final process by which toxins cause degeneration and cell death (Schanne et al., 1979; Kamakura et al., 1983). Calcium-activated proteases and other degradative enzymes are thought to destroy the cytoskeletal organization and thereby to interfere, for example, with axoplasmic flow (Kamakura et al., 1983), a function that is also inhibited by capsaicin (Gamse et al., 1982). In addition, the capacity of dorsal root ganglion cells to buffer excess intracellular calcium is apparently lower than that of other neurons (Jia and Nelson, 1986; Maggi and Meli, 1988).

It could be deduced from these considerations that different degrees of calcium accumulation, and the rapidity with which the calcium overloading of the cell can be reversed, determine whether transient defunctionalization (nonspecific desensitization) or long-lasting neurotoxicity ensues. Under circumstances in which the cell remains capable of buffering and disposing of the entering calcium, temporary nonspecific desensitization will be observed. However, when the cellular calcium loading in response to high concentrations of capsaicin is in excess of that which can be buffered and disposed of quickly, calcium accumulation will lead to ultrastructural changes and cell damage.

The observation that the neurotoxic effects of capsaicin and resiniferatoxin on cultured sensory neurons are also reduced when NaCl is replaced by sucrose, but abolished only when, in addition, external Ca^{2+} is removed as well, indicates the existence of a second mechanism of neurotoxicity (Winter et al., 1990). This other pathway requires the availability of permeant monovalent cations and anions and, under physiological conditions, appears to involve the influx of Na^+ through the capsaicin-operated cation channels; Na^+ entry is passively followed by Cl^- through a "resting leakage" route (Bevan and Szolcsányi, 1990; Winter et al., 1990). This comovement of Na^+ and Cl^- is necessary for neurotoxicity because replacement of external Cl^- with the impermeant ion glutamate abolishes those toxic effects of capsaicin and resiniferatoxin that are seen in the absence of external Ca^{2+} (Winter et al., 1990). The entry of Na^+ and Cl^- will result in a net uptake of NaCl, which is thought to be followed by an influx of water and osmotic lysis (Hogan, 1983; Bevan and Szolcsányi, 1990; Winter et al., 1990). Fiber enlargement and swelling of mitochondria indeed are prominent features of the ultrastructural changes produced by capsaicin-related compounds in sensory neurons (Joó et al., 1969, Szolcsányi et al., 1975, 1990; Hoyes and Barber, 1981; Hoyes et al., 1981; Sikri et al., 1981; Chiba et al., 1986, Marsh et al., 1987; Szallasi et al., 1989). Sensory neurons in culture can be killed by either pathway of neurotoxicity alone, but the development of damage is more rapid when both operate simultaneously as is the case under physiological conditions (Bevan and Szolcsányi, 1990; Winter et al., 1990).

b. SECONDARY DEPLETION OF PEPTIDES. Capsaicin-induced cell damage and/or defunctionalization precede the depletion of peptide markers such as substance P from sensory neurons in the rat (Lembeck and Donnerer, 1981; Gamse et al., 1982; Bittner and LaHann, 1985; Maggi et al., 1987d,e) and guinea pig (Miller et al., 1982a; Buck et al., 1983), although there are rapid changes in the immunohistochemical appearance of substance P-containing nerve fibers (Papka et al., 1984). It follows that depletion of peptides and other neuronal constitu-

ents is a secondary consequence of the neurotoxic action of capsaicin on sensory neurons of the rat and guinea pig. Consequently, it is not depletion of transmitter substances that leads to a long-lasting defunctionalization of sensory neurons as was thought in the early phases of research on capsaicin. It may be, however, that under certain circumstances peptide depletion is a factor in the later stages of the inhibitory effect of capsaicin on sensory neuron functions in the rat (Gamse et al., 1982; Maggi et al. 1987d). For instance, the onset of the inhibitory effect, which perineural capsaicin exerts on local effector functions of peripheral sensory nerve endings, parallels that of substance P depletion from afferent nerve fibers distal to the treatment site (Gamse et al., 1982). It could be inferred, therefore, that in this case peptide depletion causes the functional deficit, but the evidence for such a relationship is only correlative and not causal. There are too few data to decide whether there is a similar situation in the rabbit, in which capsaicin is less neurotoxic than in the rat or guinea pig and in which this drug apparently fails to induce degeneration or morphological damage of sensory neurons. Capsaicin, however, is capable of stimulating and defunctionalizing sensory neurons in this species (Szolcsányi, 1987). It is conceivable, therefore, that in the rabbit nonspecific desensitization to capsaicin results from depletion of the releasable pool of sensory neuropeptides (Håkanson et al., 1987; Amann and Lembeck, 1991).

5. Ruthenium red as a functional antagonist of capsaicin. The inorganic dye ruthenium red inhibits the capsaicin-induced stimulation of sensory neurons and prevents their desensitization to capsaicin in rat, guinea pig, rabbit, and other species (Amann and Maggi, 1991). At the concentration range of 0.03 to 1 μM, the dye selectively blocks capsaicin/resiniferatoxin-induced excitation of afferent neurons and leaves responses to other stimuli unaffected. Depending on the tissue under study, the selectivity for capsaicin-related compounds is lost when concentrations of ruthenium red higher than 1 to 20 μM are used (Amann and Maggi, 1991). The inhibitory effect of ruthenium red has been demonstrated on a variety of sensory neuron-mediated reflex responses to capsaicin including excitation of nociceptors as measured by depolarization of spinal ventral roots (Dray et al., 1990d), cardiovascular reflexes (Amann and Lembeck, 1989; Amann et al., 1989a, 1990a; Maggi et al., 1989a, 1990c), the Bezold-Jarisch reflex (Pethö and Szolcsányi, 1990), and motor reflexes of the urinary bladder (Maggi et al., 1989a). Activation of sensory neurons by mechanical stimuli (Maggi et al., 1989a; Dray et al., 1990d), acetylcholine, bradykinin, and 5-hydroxytryptamine (Amann and Lembeck, 1989; Amann et al., 1989a, 1990a; Dray et al., 1990d) is not affected by ruthenium red. Reflex responses to noxious heat either remain unaffected (Dray et al., 1990d) or are reduced (Amann et al., 1990a). Ruthenium red also blocks the capsaicin/resiniferatoxin-induced release of peptides from sensory nerve endings and the local effects of capsaicin that are due to peptide release. Thus, the release of substance P and/or calcitonin gene-related peptide from guinea pig lung (Amann et al., 1989b; Franco-Cereceda et al., 1990) and bronchi (Maggi et al., 1989c), guinea pig and rat urinary bladder (Maggi et al., 1988d; Amann et al., 1990c), guinea pig heart (Franco-Cereceda et al., 1989a; Maggi et al., 1989c), rat trachea (Ray et al., 1990), and rabbit ear (Amann et al., 1989a, 1990a) is inhibited by the dye. Peptide release evoked by bradykinin, nicotine, veratridine, or potassium depolarization remains unaltered by ruthenium red (Amann et al., 1989b, 1990a; Franco-Cereceda et al., 1989a; Ray et al., 1990).

Antagonism of capsaicin-, resiniferatoxin-, or piperine-evoked motor effects by ruthenium red has been observed in rat and guinea pig urinary bladder, rat vas deferens (Maggi et al., 1988b,d, 1989a, 1990c), guinea pig ileum (Chahl, 1989; Jin et al., 1989, 1990; Takaki et al., 1989, 1990), and guinea pig bronchus and heart (Franco-Cereceda et al., 1989a, 1990; Maggi et al., 1989c; Amann et al., 1990c). Motor responses to electrical field stimulation, substance P, neurokinin A, calcitonin gene-related peptide, acetylcholine, nicotine, or potassium depolarization are not inhibited by ruthenium red at concentrations of $\leq$0.5 to 1 μM, whereas higher concentrations of the dye depress muscle activity in a nonselective fashion (Maggi et al., 1988d; Chahl, 1989; Takaki et al., 1990).

Intravenous administration of ruthenium red (0.5 to 2 mg/kg = 0.6 to 2.3 μmol/kg) to the rat inhibits the Bezold-Jarisch reflex response to capsaicin but does not inhibit the reflex response to veratridine or the bradycardia caused by electrical stimulation of the peripheral stump of the cut vagal nerve (Szolcsányi et al., 1991). The capsaicin-induced increase in blood flow and vascular permeability in the rabbit skin also is prevented by intradermal administration of ruthenium red (10 nmol), whereas the vascular responses to bradykinin, N-formyl-methionyl-leucyl-phenylalanine, platelet-activating factor, histamine, and calcitonin gene-related peptide are spared (Buckley et al., 1990).

The potency of ruthenium red in preventing capsaicin desensitization is distinctly higher than that in blocking the stimulant effect of capsaicin in isolated tissues (Maggi et al. 1988b, 1989a). Furthermore, ruthenium red-induced protection from capsaicin desensitization lasts much longer after washout of the dye than does antagonism of the stimulant effect of capsaicin (Maggi et al., 1988b,d; Chahl, 1989). Although these in vitro observations may have something to do with the fact that ruthenium red is difficult to wash out from the tissue (Maggi et al., 1988d; Chahl, 1989; Takaki et al. 1989; Bleakman et al., 1990; Maggi 1991), they most likely reflect a certain aspect of ruthenium red's mechanism of action (Chahl, 1989) because the preference of the dye

to block capsaicin's desensitizing and/or neurotoxic action on sensory neurons is also seen when ruthenium red (4.3 mg/kg = 5 μmol/kg) is administered subcutaneously to rats (Amann et al., 1990b). Thus, systemic ruthenium red does not impair corneal nociception and cardiovascular reflexes in response to acute capsaicin but attenuates the long-term neurotoxic effect of capsaicin as determined by depletion of substance P and calcitonin gene-related peptide from peripheral terminals of sensory neurons in the urinary bladder and heart (Amann et al., 1990b). The capsaicin-induced defunctionalization of peripheral sensory nerve terminals as assessed by peptide release and peptide-mediated plasma protein extravasation is likewise inhibited by the dye (Amann et al., 1990b). Capsaicin-induced peptide depletion from, and defunctionalization of, the central terminals of sensory neurons, however, does not seem to be prevented by systemic ruthenium red, which suggests that the dye does not effectively enter the central nervous system (Amann et al., 1990b). Whether ruthenium red can protect from the ultrastructural changes caused by capsaicin in sensory neurons remains to be examined.

The conclusion to be drawn from the available data is that, at submicromolar/micromolar concentrations, ruthenium red acts as a specific antagonist of capsaicin. Its site and mechanism of action, however, have proved difficult to elucidate because of the multiplicity of cellular actions which the dye can exert (Amann and Maggi, 1991). Because of the dye's inhibitory actions on transmembrane Ca^{2+} fluxes and mitochondrial Ca^{2+} sequestration, it was originally suspected that ruthenium red acted by blocking the capsaicin-induced Ca^{2+} uptake at a mitochondrial level (Wood et al., 1988). Such a site of action, however, is unlikely to explain its antagonism of capsaicin for two major reasons. First, ruthenium red blocks the capsaicin-evoked opening of a nonselective cation conductance in the cell membrane of cultured sensory neurons (Dray et al., 1990d) and the resulting depolarization (Bleakman et al., 1990). Second, the intracellular penetration of ruthenium red is very poor (Maggi et al., 1988d; Chahl, 1989; Maggi, 1991) and there is pharmacological evidence that, at concentrations needed to antagonize capsaicin, ruthenium red is devoid of intracellular actions (Maggi et al., 1988d, 1989c; Amann et al., 1989a, 1990c; Franco-Cereceda et al., 1989a). If ruthenium red were to act by preventing Ca^{2+} binding to mitochondria, elevation of intracellular Ca^{2+} would be expected to interfere nonselectively with the stimulation of sensory neurons. Indeed, uncoupling of oxidative phosphorylation by cyanide inhibits capsaicin- and depolarization-evoked peptide release from sensory nerve endings in a nonselective manner, yet ruthenium red antagonizes only the response to capsaicin (Amann et al., 1990c). Furthermore, Ca^{2+} is not essential for the capsaicin-antagonistic action of ruthenium red because removal of external Ca^{2+} is without effect (Amann et al., 1989a).

The observations that ruthenium red prevents the capsaicin-induced opening of a cation channel in isolated cell membrane patches from dorsal root ganglion cultures (Dray et al., 1990d) and the capsaicin-induced depolarization of whole ganglion cells (Bleakman et al., 1990) indicate that the primary site of its action is the cell membrane. However, ruthenium red does not appear to displace the binding of [^{3}H]resiniferatoxin from membranes of dorsal root ganglia, although capsaicin does (Szallasi and Blumberg, 1990a). It follows that ruthenium red is not a competitive antagonist at the resiniferatoxin/capsaicin recognition site but is a functional antagonist of capsaicin.

In view of the interaction of ruthenium red with many Ca^{2+}-binding proteins (Charuk et al., 1990), including neuronal Ca^{2+} channels (Tapia et al., 1985), it could be inferred that the dye antagonizes capsaicin by blocking the capsaicin-operated nonselective cation channel. Indirect support for this argument comes from the finding that the ionic requirements for both the stimulant action of capsaicin on sensory neurons and the capsaicin-antagonistic action of ruthenium red are similar in that neither action requires the presence of external Ca^{2+} (Amann et al., 1989a). However, the inhibitory action of ruthenium red on the capsaicin-induced opening of cation channels does not have the characteristics typical of ion channel blockade (Dray et al., 1990d). Hence, it appears as if the dye would either interrupt the coupling between the capsaicin recognition site and the cation channels, block the binding of cations to the channels, or interfere in some other way with the activation or inactivation of the channels. In an attempt to explain why a short exposure to ruthenium red does not prevent capsaicin-induced contractions of the guinea pig isolated ileum 30 min after washout of the dye but does protect from capsaicin desensitization, Chahl (1989) has proposed a two-site model of interaction between the dye and the capsaicin receptor/cation channel complex. In this model ruthenium red is thought to bind irreversibly to a site of the complex that accounts for the prolonged protection from capsaicin desensitization and reversibly to some other site that causes only a short-lasting suppression of the stimulant action of capsaicin on sensory neurons (Chahl, 1989). The inhibitory effects of ruthenium red on the capsaicin-induced increase in the intracellular concentration of free Ca^{2+} ions (Bleakman et al., 1990; Dray et al., 1990d), intracellular accumulation of calcium (Wood et al., 1988), and inhibition of voltage-gated Ca^{2+} channels (Bleakman et al., 1990) are by all means secondary consequences of the dye's inhibitory effect on the capsaicin-evoked opening of cation channels in the cell membrane.

Taken together, ruthenium red appears to be a functional antagonist of capsaicin's stimulant and desensitiz-

ing actions on sensory neurons, although it is not clear whether these two actions are antagonized via a common pathway. The structural requirements for ruthenium red to act as a blocker of capsaicin are also unknown except that the entire molecule of ruthenium red is required (Amann and Maggi, 1991) and ruthenium chloride is ineffective (Maggi et al., 1988b).

6. Interaction with nerve growth factor. NGF is able to prevent the neurotoxic effect of capsaicin on B-type sensory neurons in the rat and guinea pig at all ages. To explain these observations one has to take into account that capsaicin disrupts the retrograde transport of NGF (Miller et al., 1982a,b) and horseradish peroxidase (Taylor et al., 1985) in the adult guinea pig and rat and that inhibition of retrograde transport precedes depletion of substance P from dorsal root ganglia; the potency of capsaicin for these two effects is identical (Miller et al., 1982b).

Systemic administration of NGF protects B-type sensory neurons from capsaicin-induced degeneration in the newborn rat (Otten et al., 1983) and counteracts capsaicin-induced depletion of substance P from sensory neurons in the adult guinea pig (Miller et al., 1982b). The site of interaction, however, between NGF and capsaicin is not clear. One possibility is that NGF supplementation circumvents the deleterious effect of capsaicin-induced inhibition of NGF transport to the sensory ganglia. However, NGF also prevents capsaicin from inhibiting the retrograde transport of horseradish peroxidase when both NGF and capsaicin are administered locally to the peripheral endings of sensory neurons (Taylor et al., 1985). This suggests that NGF can antagonize capsaicin at a peripheral site of action and argues against the ideas that the neurotoxic action of capsaicin is related to NGF deprivation of the neuronal somata or an interference with the trophic effects of NGF itself (Taylor et al., 1985). Consequently, it has been proposed that capsaicin may block the uptake of NGF by the peripheral sensory nerve endings and that NGF supplementation may overcome this action of capsaicin (Taylor et al., 1985).

The situation is profoundly different in sensory neuron cultures derived from dorsal root ganglia of adult rats. NGF is not required for the survival of these neurons but controls their responsiveness to capsaicin (Winter et al., 1988). Sensitivity to capsaicin, as assessed by the cobalt uptake stain, is retained for weeks in culture if there is an adequate supply of NGF. If, however, NGF is removed, the neurons loose their responsiveness to capsaicin within 3 to 4 days (Winter et al., 1988). This loss is reversible because full sensitivity to capsaicin is restored within 4 to 6 days after readdition of NGF. The time lag of several days suggests that NGF turns on the synthesis of cellular components, e.g., the cation channel whose presence determines the capsaicin sensitivity of sensory neurons (Winter et al., 1988). Alternatively, NGF is involved in the activation of the capsaicin-operated cation channel or in the modulation of other neuronal functions (Winter et al., 1988).

Although the in vivo observations are at present difficult to reconcile with the data from cultured sensory neurons, they are not necessarily contradictory with each other. The findings obtained from cultured sensory neurons indicate that NGF regulates the cellular expression of capsaicin sensitivity. This also may hold true for the situation in vivo but has not yet been examined. It would be worth testing whether treatment of adult rats or guinea pigs with antibodies to NGF can prevent the deleterious action of capsaicin on sensory neurons. The protective effect of NGF in vivo could be related to the maintenance of an optimal supply of NGF, which enhances neuronal resistance to damage and promotes neuronal repair processes.

7. Peripheral endings of sensory neurons as primary target of capsaicin's action—distal axonopathy induced by capsaicin in the adult rat. In view of the insensitivity of capsaicin's local excitatory action to tetrodotoxin and of other characteristics of the "dual sensory-efferent function" of afferent nerve endings, the hypothesis has been put forward that the very terminal region of sensory nerve fibers is the primary target of capsaicin's action (Jancsó, 1960, 1968; Szolcsányi, 1984b, 1988; Maggi and Meli, 1988; Maggi, 1991; Szolcsányi et al., 1991). In this model, the sensory nerve ending consists of a generator or sensory receptor region, in which stimulation produces a tetrodotoxin-resistant generator potential, and a regenerative (preterminal axonal) region which possesses voltage-gated Na^+ channels. Here the generator potential gives rise to propagated action potentials that can be blocked by tetrodotoxin. The Na^+ channels also can be activated by veratridine, and the inability of ruthenium red to block the excitatory action of veratridine has been used to add weight to the above hypothesis (Szolcsányi et al., 1991). These arguments, however, are not conclusive and inconsistent with capsaicin's ability to depolarize all parts of sensory neurons, from which it appears as if capsaicin recognition sites coupled to tetrodotoxin-resistant cation channels are present in all parts of sensory neurons. In addition, the above model does not take account of the varicose structure of many capsaicin-sensitive peptidergic nerve fibers, peptide release being thought to occur from the varicosities, i.e., from multiple sites along the terminal axons of sensory neurons (Gibbins et al., 1987; Lundberg and Saria, 1987; Weihe, 1990). Because peptide release evoked by capsaicin also is tetrodotoxin resistant (Saria et al., 1983b; Hua et al., 1986; Maggi et al., 1989c, 1990b), it is conceivable that there are multiple sites on the preterminal axon that are directly sensitive to capsaicin. From these considerations it is clear that the issue of the primary site, if any, of capsaicin's excitatory action will only be resolved by a mapping of the distribution and operationality of the

capsaicin receptor/cation channel complexes along the axons of sensory neurons.

In vivo, the route of administration of capsaicin and the existence of diffusion barriers for the drug may favour certain sites of action. Thus, some neurophysiological characteristics of the stimulant and desensitizing effects of topical capsaicin on cutaneous C-fiber polymodal nociceptors have been used to suggest a primary action of capsaicin on the peripheral endings of rat sensory neurons (Kenins, 1982). Support for this reasoning also comes from the observation that close arterial injection of capsaicin to the rabbit ear can abolish the responsiveness of polymodal nociceptors to certain, but not all, of its modalities (Szolcsányi, 1987). This finding points to a site of action on the sensory receptors themselves and argues against a site of action on the conducting preterminal part of sensory fibers (Szolcsányi, 1987). Furthermore, close arterial administration of capsaicin to a segment of the rat saphenous nerve cut at both ends, but with intact vascular supply, has been found to be rather ineffective in producing axonal excitation (G. Pethö and J. Szolcsányi, personal communication). These findings suggest that the peripheral nerve endings are the primary site of action of capsaicin when the drug is administered by the topical or close arterial route and thus is preferentially delivered to the axon terminals of sensory neurons.

Although the primary site of capsaicin's excitatory action remains to be elucidated, there is a body of circumstantial evidence indicating that the peripheral nerve terminals are much more vulnerable to the drug given systemically to adult rats than are the somata and axons of sensory neurons (Chung et al., 1985b, 1990). This segmentally different neurotoxicity could be due to differences in the access of capsaicin to the different parts of sensory neurons. Thus, although capsaicin given parenterally distributes rapidly throughout the body (Saria et al., 1982), the Schwann cell sheath (perineurium) around bundles of unmyelinated afferent nerve fibers may represent a barrier that prevents neurotoxic concentrations of capsaicin from reaching the axons (Chung et al., 1990). This assumption is in keeping with the necessity of using high (millimolar) concentrations of periaxonal capsaicin to induce ablation of afferent neurons (Gamse et al., 1982; Jancsó et al., 1987a,b; Lynn et al., 1987; Jancsó and Lawson, 1990; Pini et al., 1990).

Alternatively, the segmentally different neurotoxicity of capsaicin may be due to segmentally different capacities of resistance and repair in sensory neurons. Thus, only a few, if any, B-type neurons degenerate after systemic capsaicin treatment of adult rats (Jancsó et al., 1985b), although many somata display ultrastructural changes for several weeks (Joó et al., 1969; Szolcsányi et al., 1975; Chiba et al., 1986); neuropeptides, after an initial increase (Lembeck and Donnerer, 1981; Gamse et al., 1981b; Geppetti et al., 1988a), are depleted from dorsal root ganglia (Jessell et al., 1978; Gamse et al., 1981b; Gamse, 1982; Geppetti et al., 1988a). Likewise, no (Chung et al., 1985b, 1990), or minor (Jancsó et al., 1985b), degeneration of axons in afferent nerves or dorsal roots is noted. This indicates that somata and axons of afferent neurons in the adult rat are fairly resistant to the neurodegenerative action of capsaicin. In contrast, unmyelinated sensory nerve terminals in the periphery (Hoyes and Barber, 1981; Hoyes et al., 1981; Chung et al., 1985b, 1990) show extensive degeneration. In skin (Chung et al., 1990) and ureter (Chung et al., 1985b) 50 to 90% of all axon terminals degenerate. The result of capsaicin treatment of adult rats has been described, therefore, as "distal axonopathy" (Chung et al., 1990). Because the somata and axons do not degenerate, it seems likely that the slow neurochemical and functional recovery of sensory neurons, which is seen after capsaicin treatment of adult rats, reflects a regeneration of damaged nerve terminals.

There are additional data to suggest that the primary target of capsaicin's neurotoxic action on sensory neurons of the adult rat is the peripheral nerve terminal or a site distal to the cell body. For instance, substance P depletion from the peripheral processes of sensory neurons proceeds considerably faster after systemic capsaicin administration than after nerve section (Maggi et al., 1987e). Furthermore, NGF appears to antagonize the neurotoxic action of capsaicin at a site distal to the cell body (Miller et al., 1982a), if not at the peripheral nerve process itself (Taylor et al., 1985). In addition, studies with sensory neuron cultures from the embryonic chick (Hiura and Sakamoto, 1987a) and newborn and adult rat (Winter et al., 1988, 1990) have shown that neurites rather than somata are the preferential target of the neurotoxic action of capsaicin.

It is at present not possible to decide conclusively whether the distal axonopathy caused by systemic capsaicin treatment of adult rats arises from segmental differences in the accessibility of the drug and/or segmental differences in the vulnerability to the drug. Additional factors may have a differential bearing on the neurotoxicity of capsaicin, depending on whether the drug is administered systemically or applied locally to axons or nerve terminals of sensory neurons or to cultured sensory neurons. Thus, periaxonal capsaicin is effective in causing substantial degeneration of all segments of unmyelinated afferent neurons (Jancsó and Lawson, 1990), whereas local administration of capsaicin to the spinal cord produces extensive damage of the central terminals of sensory neurons (Palermo et al., 1981; Ribeiro-da-Silva and Coimbra, 1984) but not to their somata and distal axons (Jancsó, 1981; Gamse, 1982; Gamse et al., 1984, 1986). A better understanding of these phenomena will require investigation of the segmental distribution of the capsaicin receptor/cation channel and other determinants of the neurotoxic action

of capsaicin along somata, axons, and nerve terminals of sensory neurons. It may be recalled that the density of certain voltage-gated Ca^{2+} channels differs in different segments of sensory neurons, being especially high in axonal terminal regions (Kostyuk, 1989).

8. Ontogenetic shift in the neurotoxicity of capsaicin in the rat. The somata of B-type sensory neurons in the newborn rat are considerably more sensitive to the neurodegenerative action of systemic capsaicin than are those in the adult rat. The neurotoxic action of capsaicin has been found to be expressed at 16 days of gestation in the rat (Kirby et al., 1982) and at 15 days of gestation in the mouse (Atkinson and Chaggar, 1983). In the adult rat only the peripheral axon terminals of unmyelinated afferent fibers seem to be as vulnerable to capsaicin as those in the newborn rat (Chung et al., 1985b, 1990). This ontogenetic loss of capsaicin vulnerability does not appear to be due to a change in the number or affinity of the capsaicin recognition sites in dorsal root ganglia, as estimated by the specific binding of [^{3}H]resiniferatoxin (Szallasi and Blumberg, 1990b). Consequently, it must be other determinants of capsaicin's neurotoxic action that change with age. Although these determinants have not yet been elucidated, it could be argued that there is an ontogenetic change in the expression or activity of the capsaicin-operated membrane channels that prime the intracellular admission of toxic concentrations of cations. This speculation is based on the findings that the expression of a certain voltage-gated Ca^{2+} channel and of a tetrodotoxin-resistant Na^+ channel in sensory neurons has been found to vary with age, being typical of developing nerve cells only (Fedulova et al., 1986; Kostyuk et al., 1986; Petersen et al., 1987; Kostyuk, 1989). It is possible, therefore, that afferent neurons in the developing rat are particularly susceptible to the neurodegenerative action of capsaicin because they possess more capsaicin-operated cation channels than do afferent neurons in the adult animal in which only axon terminals remain endowed with a high density of these channels.

Because the expression of functional capsaicin receptor/cation channel complexes appears to be under the control of NGF (Winter et al., 1988), and possibly of other trophic factors, it could be inferred that downregulation of the receptor/channel complexes in the adult animal is due to the fact that the NGF requirements of sensory neurons decrease during ontogeny (Buck and Burks, 1986). Alternatively, it could be speculated that the immature sensory neurons in the newborn rat are particularly susceptible to the secondary, intracellular consequences of capsaicin's action on the cell membrane.

9. Endogenous capsaicin-like substances. The presence of a specific recognition site for capsaicin-like substances coupled to nonselective cation channels on sensory neurons raises the question as to whether certain endogenous compounds may interact physiologically with these structures. The development of a radioimmunoassay for capsaicin (Wood et al., 1990), the availability of a [^{3}H] resiniferatoxin-binding assay (Szallasi and Blumberg, 1990a), the discovery of a competitive capsaicin antagonist, capsazepine (Bevan et al., 1991; Dray et al., 1991), and the establishment of ruthenium red as a functional capsaicin antagonist (Amann and Maggi, 1991) have made it possible to search for the existence of endogenous substances that are structurally related to capsaicin or use the same transduction mechanisms as capsaicin. However, studies in which a sensitive radioimmunoassay that grossly recognizes the structural features of capsaicin required for stimulation of sensory neurons was used have failed to detect any endogenous capsaicin-like immunoreactive material in normal and inflamed tissues of the rat (Wood et al., 1990). It would appear, therefore, that there is no endogenous substance that fulfills the structural requirements that a compound has to meet in order to interact with the capsaicin recognition site on sensory neurons. This contention, however, ought to be further strengthened by experiments using the [^{3}H]resiniferatoxin assay and the competitive antagonist, capsazepine, as screening tools.

Given that at submicromolar/micromolar concentrations ruthenium red antagonizes the action of capsaicin in a specific fashion, it would seem that there are endogenous and exogenous substances that utilize a ruthenium red-blockable transduction pathway, even though they are not structurally related to capsaicin. Thus, ruthenium red has been found to reduce the stimulant actions of toluene diisocyanate (Mapp et al., 1990) and hydrogen ions (Geppetti et al., 1991) on capsaicin-sensitive afferent neurons. Similarly, nociceptor stimulation by noxious heat applied to the rabbit ear is also inhibited by ruthenium red (Amann et al., 1990a). In addition, the dye is able to antagonize sodium deoxycholate, a bile salt, in facilitating capsaicin's excitatory effect on sensory neurons (Jin and Nakayama, 1990). These observations suggest that the aforementioned stimuli activate sensory neurons by a transduction mechanism similar to that of capsaicin or can release an endogenous compound that acts like capsaicin. It remains to be examined whether other factors such as mechanical noxious stimuli, ischemia (Franco-Cereceda et al., 1989b), the vapour phase of tobacco smoke (Lundberg and Saria, 1983, 1987; Lundblad, 1984), xylene (Abelli et al., 1988), or hydrogen sulphide (Prior et al., 1990), all of which stimulate, and acrylamide (Abelli et al., 1991), which defunctionalizes capsaicin-sensitive afferent neurons, share common transduction pathways with capsaicin.

An aspect of capsaicin-sensitive neurons that may be of pathophysiological significance is their sensitivity to hydrogen ions, a property for which there is both indirect (Clarke and Davison, 1978; Cervero and McRitchie, 1982; Martling and Lundberg, 1988; Geppetti et al., 1990, 1991; Holzer et al., 1991; Raybould et al., 1991) and direct

(Bevan and Yeats, 1989) evidence. Studies of rat dorsal root ganglion cultures have demonstrated that lowering the pH to <6.4 activates a long-lasting inward current in about 30 to 50% of the neurons. This proton-evoked current has a reversal potential of approximately 0 mV which is indistinguishable from that of the capsaicin-evoked inward current (Bevan and Yeats, 1989). Furthermore, the neurons that respond to hydrogen ions with a long-lasting inward current appear to be identical with those neurons that are killed by prolonged exposure to micromolar concentrations of capsaicin (Bevan and Yeats, 1989). In addition, protons have been found to activate a short-lasting Na^+ conductance in isolated sensory neurons (Krishtal and Pidoplichko, 1980, 1981), which most probably represents a Ca^{2+} channel that is transformed to a Na^+ channel in the presence of protons (Konnerth et al., 1987; Kostyuk, 1989). This short-lasting inward current evoked by hydrogen ions predominates in the smaller trigeminal ganglion neurons (Krishtal and Pidoplichko, 1981) but is not confined to the population of capsaicin-sensitive neurons cultured from dorsal root ganglia (Bevan and Yeats, 1989). Future work will have to reveal the exact relationships between the inward currents induced by hydrogen ions and those induced by capsaicin. A close examination of any possible transitions between known cation conductances and those evoked by capsaicin also is needed.

C. Mechanisms of the Cell-nonselective Effects

Apart from the activation of a cation conductance in certain sensory neurons, capsaicin exerts other actions on sensory as well as nonsensory neurons and nonneuronal cells, which seem to be unrelated to its selective stimulant, desensitizing, and neurotoxic effects on thin sensory neurons. Although these cell-nonselective actions of capsaicin have not been investigated systematically, it is possible to identify some common traits shared by them. Thus, the nonselective effects of capsaicin can be shown repetitively and do not exhibit desensitization and are not related to cell toxicity. Whereas the sensory neuron-selective effects are produced by nanomolar (>10 nM) concentrations of capsaicin (EC_{50} approximately 0.2 μM; Marsh et al., 1987; Wood et al., 1988; Amann, 1990; Winter et al., 1990), it is typically micromolar (>3 μM) concentrations of capsaicin that are required to cause its cell-nonselective effects (Bevan and Szolcsányi, 1990). Precise estimations of the potency of capsaicin in this respect are not available.

With respect to membrane mechanisms, inhibition of voltage-gated Na^+ and K^+ channels is the most common component in the nonselective actions of capsaicin. The drug is able to influence the action potential in all sensory neurons of the rat, guinea pig, and chick (Godfraind et al., 1981; Petersen et al., 1987; Szolcsányi, 1990). Capsaicin prolongs the duration of the action potential in sensory neurons of the rat and chick (Godfraind et al., 1981) and in giant neurons of the snail (Erdélyi et al., 1987). This effect of capsaicin may be related to an inhibition of both the Na^+ inward and K^+ outward current as seen in guinea pig and chick sensory neurons (Petersen et al., 1987; Bevan and Forbes, 1988; Szolcsányi, 1990), rat sympathetic neurons (Bevan et al., 1987), snail neurons (Erdélyi et al., 1987), and crayfish giant axons (Yamanaka et al., 1984). Blockade of a certain component of the outward K^+ current, with some inhibition of the Na^+ inward current, also is observed in myelinated nerve fibers of the frog sciatic nerve (Dubois, 1982). This inhibition of membrane currents is likely to account for a capsaicin-induced transient blockade of nerve conduction as has been shown in rat A-fiber afferent neurons (Baranowski et al., 1986; Marsh et al., 1987), cat sympathetic nerve fibers (Such and Jancsó, 1986), and crayfish giant axons (Yamanaka et al., 1984).

Although the nonselective effects of capsaicin on voltage-dependent ion conductances in the cell membrane are fairly consistent in vertebrate and nonvertebrate species, there is much variation in the effect of capsaicin on the resting cell membrane. Rat and chick sensory neurons respond to capsaicin with a hyperpolarization that is blocked by removal of extracellular Cl^- (Godfraind et al., 1981). In the crayfish giant axon (Yamanaka et al., 1984) and in the giant amoeba (Foster et al., 1981) capsaicin is without effect on the resting potential, although in the amoeba capsaicin reduces the input resistance of the membrane (Foster et al. 1981). In contrast, capsaicin has been reported to depolarize giant neurons of the snail and to inhibit voltage-gated Ca^{2+} inward currents in these neurons (Erdélyi et al., 1987).

It is conceivable that the inhibitory effect of capsaicin on the neuronal Ca^{2+} conductance bears a relation to the capsaicin-induced inhibition of Ca^{2+} uptake in mouse neuroblastoma cells and rat aortic smooth muscle cells (Monsereenusorn and Kongsamut, 1985). Whether the inhibitory effects of capsaicin and piperine on cardiac muscle (Zernig et al., 1984; Franco-Cereceda and Lundberg, 1988) and visceral smooth muscle (Holzer and Lembeck, 1979; Barthó et al., 1982b, 1987; Maggi et al., 1987c, 1989b; Takaki et al., 1990) arise from a similar action has not yet been examined. In contrast, the contractile effect of capsaicin on vascular smooth muscle (Donnerer and Lembeck, 1982; Duckles, 1986; Saito et al., 1988; Bény et al., 1989; Holzer et al., 1990b) seems to be associated with an influx of extracellular Ca^{2+} (Edvinsson et al., 1990).

The molecular mechanism of capsaicin's cell-nonselective effects remains to be disclosed. Because many of these actions manifest themselves in a kind of membrane stabilization, it is conceivable that they are related to the lipophilicity of the drug. Owing to this chemical property, capsaicin could interact directly with the cell membrane and thereby influence membrane fluidity and ion permeability.

TABLE 3

Characteristics and mechanisms of capsaicin's actions on excitable cells

A. Cell-nonselective effects of capsaicin

1. *Targets:* excitable cells of vertebrate and invertebrate species (myelinated and unmyelinated sensory neurons, sympathetic neurons, cardiac muscle, vascular smooth muscle, visceral smooth muscle)
2. *Phenomenology of action*
   a. Typically depression of excitability (except in vascular smooth muscle) and inhibition of nerve conduction
   b. Effect to be shown repetitively (not desensitizing)
   c. Nonneurotoxic
   d. Unaffected by prior ablation of sensory neurons
3. *Potency:* species and tissue differences; in many cases EC_{50} probably >10 μM
4. *Mechanisms of action:* in most instances (except vascular smooth muscle) stabilization of the cell membrane: reduction of both the outward potassium conductance and the inward current of the action potential

B. Sensory neuron-selective effects of capsaicin

1. *Targets:* primary afferent neurons of mammalian species having small-diameter somata and unmyelinated (C-) or thinly myelinated (Aδ-) fibers
2. *Phenomenology of action*
   Sequence of effects consisting of:
   a. Dose-dependent excitation
   b. Capsaicin-specific (low doses) or capsaicin-nonspecific (moderate to high doses) desensitization
   c. Blockade of nerve conduction
   d. Neurotoxic alterations (moderate to high doses)
3. *Potency:* species differences; in the rat EC_{50} in the range of 0.1–0.3 μM
4. *Mechanisms of action*
   a. Occupation of a specific recognition site ("receptor") on the cell membrane
   b. Opening of nonselective cation channels (insensitive to tetrodotoxin and blockers of voltage-dependent Ca^{2+} channels), influx of Na^+, Ca^{2+}, and other cations, depolarization
   c. Increase in the intracellular concentration of Ca^{2+} and Na^+
   d. Peptide release
   e. Activation of a Ca^{2+}-dependent outward current and long-lasting inhibition of voltage-gated Ca^{2+} channels
   f. Desensitization (functional refractoriness)
      Specific desensitization due to inhibition of transduction mechanisms utilized by capsaicin only
      Nonspecific desensitization due to general neuronal defunctionalization, probably reflecting a mild and reversible form of neurotoxicity
   g. Neurotoxicity (ultrastructural damage of intracellular organization) due to intracellular accumulation of calcium and NaCl, either slowly reversible (no somatic degeneration) or irreversible (degeneration of neuronal soma), associated with quick defunctionalization and delayed depletion of cellular constituents and peptide transmitters

D. Summary: Mechanisms of Action

1. Sensory neuron-selective actions. It is now possible to formulate an overall hypothesis explaining the principal features of the effects of capsaicin on thin sensory neurons: excitation, desensitization, neurotoxicity, and selectivity (table 3). It has previously been speculated that the unique sensitivity of certain afferent neurons to capsaicin is due to a particular functional makeup of their cell membrane or to their possession of free nerve endings (Buck and Burks, 1986). These well-taken arguments, however, have been superseded by a concept that explains the sensory neuron-selective effects of capsaicin, i.e., by the identification of a specific membrane recognition site that is coupled to cation channels in the cell membrane (table 3).

Direct evidence for a capsaicin or "vanilloid" (Szallasi and Blumberg, 1990b) recognition site on thin sensory neurons comes from binding studies in which [^{3}H]resiniferatoxin is used as the test ligand (Szallasi and Blumberg, 1990a) and from the development of a specific and competitive receptor antagonist, capsazepine (Bevan et al., 1991; Dray et al., 1991). The molecular structure of the capsaicin/vanilloid receptor and its orientation in the cell membrane remain to be elucidated.

Further, although indirect, support for the presence of such a recognition site is provided by (*a*) the existence of a structure-activity relationship for capsaicin-like activity (Szolcsányi and Jancsó-Gábor, 1975, 1976; Szolcsányi, 1982; Hayes et al., 1984b; Szallasi and Blumberg, 1990b), (*b*) the ability to label the putative capsaicin recognition site with structurally related and long-acting photoaffinity probes (James et al., 1988), (*c*) the remarkable cell selectivity of capsaicin's actions on thin sensory neurons, (*d*) the ability of capsaicin to activate single cation channels in membrane patches from dorsal root ganglion neurons (Forbes and Bevan, 1988), and (*e*) the cellular regulation of the responsiveness to capsaicin by NGF (Winter et al., 1988).

Occupation of the capsaicin recognition site leads to the opening of nonselective cation channels in the cell membrane that are unique in that they are not affected by tetrodotoxin or blockers of voltage-dependent Ca^{2+} channels. Opening of these channels admits Na^+, Ca^{2+}, and other cations to the cytoplasm and causes depolarization. The increase in the intracellular Ca^{2+} concentration inhibits voltage-dependent Ca^{2+} channels and activates Ca^{2+}-dependent K^+ channels and Ca^{2+}-dependent

intracellular enzymes (table 3). All of these effects of capsaicin depend on the applied concentration of the drug, and it appears that the magnitude of the primary action on the cell membrane determines the sequence of some secondary effects such as nonspecific desensitization and neurotoxicity. In contrast, specific desensitization to capsaicin, which implies inactivation of the cellular transduction mechanism that is utilized by capsaicin-like drugs only, may occur in the absence of an excitatory action on sensory nerve endings (Dray et al., 1990b,c). Nonspecific desensitization reflects a general impairment of sensory neuron function and, as judged by its ionic requirements, is probably the result of a reversible neurotoxic action of the drug (table 3).

The neurotoxic action of high concentrations of capsaicin appears to arise from the intracellular accumulation of calcium and NaCl and, in this respect, resembles neurotoxicity induced by activation of, for example, excitatory amino acid receptors (Garthwaite et al., 1986; Choi, 1987). This process gives rise not only to defunctionalization but also to ultrastructural and degenerative changes of sensory neurons which, in turn, explain the long duration of capsaicin's neurotoxic action. Depletion of peptides and other constituents of sensory neurons is probably a consequence of the neurotoxic action of capsaicin but is not the cause of defunctionalization. If the somata of sensory neurons do not degenerate, the manifestations of neurotoxicity may be slowly reversible, whereas degeneration of the somata results in an irreversible neurotoxic effect (table 3).

2. Cell-nonselective actions. It is important to realize that, apart from its sensory neuron-selective effects, capsaicin also exerts cell-nonselective effects on a variety of excitable cells. These nonselective effects do not seem to be mediated by a specific recognition site but may be due to physicochemical interactions of the lipophilic capsaicin molecule with the cell membrane, especially when high concentrations of the drug are used. Unlike the sensory neuron-selective effects, the cell-nonselective effects of capsaicin do not show desensitization and do not result in neurotoxicity (table 3).

IV. Capsaicin as a Pharmacological Tool

A. Caveats to Be Considered

The sensory neuron-selective effects of capsaicin exhibit a high degree of selectivity for a group of primary afferent neurons with unmyelinated and thinly myelinated nerve fibers. This observation has made capsaicin an important, if not indispensable, tool with which to investigate the neuroanatomical, neurochemical, and functional characteristics of afferent neurons. It is warranted that further elucidation of its targets and mechanisms of action will further improve the potential of capsaicin as a selective probe for establishing the participation of sensory neurons in physiological processes. In the investigation of sensory neuron functions both the acute excitatory and the long-term neurotoxic actions of capsaicin have been, and can be, made use of. The selectivity of capsaicin for sensory neurons, however, is only relative and there are a number of limitations to be considered when capsaicin is used as a pharmacological tool. The major caveats that have to be considered in this respect are listed in table 4.

1. The group of capsaicin-sensitive primary afferent neurons is not identical with any particular population of afferent neurons that have been classified according to morphological, neurochemical, or functional criteria.

2. The degree of selectivity differs considerably for the acute and long-term actions of capsaicin. This is because, when acutely administered, the drug exerts both sensory neuron-selective and cell-nonselective effects; the acute effects of capsaicin display the least selectivity for sensory neurons (table 4). In many instances, however, the cell-nonselective effects can easily be differentiated from the sensory neuron-selective actions of capsaicin because the nonselective effects can be shown repetitively, do not exhibit desensitization, and remain unaltered after ablation of capsaicin-sensitive neurons.

3. Another limitation has to do with the acute excitatory effect of capsaicin on sensory neurons. Depending on their magnitude, the manifestations of sensory neuron stimulation can temporarily override or obscure the function of other systems such that false-positive evidence for an involvement of capsaicin-sensitive sensory neurons in these systems may be obtained. This problem applies particularly to the gastrointestinal system in which peptide transmitters such as substance P are utilized by both primary afferent and enteric neurons. After having been released by capsaicin from sensory nerve endings in the gut, these peptides may also activate and desensitize receptors that are the physiological targets of peptides released from enteric neurons and, thereby, temporarily interrupt enteric reflex pathways (Barthó et al., 1982a; Holzer et al., 1989; Jin et al., 1990).

4. There are also restrictions in the sensory neuron selectivity of the long-term neurotoxic effect of capsaicin because the capsaicin-induced ablation of C-fiber neurons results in changes in the afferent neuron system itself and in the cellular systems that are functionally connected to these neurons. Complete reorganization of sensory pathways and associated systems appears to take place following treatment of newborn rats with a neurotoxic dose of capsaicin. It is not yet clear whether a similar extent of reorganization occurs when adult rats are treated with systemic capsaicin or whether the neuronal reorganization seen in rats treated as neonates is related to the plasticity of the still immature nervous system.

5. Certain long-term actions of capsaicin on nonsensory neural systems, such as thermosensitive neurons in the hypothalamus, some neurons in other brain areas, and some neurons of the enteric nervous system, cannot

TABLE 4

Capsaicin as a pharmacological tool: Caveats to be considered

A. Limitations in the classification of capsaicin-sensitive afferent neurons
1. *Not all* unmyelinated (C-fiber) or thinly myelinated (Aδ-fiber) afferent neurons are capsaicin sensitive
2. *Not all* afferent neurons containing substance P, CGRP, and other peptide markers are capsaicin sensitive
3. Capsaicin-sensitive afferent neurons are heterogeneous in terms of their sensory modality
4. The group of capsaicin-sensitive afferent neurons are not identical with any group of afferent neurons defined according to morphological, neurochemical, or functional criteria

B. Acute effects of capsaicin are less selective for thin sensory neurons than its long-term effects
1. Sensory neuron-selective effects of capsaicin may be obscured by its cell-nonselective effects
2. Sensory neuron-selective effects of capsaicin can temporarily override the function of other systems, which may give rise to false positive evidence for an involvement of thin afferent neurons in the function under study

C. Limitations in the sensory neuron selectivity of the long-term neurotoxic effect of capsaicin
1. Secondary alterations in the afferent system itself and in the cellular systems that are functionally connected to capsaicin-sensitive afferent neurons
2. Certain neurons of the central nervous system (preoptic region of the hypothalamus and other nuclei) and perhaps of the enteric nervous system may be susceptible to the neurotoxic effect of capsaicin

D. Age-dependent differences in sensory neuron sensitivity to capsaicin
1. Treatment of newborn rats with a neurotoxic dose of capsaicin leads to degeneration of the majority of thin afferent neurons, but in the animals grown to adulthood there is no change in the different types of afferent nerve fibers
2. Treatment of adult rats with systemic capsaicin is neurotoxic to the majority of thin afferent neurons but does not necessarily cause degeneration of the whole neuron. Certain receptor types, such as polymodal nociceptors, are missing in afferent nerves

E. Strain and species differences in the sensory neuron sensitivity to capsaicin
1. There are subtle quantitative differences in the manifestations of the neurotoxic effect of capsaicin among different strains of rats
2. There are pronounced differences in sensory neuron sensitivity to capsaicin between different mammalian species. Primates, dogs, rats, mice, and guinea pigs are very sensitive, whereas the rabbit and hamster are clearly less sensitive
3. Nonmammalian species appear to be essentially unresponsive to the sensory neuron selective effects of capsaicin

be explained as being consequences of sensory neuron ablation. Confirmation and clarification of this issue is of particular importance for the future value of capsaicin as a research tool and for the validity of the conclusions that have been drawn from the long-term neurotoxic actions of capsaicin.

6. Special attention needs to be given to age, strain, and species differences in the sensory neuron sensitivity to capsaicin that may be related in part to different degrees of sensory neuron selectivity of the drug. In the rat, there are obvious differences in the neurotoxic action of capsaicin, depending upon whether the drug is given to newborn or adult animals. Whereas there is a loss of polymodal nociceptors after treatment of adult rats, there is apparently no change in the types of nociceptors after treatment of newborn rats, although most unmyelinated afferent nerve fibers have degenerated. The disparity of these observations is not understood but throws some doubt on the value of neonatal capsaicin treatment in the functional investigation of sensory neurons. The profound alterations in sensory pathways may be another unfavourable feature of neonatal capsaicin treatment.

Taken together, there are a number of factors that limit the selectivity and usefulness of capsaicin as a research tool for the investigation of sensory neurons (table 4). Many of these restrictions, however, can be overcome by the use of appropriate controls and by a careful consideration of the advantages and disadvantages associated with the different experimental uses of capsaicin.

B. Routes of Administration

Systemic treatment of small rodents with neurotoxic doses of capsaicin has been the most frequent method used when examining the anatomical, neurochemical, and functional alterations induced in capsaicin-sensitive afferent neurons. This approach ablates all neurons sensitive and accessible to the drug. Alternative routes of considerable potential are *periaxonal* treatment of afferent nerve fibers and *local (topical)* application of the drug to the receptive fields under study. These routes of administration enable the investigator to selectively ablate the sensory projections under study and, thereby, to avoid the selectivity problems associated with systemic administration of capsaicin. In the adult rat, periaxonal application of capsaicin causes substantial degeneration of C-fiber afferent neurons (Lynn et al., 1987; Jancsó and Lawson, 1990) and, in this respect, is considerably more effective than is systemic administration (Chung et al., 1985b, 1990; Jancsó et al., 1985b). Even in a species such as the rabbit which is comparatively resistant to the drug, it is possible to both induce loss of function and cause depletion of peptides from sensory neurons after their exposure to periaxonal capsaicin (Gamse et al., 1982; Lynn and Shakhanbeh, 1988); systemic capsaicin administration is less effective in this respect (Tervo, 1981; Lynn et al., 1984; Maggi et al., 1987b). Thus, periaxonal administration of capsaicin combines the advantage of producing an effective ablation of neurons with a high degree of topical and C-fiber selectivity.

Acknowledgments. The author is indebted to Drs. János Szolcsányi, Gábor Jancsó, Carlo A. Maggi, Robert E. Stitzel, Rainer Amann, Irmgard Th. Lippe, Josef Donnerer, and Ulrike Holzer-Petsche for their critical reading of, and most helpful comments concerning, the manuscript. Dr. Ulrike Holzer-Petsche prepared the graph.

REFERENCES

ABELLI, L., CONTE, B., SOMMA, V., MAGGI, C. A., GIULIANI, S., GEPPETTI, P., ALESSANDRI, M., THEODORSSON, E., AND MELI, A.: The contribution of capsaicin-sensitive sensory nerves to xylene-induced visceral pain in conscious, freely moving rats. Naunyn Schmiedebergs Arch. Pharmacol. **337:** 545–551, 1988.

ABELLI, L., FERRI, G.-L., ASTOLFI, M., CONTE, B., GEPPETTI, P., PARLANI, M., DAHL, D., POLAK, J. M., AND MAGGI, C. A.: Acrylamide-induced visceral neuropathy: evidence for the involvement of capsaicin-sensitive nerves of the rat urinary bladder. Neuroscience, **41:** 311–321, 1991.

ABERDEEN, J., CORR, L., MILNER, P., LINCOLN, J., AND BURNSTOCK, G.: Marked increases in calcitonin gene-related peptide-containing nerves in the developing rat following long-term sympathectomy with guanethidine. Neuroscience **35:** 175–184, 1990.

AGARWAL, R. C., AND BHIDE, S. V.: Histopathological studies on toxicity of chilli (capsaicin) in Syrian golden hamsters. Ind. J. Exp. Biol. **26:** 377–382, 1988.

AHLSTEDT, S., ALVING, K., HESSELMAR, B., AND OLAISSON, E.: Enhancement of the bronchial reactivity in immunized rats by neonatal treatment with capsaicin. Int. Arch. Allergy Appl. Immunol. **80:** 262–266, 1986.

AINSWORTH, A., HALL, P., WALL, P. D., ALLT, G., MACKENZIE, M. L., GIBSON, S., AND POLAK, J. M.: Effects of capsaicin applied locally to adult peripheral nerve. II. Anatomy and enzyme and peptide chemistry of peripheral nerve and spinal cord. Pain **11:** 379–388, 1981.

AKAGI, H., OTSUKA, M., AND YANAGISAWA, M.: Identification by high-performance liquid chromatography of immunoreactive substance P released from isolated rat spinal cord. Neurosci. Lett. **20:** 259–263, 1980.

ALBER, G., SCHEUBER, P. H., RECK, B., SAILER-KRAMER, B., HARTMANN, A., AND HAMMER, D. K.: Role of substance P in immediate-type skin reactions induced by staphylococcal enterotoxin B in unsensitized monkeys. J. Allergy Clin. Immunol. **84:** 880–885, 1989.

ALVING, K., MATRAN, R., AND LUNDBERG, J. M.: Capsaicin-induced local effector responses, autonomic reflexes and sensory neuropeptide depletion in the pig. Naunyn Schmiedebergs Arch. Pharmacol. **343:** 37–45, 1991.

AMANN, R.: Desensitization of capsaicin-evoked neuropeptide release—influence of Ca^{2+} and temperature. Naunyn Schmiedebergs Arch. Pharmacol. **342:** 671–676, 1990.

AMANN, R., DONNERER, J., AND LEMBECK, F.: Capsaicin-induced stimulation of polymodal nociceptors is antagonized by ruthenium red independently of extracellular calcium. Neuroscience **32:** 255–259, 1989a.

AMANN, R., DONNERER, J., AND LEMBECK, F.: Ruthenium red selectively inhibits capsaicin-induced release of calcitonin gene-related peptide from the isolated perfused guinea pig lung. Neurosci. Lett. **101:** 311–315, 1989b.

AMANN, R., DONNERER, J., AND LEMBECK, F.: Activation of primary afferent neurons by thermal stimulation. Influence of ruthenium red. Naunyn Schmiedebergs Arch. Pharmacol. **341:** 106–113, 1990a.

AMANN, R., DONNERER, J., MAGGI, C. A., GIULIANI, S., DELBIANCO, E., WEIHE, E., AND LEMBECK, F.: Capsaicin desensitization *in vivo* is inhibited by ruthenium red. Eur. J. Pharmacol. **186:** 169–175, 1990b.

AMANN, R., AND LEMBECK, F.: Capsaicin sensitive afferent neurons from peripheral glucose receptors mediate the insulin-induced increase in adrenaline secretion. Naunyn Schmiedebergs Arch. Pharmacol. **334:** 71–76, 1986.

AMANN, R., AND LEMBECK, F.: Stress induced ACTH release in capsaicin treated rats. Br. J. Pharmacol. **90:** 727–731, 1987.

AMANN, R., AND LEMBECK, F.: Ruthenium red selectively prevents capsaicin-induced nociceptor stimulation. Eur. J. Pharmacol. **161:** 227–229, 1989.

AMANN, R., AND LEMBECK, F.: Capsaicin-induced desensitization in rat and rabbit. Ann. NY Acad. Sci., in press, 1991.

AMANN, R., AND MAGGI, C. A.: Ruthenium red as a capsaicin antagonist. Life Sci., in press, 1991.

AMANN, R., MAGGI, C. A., GIULIANI, S., DONNERER, J., AND LEMBECK, F.: Effects of carbonyl cyanide para-trichloromethoxyphenylhydrazone (CCCP) and of ruthenium red on capsaicin-evoked neuropeptide release from peripheral terminals of primary afferent neurones. Naunyn Schmiedebergs Arch. Pharmacol. **341:** 534–537, 1990c.

AMANN, R., SKOFITSCH, G., AND LEMBECK, F.: Species-related differences in the capsaicin-sensitive innervation of the rat and guinea-pig ureter. Naunyn Schmiedebergs Arch. Pharmacol. **338:** 407–410, 1988.

ANAND, P., BLOOM, S. R., AND MCGREGOR, G. P.: Topical capsaicin pretreatment inhibits axon reflex vasodilatation caused by somatostatin and vasoactive intestinal polypeptide in human skin. Br. J. Pharmacol. **78:** 665–669, 1983.

ANAND, P., GIBSON, S. J., SCARAVILLI, F., BLANK, M. A., MCGREGOR, G. P., APPENZELLER, O., DHITAL, K., POLAK, J. M., AND BLOOM, S. R.: Studies of vasoactive intestinal polypeptide expression in injured peripheral neurons using capsaicin, sympathectomy and *mf* mutant rats. Neurosci. Lett. **118:** 61–66, 1990.

ANDOH, R., SAKURADA, S., SATO, T., TAKAHASHI, M., AND KISARA, K.: Potentiating effects of prostaglandin E_2 on bradykinin and capsaicin responses in medial thalamic nociceptive neurons. Jpn. J. Pharmacol. **32:** 81–89, 1982.

ARVIDSSON, J., AND YGGE, J.: A quantitative study of the effects of neonatal capsaicin treatment and of subsequent peripheral nerve transection in the adult rat. Brain Res. **397:** 130–136, 1986.

ATKINSON, M. E., AND CHAGGAR, J. S.: The effects of prenatal capsaicin on the distribution of substance P in developing primary afferent neurons. Neurosci. Lett. **35:** 25–29, 1983.

AULT, B., AND EVANS, H.: Depolarizing action of capsaicin on isolated dorsal root fibres of the rat. J. Physiol. (Lond.) **306:** 22P-23P, 1980.

BACCAGLINI, P. I., AND HOGAN, P. G.: Some rat sensory neurons in culture express characteristics of differentiated pain sensory cells. Proc. Natl. Acad. Sci. USA **80:** 594–598, 1983.

BARANOWSKI, R., LYNN, B., AND PINI, A.: The effects of locally applied capsaicin on conduction in cutaneous nerves of four mammalian species. Br. J. Pharmacol. **89:** 267–276, 1986.

BARTHÓ, L., AND HOLZER, P.: Search for a physiological role of substance P in gastrointestinal motility. Neuroscience **16:** 1–32, 1985.

BARTHÓ, L., HOLZER, P., LEMBECK, F., AND SZOLCSÁNYI, J.: Evidence that the contractile response of the guinea-pig ileum to capsaicin is due to release of substance P. J. Physiol. (Lond.) **332:** 157–167, 1982a.

BARTHÓ, L., PETHÖ, G., ANTAL, A., HOLZER, P., AND SZOLCSÁNYI, J.: Two types of relaxation due to capsaicin in the guinea-pig isolated ileum. Neurosci. Lett. **31:** 146–150, 1987.

BARTHÓ, L., SEBÖK, B., AND SZOLCSÁNYI, J.: Indirect evidence for the inhibition of enteric substance P neurones by opiate agonists but not by capsaicin. Eur. J. Pharmacol. **77:** 273–279, 1982b.

BARTHÓ, L., STEIN, C., AND HERZ, A.: Involvement of capsaicin-sensitive neurones in hyperalgesia and enhanced opioid antinociception in inflammation. Naunyn Schmiedebergs Arch. Pharmacol. **342:** 666–670, 1990.

BARTHÓ, L., AND SZOLCSÁNYI, J.: The site of action of capsaicin on the guinea-pig isolated ileum. Naunyn Schmiedebergs Arch. Pharmacol. **305:** 75–81, 1978.

BARTHÓ, L., AND SZOLCSÁNYI, J.: The mechanism of the motor response to periarterial nerve stimulation in the small intestine of the rabbit. Br. J. Pharmacol. **70:** 193–195, 1980.

BENNETT, T., AND GARDINER, S. M.: Neonatal capsaicin treatment impairs vasopressin-mediated blood pressure recovery following acute hypotension. Br. J. Pharmacol. **81:** 341–345, 1984.

BENNETT, T., AND GARDINER, S. M.: Neonatal treatment with capsaicin impairs hormonal regulation of blood pressure in adult, water-deprived, Long-Evans but not Brattleboro rats. Neurosci. Lett. **63:** 131–134, 1986.

BÉNY, J.-L., BRUNET, P. C., AND HUGGEL, H.: Effects of substance P, calcitonin gene-related peptide and capsaicin on tension and membrane potential of pig coronary artery *in vitro*. Regul. Pept. **25:** 25–36, 1989.

BERGSTROM, L., HAMMOND, D. L., GO, V. L. W., AND YAKSH, T. L.: Concurrent measurement of substance P and serotonin in spinal superfusates: failure of capsaicin and *p*-chloroamphetamine to co-release. Brain Res. **270:** 181–184, 1983.

BERKENBOSCH, F., SCHIPPER, J., AND TILDERS, F. J. H.: Corticotropin-releasing factor immunostaining in the rat spinal cord and medulla oblongata: an unexpected form of cross-reactivity with substance P. Brain Res. **399:** 87–96, 1986.

BERNSTEIN, J. E., BICKERS, D. R., DAHL, M. V., AND ROSHAL, J. Y.: Treatment of chronic postherpetic neuralgia with topical capsaicin: a preliminary study. J. Am. Acad. Dermatol. **17:** 93–96, 1987.

BERNSTEIN, J. E., PARISH, R. C., RAPAPORT, M., ROSENBAUM, M. M., AND ROENIGK, H. H.: Effect of topically applied capsaicin on moderate and severe psoriasis vulgaris. J. Am. Acad. Dermatol. **15:** 504–507, 1986.

BERNSTEIN, J. E., SWIFT, R. M., SOLTANI, K., AND LORINCZ, A. L.: Inhibition of axon reflex vasodilatation by topically applied capsaicin. J. Invest. Dermatol. **76:** 394–395, 1981.

BETTANEY, J., DRAY, A., AND FORSTER, P.: Calcium and the effects of capsaicin on afferent fibres in a neonatal rat isolated spinal cord-tail preparation. J. Physiol. (Lond.) **406:** 37P, 1988.

BEVAN, S. J., AND FORBES, C. A.: Membrane effects of capsaicin on rat dorsal root ganglion neurones in cell culture. J. Physiol. (Lond.) **398:** 28P, 1988.

BEVAN, S., HOTHI, S., HUGHES, G. A., JAMES, I. F., RANG, H. P., SHAH, K., WALPOLE, C. S. J., AND YEATS, J. C.: Development of a competitive antagonist for the sensory neurone excitant, capsaicin. Br. J. Pharmacol., 3102: 77P, 1991.

BEVAN, S. J., JAMES, I. F., RANG, H. P., WINTER, J., AND WOOD, J. N.: The mechanism of action of capsaicin—a sensory neurotoxin. *In* Neurotoxins and Their Pharmacological Implications, ed. by P. Jenner, pp. 261–277, Raven Press, New York, 1987.

BEVAN, S., AND SZOLCSÁNYI, J.: Sensory neuron-specific actions of capsaicin—mechanisms and applications. Trends Pharmacol. Sci. **11:** 330–333, 1990.

BEVAN, S., AND YEATS, J. C.: Protons activate a sustained inward current in a sub-population of rat isolated dorsal root ganglion (DRG) neurones. J. Physiol. (Lond.) **417:** 81P, 1989.

BITTNER, M. A., AND LAHANN, T. R.: Biphasic time-course of capsaicin-induced substance P depletion: failure to correlate with thermal analgesia in the rat. Brain Res. **322:** 305–309, 1985.

BJERRING, P., ARENDT-NIELSEN, L., AND SODERBERG, U.: Argon laser induced cutaneous sensory and pain thresholds in post-herpetic neuralgia. Quantitative modulation by topical capsaicin. Acta Dermatol. Venereol. **70:** 121–125, 1990.

BLEAKMAN, D., BRORSON, J. R., AND MILLER, R. J.: The effect of capsaicin on voltage-gated calcium currents and calcium signals in cultured dorsal root ganglion cells. Br. J. Pharmacol. **101:** 423–431, 1990.

BODNAR, R. J., KIRCHGESSNER, A., NILAVER, G., MULHERN, J., AND ZIMMERMAN, E. A.: Intraventricular capsaicin: alterations in analgesic responsivity without depletion of substance P. Neuroscience **7:** 631–638, 1982.

BOND, S. M., CERVERO, F., AND MCQUEEN, D. S.: Influence of neonatally administered capsaicin on baroreceptor and chemoreceptor reflexes in the adult rat. Br. J. Pharmacol. **77:** 517–524, 1982.

BRAGA, P. C., BIELLA, G., AND TIENGO, M.: Effects of capsaicin peripherally applied on spontaneous and evoked activity of thalamic nociceptive neurons in rat. Med. Sci. Res. 15: 1511–1512, 1987.
BRAND, L. M., SKARE, K. L., LOOMANS, M. E., RELLER, H. H., SCHWEN, R. J., LADE, D. A., BOHNE, R. L., MADDIN, C. S., MOOREHEAD, D. P., FANELLI, R., CHIABRANDO, C., CASTELLI, M. G., AND TAI, H. H.: Anti-inflammatory pharmacology and mechanism of the orally active capsaicin analogs, NE-19550 and NE-28345. Agents Actions 31: 329–340, 1990.
BRUGGER, F., EVANS, R. H., AND HAWKINS, N. S.: Effects of N-methyl-D-aspartate antagonists and spantide on spinal reflexes and responses to substance P and capsaicin in isolated spinal cord preparations from mouse and rat. Neuroscience 36: 611–622, 1990.
BUCK, S. H., AND BURKS, T. F.: The neuropharmacology of capsaicin—review of some recent observations. Pharmacol Rev 38: 179–226, 1986.
BUCK, S. H., MILLER, M. S., AND BURKS, T. F.: Depletion of primary afferent substance P by capsaicin and dihydrocapsaicin without altered thermal sensitivity in rats. Brain Res. 233: 216–220, 1982.
BUCK, S. H., WALSH, J. H., DAVIS, T. P., BROWN, M. R., YAMAMURA, H. I., AND BURKS, T. F.: Characterization of the peptide and sensory neurotoxic effects of capsaicin in the guinea pig. J. Neurosci. 3: 2064–2074, 1983.
BUCKLEY, T. L., BRAIN, S. D., AND WILLIAMS, T. J.: Ruthenium red selectively inhibits oedema formation and increased blood flow induced by capsaicin in rabbit skin. Br. J. Pharmacol. 99: 7–8, 1990.
BUCSICS, A., AND LEMBECK, F.: In vitro release of substance P from spinal cord slices by capsaicin congeners. Eur. J. Pharmacol. 71: 71–77, 1981.
BUCSICS, A., MAYER, N., PABST, M. A., AND LEMBECK, F.: Distribution of spinal cord nerve endings containing various neurotransmitters on a continuous density gradient. J. Neurochem. 42: 692–697, 1984.
BUCSICS, A., SUTTER, D., JANCSÓ, G., AND LEMBECK, F.: Quantitative assay of capsaicin-sensitive thiamine monophosphatase and β-glycerophosphatase activity in rodent spinal cord. J. Neurosci. Meth. 24: 155–162, 1988.
BYNKE, G.: Capsaicin pretreatment prevents disruption of the blood-aqueous barrier in the rabbit eye. Invest. Ophthalmol. Vis. Sci. 24: 744–748, 1983.
CAMRAS, C. B., AND BITO, L. Z.: The pathophysiological effects of nitrogen mustard on the rabbit eye. II. The inhibition of the initial hypertensive phase by capsaicin and the apparent role of substance P. Invest. Ophthalmol. Vis. Sci. 19: 423–428, 1980.
CARPENTER, S. E., AND LYNN, B.: Vascular and sensory responses of human skin to mild injury after topical treatment with capsaicin. Br. J. Pharmacol. 73: 755–758, 1981.
CARR, P. A., YAMAMOTO, T., AND NAGY, J. I.: Calcitonin gene-related peptide in primary afferent neurons of rat: co-existence with fluoride-resistant acid phosphatase and depletion by neonatal capsaicin. Neuroscience 36: 751–760, 1990.
CERVERO, F., AND MCRITCHIE, H. A.: Neonatal capsaicin and thermal nociception: a paradox. Brain Res. 215: 414–418, 1981.
CERVERO, F., AND MCRITCHIE, H. A.: Neonatal capsaicin does not affect unmyelinated efferent fibers of the autonomic nervous system: functional evidence. Brain Res. 239: 283–288, 1982.
CERVERO, F., AND PLENDERLEITH, M. B.: Spinal cord sensory systems after neonatal capsaicin. Acta Physiol. Hung. 69: 393–401, 1987.
CERVERO, F., SCHOUENBORG, J., SJÖLUND, B. H., AND WADDELL, P. J.: Cutaneous inputs to dorsal horn neurones in adult rats treated at birth with capsaicin. Brain Res. 301: 47–57, 1984.
CERVERO, F., AND SHARKEY, K. A.: An electrophysiological and anatomical study of intestinal afferent fibres in the rat. J. Physiol. (Lond.) 401: 381–397, 1988.
CHAHL, L. A.: Evidence that the contractile response of the guinea-pig ileum to capsaicin is due to substance P release. Naunyn Schmiedebergs Arch. Pharmacol. 319: 212–215, 1982.
CHAHL, L. A.: Antidromic vasodilatation and neurogenic inflammation. Pharmacol. Ther. 37: 275–300, 1988.
CHAHL, L. A.: The effects of ruthenium red on the response of guinea-pig ileum to capsaicin. Eur. J. Pharmacol. 169: 241–247, 1989.
CHARUK, J. H. M., PIRRAGLIA, C. A., AND REITHMEIER, R. A. F.: Interaction of ruthenium red with Ca^{2+}-binding proteins. Anal. Biochem. 188: 123–131, 1990.
CHÉRY-CROZE, S., BOSSHARD, A., MARTIN, H., CUBER, J. C., CHARNAY, Y., AND CHAYVIALLE, J. A.: Peptide immunocytochemistry in afferent neurons from lower gut in rats. Peptides 9: 873–881, 1989.
CHÉRY-CROZE, S., GODINOT, F., JOURDAN, G., BERNARD, C., AND CHAYVIALLE, J. A.: Capsaicin in adult frogs: effects on nociceptive responses to cutaneous stimuli and on nervous tissue concentrations of immunoreactive substance P, somatostatin and cholecystokinin. Naunyn Schmiedebergs Arch. Pharmacol. 331: 159–165, 1985.
CHIBA, T., MASUKO, S., AND KAWANO, H.: Correlation of mitochondrial swelling after capsaicin treatment and substance P and somatostatin immunoreactivity in small neurons of dorsal root ganglion in the rat. Neurosci. Lett. 64: 311–316, 1986.
CHOI, D. W.: Ionic dependence of glutamate neurotoxicity. J. Neurosci. 7: 369–379, 1987.
CHUDAPONGSE, P., AND JANTHASOOT, W.: Mechanism of the inhibitory action of capsaicin on energy metabolism by rat liver mitochondria. Biochem. Pharmacol. 30: 735–740, 1981.
CHUNG, J. M., LEE, K. H., HORI, Y., AND WILLIS, W. D.: Effects of capsaicin applied to a peripheral nerve on the responses of primate spinothalamic tract cells. Brain Res, 329: 27–38, 1985a.
CHUNG, K., KLEIN, C. M., AND COGGESHALL, R. E.: The receptive part of the primary afferent axon is most vulnerable to systemic capsaicin in adult rats. Brain Res. 511: 222–226, 1990.
CHUNG, K., SCHWEN, R. J., AND COGGESHALL, R. E.: Ureteral axon damage following subcutaneous administration of capsaicin in adult rats. Neurosci. Lett. 53: 221–226, 1985b.
CLARKE, G. D., AND DAVISON, J. S.: Mucosal receptors in the gastric antrum and small intestine of the rat with afferent fibres in the cervical vagus. J. Physiol. (Lond.) 284: 55–67, 1978.
CODERRE, T. J., ABBOTT, F. V., AND MELZACK, R.: Behavioral evidence in rats for a peptidergic-noradrenergic interaction in cutaneous sensory and vascular function. Neurosci. Lett. 47: 113–118, 1984.
COLERIDGE, H. M., AND COLERIDGE, J. C. G.: Impulse activity in afferent vagal C-fibers with endings in the intrapulmonary airways of dogs. Respir. Physiol. 29: 125–142, 1977.
COLERIDGE, J. C. G., AND COLERIDGE, H. M.: Afferent vagal C fibre innervation of the lung and airways and its functional significance. Rev. Physiol. Biochem. Pharmacol. 99: 1–110, 1984.
COLLIER, J. G., AND FULLER, R. W.: Capsaicin inhalation in man and the effects of sodium cromoglycate. Br. J. Pharmacol. 81: 113–117, 1984.
COSTA, M., FURNESS, J. B., AND GIBBINS, I. L.: Coexistence of neuronal messengers: a new principle in chemical transmission: chemical coding of enteric neurons. Prog. Brain Res. 68: 217–239, 1986.
CRAYTON, S. C., MITCHELL, J. H., AND PAYNE, F. C.: Reflex cardiovascular response during injection of capsaicin into skeletal muscle. Am. J. Physiol. 240: H315-H319, 1981.
CUI, J., ZAROR-BEHRENS, G., AND HIMMS-HAGEN, J.: Capsaicin desensitization induces atrophy of brown adipose tissue in rats. Am. J. Physiol. 259: R324-R332, 1990.
CULP, W. J., OCHOA, J., CLINE, M., AND DOTSON, R.: Mechanical hyperalgesia induced by capsaicin—cross modality threshold modulation in human C-nociceptors. Brain 112: 1317–1331, 1989.
DANIEL, E. E., NAGAHMA, M., SATO, H., JURY, J., AND BOWKER, P.: Immunochemical studies on substance P release from muscularis mucosa of opossum esophagus. *In* Substance P and Neurokinins, ed. by J. L. Henry, R. Couture, A. C. Cuello, G. Pelletier, R. Quirion, and D. Regoli, pp. 266–269, Springer, New York, 1987.
DAWBARN, D., HARMAR, A. J., AND PYCOCK, C. J.: Intranigral injection of capsaicin enhances motor activity and depletes nigral 5-hydroxytryptamine but not substance P. Neuropharmacology 20: 341–346, 1981.
DE, A. K., AND GHOSH, J. J.: Short- and long-term effects of capsaicin on the pulmonary anti-oxidant enzyme system in rats. Phytother. Res. 3: 184–187, 1989.
DECKER, M. W., TOWLE, A. C., BISSETTE, G., MUELLER, R. A., LAUDER, J. M., AND NEMEROFF, C. B.: Bombesin-like immunoreactivity in the central nervous system of capsaicin-treated rats: a radioimmunoassay and immunohistochemical study. Brain Res. 342: 1–8, 1985.
DELLA, N. G., PAPKA, R. E., FURNESS, J. B., AND COSTA, M.: Vasoactive intestinal peptide-like immunoreactivity in nerves associated with the cardiovascular system of guinea pigs. Neuroscience 9: 605–619, 1983.
DE VRIES, D. J., AND BLUMBERG, P. M.: Thermoregulatory effects of resiniferatoxin in the mouse: comparison with capsaicin. Life Sci. 44: 711–715, 1989.
DIB, B.: Dissociation between peripheral and central heat loss mechanisms induced by neonatal capsaicin. Behav. Neurosci. 97: 822–829, 1983.
DICKENSON, A., ASHWOOD, N., SULLIVAN, A. F., JAMES, I., AND DRAY, A.: Antinociception produced by capsaicin: spinal or peripheral mechanism? Eur. J. Pharmacol. 187: 225–233, 1990a.
DICKENSON, A., HUGHES, C., RUEFF, A., AND DRAY, A.: A spinal mechanism of action is involved in the antinociception produced by the capsaicin analogue NE 19550 (olvanil). Pain 43: 353–362, 1990b.
DIEZ GUERRA, F. J., ZAIDI, M., BEVIS, P., MACINTYRE, I., AND EMSON, P. C.: Evidence for release of calcitonin gene-related peptide and neurokinin A from sensory nerve endings *in vivo*. Neuroscience 25: 839–846, 1988.
DINH, T. T., AND RITTER, S.: Capsaicin induces neuronal degeneration in midbrain and forebrain structures in neonatally treated rats. Soc. Neurosci. Abstr. 13: 1684, 1987.
DOCHERTY, R. J., ROBERTSON, B., AND BEVAN, S.: Capsaicin causes prolonged inhibition of voltage-activated calcium currents in adult rat dorsal root ganglion neurons in culture. Neuroscience 40: 513–521, 1991.
DON, P. C.: Topical capsaicin for treatment of neuralgia associated with herpes zoster infection. J. Am. Acad. Dermatol. 18: 1135, 1988.
DONNERER, J., AND AMANN, R.: Capsaicin-evoked neuropeptide release is not dependent on membrane potential changes. Neurosci. Lett. 117: 331–334, 1990.
DONNERER, J., BARTHÓ, L., HOLZER, P., AND LEMBECK, F.: Intestinal peristalsis associated with release of immunoreactive substance P. Neuroscience 11: 913–918, 1984.
DONNERER, J., EGLEZOS, A., AND HELME, R. D.: Neuroendocrine and immune function in the capsaicin-treated rat: evidence for afferent neural modulation *in vivo*. *In* The Neuroendocrine-Immune Network, ed. by S. Freier, pp. 69–83, CRC Press, Boca Raton, FL, 1990.
DONNERER, J., AND LEMBECK, F.: Analysis of the effects of intravenously injected capsaicin in the rat. Naunyn Schmiedebergs Arch. Pharmacol. 320: 54–57, 1982.
DONNERER, J., AND LEMBECK, F.: Heat loss reaction to capsaicin through a peripheral site of action. Br. J. Pharmacol. 79: 710–723, 1983.

DONNERER, J., AND LEMBECK, F.: Different control of the adrenocorticotropin-corticosterone response and of prolactin secretion during cold stress, anesthesia, surgery, and nicotine injection in the rat: involvement of capsaicin-sensitive sensory neurons. Endocrinology **126:** 921–926, 1990.

DONNERER, J., SCHULIGOI, R., AND LEMBECK, F.: Influence of capsaicin-induced denervation on neurogenic and humoral control of arterial pressure. Naunyn Schmiedebergs Arch. Pharmacol. **340:** 740–743, 1989.

DOUCETTE, R., THERIAULT, E., AND DIAMOND, J.: Regionally selective elimination of cutaneous thermal nociception in rats by neonatal capsaicin. J. Comp. Neurol. **261:** 583–591, 1987.

DRAY, A., BETTANEY, J., AND FORSTER, P.: Capsaicin desensitization of peripheral nociceptive fibers does not impair sensitivity to other noxious stimuli. Neurosci. Lett. **99:** 50–54, 1989a.

DRAY, A., BETTANEY, J., AND FORSTER, P.: Resiniferatoxin, a potent capsaicin-like stimulator of peripheral nociceptors in the neonatal rat tail *in vitro*. Br. J. Pharmacol. **99:** 323–326, 1990a.

DRAY, A., BETTANEY, J., AND FORSTER, P.: Actions of capsaicin on peripheral nociceptors of the neonatal rat spinal cord-tail *in vitro*: dependence of extracellular ions and independence of second messengers. Br. J. Pharmacol. **101:** 727–733, 1990b.

DRAY, A., BETTANEY, J., RUEFF, A., WALPOLE, C., AND WRIGGLESWORTH, R.: NE-19550 and NE-21610, antinociceptive capsaicin analogues: studies on nociceptive fibres of the neonatal rat tail *in vitro*. Eur. J. Pharmacol. **181:** 289–293, 1990c.

DRAY, A., CAMPBELL, E. A., HUGHES, G. A., PATEL, I. A., PERKINS, M. N., RANG, H. P., RUEFF, A., SENO, N., URBAN, L., AND WALPOLE, C. S. J.: Antagonism of capsaicin-induced activation of C-fibres by a selective capsaicin antagonist, capsazepine. Br. J. Pharmacol., **102:** 78P, 1991.

DRAY, A., FORBES, C. A., AND BURGESS, G. M.: Ruthenium red blocks the capsaicin-induced increase in intracellular calcium and activation of membrane currents in sensory neurones as well as the activation of peripheral nociceptors *in vitro*. Neurosci. Lett. **110:** 52–59, 1990d.

DRAY, A., HANKINS, M. W., AND YEATS, J. C.: Desensitization and capsaicin-induced release of substance P-like immunoreactivity from guinea-pig ureter *in vitro*. Neuroscience **31:** 479–483, 1989b.

DUBOIS, J. M.: Capsaicin blocks one class of K^+ channels in the frog node of Ranvier. Brain Res. **245:** 372–375, 1982.

DUCKLES, S. P.: Effects of capsaicin on vascular smooth muscle. Naunyn Schmiedebergs Arch. Pharmacol. **333:** 59–64, 1986.

DUN, N. J., AND KIRALY, M.: Capsaicin causes release of a substance P-like peptide in guinea-pig inferior mesenteric ganglia. J. Physiol. (Lond.) **340:** 107–120, 1983.

EDVINSSON, L., JANSEN, I., KINGMAN, T. A., AND MCCULLOCH, J.: Cerebrovascular responses to capsaicin *in vitro* and *in situ*. Br. J. Pharmacol. **100:** 312–318, 1990.

EIMERL, D., AND PAPIR-KRICHELI, D.: Epidural capsaicin produces prolonged segmental analgesia in the rat. Exp. Neurol. **97:** 169–178, 1987.

EL-FAKAHANY, E., AND RICHELSON, E.: Temperature dependence of muscarinic acetylcholine receptor activation, desensitization, and resensitization. J. Neurochem. **34:** 1288–1295, 1980.

ERDÉLYI, L., SUCH, G., AND JANCSÓ, G.: Intracellular and voltage clamp studies of capsaicin induced effects on a sensory neuron model. Acta Physiol. Hung. **69:** 481–492, 1987.

ESPLUGUES, J. V., RAMOS, E. G., GIL, L., AND ESPLUGUES, J.: Influence of capsaicin-sensitive afferent neurones on the acid secretory responses of the rat stomach *in vivo*. Br. J. Pharmacol. **100:** 491–496, 1990.

ESPLUGUES, J. V., WHITTLE, B. J. R., AND MONCADA, S.: Local opioid-sensitive afferent sensory neurones in the modulation of gastric damage induced by Paf. Br. J. Pharmacol. **97:** 579–585, 1989.

EVANGELISTA, S., MAGGI, C. A., AND MELI, A.: Evidence for a role of adrenals in the capsaicin-sensitive "gastric defence mechanism" in rats. Proc. Soc. Exp. Biol. Med. **182:** 568–569, 1986.

FAULKNER, D. C., AND GROWCOTT, J. W.: Effects of neonatal capsaicin administration on the nociceptive response of the rat to mechanical and chemical stimuli. J. Pharm. Pharmacol. **32:** 656–657, 1980.

FEDULOVA, S. A., KOSTYUK, P. G., AND VESELOVSKY, N. S.: Changes in ionic mechanisms of electrical excitability of the somatic membrane of rat's dorsal root ganglion neurons during ontogenesis. Correlation between inward currents densities. Neurophysiology (Kiev) **18:** 820–827, 1986.

FEHÉR, E., AND VAJDA, J.: Effect of capsaicin on the nerve elements of the small intestine. Acta Morphol. Acad. Sci. Hung. **30:** 57–63, 1982.

FERRI, G. L., SABANI, A., ABELLI, L., POLAK, J. M., DAHL, D., AND PORTIER, M. M.: Neuronal intermediate filaments in rat dorsal root ganglia—differential distribution of peripherin and neurofilament protein immunoreactivity and effect of capsaicin. Brain Res. **515:** 331–335, 1990.

FITZGERALD, M.: Alterations in the ipsi- and contralateral afferent inputs of dorsal horn cells produced by capsaicin treatment of one sciatic nerve in the rat. Brain Res. **248:** 92–107, 1982.

FITZGERALD, M.: Capsaicin and sensory neurones—a review. Pain **15:** 109–130, 1983.

FITZGERALD, M., AND WOOLF, C. J.: The time course and specificity of the changes in the behavioural and dorsal horn cell responses to noxious stimuli following peripheral nerve capsaicin treatment in the rat. Neuroscience **9:** 2051–2056, 1982.

FLYNN, D. L., RAFFERTY, M. F., AND BOCTOR, A. M.: Inhibition of human neutrophil 5-lipoxygenase activity by gingerdione, shogaol, capsaicin and related pungent compounds. Prostaglandins Leuk. Med. **24:** 195–198, 1986.

FORBES, C. A., AND BEVAN, S. J.: Properties of single capsaicin-activated channels. Soc. Neurosci. Abstr. **14:** 642, 1988.

FORSTER, E. R., GREEN, T., ELLIOT, M., BREMNER, A., AND DOCKRAY, G. J.: Gastric emptying in rats—role of afferent neurons and cholecystokinin. Am. J. Physiol. **258:** G552-G556, 1990.

FOSTER, R. W., AND RAMAGE, A. G.: The action of some chemical irritants on somatosensory receptors of the cat. Neuropharmacology **20:** 191–198, 1981.

FOSTER, R. W., WESTON, A. H., AND WESTON, K. M.: Some effects of chemical irritants on the membrane of the giant amoeba. Br. J. Pharmacol. **74:** 333–339, 1981.

FRANCO-CERECEDA, A., BENGTSSON, L., AND LUNDBERG, J. M.: Inotropic effects of calcitonin gene-related peptide, vasoactive intestinal polypeptide and somatostatin on the human right atrium *in vitro*. Eur. J. Pharmacol. **134:** 69–76, 1987a.

FRANCO-CERECEDA, A., HENKE, H., LUNDBERG, J. M., PETERMANN, J. B., HÖKFELT, T., AND FISCHER, J. A.: Calcitonin gene-related peptide (CGRP) in capsaicin-sensitive substance P-immunoreactive sensory neurons in animals and man: distribution and release by capsaicin. Peptides **8:** 399–410, 1987b.

FRANCO-CERECEDA, A., LOU, Y.-P., AND LUNDBERG, J. M.: Ruthenium red differentiates between capsaicin and nicotine effects on cardiac sensory nerves. Acta Physiol. Scand. **137:** 457–458, 1989a.

FRANCO-CERECEDA, A., LOU, Y.-P., AND LUNDBERG, J. M.: Resiniferatoxin-evoked CGRP release and bronchoconstriction in the guinea-pig lung are inhibited by ruthenium red. Eur. J. Pharmacol. **187:** 291–292, 1990.

FRANCO-CERECEDA, A., AND LUNDBERG, J. M.: Actions of calcitonin gene-related peptide and tachykinins in relation to the contractile effects of capsaicin in the guinea-pig and rat heart *in vitro*. Naunyn Schmiedebergs Arch. Pharmacol. **337:** 649–655, 1988.

FRANCO-CERECEDA, A., AND LUNDBERG, J. M.: Post-occlusive reactive hyperaemia in the heart, skeletal muscle and skin of control and capsaicin-pretreated pigs. Acta Physiol. Scand. **137:** 271–277, 1989.

FRANCO-CERECEDA, A., RUDEHILL, A., AND LUNDBERG, J. M.: Calcitonin gene-related peptide but not substance P mimics capsaicin-induced coronary vasodilatation in the pig. Eur. J. Pharmacol. **142:** 235–243, 1987c.

FRANCO-CERECEDA, A., SARIA, A., AND LUNDBERG, J. M.: Differential release of calcitonin gene-related peptide and neuropeptide Y from the isolated heart by capsaicin, ischaemia, nicotine, bradykinin and ouabain. Acta Physiol. Scand. **135:** 173–188, 1989b.

FRIED, K., ALDSKOGIUS, H., AND HILDEBRAND, C.: Proportion of unmyelinated axons in rat molar and incisor tooth pulps following neonatal capsaicin treatment and/or sympathectomy. Brain Res. **463:** 118–123, 1988.

FUJITA, S., SHIMIZU, T., IZUMI, K., FUKUDA, T., SAMESHIMA, M., AND OHBA, N.: Capsaicin-induced neuroparalytic keratitis-like corneal changes in the mouse. Exp. Eye Res. **38:** 165–175, 1984.

FURNESS, J. B., PAPKA, R. E., DELLA, N. G., COSTA, M., AND ESKAY, R. L.: Substance P-like immunoreactivity in nerves associated with the vascular system of guinea-pigs. Neuroscience **7:** 447–459, 1982.

GAMSE, R.: Capsaicin and nociception in the rat and mouse. Possible role of substance P. Naunyn Schmiedebergs Arch. Pharmacol. **320:** 205–216, 1982.

GAMSE, R., HOLZER, P., AND LEMBECK, F.: Decrease of substance P in primary sensory neurones and impairment of neurogenic plasma extravasation by capsaicin. Br. J. Pharmacol. **68:** 207–213, 1980.

GAMSE, R., HOLZER, P., AND LEMBECK, F.: Indirect evidence for presynaptic location of opiate receptors on chemosensitive primary sensory neurones. Naunyn Schmiedebergs Arch. Pharmacol. **308:** 281–285, 1979a.

GAMSE, R., JANCSÓ, G., AND KIRÁLY, E.: Intracisternal capsaicin: a novel approach for studying nociceptive sensory neurons. *In* Antidromic Vasodilatation and Neurogenic Inflammation, ed. by L. A. Chahl, J. Szolcsányi, and F. Lembeck, pp. 93–106, Akadémiai Kiadó, Budapest, 1984.

GAMSE, R., LACKNER, D., GAMSE, G., AND LEEMAN, S. E.: Effect of capsaicin pretreatment on capsaicin-evoked release of immunoreactive somatostatin and substance P from primary sensory neurons. Naunyn Schmiedebergs Arch. Pharmacol. **316:** 38–41, 1981a.

GAMSE, R., LEEMAN, S. E., HOLZER, P., AND LEMBECK, F.: Differential effects of capsaicin on the content of somatostatin, substance P, and neurotensin in the nervous system of the rat. Naunyn Schmiedebergs Arch. Pharmacol. **317:** 140–148, 1981b.

GAMSE, R., MOLNAR, A., AND LEMBECK, F.: Substance P release from spinal cord slices by capsaicin. Life Sci. **25:** 629–636, 1979b.

GAMSE, R., PETSCHE, U., LEMBECK, F., AND JANCSÓ, G.: Capsaicin applied to peripheral nerve inhibits axoplasmic transport of substance P and somatostatin. Brain Res. **239:** 447–462, 1982.

GAMSE, R., SARIA, A., LUNDBERG, J. M., AND THEODORSSON-NORHEIM, E.: Behavioral and neurochemical changes after intracisternal capsaicin treatment of the guinea pig. Neurosci. Lett. **64:** 287–292, 1986.

GAMSE, R., WAX, A., ZIGMOND, R. E., AND LEEMAN, S. E.: Immunoreactive substance P in sympathetic ganglia: distribution and sensitivity towards capsaicin. Neuroscience **6:** 437–441, 1981c.

GANNETT, P. M., NAGEL, D. L., REILLY, P. J., LAWSON, T., SHARPE, J., AND TOTH, P.: The capsaicinoids: their separation, synthesis, and mutagenicity. J. Org. Chem. **53:** 1064–1071, 1988.

GARDINER, S. M., BENNETT, T., AND O'NEILL, T. P.: Synthetic capsaicin

reversibly impairs vasopressin-mediated blood pressure recovery. Am. J. Physiol. 257: R1429-R1435, 1989.
GARTHWAITE, G., HAJOS, F., AND GARTHWAITE, J.: Ionic requirements for neurotoxic effects of excitatory amino acid analogues in rat cerebellar slices. Neuroscience 18: 437–447, 1986.
GAZELIUS, B., BRODIN, E., KAHAN, T., PANOPOULOS, P., AND OLGART, L.: Local application of capsaicin to a peripheral nerve: effects on substance P and noradrenaline levels in peripheral nerve endings and vascular responses to nerve stimulation. *In* Substance P—Dublin 1983, ed. by P. Skrabanek and D. Powell, pp. 241–242, Boole Press, Dublin, 1983.
GEISTHÖVEL, E., LUDWIG, O., AND SIMON, E.: Capsaicin fails to produce disturbances of autonomic heat and cold defence in an avian species (Anas platyrhynchos). Pflügers Arch. 406: 343–350, 1986.
GEPPETTI, P., FRILLI, S., RENZI, D., SANTICIOLI, P., MAGGI, C. A., THEODORSSON, E., AND FANCIULLACCI, M.: Distribution of calcitonin gene-related peptide-like immunoreactivity in various rat tissues: correlation with substance P and other tachykinins and sensitivity to capsaicin. Regul. Pept. 23: 289–298, 1988a.
GEPPETTI, P., FUSCO, B., MARABINI, S., MAGGI, C. A., FANCIULLACCI, M., AND SICUTERI, F.: Secretion, pain and sneezing induced by the application of capsaicin to the nasal mucosa in man. Br. J. Pharmacol. 93: 509–514, 1988b.
GEPPETTI, P., MAGGI, C. A., PERRETTI, F., FRILLI, S., AND MANZINI, S.: Simultaneous release by bradykinin of substance P- and calcitonin gene-related peptide immunoreactivities from capsaicin-sensitive structures in guinea-pig heart. Br. J. Pharmacol. 94: 288–290, 1988c.
GEPPETTI, P., TRAMONTANA, M., EVANGELISTA, S., RENZI, D., MAGGI, C. A., FUSCO, B. M., AND DEL BIANCO, E.: Differential effect on neuropeptide release of different concentrations of hydrogenions on afferent and intrinsic neurons of the rat stomach. Gastroenterology, in press, 1991.
GEPPETTI, P., TRAMONTANA, M., PATACCHINI, R., DEL BIANCO, E., SANTICIOLI, P., AND MAGGI, C. A.: Neurochemical evidence for the activation of the 'efferent' function of capsaicin-sensitive nerves by lowering of the pH in the guinea-pig urinary bladder. Neurosci. Lett. 114: 101–106, 1990.
GIBBINS, I. L., FURNESS, J. B., AND COSTA, M.: Pathway-specific patterns of the co-existence of substance P, calcitonin gene-related peptide, cholecystokinin and dynorphin in neurons of the dorsal root ganglia of the guinea-pig. Cell Tissue Res. 248: 417–437, 1987.
GIBBINS, I. L., FURNESS, J. B., COSTA, M., MACINTYRE, I., HILLYARD, C. J., AND GIRGIS, S.: Co-localization of calcitonin gene-related peptide-like immunoreactivity with substance P in cutaneous, vascular and visceral sensory neurons of guinea pigs. Neurosci. Lett. 57: 125–130, 1985.
GIBSON, S. J., MCGREGOR, G., BLOOM, S. R., POLAK, J. M., AND WALL, P. D.: Local application of capsaicin to one sciatic nerve of the adult rat induces a marked depletion in the peptide content of the lumbat dorsal horn. Neuroscience 7: 3153–3162, 1982.
GLINSUKON, T., STITMUNNAITHUM, V., TOSKULKAO, C., BARANAWUTI, T., AND TANGKRISANAVINONT, V.: Acute toxicity of capsaicin in several animal species. Toxicon 18: 215–220, 1980.
GODFRAIND, J. M., JESSELL, T. M., KELLY, J. S., MCBURNEY, R. N., MUDGE, A. W., AND YAMAMOTO, M.: Capsaicin prolongs action potential duration in cultured sensory neurones. J. Physiol. (Lond.) 312: 32P-33P, 1981.
GREEN, B. G.: Sensory interactions between capsaicin and temperature in the oral cavity. Chem. Senses 11: 371–382, 1986.
GREEN, B. G.: Capsaicin sensitization and desensitization on the tongue produced by brief exposures to a low concentration. Neurosci. Lett. 107: 173–178, 1989.
GREEN, T., AND DOCKRAY, G. J.: Characterization of the peptidergic afferent innervation of the stomach in the rat, mouse and guinea-pig. Neuroscience 25: 181–193, 1988.
HÄBLER, H.-J., JÄNIG, W., AND KOLTZENBURG, M.: Activation of unmyelinated afferents by mechanical stimuli and inflammation of the urinary bladder. J. Physiol. (Lond.) 425: 545–563, 1990.
HAJÓS, M., ENGBERG, G., AND ELAM, M.: Reduced responsiveness of locus coeruleus neurons to cutaneous thermal stimuli in capsaicin-treated rats. Neurosci. Lett. 70: 382–387, 1986a.
HAJÓS, M., OBÁL, F., JANCSÓ, G., AND OBÁL, F.: The capsaicin sensitivity of the preoptic region is preserved in adult rats pretreated as neonates, but lost in rats pretreated as adults. Naunyn Schmiedebergs Arch. Pharmacol. 324: 219–222, 1983.
HAJÓS, M., SVENSSON, K., NISSBRANDT, H., OBÁL, F., AND CARLSSON, A.: Effects of capsaicin on central monoaminergic mechanisms in the rat. J. Neural Transm. 66: 221–242, 1986b.
HÅKANSON, R., BEDING, B., EKMAN, R., HEILIG, M., WAHLESTEDT, C., AND SUNDLER, F.: Multiple tachykinin pools in sensory nerve fibres in the rabbit iris. Neuroscience 21: 943–950, 1987.
HAMMOND, D. L., AND RUDA, M. A.: Developmental alterations in thermal nociceptive threshold and the distribution of immunoreactive calcitonin gene-related peptide and substance P after neonatal administration of capsaicin in the rat. Neurosci. Lett. 97: 57–62, 1989.
HAMON, M., GALLISSOT, M. C., MENARD, F., GOZLAN, H., BOURGOIN, S., AND VERGE, D.: 5-HT_3 receptor binding sites are on capsaicin-sensitive fibres in the rat spinal cord. Eur. J. Pharmacol. 164: 315–322, 1989.
HANDWERKER, H. O., HOLZER-PETSCHE, U., HEYM, C., AND WELK, E.: C-fibre functions after topical application of capsaicin to a peripheral nerve and after neonatal capsaicin treatment. *In* Antidromic Vasodilatation and Neurogenic Inflammation, ed. by L. A. Chahl, J. Szolcsányi, and F. Lembeck, pp. 57–78, Akadémiai Kiadó, Budapest, 1984.
HARMAR, A., SCHOFIELD, J. G., AND KEEN, P.: Substance P biosynthesis in dorsal root ganglia: an immunohistochemical study of [^{35}S]methionine and [^{3}H]proline incorporation *in vitro*. Neuroscience 6: 1917–1922, 1981.
HARPER, A. A., AND LAWSON, S. N.: Conduction velocity is related to morphological cell type in rat dorsal root ganglion neurones. J. Physiol. (Lond.) 359: 31–46, 1985.
HARRIS, N. C., AND RYALL, R. W.: Mustard oil excites but does not inhibit nociceptive dorsal horn neurones in the rat: a presumed effect on A fibres. Br. J. Pharmacol. 94: 180–184, 1988.
HARTI, G.: Wirkung von Capsaicin auf afferente peptiderge Nervenfasern: Immunohistochemische Untersuchungen an Ratten, Meerschweinchen und Tauben. Dr. rer. nat. thesis, University of Giessen, 1988.
HARTI, G., SHARKEY, K. A., AND PIERAU, F.-K.: Effects of capsaicin in rat and pigeon on peripheral nerves containing substance P and calcitonin gene-related peptide. Cell Tissue Res. 256: 465–474, 1989.
HARTI, G., SHARKEY, K. A., RÖSSLER, W., AND PIERAU, F.-K.: Distribution of substance P (SP) and calcitonin gene-related peptide (CGRP) and the effect of capsaicin in sensory neurones of rat and pigeon. Neuroscience 22 (suppl): S322, 1987.
HARTUNG, M., LEAH, J., AND ZIMMERMANN, M.: The excitation of cutaneous nerve endings in a neuroma by capsaicin. Brain Res. 499: 363–366, 1989.
HAYES, A. G., HAWCOCK, A. B., AND HILL, R. G.: The depolarising action of capsaicin on rat isolated sciatic nerve. Life Sci. 35: 1561–1568, 1984a.
HAYES, A. G., OXFORD, A., REYNOLDS, M., SHINGLER, A. H., SKINGLE, M., SMITH, C., AND TYERS, M. B.: The effects of a series of capsaicin analogues on nociception and body temperature in the rat. Life Sci. 34: 1241–1248, 1984b.
HAYES, A. G., SCADDING, J. W., SKINGLE, M., AND TYERS, M. B.: Effects of neonatal administration of capsaicin on nociceptive thresholds in the mouse and rat. J. Pharm. Pharmacol. 33: 183–185, 1981a.
HAYES, A. G., SKINGLE, M., AND TYERS, M. B.: Effect of single doses of capsaicin on nociceptive thresholds in the rodent. Neuropharmacology 20: 505–511, 1981b.
HAYES, A. G., AND TYERS, M. B.: Effects of capsaicin on nociceptive heat, pressure and chemical thresholds and on substance P levels in the rat. Brain Res. 189: 561–564, 1980.
HE, X., SCHEPELMANN, K., SCHAIBLE, H.-G., AND SCHMIDT, R. F.: Capsaicin inhibits responses of fine afferents from the knee joint of the cat to mechanical and chemical stimuli. Brain Res. 530: 147–150, 1990.
HE, X., SCHMIDT, R. F., AND SCHMITTNER, H.: Effects of capsaicin on articular afferents of the cat's knee joint. Agents Actions 25: 222–224, 1988.
HELKE, C. J., DIMICCO, J. A., JACOBOWITZ, D. M., AND KOPIN, I. J.: Effect of capsaicin administration to neonatal rats on the substance P content of discrete CNS regions. Brain Res. 222: 428–431, 1981a.
HELKE, C. J., JACOBOWITZ, D. M., AND THOA, N. B.: Capsaicin and potassium evoked substance P release from the nucleus tractus solitarius and spinal trigeminal nucleus *in vitro*. Life Sci. 29: 1779–1785, 1981b.
HELME, R. D., EGLEZOS, A., DANDIE, G. W., ANDREWS, P. V., AND BOYD, R. L.: The effect of substance P on the regional lymph node antibody response to antigenic stimulation in capsaicin-pretreated rats. J. Immunol. 139: 3470–3473, 1987.
HEYMAN, I., AND RANG, H. P.: Depolarizing responses to capsaicin in a subpopulation of rat dorsal root ganglion cells. Neurosci. Lett. 56: 69–75, 1985.
HIURA, A., AND ISHIZUKA, H.: Changes in features of degenerating primary sensory neurons with time after capsaicin treatment. Acta Neuropathol. 78: 35–46, 1989.
HIURA, A., ISHIZUKA, H., AND SAKAMOTO, Y.: Electron microscopic study of the effect of capsaicin on the mouse chorda tympani nerves. Arch. Oral Biol. 35: 913–916, 1990a.
HIURA, A., AND SAKAMOTO, Y.: Effect of capsaicin on neurites of cultured dorsal root ganglia and isolated neurons of chick embryos. Neurosci. Lett. 73: 237–241, 1987a.
HIURA, A., AND SAKAMOTO, Y.: Quantitative estimation of the effect of capsaicin on the mouse primary sensory neurons. Neurosci. Lett. 76: 101–106, 1987b.
HIURA, A., VILLALOBOS, E. L., AND ISHIZUKA, H.: The action of capsaicin on primary afferent central terminals in the superficial dorsal horn of newborn mice. Arch. Histol. Cytol. 53: 455–466, 1990b.
HOGAN, P.: Expression of markers for pain sensory neurones in cell culture. Ph. D. thesis, Harvard University, Boston, 1983.
HÖGYES, A.: Beiträge zur physiologischen Wirkung der Bestandteile des Capsicum annuum. Arch. Exp. Pathol. Pharmakol. 9: 117–130, 1878.
HOHEISEL, U., AND MENSE, S.: Non-myelinated afferent fibres do not originate exclusively from the smallest dorsal root ganglion cells in the cat. Neurosci. Lett. 72: 153–157, 1986.
HOLJE, H., HILDEBRAND, C., AND FRIED, K.: Proportion of unmyelinated axons in the rat inferior alveolar nerve and mandibular molar pulps after neonatal administration of capsaicin. Brain Res. 266: 133–136, 1983.
HOLZ, G. G., DUNLAP, K., AND KREAM, R. M.: Characterization of the electrically evoked release of substance P from dorsal root ganglion neurons: methods and dihydropyridine sensitivity. J. Neurosci. 8: 463–471, 1988.
HOLZER, P.: Characterization of the stimulus-induced release of immunoreactive substance P from the myenteric plexus of the guinea-pig small intestine. Brain Res. 297: 127–136, 1984.

HOLZER, P.: Capsaicin-sensitive afferent neurones and gastrointestinal propulsion in the rat. Naunyn Schmiedebergs Arch. Pharmacol. **332:** 62–65, 1986.
HOLZER, P.: Local effector functions of capsaicin-sensitive sensory nerve endings: involvement of tachykinins, calcitonin gene-related peptide and other neuropeptides. Neuroscience **24:** 739–768, 1988.
HOLZER, P., BARTHÓ, L., MATUSÁK, O., AND BAUER, V.: Calcitonin gene-related peptide action on intestinal circular muscle. Am. J. Physiol. **256:** G546-G552, 1989.
HOLZER, P., BUCSICS, A., AND LEMBECK, F.: Distribution of capsaicin-sensitive nerve fibres containing immunoreactive substance P in cutaneous and visceral tissues of the rat. Neurosci. Lett. **31:** 253–257, 1982.
HOLZER, P., GAMSE, R., AND LEMBECK, F.: Distribution of substance P in the rat gastrointestinal tract—lack of effect of capsaicin pretreatment. Eur. J. Pharmacol. **61:** 303–307, 1980.
HOLZER, P., JURNA, I., GAMSE, R., AND LEMBECK, F.: Nociceptive threshold after neonatal capsaicin treatment. Eur. J. Pharmacol. **58:** 511–514, 1979.
HOLZER, P., AND LEMBECK, F.: Longitudinal contraction of isolated guinea-pig ileum induced by rapid cooling. Naunyn Schmiedebergs Arch. Pharmacol. **310:** 169–174, 1979.
HOLZER, P., LIPPE, I. TH., AND HOLZER-PETSCHE, U.: Inhibition of gastrointestinal transit due to surgical trauma or peritoneal irritation is reduced in capsaicin-treated rats. Gastroenterology **91:** 360–363, 1986.
HOLZER, P., LIVINGSTON, E. H., AND GUTH, P. H.: Sensory neurons signal for an increase in rat gastric mucosal blood flow in the face of pending acid injury. Gastroenterology, in press, 1991.
HOLZER, P., PABST, M. A., LIPPE, I. TH., PESKAR, B. M., PESKAR, B. A., LIVINGSTON, E. H., AND GUTH, P. H.: Afferent nerve-mediated protection against deep mucosal damage in the rat stomach. Gastroenterology **98:** 838–848, 1990a.
HOLZER, P., PESKAR, B. M., PESKAR, B. A., AND AMANN, R.: Release of calcitonin gene-related peptide induced by capsaicin in the vascularly perfused rat stomach. Neurosci. Lett. **108:** 195–200, 1990b.
HOLZER, P., AND SAMETZ, W.: Gastric mucosal protection against ulcerogenic factors in the rat mediated by capsaicin-sensitive afferent neurons. Gastroenterology **91:** 975–981, 1986.
HOLZER, P., SARIA, A., SKOFITSCH, G., AND LEMBECK, F.: Increase in tissue concentrations of histamine and 5-hydroxytryptamine following capsaicin treatment of newborn rats. Life Sci. **29:** 1099–1105, 1981.
HOLZER-PETSCHE, U., AND LEMBECK, F.: Systemic capsaicin treatment impairs the micturition reflex in the rat. Br. J. Pharmacol. **83:** 935–941, 1984.
HOLZER-PETSCHE, U., RINNER, I., AND LEMBECK, F.: Distribution of choline acetyltransferase activity in rat spinal cord—influence of primary afferents? J. Neural Transm. **66:** 85–92, 1986.
HORI, T.: Thermosensitivity of preoptic and anterior hypothalamic neurons in the capsaicin-desensitized rat. Pflügers Arch. **389:** 297–299, 1981.
HORI, T.: Capsaicin and central control of thermoregulation. Pharmacol. Ther. **26:** 389–416, 1984.
HORI, T., AND TSUZUKI, S.: Thermoregulation in adult rats which have been treated with capsaicin as neonates. Pflügers Arch. **390:** 219–223, 1981.
HORVÁTH, K. J., JANCSÓ, G., AND WOLLEMANN, M.: The effect of calcium on the capsaicin activation of adenylate cyclase in rat brain. Brain Res. **179:** 401–403, 1979.
HOTTENSTEIN, O. D., PAWLIK, W. W., REMAK, G., AND JACOBSON, E. D.: Capsaicin-sensitive nerves modulate resting blood flow and vascular tone in rat gut. Naunyn Schmiedebergs Arch. Pharmacol. **343:** 179–184, 1991.
HOYES, A. D., AND BARBER, P.: Degeneration of axons in the ureteric and duodenal nerve plexuses of the adult rat following *in vivo* treatment with capsaicin. Neurosci. Lett. **25:** 19–24, 1981.
HOYES, A. D., BARBER, P., AND JAGESSAR, H.: Effect of capsaicin on the intraperitoneal axons of the rat trachea. Neurosci. Lett. **26:** 329–334, 1981.
HUA, X.-Y., AND LUNDBERG, J. M.: Dual capsaicin effects on ureteric motility: low dose inhibition mediated by calcitonin gene-related peptide and high dose stimulation by tachykinins? Acta Physiol. Scand. **128:** 453–465, 1986.
HUA, X.-Y., SARIA, A., GAMSE, R., THEODORSSON-NORHEIM, E., BRODIN, E., AND LUNDBERG, J. M.: Capsaicin induced release of multiple tachykinins (substance P, neurokinin A and eledoisin-like material) from guinea-pig spinal cord and ureter. Neuroscience **19:** 313–319, 1986.
HUA, X.-Y., THEODORSSON-NORHEIM, E., BRODIN, E., LUNDBERG, J. M., AND HÖKFELT, T.: Multiple tachykinins (neurokinin A, neuropeptide K and substance P) in capsaicin-sensitive sensory neurons in the guinea-pig. Regul. Pept. **13:** 1–19, 1985.
INOMATA, K., AND NASU, F.: Effects of neonatal capsaicin treatment on thiamine monophosphatase (TMPase) activity in the substantia gelatinosa of the spinal cord. Int. J. Dev. Neurosci. **2:** 307–311, 1984.
JAMES, I. F., WALPOLE, C. S. J., HIXON, J., WOOD, J. N., AND WRIGGLESWORTH, R.: Long-lasting agonist activity produced by a capsaicin-like photoaffinity probe. Mol. Pharmacol. **33:** 643–649, 1988.
JANCSÓ, G.: Selective degeneration of chemosensitive primary sensory neurones induced by capsaicin: glial changes. Cell Tissue Res. **195:** 145–152, 1978.
JANCSÓ, G.: Intracisternal capsaicin: selective degeneration of chemosensitive primary sensory afferents in the adult rat. Neurosci. Lett. **27:** 41–45, 1981.
JANCSÓ, G.: Sensory nerves as modulators of inflammatory reactions. *In* Antidromic Vasodilatation and Neurogenic Inflammation, ed. by L. A. Chahl, J. Szolcsányi, and F. Lembeck, pp. 207–222, Akadémiai Kiadó, Budapest, 1984.
JANCSÓ, G., FERENCSIK, M., SUCH, G., KIRÁLY, E., NAGY, A., AND BUJDOSÓ, M.: Morphological effects of capsaicin and its analogues in newborn and adult mammals. *In* Tachykinin Antagonists, ed. by R. Håkanson and F. Sundler, pp. 35–44, Elsevier, Amsterdam, 1985a.
JANCSÓ, G., HÖKFELT, T., LUNDBERG, J. M., KIRÁLY, E., HALÁSZ, N., NILSSON, G., TERENIUS, L., REHFELD, J., STEINBUSCH, H., VERHOFSTAD, A. E. R., SAID, S., AND BROWN, M.: Immunohistochemical studies on the effect of capsaicin on spinal and medullary peptide and monoamine neurons using antisera to substance P, gastrin/CCK, somatostatin, VIP, enkephalin, neurotensin and 5-hydroxytryptamine. J. Neurocytol. **10:** 963–980, 1981.
JANCSÓ, G., AND JANCSÓ-GÁBOR, A.: Effect of capsaicin on morphine analgesia—possible involvement of hypothalamic structures. Naunyn Schmiedebergs Arch. Pharmacol. **311:** 285–288, 1980.
JANCSÓ, G., KARCSÚ, S., KIRÁLY, E., SZEBENI, A., TÓTH, L., BÁCSY, E., JOÓ, F., AND PÁRDUCZ, A.: Neurotoxin induced nerve cell degeneration: possible involvement of calcium. Brain Res. **295:** 211–216, 1984.
JANCSÓ, G., AND KIRÁLY, E.: Distribution of chemosensitive primary sensory afferents in the central nervous system of the rat. J. Comp. Neurol. **190:** 781–792, 1980.
JANCSÓ, G., AND KIRÁLY, E.: Sensory neurotoxins: chemically induced selective destruction of primary sensory neurons. Brain Res. **210:** 83–89, 1981.
JANCSÓ, G., AND KIRÁLY, E.: Regeneration of peptidergic sensory nerves in the rat skin. Acta Physiol. Acad. Sci. Hung. **63:** 239–240, 1984.
JANCSÓ, G., KIRÁLY, E., AND JANCSÓ-GÁBOR, A.: Pharmacologically induced selective degeneration of chemosensitive primary sensory neurones. Nature **270:** 741–743, 1977.
JANCSÓ, G., KIRÁLY, E., AND JANCSÓ-GÁBOR, A.: Chemosensitive pain fibres and inflammation. Int. J. Tissue React. **2:** 57–66, 1980a.
JANCSÓ, G., KIRÁLY, E., AND JANCSÓ-GÁBOR, A.: Direct evidence for an axonal site of action of capsaicin. Naunyn Schmiedebergs Arch. Pharmacol. **313:** 91–94, 1980b.
JANCSÓ, G., KIRÁLY, E., JOÓ, F., SUCH, G., AND NAGY, A.: Selective degeneration by capsaicin of a subpopulation of primary sensory neurons in the adult rat. Neurosci. Lett. **59:** 209–214, 1985b.
JANCSÓ, G., KIRÁLY, E., SUCH, G., JOÓ, F., AND NAGY, A.: Neurotoxic effect of capsaicin in mammals. Acta Physiol. Hung. **69:** 295–313, 1987a.
JANCSÓ, G., AND KNYIHÁR, E.: Functional linkage between nociception and fluoride-resistant acid phosphatase activity in the Rolando substance. Neurobiology **5:** 42–43, 1975.
JANCSÓ, G., AND LAWSON, S. N.: Ganglionic changes associated with transganglionic degeneration of capsaicin-sensitive primary sensory afferents: a quantitative morphometric and immunohistochemical study. Regul. Pept. **22:** 97, 1988.
JANCSÓ, G., AND LAWSON, S. N.: Transganglionic degeneration of capsaicin-sensitive C-fiber primary afferent terminals. Neuroscience **39:** 501–511, 1990.
JANCSÓ, G., AND MAGGI, C. A.: Distribution of capsaicin-sensitive urinary bladder afferents in the rat spinal cord. Brain Res. **418:** 371–376, 1987.
JANCSÓ, G., SÁVAY, G., AND KIRÁLY, E.: Appearance of biochemically detectable ionic calcium in degenerating primary sensory neurons. Acta Histochem. **62:** 165–169, 1978.
JANCSÓ, G., AND SUCH, G.: Effects of capsaicin applied perineurally to the vagus nerve on cardiovascular and respiratory functions in the cat. J. Physiol. (Lond.) **341:** 359–370, 1983.
JANCSÓ, G., AND SUCH, G.: Evidence for a capsaicin-sensitive vasomotor mechanism in the ventral medullary chemosensitive area of the cat. Naunyn Schmiedebergs Arch. Pharmacol. **329:** 56–62, 1985.
JANCSÓ, G., SUCH, G., AND RÖDEL, C.: A new approach to selective regional analgesia. *In* Trends in Cluster Headache, ed. by F. Sicuteri, L. Vecchiet, and M. Fanciullacci, pp. 59–68, Elsevier, Amsterdam, 1987b.
JANCSÓ, N.: Role of the nerve terminals in the mechanism of inflammatory reactions. Bull. Millard Fillmore Hosp. 7:53–77, 1960.
JANCSÓ, N.: Desensitization with capsaicin and related acylamides as a tool for studying the function of pain receptors. *In* Pharmacology of Pain, ed. by R. K. S. Lim, pp. 33–55, Oxford, Pergamon Press, 1968.
JANCSÓ, N., JANCSÓ-GÁBOR, A., AND SZOLCSÁNYI, J.: Direct evidence for neurogenic inflammation and its prevention by denervation and by pretreatment with capsaicin. Br. J. Pharmacol. **31:** 138–151, 1967.
JANCSÓ, N., JANCSÓ-GÁBOR, A., AND SZOLCSÁNYI, J.: The role of sensory nerve endings in neurogenic inflammation induced in human skin and in the eye and paw of the rat. Br. J. Pharmacol. **33:** 32–41, 1968.
JANCSÓ-GÁBOR, A.: Anaesthesia-like condition and/or potentiation of hexobarbital sleep produced by pungent agents in normal and capsaicin-desensitized rats. Acta Physiol. Acad. Sci. Hung. **55:** 57–62, 1980.
JANCSÓ-GÁBOR, A., SZOLCSÁNYI, J., AND JANCSÓ, N.: Irreversible impairment of thermoregulation induced by capsaicin and similar pungent substances. J. Physiol. (Lond.) **206:** 495–507, 1970.
JESSELL, T. M., IVERSEN, L. L., AND CUELLO, A. C.: Capsaicin-induced depletion of substance P from primary sensory neurones. Brain Res. **152:** 183–188, 1978.
JHAMANDAS, K., YAKSH, T. L., HARTY, G., SZOLCSÁNYI, J., AND GO, V. L. W.: Action of intrathecal capsaicin and its structural analogues on the content and release of spinal substance P: selective action and relationship to analgesia. Brain Res. **306:** 215–225, 1984.
JIA, M., AND NELSON, P. G.: Calcium currents and transmitter output in cultured spinal cord and dorsal root ganglion neurons. J. Neurophysiol. **56:** 1257–1267, 1986.

JIN, J.-G., AND NAKAYAMA, S.: Bile salt potentiates the action of capsaicin on sensory neurones of guinea-pig ileum. Neurosci. Lett. **109:** 88–91, 1990.

JIN, J.-G., TAKAKI, M., AND NAKAYAMA, S.: Ruthenium red prevents capsaicin-induced neurotoxic action on sensory fibers of the guinea pig ileum. Neurosci. Lett. **106:** 152–156, 1989.

JIN, J.-G., TAKAKI, M., AND NAKAYAMA, S.: Inhibitory effect of capsaicin on the ascending pathway of the guinea-pig ileum and antagonism of this effect by ruthenium red. Eur. J. Pharmacol. **180:** 13–19, 1990.

JOÓ, F., SZOLCSÁNYI, J., AND JANCSÓ-GÁBOR, A.: Mitochondrial alterations in the spinal ganglion cells of the rat accompanying the long-lasting sensory disturbance induced by capsaicin. Life Sci. **8:** 621–626, 1969.

JU, G., HÖKFELT, T., FISCHER, J. A., FREY, P., REHFELD, J. F., AND DOCKRAY, G. J.: Does cholecystokinin-like immunoreactivity in rat primary sensory neurons represent calcitonin gene-related peptide? Neurosci. Lett. **68:** 305–310, 1986.

JUAN, H., LEMBECK, F., SEEWANN, S., AND HACK, U.: Nociceptor stimulation and PGE release by capsaicin. Naunyn Schmiedebergs Arch. Pharmacol. **312:** 139–143, 1980.

KAI-KAI, M. A., ANDERTON, B. H., AND KEEN, P.: A quantitative analysis of the interrelationships between subpopulations of rat sensory neurons containing arginine vasopressin or oxytocin and those containing substance P, fluoride-resistant acid phosphatase or neurofilament protein. Neuroscience **18:** 475–486, 1986.

KAMAKURA, K., ISHIURA, S., SUGITA, H., AND TOYOKURA, Y.: Identification of Ca^{2+}-activated neutral protease in the peripheral nerve and its effect on neurofilament degeneration. J. Neurochem. **40:** 908–913, 1983.

KANUI, T. I., HOLE, K., AND MIARON, J. O.: Nociception in crocodiles: capsaicin instillation, formalin and hot plate tests. Zool. Sci. **7:** 537–540, 1990.

KASHIBA, H., SENBA, E., UEDA, Y., AND TOHYAMA, M.: Relative sparing of calcitonin gene-related peptide-containing primary sensory neurons following neonatal capsaicin treatment in the rat. Peptides **11:** 491–496, 1990a.

KASHIBA, H., SENBA, E., UEDA, Y., AND TOHYAMA, M.: Calbindin D28k-containing splanchnic and cutaneous dorsal root ganglion neurons of the rat. Brain Res. **528:** 311–316, 1990b.

KAUFMAN, M. P., IWAMOTO, G. A., LONGHURST, J. C., AND MITCHELL, J. H.: Effects of capsaicin and bradykinin on afferent fibers with endings in skeletal muscle. Circ. Res. **50:** 133–139, 1982.

KEEN, P., TULLO, A. B., BLYTH, W. A., AND HILL, T. J.: Substance P in the mouse cornea: effects of chemical and surgical denervation. Neurosci. Lett. **29:** 231–235, 1982.

KENINS, P.: Responses of single nerve fibres to capsaicin applied to the skin. Neurosci. Lett. **29:** 83–88, 1982.

KENINS, P., HURLEY, J. V., AND BELL, C.: The role of substance P in the axon reflex in the rat. Br. J. Dermatol. **111:** 551–559, 1984.

KI, P., NEGULESCO, J. A., AND MURNANE, M.: Decreased total serum, myocardial and aortic cholesterol levels following capsaicine treatment. IRCS Med. Sci. **10:** 446–447, 1982.

KIRÁLY, E., JANCSÓ, G., AND HAJÓS, M.: Possible morphological correlates of capsaicin desensitization. Brain Res. **540:** 279–282, 1991.

KIRBY, M. L., GALE, T. F., AND MATTIO, T. G.: Effects of prenatal capsaicin treatment on fetal spontaneous activity, opiate receptor binding, and acid phosphatase in the spinal cord. Exp. Neurol. **76:** 298–308, 1982.

KIRCHGESSNER, A. L., DODD, J., AND GERSHON, M. D.: Markers shared between dorsal root and enteric ganglia. J. Comp. Neurol. **276:** 607–621, 1988.

KJARTANSSON, J., DALSGAARD, C. J., AND JONSSON, C. E.: Decreased survival of experimental critical flaps in rats after sensory denervation with capsaicin. Plast. Reconstr. Surg. **79:** 218–221, 1987.

KNYAZEV, G. G., KNYAZEVA, G. B., AND NIKIFOROV, A. F.: Neuroparalytic keratitis and capsaicin. Acta Physiol. Hung. **75:** 29–34, 1990.

KONIETZNY, F., AND HENSEL, H.: The effect of capsaicin on the response characteristics of human C-polymodal nociceptors. J. Therm. Biol. **8:** 213–215, 1983.

KONNERTH, A., LUX, H. D., AND MORAD, M.: Proton-induced transformation of calcium channel in chick dorsal root ganglion cells. J. Physiol. (Lond.) **386:** 603–633, 1987.

KOSTYUK, P. G.: Diversity of calcium ion channels in cellular membranes. Neuroscience **28:** 253–261, 1989.

KOSTYUK, P. G., FEDULOVA, S. A., AND VESELOVSKY, N. S.: Changes in ionic mechanisms of electrical excitability of the somatic membrane of rat dorsal root ganglion neurons during ontogenesis. Distribution of ionic channels of inward current. Neurophysiology (Kiev) **18:** 813–820, 1986.

KRISHTAL, O. A., AND PIDOPLICHKO, V. I.: A receptor for protons in the nerve cell membrane. Neuroscience **5:** 2325–2327, 1980.

KRISHTAL, O. A., AND PIDOPLICHKO, V. I.: A 'receptor' for protons in small neurons of trigeminal ganglia: possible role in nociception. Neurosci. Lett. **24:** 243–246, 1981.

LADENHEIM, E. E., SPETH, R. C., AND RITTER, R. C.: Reduction of CCK-8 binding in the nucleus of the solitary tract of capsaicin pretreated rats. Soc. Neurosci. Abstr. **12:** 828, 1986.

LADURON, P. M.: Axonal transport of opiate receptors in capsaicin-sensitive neurones. Brain Res. **294:** 157–160, 1984.

LAMOTTE, R. H., SIMONE, D. A., BAUMANN, T. K., SHAIN, C. N., AND ALREJA, M.: Hypothesis for novel classes of chemoreceptors mediating chemogenic pain and itch. *In* Pain Research and Clinical Management, ed. by R. Dubner, G. F. Gebhart, and M. R. Bond, pp. 529–535, Elsevier, Amsterdam, 1988.

LANG, E., NOVAK, A., REEH, P. W., AND HANDWERKER, H. O.: Chemosensitivity of fine afferents from rat skin *in vitro*. J. Neurophysiol. **63:** 887–901, 1990.

LAWSON, S. N., AND HARPER, A. A.: A Neonatal capsaicin is not a specific neurotoxin for sensory C-fibres or small dark cells of rat dorsal root ganglia. *In* Antidromic Vasodilatation and Neurogenic Inflammation, ed. by L. A. Chahl, J. Szolcsányi, and F. Lembeck, pp. 111–116, Akadémiai Kiadó, Budapest, 1984.

LAWSON, S. N., HARPER, A. A., GARSON, J. A., AND ANDERTON, B. H.: A monoclonal antibody against neurofilament protein specifically labels a subpopulation of rat sensory neurones. J. Comp. Neurol. **228:** 262–272, 1984.

LAWSON, S. N., AND NICKELS, S. M.: The use of morphometric techniques to analyse the effects of neonatal capsaicin treatment on rat dorsal root ganglia and dorsal roots. J. Physiol. (Lond.) **303:** 12P, 1980.

LAWSON, T., AND GANNETT, P.: The mutagenicity of capsaicin and dihydrocapsaicin in V79 cells. Cancer Lett. **48:** 109–113, 1989.

LEMBECK, F.: A network of defense. *In* Substance P and Neurokinins, ed. by J. L. Henry, R. Couture, A. C. Cuello, G. Pelletier, R. Quirion, and D. Regoli, pp. 380–387, Springer, New York, 1987a.

LEMBECK, F.: Columbus, Capsicum and capsaicin: past, present and future. Acta Physiol. Hung. **69:** 265–273, 1987b.

LEMBECK, F.: The 1988 Ulf von Euler lecture. Substance P: from extract to excitement. Acta Physiol. Scand. **133:** 435–454, 1988.

LEMBECK, F., AND DONNERER, J.: Time course of capsaicin-induced functional impairments in comparison with changes in neuronal substance P content. Naunyn Schmiedebergs Arch. Pharmacol. **316:** 240–243, 1981.

LEMBECK, F., AND HOLZER, P.: Substance P as neurogenic mediator of antidromic vasodilation and neurogenic plasma extravasation. Naunyn Schmiedebergs Arch. Pharmacol. **310:** 175–183, 1979.

LEMBECK, F., AND SKOFITSCH, G.: Visceral pain reflex after pretreatment with capsaicin and morphine. Naunyn Schmiedebergs Arch. Pharmacol. **321:** 116–122, 1982.

LONGHURST, J. C., ASHTON, J. H., AND IWAMOTO, G. A.: Cardiovascular reflexes resulting from capsaicin-stimulated gastric receptors in anesthetized dogs. Circ. Res. **46:** 780–788, 1980.

LONGHURST, J. C., KAUFMAN, M. P., ORDWAY, G. A., AND MUSCH, T. I.: Effects of bradykinin and capsaicin on endings of afferent fibers from abdominal visceral organs. Am. J. Physiol. **247:** R552-R559, 1984.

LOREZ, H. P., HAEUSLER, G., AND AEPPLI, L.: Substance P neurones in medullary baroreflex areas and baroreflex areas and baroreflex function of capsaicin-treated rats. Comparison with other primary afferent systems. Neuroscience **8:** 507–523, 1983.

LOU, Y. P., FRANCO-CERECEDA, A., AND LUNDBERG, J. M.: Omega-conotoxin inhibits CGRP release and bronchoconstriction evoked by a low concentration of capsaicin. Acta Physiol. Scand. **141:** 135–136, 1991.

LUNDBERG, J. M., FRANCO-CERECEDA, A., HUA, X.-Y., HÖKFELT, T., AND FISCHER, J.: Co-existence of substance P and calcitonin gene-related peptide immunoreactivities in sensory nerves in relation to cardiovascular and bronchoconstrictor effects of capsaicin. Eur. J. Pharmacol. **108:** 315–319, 1985.

LUNDBERG, J. M., AND SARIA, A.: Capsaicin induced desensitization of the airway mucosa to cigarette smoke, mechanical and chemical irritants. Nature **302:** 251–253, 1983.

LUNDBERG, J. M., AND SARIA, A.: Polypeptide-containing neurons in airway smooth muscle. Annu. Rev. Physiol. **49:** 557–572, 1987.

LUNDBLAD, L.: Protective reflexes and vascular effects in the nasal mucosa elicited by activation of capsaicin-sensitive substance P-immunoreactive trigeminal neurons. Acta Physiol. Scand. Suppl. **529:** 1–42, 1984.

LUNDBLAD, L., LUNDBERG, J. M., ÅNGGARD, A., AND ZETTERSTRÖM, D.: Capsaicin-sensitive nerves and the cutaneous allergy reaction in man. Possible involvement of sensory neuropeptides in the flare reaction. Allergy **42:** 20–25, 1987.

LUTHMAN, J., STROMBERG, I., BRODIN, E., AND JONSSON, G.: Capsaicin treatment to developing rats induces increase of noradrenaline levels in the iris without affecting the adrenergic terminal density. Int. J. Dev. Neurosci. **7:** 613–620, 1989.

LYNN, B.: Effect of neonatal treatment with capsaicin on the numbers and properties of cutaneous afferent units from the hairy skin of the rat. Brain Res. **322:** 255–260, 1984.

LYNN, B.: Capsaicin: actions on nociceptive C-fibres and therapeutical potential. Pain **41:** 61–69, 1990.

LYNN, B., CARPENTER, S. E., AND PINI, A.: Capsaicin and cutaneous afferents. *In* Antidromic Vasodilatation and Neurogenic Inflammation, ed. by L. A. Chahl, J. Szolcsányi, and F. Lembeck, pp. 83–92, Akadémiai Kiadó, Budapest, 1984.

LYNN, B., AND PINI, A.: Long-term block of afferent C-fibres following capsaicin treatment in the rat. J. Physiol. (Lond.) **362:** 198, 1985.

LYNN, B., PINI, A., AND BARANOWSKI, R.: Injury of somatosensory afferents by capsaicin: selectivity and failure to regenerate. *In* Effects of Injury on Trigeminal and Spinal Somatosensory Systems, ed. by L. M. Pubols, and B. Sessle, pp. 115–124, Alan R. Liss, New York, 1987.

LYNN, B., AND SHAKHANBEH, J.: Substance P content of the skin, neurogenic inflammation and numbers of C-fibres following capsaicin application to a cutaneous nerve in the rabbit. Neuroscience **24:** 769–776, 1988.

MACLEAN, D. D.: Abrogation of peripheral cholecystokinin-satiety in the capsaicin-treated rat. Regul. Pept. **11:** 321–333, 1985.

MAGGI, C. A.: Capsaicin-sensitive nerves in the gastrointestinal tract. Arch. Int. Pharmacodyn. Ther. **303:** 157–166, 1990.
MAGGI, C. A.: Capsaicin and primary afferent neurons: from basic science to human therapy? J. Auton. Nerv. Syst., in press, 1991.
MAGGI, C. A., ASTOLFI, M., DONNERER, J., AND AMANN, R.: Which mechanisms account for the sensory neuron blocking action of capsaicin on primary afferents in the rat urinary bladder? Neurosci. Lett. **110:** 267–272, 1990a.
MAGGI, C. A., BORSINI, F., SANTICIOLI, P., GEPPETTI, P., ABELLI, L., EVANGELISTA, S., MANZINI, S., THEODORSSON-NORHEIM, E., SOMMA, V., AMENTA, F., BACCIARELLI, C., AND MELI, A.: Cutaneous lesions in capsaicin-pretreated rats. A trophic role of capsaicin-sensitive afferents? Naunyn Schmiedebergs Arch. Pharmacol. **336:** 538–545, 1987a.
MAGGI, C. A., GIULIANI, S., AND MELI, A.: Effect of ruthenium red on responses mediated by activation of capsaicin-sensitive nerves of the rat urinary bladder. Naunyn Schmiedebergs Arch. Pharmacol. **340:** 541–546, 1989a.
MAGGI, C. A., GIULIANI, S., SANTICIOLI, P., ABELLI, L., GEPPETTI, P., SOMMA, V., RENZI, D., AND MELI, A.: Species-related variations in the effects of capsaicin on urinary bladder functions: relation to bladder content of substance P-like immunoreactivity. Naunyn Schmiedebergs Arch. Pharmacol. **336:** 546–555, 1987b.
MAGGI, C. A., GIULIANI, S., SANTICIOLI, P., TRAMONTANA, M., AND MELI, A.: Effect of omega conotoxin on reflex responses mediated by activation of capsaicin-sensitive nerves of the rat urinary bladder and peptide release from the rat spinal cord. Neuroscience **34:** 243–250, 1990b.
MAGGI, C. A., LIPPE, I. TH., GIULIANI, S., ABELLI, L., SOMMA, V., GEPPETTI, P., JANCSÓ, G., SANTICIOLI, P., AND MELI, A.: Topical versus systemic capsaicin desensitization: specific and unspecific effects as indicated by modification of reflex micturition in rats. Neuroscience **31:** 745–756, 1989b.
MAGGI, C. A., MANZINI, S., GIULIANI, S., SANTICIOLI, S., AND MELI, A.: Extrinsic origin of the capsaicin-sensitive innervation of rat duodenum: possible involvement of calcitonin gene-related peptide (CGRP) in the capsaicin-induced activation of intramural non-adrenergic non-cholinergic ('purinergic'?) neurons. Naunyn Schmiedebergs Arch. Pharmacol. **334:** 172–180, 1986.
MAGGI, C. A., AND MELI, A.: The role of neuropeptides in the regulation of the micturition reflex. J. Auton. Pharmacol. **6:** 133–162, 1986.
MAGGI, C. A., AND MELI, A.: The sensory-efferent function of capsaicin-sensitive sensory neurons. Gen. Pharmacol. **19:** 1–43, 1988.
MAGGI, C. A., MELI, A., AND SANTICIOLI, P.: Four motor effects of capsaicin on guinea-pig distal colon. Br. J. Pharmacol. **90:** 651–660, 1987c.
MAGGI, C. A., PATACCHINI, R., GIULIANI, S., SANTICIOLI, P., AND MELI, A.: Evidence for two independent modes of activation of the 'efferent' function of capsaicin-sensitive nerves. Eur. J. Pharmacol. **156:** 367–374, 1988a.
MAGGI, C. A., PATACCHINI, R., SANTICIOLI, P., GIULIANI, S., DEL BIANCO, E., GEPPETTI, P., AND MELI, A.: The efferent function of capsaicin-sensitive nerves—ruthenium red discriminates between different mechanisms of activation. Eur. J. Pharmacol. **170:** 167–177, 1989c.
MAGGI, C. A., PATACCHINI, R., SANTICIOLI, P., GIULIANI, S., GEPPETTI, P., AND MELI, A.: Protective action of ruthenium red toward capsaicin desensitization of sensory fibers. Neurosci. Lett. **88:** 201–205, 1988b.
MAGGI, C. A., PATACCHINI, R., SANTICIOLI, P., LIPPE, I. TH., GIULIANI, S., GEPPETTI, P., DEL BIANCO, E., SELLERI, S., AND MELI, A.: The effect of omega conotoxin GVIA, a peptide modulator of the N-type voltage sensitive calcium channels, on motor responses produced by activation of efferent and sensory nerves in mammalian smooth muscle. Naunyn Schmiedebergs Arch. Pharmacol. **338:** 107–113, 1988c.
MAGGI, C. A., PATACCHINI, R., TRAMONTANA, M., AMANN, R., GIULIANI, S., AND SANTICIOLI, P.: Similarities and differences in the action of resiniferatoxin and capsaicin on central and peripheral endings of primary sensory neurons. Neuroscience **37:** 531–539, 1990c.
MAGGI, C. A., SANTICIOLI, P., DEL BIANCO, E., GEPPETTI, P., BARBANTI, G., TURINI, D., AND MELI, A.: Release of VIP- but not CGRP-like immunoreactivity by capsaicin from the human isolated small intestine. Neurosci. Lett. **98:** 317–320, 1989d.
MAGGI, C. A., SANTICIOLI, P., GEPPETTI, P., FURIO, M., FRILLI, S., CONTE, B., FANCIULLACCI, M., GIULIANI, S., AND MELI, A.: The contribution of capsaicin-sensitive innervation to activation of the spinal vesicovesical reflex in rats: relationship between substance P levels in the urinary bladder and the sensory-efferent function of capsaicin-sensitive sensory neurons. Brain Res. **415:** 1–13, 1987d.
MAGGI, C. A., SANTICIOLI, P., GEPPETTI, P., GIULIANI, S., PATACCHINI, R., FRILLI, S., GRASSI, J., AND MELI, A.: Involvement of a peripheral site of action in the early phase of neuropeptide depletion following capsaicin desensitization. Brain Res. **436:** 402–406, 1987e.
MAGGI, C. A., SANTICIOLI, P., GEPPETTI, P., PARLANI, M., ASTOLFI, M., DEL BIANCO, E., PATACCHINI, R., GIULIANI, S., AND MELI, A.: The effect of calcium free medium and nifedipine on the release of substance P-like immunoreactivity and contractions induced by capsaicin in the isolated guinea pig and rat bladder. Gen. Pharmacol. **20:** 445–456, 1989e.
MAGGI, C. A., SANTICIOLI, P., GEPPETTI, P., PARLANI, M., ASTOLFI, M., PRADELLES, P., PATACCHINI, R., AND MELI, A.: The antagonism induced by ruthenium red of the actions of capsaicin on the peripheral terminals of sensory neurons: further studies. Eur. J. Pharmacol. **154:** 1–10, 1988d.
MAGGI, C. A., SANTICIOLI, P., GEPPETTI, P., PATACCHINI, R., DEL BIANCO, E., AND MELI, A.: Calcium and capsaicin-induced substance P release from peripheral terminals of primary sensory neurons. Regul. Pept. **22:** 117, 1988e.
MAGGI, C. A., SANTICIOLI, P., AND MELI, A.: The effects of capsaicin on rat urinary bladder motility *in vivo*. Eur. J. Pharmacol. **103:** 41–50, 1984.
MAGGI, C. A., SANTICIOLI, P., PATACCHINI, R., GEPPETTI, P., GIULIANI, S., ASTOLFI, M., BALDI, E., PARLANI, M., THEODORSSON, E., FUSCO, B., AND MELI, A.: Regional differences in the motor response to capsaicin in the guinea-pig urinary bladder: relative role of pre- and postjunctional factors related to neuropeptide-containing sensory nerves. Neuroscience **27:** 675–688, 1988f.
MAGGIO, J. E., AND HUNTER, J. C.: Regional distribution of kassinin-like immunoreactivity in rat central and peripheral tissues and the effect of capsaicin. Brain Res. **307:** 370–373, 1984.
MANZINI, S., MAGGI, C. A., GEPPETTI, P., AND BACCIARELLI, C.: Capsaicin desensitization protects from antigen-induced bronchospasm in conscious guinea-pigs. Eur. J. Pharmacol. **138:** 307–308, 1987.
MANZINI, S., TRAMONTANA, M., PAGE, C. P., AND PERRETTI, F.: Motor effects of capsaicin-sensitive nerves in rabbit isolated bronchus. Eur. J. Pharmacol. **183:** 1696, 1990.
MAPP, C. E., CHITANO, P., FABBRI, L. M., PATACCHINI, R., AND MAGGI, C. A.: Pharmacological modulation of the contractile response to toluene diisocyanate in the rat isolated urinary bladder. Br. J. Pharmacol. **100:** 886–888, 1990.
MARABINI, S., CIABATTI, G., POLLI, G., FUSCO, B. M., GEPPETTI, P., MAGGI, C. A., FANCIULLACCI, M., AND SICUTERI, F.: Effect of topical nasal treatment with capsaicin in vasomotor rhinitis. Regul. Pept. **22:** 121, 1988.
MARLEY, P., AND LIVETT, B. G.: Neuropeptides in the autonomic nervous system. CRC Crit. Rev. Clin. Neurobiol. **1:** 201–283, 1985.
MARLIER, L., RAJAOFETRA, N., POULAT, P., AND PRIVAT, A.: Modification of serotonergic innervation of the rat spinal cord dorsal horn after neonatal capsaicin treatment. J. Neurosci. Res. **25:** 112–118, 1990.
MARSH, S. J., STANSFELD, C. E., BROWN, D. A., DAVEY, R., AND MCCARTHY, D.: The mechanism of action of capsaicin on sensory C-type neurons and their axons *in vitro*. Neuroscience **23:** 275–290, 1987.
MARTIN, H. A., BASBAUM, A. I., KWIAT, G. C., GOETZL, E. J., AND LEVINE, J. D.: Leukotriene and prostaglandin sensitization of cutaneous high threshold C- and A-delta mechanonociceptors in the hairy skin of rat hindlimbs. Neuroscience **22:** 651–659, 1987.
MARTLING, C.-R., AND LUNDBERG, J. M.: Capsaicin sensitive afferents contribute to acute airway edema following tracheal instillation of hydrochloric acid or gastric juice in the rat. Anesthesiology **68:** 350–356, 1988.
MASON, R. J., AND MARUNIAK, J. A.: Bahavioral and physiological effects of capsaicin in red-winged blackbirds. Pharmacol. Biochem. Behav. **19:** 857–862, 1983.
MATRAN, R., ALVING, K., AND LUNDBERG, J. M.: Cigarette smoke, nicotine and capsaicin aerosol-induced vasodilatation in pig respiratory mucosa. Br. J. Pharmacol. **100:** 535–541, 1990.
MATRAN, R., ALVING, K., MARTLING, C.-R., LACROIX, J. S., AND LUNDBERG, J. M.: Effects of neuropeptides and capsaicin on tracheobronchial blood flow of the pig. Acta Physiol. Scand. **135:** 335–342, 1989.
MATSUMIYA, T., SAWA, A., AND OKA, T.: The effect of capsaicin on C-fiber reflex and heat evoked discharge in the acute spinal cat. Tokai J. Exp. Clin. Med. **8:** 325–332, 1983.
MATTHEWS, M. R., AND CUELLO, A. C.: Substance P-immunoreactive peripheral branches of sensory neurons innervate guinea-pig sympathetic neurons. Proc. Natl. Acad. Sci. USA **79:** 1668–1672, 1982.
MATUCCI-CERINIC, M., MARABINI, S., JANTSCH, S., CAGNONI, M., AND PARTSCH, G.: Effects of capsaicin on the metabolism of rheumatoid arthritis synoviocytes *in vitro*. Ann. Rheum. Dis. **49:** 598–602, 1990.
MCCANN, M. J., VERBALIS, J. G., AND STRICKER, E. M.: Capsaicin pretreatment attenuates multiple responses to cholecystokinin in rats. J. Auton. Nerv. Syst. **23:** 265–271, 1988.
MCCUSKER, M. T., CHUNG, K. F., ROBERTS, N. M., AND BARNES, P. J.: Effect of topical capsaicin on the cutaneous responses to inflammatory mediators and to antigen in man. J. Allergy Clin. Immunol. **83:** 1118–1123, 1989.
MCDOUGAL, D. B., YUAN, M. J. C., DARGAR, R. V., AND JOHNSON, E. M.: Neonatal capsaicin and guanethidine and axonally transported organelle-specific enzymes in sciatic nerve and in sympathetic and dorsal root ganglia. J. Neurosci. **3:** 124–132, 1983.
MCDOUGAL, D. B., YUAN, M. J. C., AND JOHNSON, E. M.: Effect of capsaicin upon fluoride sensitive acid phosphatases in selected ganglia and spinal cord and upon neuronal size and number in dorsal root ganglion. Brain Res. **331:** 63–70, 1985.
MCGREGOR, G. P., AND CONLON, J. M.: Regulatory peptide and serotonin content and brush-border enzyme activity in the rat gastrointestinal tract following neonatal treatment with capsaicin: lack of effect on epithelial markers. Regul. Pept. **32:** 109–119, 1991.
MCMAHON, S., AND KOLTZENBURG, M.: The changing role of primary afferent neurones in pain. Pain **43:** 269–272, 1990.
MCMAHON, S. B., WALL, P. D., GRANUM, S. L., AND WEBSTER, K. E.: The effect of capsaicin applied to peripheral nerves on responses of a group of lamina I cells in adult rats. J. Comp. Neurol. **227:** 393–400, 1984.
MCNEILL, D. L., CARLTON, S. M., COGGESHALL, R. E., AND HULSEBOSCH, C. E.: Denervation-induced intraspinal synaptogenesis of calcitonin gene-related peptide containing primary afferent terminals. J. Comp. Neurol. **296:** 263–268, 1990.
MICEVYCH, P. E., YAKSH, T. L., AND SZOLCSÁNYI, J.: Effect of intrathecal capsaicin analogues on the immunofluorescence of peptides and serotonin in the dorsal horn in rats. Neuroscience **8:** 123–131, 1983.

MILLER, M. S., BRENDEL, K., BURKS, T. F., AND SIPES, I. G.: Interaction of capsaicinoids with drug-metabolizing systems—relationship to toxicity. Biochem. Pharmacol. 32: 547–551, 1983.
MILLER, M. S., BUCK, S. H., SIPES, I. G., AND BURKS, T. F.: Capsaicinoid-induced local and systemic antinociception without substance P depletion. Brain Res. 244: 193–197, 1982a.
MILLER, M. S., BUCK, S. H., SIPES, I. G., YAMAMURA, H. I., AND BURKS, T. F.: Regulation of substance P by nerve growth factor: disruption by capsaicin. Brain Res. 250: 193–196, 1982b.
MIYAUCHI, T., ISHIKAWA, T., SGISHITA, Y., SAITO, A., AND GOTO, K.: Involvement of calcitonin gene-related peptide in the positive chronotropic and inotropic effects of piperine and development of cross-tachyphylaxis between piperine and capsaicin in the isolated rat atria. J. Pharmacol. Exp. Ther. 248: 816–824, 1989.
MODLY, C. E., DAS, M., DON, P. S. C., MARCELO, C. L., MUKHTAR, H., AND BICKERS, D. R.: Capsaicin as an *in vitro* inhibitor of benzo(a)pyrene metabolism and its DNA binding in human and murine keratinocytes. Drug Metab. Dispos. 14: 413–416, 1986.
MONSEREENUSORN, Y., AND KONGSAMUT, S.: Inhibition of calcium uptake by capsaicin. Res. Commun. Chem. Pathol. Pharmacol. 47: 453–456, 1985.
MOODY, T. W., THOA, N. B., O'DONOHUE, T. L., AND JACOBOWITZ, D. M.: Bombesin-like peptides in rat spinal cord: biochemical characterization, localization, and mechanism of release. Life Sci. 29: 2273–2279, 1981.
MORITOKI, H., TAKASE, H., AND TANIOKA, A.: Dual effects of capsaicin on responses of the rabbit ear artery to field stimulation. Br. J. Pharmacol. 99: 152–156, 1990.
MORTON, C. R., AND CHAHL, L. A.: Pharmacology of the neurogenic oedema response to electrical stimulation of the saphenous nerve in the rat. Naunyn Schmiedebergs Arch. Pharmacol. 314: 271–276, 1980.
MUELLER, G. P.: Beta-endorphin immunoreactivity in rat plasma: variations in response to different physical stimuli. Life Sci. 29: 1669–1674, 1981.
MÜNTENER, M.: Muscle fiber transformation in capsaicin-treated rats. Exp. Neurol. 88: 205–214, 1985.
MURALIDHARA, N., AND NARASIMHAMURTHY, K.: Non-mutagenicity of capsaicin in albino mice. Food Chem. Toxicol. 26: 955–958, 1988.
MUSSAP, C. J., LEW, R., AND BURCHER, E.: The autoradiographic distribution of substance P binding sites in guinea-pig vas deferens is altered by capsaicin pretreatment. Eur. J. Pharmacol. 168: 337–345, 1989.
NAGABHUSHAN, M., AND BHIDE, S. V.: Mutagenicity of chili extract and capsaicin in short-term tests. Environ. Mutagen. 7: 881–888, 1985.
NAGY, J. I.: Capsaicin: a chemical probe for sensory neuron mechanisms. *In* Handbook of Psychopharmacology, ed. by L. L. Iversen, S. D. Iversen, and S. H. Snyder, vol. 15, pp. 185–235, Plenum, New York, 1982.
NAGY, J. I., AND DADDONA, P. E.: Anatomical and cytochemical relationships of adenosine deaminase-containing primary afferent neurons in the rat. Neuroscience 15: 799–813, 1985.
NAGY, J. I., EMSON, P. C., AND IVERSEN, L. L.: A re-evaluation of the neurochemical and antinociceptive effects of intrathecal capsaicin in the rat. Brain Res. 211: 497–502, 1981a.
NAGY, J. I., AND HUNT, S. P.: The termination of primary afferents within the rat dorsal horn: evidence for rearrangement following capsaicin treatment. J. Comp. Neurol. 218: 145–158, 1983.
NAGY, J. I., HUNT, S. P., IVERSEN, L. L., AND EMSON, P. C.: Biochemical and anatomical observations on the degeneration of peptide-containing primary afferent neurons after neonatal capsaicin. Neuroscience 6: 1923–1934, 1981b.
NAGY, J. I., IVERSEN, L. L., GOEDERT, M., CHAPMAN, D., AND HUNT, S. P.: Dose-dependent effects of capsaicin on primary sensory neurons in the neonatal rat. J. Neurosci. 3: 399–406, 1983.
NAGY, J. I., AND VAN DER KOY, D.: Effects of neonatal capsaicin treatment on nociceptive thresholds in the rat. J. Neurosci. 3: 1145–1150, 1983.
NAGY, J. I., VINCENT, S. R., STAINES, W. M. A., FIBIGER, H. C., REISINE, T. D., AND YAMAMURA, H. I.: Neurotoxic action of capsaicin on spinal substance P neurons. Brain Res. 186: 435–444, 1980.
NEGULESCO, J. A., YOUNG, R. M., AND KI, P.: Capsaicin lowers plasma cholesterol and triglycerides of lagomorpha. Artery 12: 301–311, 1983.
NIELSCH, U., AND KEEN, P.: Effects of neonatal 6-hydroxydopamine administration on different substance P-containing sensory neurones. Eur. J. Pharmacol. 138: 193–197, 1987.
NILSSON, G.: Modulation of the immune response in rat by utilizing the neurotoxin capsaicin. Acta Universitatis Upsaliensis, Uppsala, 1989.
NORTHAM, W. J., AND JONES, D. J.: Comparison of capsaicin and substance P induced cyclic AMP accumulation in spinal cord tissue slices. Life Sci. 35: 293–302, 1984.
OBÁL, F., JANCSÓ, G., JANCSÓ-GÁBOR, A., AND OBÁL, F.: Vasodilatation on preoptic heating in capsaicin-treated rats. Experientia 39: 221–223, 1983.
OGILVY, C. S., SILVERBERG, K. R., AND BORGES, L. F.: Sprouting of corneal sensory fibers in rats treated at birth with capsaicin. Invest. Ophthalmol. Vis. Sci. 32: 112–121, 1991.
ORDWAY, G. A., AND LONGHURST, J. C.: Cardiovascular reflexes arising from the gallbladder of the cat. Effects of capsaicin, bradykinin, and distension. Circ. Res. 52: 26–35, 1983.
OSBORNE, P., AND CAMPBELL, G.: A pharmacological and immunohistochemical study of the splanchnic innervation of ileal longitudinal muscle in the toad Bufo marinus. Naunyn Schmiedebergs Arch. Pharmacol. 334: 210–217, 1986.
OTTEN, U., LOREZ, H. P., AND BUSINGER, F.: Nerve growth factor antagonizes the neurotoxic action of capsaicin on primary sensory neurones. Nature 301: 515–517, 1983.
PALERMO, N. N., BROWN, H. K., AND SMITH, D. L.: Selective neurotoxic action of capsaicin on glomerular C-type terminals in rat SG. Brain Res. 207: 506–510, 1981.
PANERAI, A. E., MARTINI, A., LOCATELLI, V., AND MANTEGAZZA, P.: Capsaicin decreases b-endorphin hypothalamic concentrations in the rat. Pharmacol. Res. Commun. 15: 825–832, 1983.
PAPKA, R. E., FURNESS, J. B., DELLA, N. G., MURPHY, R., AND COSTA, M.: Time course of effect of capsaicin on ultrastructure and histochemistry of substance P-immunoreactive nerves associated with the cardiovascular system of the guinea-pig. Neuroscience 12: 1277–1292, 1984.
PATACCHINI, R., MAGGI, C. A., AND MELI, A.: Capsaicin-like activity of some natural pungent substances on peripheral endings of visceral primary afferents. Naunyn Schmiedebergs Arch. Pharmacol. 342: 72–77, 1990.
PATACCHINI, R., SANTICIOLI, P., GIULIANI, S., MAGGI, C. A., AND MELI, A.: Cadmium chloride induces contractions of the rat isolated urinary bladder by activation of capsaicin-sensitive sensory nerves. Eur. J. Pharmacol. 148: 449–452, 1988.
PERTOVAARA, A.: Collateral sprouting of nociceptive C-fibers after cut or capsaicin treatment of the sciatic nerve in adult rats. Neurosci. Lett. 90: 248–253, 1988.
PETERSEN, M., PIERAU, F.-K., AND WEYRICH, M.: The influence of capsaicin on membrane currents in dorsal root ganglion neurones of guinea-pig and chicken. Pflügers Arch. 409: 403–410, 1987.
PETERSEN, M., WAGNER, G., AND PIERAU, F.-K.: Modulation of calcium-currents by capsaicin in a subpopulation of sensory neurones of guinea pig. Naunyn Schmiedebergs Arch. Pharmacol. 339: 184–191, 1989.
PETHŐ, G., AND SZOLCSÁNYI, J.: Systemically applied ruthenium red inhibits the stimulation of sensory receptors by capsaicin. Acta. Physiol. Hung. 75 (Suppl.): 235–236, 1990.
PETSCHE, U., FLEISCHER, E., LEMBECK, F., AND HANDWERKER, H. O.: The effect of capsaicin application to a peripheral nerve on impulse conduction in functionally identified afferent nerve fibres. Brain Res. 265: 233–240, 1983.
PFEIFFER, C. J., AND EVANGELISTA, S.: Gastric and jejunal ultrastructure in capsaicin-treated rats with and without experimental ulcer. *In* Sensory Nerves and Neuropeptides in Gastroenterology: From Basic Science to Clinical Perspectives, ed. by M. Costa, C. Surrenti, S. Gorini, C. A. Maggi, and A. Meli, in press, Plenum, London, 1991.
PIERAU, F.-K., SANN, H., HARTI, G., AND GAMSE, R.: Neuropeptides in sensory neurones of pigeons and the insensitivity of avians to capsaicin. In Fine nerve fibres and pain, ed. by R. F. Schmidt, H.-G. Schaible, and C. Vahle-Hinz, pp. 213–223, VHC, Weinheim, Germany, 1987.
PIERAU, F.-K., AND SZOLCSÁNYI, J.: Neurogenic inflammation: axon reflex in pigs. Agents Actions 26: 231–232, 1989.
PINI, A.: Effects of capsaicin on conduction in a cutaneous nerve of the rat. J. Physiol. (Lond.) 338: 60P-61P, 1983.
PINI, A., BARANOWSKI, R., AND LYNN, B.: Long-term reduction in the number of C-fibre nociceptors following capsaicin treatment of a cutaneous nerve in adult rats. Eur. J. Neurosci. 2: 89–97, 1990.
POHL, M., BENOLIEL, J. J., BOURGOIN, S., LOMBARD, M. C., MAUBORGNE, A., TAQUET, H., CARAYON, A., BESSON, J. M., CESSELIN, F., AND HAMON, M.: Regional distribution of calcitonin gene-related peptide-like, substance P-like, cholecystokinin-like, Met^5-enkephalin-like, and dynorphin A (1–8)-like materials in the spinal cord and dorsal root ganglia of adult rats—effects of dorsal rhizotomy and neonatal capsaicin. J. Neurochem. 55: 1122–1130, 1990.
PÓRSZÁSZ, J., AND JANCSÓ, N.: Studies on the action potentials of sensory nerves in animals desensitized with capsaicin. Acta Physiol. Acad. Sci. Hung. 16: 299–306, 1959.
PRIESTLEY, J. V., BRAMWELL, S., BUTCHER, L. L., AND CUELLO, A. C.: Effect of capsaicin on neuropeptides in areas of termination of primary sensory neurons. Neurochem. Int. 4: 57–65, 1982.
PRIOR, M., GREEN, F., LOPEZ, A., BALU, A., DE SANCTIS, G. T., AND FICK, G.: Capsaicin pretreatment modifies hydrogen sulphide-induced pulmonary injury in rats. Toxicol. Pathol. 18: 279–288, 1990.
RABE, L. S., BUCK, S. H., MORENO, L., BURKS, T. F., AND DAFNY, N.: Neurophysiological and thermoregulatory effects of capsaicin. Brain. Res. Bull. 5: 755–758, 1980.
RAY, N. J., JONES, A. J., AND KEEN, P.: Ruthenium red: selective, reversible inhibition of capsaicin-stimulated substance P release from primary afferent neurons in rat trachea. Br. J. Pharmacol. 99: 186P, 1990.
RAYBOULD, H. E., EYSSELEIN, V. E., STERNINI, C., AND HOLZER, P.: Spinal afferent neurons to the upper gastrointestinal tract: selective ablation by perineural capsaicin attenuates gastric mucosal protection against acid-induced injury. Neuroscience, in press, 1991.
RAYBOULD, H. E., AND TACHÉ, Y.: Cholecystokinin inhibits gastric motility and emptying via a capsaicin-sensitive vagal pathway in rats. Am. J. Physiol. 255: G242-G246, 1988.
RAYBOULD, H. E., AND TACHÉ, Y.: Capsaicin-sensitive vagal afferent fibers and stimulation of gastric acid secretion in anesthetized rats. Eur. J. Pharmacol. 167: 237–243, 1989.
RAYNER, H. C., ATKINS, R. C., AND WESTERMAN, R. A.: Relief of local stump pain by capsaicin cream. Lancet 2: 1276–1277, 1989.
RÉTHELYI, M., SALIM, M. Z., AND JANCSÓ, G.: Altered distribution of dorsal root

fibers in the rat following neonatal capsaicin treatment. Neuroscience **18:** 749–761, 1986.
RIBEIRO-DA-SILVA, A., AND COIMBRA, A.: Capsaicin causes selective damage to type I synaptic glomeruli in rat substantia gelatinosa. Brain Res. **290:** 380–383, 1984.
RITTER, S., AND DINH, T. T.: Capsaicin-induced neuronal degeneration: silver impregnation of cell bodies, axons, and terminals in the central nervous system of the adult rat. J. Comp. Neurol. **271:** 79–90, 1988.
RITTER, S., AND DINH, T. T.: Capsaicin-induced neuronal degeneration in the brain and retina of preweanling rats. J. Comp. Neurol. **296:** 447–461, 1990.
RODRIGUEZ-SIERRA, J. E., SKOFITSCH, G., KOMISARUK, B. R., AND JACOBOWITZ, D. M.: Abolition of vagino-cervical stimulation-induced analgesia by capsaicin administration to neonatal, but not adult rats. Physiol. Behav. **44:** 267–272, 1988.
ROGERS, L. R.: Reactions of free-ranging black bears to capsaicin spray repellent. Wildl. Soc. Bull. **12:** 59–61, 1984.
ROSS, D. R., AND VARIPAPA, R. J.: Treatment of painful diabetic neuropathy with topical capsaicin. N. Engl. J. Med. **321:** 474–475, 1989.
ROZIN, P., MARK, M., AND SCHILLER, D.: The role of desensitization to capsaicin in chili pepper ingestion and preference. Chem. Senses **6:** 23–31, 1981.
RÓZSA, Z., AND JACOBSON, E. D.: Capsaicin-sensitive nerves are involved in bile-oleate induced intestinal hyperemia. Am. J. Physiol. **256:** G476-G481, 1989.
RUSSELL, L. C., AND BURCHIEL, K. J.: Neurophysiological effects of capsaicin. Brain Res. Rev. **8:** 165–176, 1984.
SAITO, A., MASAKI, T., LEE, T. J.-F., AND GOTO, T.: Effects of capsaicin on the contractility and peptide-containing nerves of large cerebral arteries of the cat. Arch. Int. Pharmacodyn. Exp. Ther. **295:** 194–203, 1988.
SALT, T. E., CROZIER, C. S., AND HILL, R. G.: The effects of capsaicin pretreatment on the responses of single neurons to sensory stimuli in the trigeminal nucleus caudalis of the rat: evidence against a role for substance P as the neurotransmitter serving thermal nociception. Neuroscience **7:** 1141–1148, 1982.
SALT, T. E., AND HILL, R. G.: The effect of microiontophoretically applied capsaicin and substance P on single neurones in the rat and cat brain. Neurosci. Lett. **20:** 329–334, 1980.
SALT, T. E., AND HILL, R. G.: Neurotransmitter candidates of somatosensory primary afferent fibres. Neuroscience **10:** 1083–1103, 1983.
SANN, H., HARTI, G., PIERAU, F.-K., AND SIMON, E.: Effect of capsaicin upon afferent and efferent mechanisms of nociception and temperature regulation in birds. Can. J. Physiol. Pharmacol. **65:** 1347–1354, 1987.
SANTICIOLI, P., MAGGI, C. A., AND MELI, A.: The effect of capsaicin pretreatment on the cystometrograms of urethane anesthetized rats. J. Urol. **133:** 700–703, 1985.
SANTICIOLI, P., PATACCHINI, R., MAGGI, C. A., AND MELI, A.: Exposure to calcium-free medium protects sensory fibers by capsaicin desensitization. Neurosci. Lett. **80:** 167–172, 1987.
SAPORTA, S.: Loss of spinothalamic tract neurons following neonatal treatment of rats with the neurotoxin capsaicin. Somatosens. Res. **4:** 153–173, 1986.
SARIA, A.: Substance P in sensory nerve fibres contributes to the development of oedema in the rat hind paw after thermal injury. Br. J. Pharmacol. **82:** 217–222, 1984.
SARIA, A., GAMSE, R., PETERMANN, J., FISCHER, J. A., THEODORSSON-NORHEIM, E., AND LUNDBERG, J. M.: Simultaneous release of several tachykinins and calcitonin gene-related peptide from rat spinal cord slices. Neurosci. Lett. **63:** 310–314, 1986.
SARIA, A., LUNDBERG, J. M., HUA, X.-Y., AND LEMBECK, F.: Capsaicin-induced substance P release and sensory control of vascular permeability in the guinea-pig ureter. Neurosci. Lett. **41:** 167–172, 1983a.
SARIA, A., LUNDBERG, J. M., SKOFITSCH, G., AND LEMBECK, F.: Vascular protein leakage in various tissues induced by substance P, capsaicin, bradykinin, serotonin, histamine and by antigen challenge. Naunyn Schmiedebergs Arch. Pharmacol. **324:** 212–218, 1983b.
SARIA, A., MARTLING, C.-R., YAN, Z., THEODORSSON-NORHEIM, E., GAMSE, R., AND LUNDBERG, J. M.: Release of multiple tachykinins from capsaicin-sensitive sensory nerves in the lung by bradykinin, histamine, dimethylphenyl piperazinium, and vagal nerve stimulation. Am. Rev. Respir. Dis. **137:** 1330–1335, 1988.
SARIA, A., SKOFITSCH, G., AND LEMBECK, F.: Distribution of capsaicin in rat tissues after systemic administration. J. Pharm. Pharmacol. **34:** 273–275, 1982.
SARIA, A., AND WOLF, G.: Beneficial effect of topically applied capsaicin in the treatment of hyperreactive rhinopathy. Regul. Pept. **22:** 167, 1988.
SAUMET, J.-L., AND DUCLAUX, R.: Analgesia induced by neonatal capsaicin treatment in rats. Pharmacol. Biochem. Behav. **16:** 241–243, 1982.
SAVITHA, G., PANCHANATHAN, S., AND SALIMATH, B. P.: Capsaicin inhibits calmodulin-mediated oxidative burst in rat macrophages. Cell. Signal. **2:** 577–585, 1990.
SCADDING, J. W.: The permanent anatomical effects of neonatal capsaicin on somatosensory nerves. J. Anat. **131:** 473–484, 1980.
SCHAIBLE, H.-G., AND SCHMIDT, R. F.: Time course of mechanosensitivity changes in articular afferents during a developing arthritis. J. Neurophysiol. **60:** 2180–2195, 1988.
SCHANNE, F. A. X., KANE, A. B., YOUNG, E. E., AND FARBER, J. L.: Calcium dependence of toxic cell death: a final common pathway. Science **206:** 700–702, 1979.
SHARKEY, K. A., WILLIAMS, R. G., AND DOCKRAY, G. J.: Sensory substance P innervation of the stomach and pancreas. Demonstration of capsaicin-sensitive sensory neurons in the rat by combined immunohistochemistry and retrograde tracing. Gastroenterology **87:** 914–921, 1984.
SHARKEY, K. A., WILLIAMS, R. G., SCHULTZBERG, M., AND DOCKRAY, G. J.: Sensory substance P innervation of the urinary bladder: possible site of action of capsaicin in causing urine retention in rats. Neuroscience **10:** 861–868, 1983.
SHIMIZU, T., FUJITA, S., IZUMI, K., KOJA, T., OHBA, N., FUKUDA, T.: Corneal lesions induced by the systemic administration of capsaicin in neonatal mice and rats. Naunyn Schmiedebergs Arch. Pharmacol. **326:** 347–351, 1984.
SHIMIZU, T., IZUMI, K., FUJITA, S., KOJA, T., SORIMACHI, M., OHBA, N., AND FUKUDA, T.: Capsaicin-induced corneal lesions in mice and the effects of chemical sympathectomy. J. Pharmacol. Exp. Ther. **243:** 690–695, 1987.
SHORTLAND, P., MOLANDER, C., WOOLF, C. J., AND FITZGERALD, M.: Neonatal capsaicin treatment induces invasion of the substantia gelatinosa by the terminal arborizations of hair follicle afferents in the rat dorsal horn. J. Comp. Neurol. **296:** 23–31, 1990.
SICUTERI, F., FUSCO, B. M., MARABINI, S., CAMPAGNOLO, V., MAGGI, C. A., GEPPETTI, P., AND FANCIULLACCI, M.: Beneficial effect of capsaicin application to the nasal mucosa in cluster headache. Clin. J. Pain **5:** 49–53, 1989.
SIGRIST, S., FRANCO-CERECEDA, A., MUFF, R., HENKE, H., LUNDBERG, J. M., AND FISCHER, J. A.: Specific receptor and cardiovascular effects of calcitonin gene-related peptide. Endocrinology **119:** 381–389, 1986.
SIKRI, K. L., HOYES, A. D., BARBER, P., AND JAGESSAR, H.: Substance P-like immunoreactivity in the intramural nerve plexuses of the guinea-pig ureter: a light and electron microscopical study. J. Anat. **133:** 425–442, 1981.
SIMONE, D. A., BAUMANN, T. K., AND LAMOTTE, R. H.: Dose-dependent pain and mechanical hyperalgesia in humans after intradermal injection of capsaicin. Pain **38:** 99–107, 1989.
SIMONE, D. A., NGEOW, J. Y. F., PUTTERMAN, G. J., AND LAMOTTE, R. H.: Hyperalgesia to heat after intradermal injection of capsaicin. Brain Res. **418:** 201–203, 1987.
SINGER, E. A., AND PLACHETA, P.: Reduction of ^{3}H-muscimol binding sites in rat dorsal spinal cord after neonatal capsaicin treatment. Brain Res. **202:** 484–487, 1980.
SINGER, E. A., SPERK, G., AND SCHMID, R.: Capsaicin does not change tissue levels of glutamic acid, its uptake, or release in the rat spinal cord. J. Neurochem. **38:** 1383–1386, 1982.
SIZER, F., AND HARRIS, N.: The influence of common food additives and temperature on threshold perception of capsaicin. Chem. Senses **10:** 279–286, 1985.
SKOFITSCH, G., DONNERER, J., PETRONIJEVIC, S., SARIA, A., AND LEMBECK, F.: Release of histamine by neuropeptides from the perfused rat hindquarter. Naunyn Schmiedebergs Arch. Pharmacol. **322:** 153–157, 1983.
SKOFITSCH, G., AND JACOBOWITZ, D. M.: Galanin-like immunoreactivity in capsaicin-sensitive sensory neurons and ganglia. Brain Res. Bull. **15:** 191–195, 1985a.
SKOFITSCH, G., AND JACOBOWITZ, D. M.: Calcitonin gene-related peptide coexists with substance P in capsaicin sensitive neurons and sensory ganglia of the rat. Peptides **6:** 747–754, 1985b.
SKOFITSCH, G., ZAMIR, N., HELKE, C. J., SAVITT, J. M., AND JACOBOWITZ, D. M.: Corticotropin releasing factor-like immunoreactivity in sensory ganglia and capsaicin sensitive neurons of the rat central nervous system: colocalization with other neuropeptides. Peptides **6:** 307–318, 1985.
SOUKUP, T., AND JANCSÓ, G.: Development of muscle stretch receptors in the rat is not affected by neonatal capsaicin treatment. Acta Physiol. Hung. **69:** 527–532, 1987.
SOUTH, E. H., AND RITTER, R. C.: Overconsumption of preferred foods following capsaicin pretreatment of the area postrema and adjacent nucleus of the solitary tract. Brain Res. **288:** 243–251, 1983.
SOUTH, E. H., AND RITTER, R. C.: Capsaicin application to central or peripheral vagal fibers attenuates CCK satiety. Peptides **9:** 601–612, 1988.
SRINIVASAN, M. R., AND SATYANARAYANA, M. N.: Effect of capsaicin on skeletal muscle lipoprotein lipase in rats fed high fat diet. Indian J. Exp. Biol. **27:** 910–912, 1989.
STEIN, R. D., GENOVESI, S., DEMAREST, K. T., AND WEAVER, L. C.: Capsaicin treatment attenuates the reflex excitation of sympathetic activity caused by chemical stimulation of intestinal afferent nerves. Brain Res. **397:** 145–151, 1986.
STJÄRNE, P., LUNDBLAD, L., LUNDBERG, J. M., AND ÅNGGARD, A.: Capsaicin and nicotine-sensitive afferent neurones and nasal secretion in healthy human volunteers and in patients with vasomotor rhinitis. Br. J. Pharmacol. **96:** 693–701, 1989.
STERNINI, C., REEVE, J. R., AND BRECHA, N.: Distribution and characterization of calcitonin gene-related peptide immunoreactivity in the digestive system of normal and capsaicin-treated rats. Gastroenterology **93:** 852–862, 1987.
SU, H. C., BISHOP, A. E., POWER, R. F., HAMADA, Y., AND POLAK, J. M.: Dual intrinsic and extrinsic origins of CGRP- and NPY-immunoreactive nerves of rat gut and pancreas. J. Neurosci. **7:** 2674–2687, 1987.
SUCH, G., AND JANCSÓ, G.: Axonal effects of capsaicin: an electrophysiological study. Acta Physiol. Hung. **67:** 53–63, 1986.
SZALLASI, A., AND BLUMBERG, P. M.: Resiniferatoxin, a phorbol-related diterpene, acts as an ultrapotent analog of capsaicin, the irritant constituent in red pepper. Neuroscience **30:** 515–520, 1989.
SZALLASI, A., AND BLUMBERG, P. M.: Specific binding of resiniferatoxin, an ultrapotent capsaicin analog, by dorsal root ganglion membranes. Brain Res. **524:** 106–111, 1990a.

SZALLASI, A., AND BLUMBERG, P. M.: Resiniferatoxin and its analogs provide novel insights into the pharmacology of the vanilloid (capsaicin) receptor. Life Sci. **47:** 1399–1408, 1990b.

SZALLASI, A., JOO, F., AND BLUMBERG, P. M.: Duration of desensitization and ultrastructural changes in dorsal root ganglia in rats treated with resiniferatoxin, an ultrapotent capsaicin analog. Brain Res. **503:** 68–72, 1989.

SZALLASI, A., SZALLASI, Z., AND BLUMBERG, P. M.: Permanent effects of neonatally administered resiniferatoxin in the rat. Brain Res. **537:** 182–186, 1990.

SZIKSZAY, M., AND LONDON, E. D.: Effects of subacute capsaicin treatment on local cerebral glucose utilization in the rat. Neuroscience **25:** 917–923, 1988.

SZIKSZAY, M., OBÁL, F., AND OBÁL, F.: Dose-response relationships in the thermoregulatory effects of capsaicin. Naunyn Schmiedebergs Arch. Pharmacol. **320:** 97–100, 1982.

SZOLCSÁNYI, J.: A pharmacological approach to elucidation of the role of different nerve fibres and receptor endings in mediation of pain. J. Physiol. (Paris) **73:** 251–259, 1977.

SZOLCSÁNYI, J.: Capsaicin type pungent agents producing pyrexia. *In* Pyretics and Antipyretics, Handbook of Experimental Pharmacology, ed. by A. S. Milton, vol. 60, pp. 437–478, Springer, Berlin, 1982.

SZOLCSÁNYI, J.: Disturbances of thermoregulation induced by capsaicin. J. Therm. Biol. **8:** 207–212, 1983a.

SZOLCSÁNYI, J.: Tetrodotoxin-resistant non-cholinergic neurogenic contraction evoked by capsaicinoids and piperine on the guinea-pig trachea. Neurosci. Lett. **42:** 83–88, 1983b.

SZOLCSÁNYI, J.: Capsaicin and neurogenic inflammation: history and early findings. *In* Antidromic Vasodilatation and Neurogenic Inflammation, ed. by L. A. Chahl, J. Szolcsányi, and F. Lembeck, pp. 7–25, Akadémiai Kiadó, Budapest, 1984a.

SZOLCSÁNYI, J.: Capsaicin-sensitive chemoceptive neural system with dual sensory-efferent function. *In* Antidromic Vasodilatation and Neurogenic Inflammation, ed. by L. A. Chahl, J. Szolcsányi, and F. Lembeck, pp. 27–52, Akadémiai Kiadó, Budapest, 1984b.

SZOLCSÁNYI, J.: Sensory receptors and the antinociceptive effects of capsaicin. *In* Tachykinin Antagonists, ed. by R. Håkanson and F. Sundler, pp. 45–54, Elsevier, Amsterdam, 1985.

SZOLCSÁNYI, J.: Selective responsiveness of polymodal nociceptors of the rabbit ear to capsaicin, bradykinin and ultra-violet irradiation. J. Physiol. (Lond.) **388:** 9–23, 1987.

SZOLCSÁNYI, J.: Antidromic vasodilatation and neurogenic inflammation. Agents Actions **23:** 4–11, 1988.

SZOLCSÁNYI, J.: Capsaicin, irritation, and desensitization. Neurophysiological basis and future perspectives. *In* Irritation, Chemical Senses, ed. by B. G. Green, J. R. Mason, and M. R. Kare, vol. 2, pp. 141–168, Marcel Dekker, New York and Basel, 1990.

SZOLCSÁNYI, J., ANTON, F., REEH, P. W., AND HANDWERKER, H. O.: Selective excitation by capsaicin of mechano-heat sensitive nociceptors in rat skin. Brain Res. **446:** 262–268, 1988.

SZOLCSÁNYI, J., AND BARTHÓ, L.: New type of nerve-mediated cholinergic contractions of the guinea-pig small intestine and its selective blockade by capsaicin. Naunyn Schmiedebergs Arch. Pharmacol. **305:** 83–90, 1978.

SZOLCSÁNYI, J., AND BARTHÓ, L.: Impaired defense mechanism to peptic ulcer in the capsaicin-desensitized rat. *In* Gastrointestinal Defense Mechanisms, ed. by G. Mózsik, O. Hänninen, and T. Jávor, Advances in Physiological Sciences, vol. 15, pp. 39–51, Pergamon Press, Oxford; Akadémiai Kiadó, Budapest, 1981.

SZOLCSÁNYI, J., AND BARTHÓ, L.: Capsaicin-sensitive non-cholinergic excitatory innervation of the guinea-pig tracheobronchial smooth muscle. Neurosci. Lett. **34:** 247–252, 1982.

SZOLCSÁNYI, J., BARTHÓ, L., AND PETHŐ, G.: Capsaicin-sensitive bronchopulmonary receptors with dual sensory-efferent function: mode of action of capsaicin antagonists. Acta Physiol. Hung., in press, 1991.

SZOLCSÁNYI, J., AND JANCSÓ-GÁBOR, A.: Sensory effects of capsaicin congeners. I. Relationship between chemical structure and pain-producing potency of pungent agents. Drug Res. **25:** 1877–1881, 1975.

SZOLCSÁNYI, J., AND JANCSÓ-GÁBOR, A.: Sensory effects of capsaicin congeners. II. Importance of chemical structure and pungency in desensitizing activity of capsaicin-type compounds. Drug Res. **26:** 33–37, 1976.

SZOLCSÁNYI, J., JANCSÓ-GÁBOR, A., AND JOÓ, F.: Functional and fine structural characteristics of the sensory neuron blocking effect of capsaicin. Naunyn Schmiedebergs Arch. Pharmacol. **287:** 157–169, 1975.

SZOLCSÁNYI, J., JOÓ, F., AND JANCSÓ-GÁBOR, A.: Mitochondrial changes in preoptic neurones after capsaicin desensitization of the hypothalamic thermodetectors in rats. Nature **229:** 116–117, 1971.

SZOLCSÁNYI, J., SANN, H., AND PIERAU, F.-K.: Nociception in pigeons is not impaired by capsaicin. Pain **27:** 247–260, 1986.

SZOLCSÁNYI, J., SZALLASI, A., SZALLASI, Z., JOO, F., AND BLUMBERG, P. M.: Resiniferatoxin: an ultrapotent selective modulator of capsaicin-sensitive primary afferent neurons. J. Pharmacol. Exp. Ther. **255:** 923–928, 1990.

TAKAKI, M., JIN, J.-G., LU, Y.-F., AND NAKAYAMA, S.: Effects of piperine on the motility of the isolated guinea-pig ileum: comparison with capsaicin. Eur. J. Pharmacol. **186:** 71–77, 1990.

TAKAKI, M., JIN, J.-G., AND NAKAYAMA, S.: Ruthenium red antagonism of the effect of capsaicin on the motility of the isolated guinea-pig ileum. Eur. J. Pharmacol. **174:** 57–62, 1989.

TAKAKI, M., AND NAKAYAMA, S.: Effects of capsaicin on myenteric neurons of the guinea pig ileum. Neurosci. Lett. **105:** 125–130, 1989.

TAKANO, Y., NAGASHIMA, A., KAMIYA, H., KUROSAWA, M., AND SATO, A.: Well-maintained reflex responses of sympathetic nerve activity to stimulation of baroreceptor, chemoreceptor and cutaneous mechanoreceptors in neonatal capsaicin-treated rats. Brain Res. **445:** 188–192, 1988.

TAPIA, R., ARIAS, C., AND MORALES, E.: Binding of lanthanum ions and ruthenium red to synaptosomes and its effects on neurotransmitter release. J. Neurochem. **45:** 1464–1470, 1985.

TAYLOR, D. C. M., PIERAU, F.-K., AND SZOLCSÁNYI, J.: Long lasting inhibition of horseradish peroxidase (HRP) transport in sensory nerves induced by capsaicin pretreatment of the receptive field. Brain Res. **298:** 45–49, 1984.

TAYLOR, D. C. M., PIERAU, F.-K., AND SZOLCSÁNYI, J.: Capsaicin-induced inhibition of axoplasmic transport is prevented by nerve growth factor. Cell Tissue Res. **240:** 569–573, 1985.

TERRENGHI, G., ZHANG, S. Q., UNGER, W. G., AND POLAK, J. M.: Morphological changes of sensory CGRP-immunoreactive and sympathetic nerves in peripheral tissues following chronic denervation. Histochemistry **86:** 89–95, 1986.

TERVO, K.: Effect of prolonged and neonatal capsaicin treatments on the substance P immunoreactive nerves in the rabbit eye and spinal cord. Acta Ophthalmol. **59:** 737–746, 1981.

THÉRIAULT, E., OTSUKA, M., AND JESSELL, T.: Capsaicin-evoked release of substance P from primary sensory neurons. Brain Res. **170:** 209–213, 1979.

TODA, N., USUI, H., NISHINO, N., AND FUJIWARA, M.: Cardiovascular effects of capsaicin in dogs and rabbits. J. Pharmacol. Exp. Ther. **181:** 512–519, 1972.

TÓTH-KÁSA, I., JANCSÓ, G., BOGNÁR, A., HUSZ, S., AND OBÁL, F.: Capsaicin prevents histamine-induced itching. Int. J. Clin. Pharmacol. Res. **6:** 163–170, 1986.

TÓTH-KÁSA, I., JANCSÓ, G., OBÁL, F., HUSZ, S., AND SIMON, N.: Involvement of sensory nerve endings in cold and heat urticaria. J. Invest. Dermatol. **80:** 34–36, 1983.

TRAD, K. S., HARMON, J. W., FERNICOLA, M. T., HAKKI, F. Z., RAO, J. S., DZIKI, A. J., AND BASS, B. L.: Evidence for a role of capsaicin-sensitive mucosal afferent nerves in the regulation of esophageal blood flow in rabbits. Gastroenterology **98:** A139, 1990.

TRAURIG, H., PAPKA, R. E., SARIA, A., AND LEMBECK, F.: Substance P immunoreactivity in the rat mammary nipple and the effects of capsaicin treatment on lactation. Naunyn Schmiedebergs Arch. Pharmacol. **328:** 1–8, 1984a.

TRAURIG, H., SARIA, A., AND LEMBECK, F.: The effects of neonatal capsaicin treatment on growth and subsequent reproductive function in the rat. Naunyn Schmiedebergs Arch. Pharmacol. **327:** 254–259, 1984b.

TSUNOO, A., KONISHI, S., AND OTSUKA, M.: Substance P as an excitatory transmitter of primary afferent neurons in guinea-pig sympathetic ganglia. Neuroscience **7:** 2025–2037, 1982.

UEDA, N., MURAMATSU, I., AND FUJIWARA, M.: Capsaicin and bradykinin-induced substance P-ergic responses in the iris sphincter muscle of the rabbit. J. Pharmacol. Exp. Ther. **230:** 469–473, 1984.

URBÁN, L., WILLETTS, J., RANDIC, M., AND PAPKA, R. E.: The acute and chronic effects of capsaicin on slow excitatory transmission in rat dorsal horn. Brain Res. **330:** 390–396, 1985.

VARRO, A., GREEN, T., HOLMES, S., AND DOCKRAY, G. J.: Calcitonin gene-related peptide in visceral afferent nerve fibres: quantification by radioimmunoassay and determination of axonal transport rates. Neuroscience **26:** 927–932, 1988.

VIRUS, R. M., KNUEPFER, M. M., MCMANUS, D. Q., BRODY, M. J., AND GEBHART, G. F.: Capsaicin treatment in adult Wistar-Kyoto and spontaneously hypertensive rats: effects on nociceptive behavior and cardiovascular regulation. Eur. J. Pharmacol. **72:** 209–217, 1981.

VIRUS, R. M., MCMANUS, D. Q., AND GEBHART, G. F.: Capsaicin treatment in adult Wistar-Kyoto and spontaneously hypertensive rats: effects on substance P contents of peripheral and central nervous system. Eur. J. Pharmacol. **81:** 67–73, 1982.

VIRUS, R. M., MCMANUS, D. Q., AND GEBHART, G. F.: Capsaicin treatment in adult Wistar-Kyoto and spontaneously hypertensive rats: neurochemical effects in the spinal cord. Eur. J. Pharmacol. **92:** 1–8, 1983.

WADDELL, P. J., AND LAWSON, S. N.: The C-fibre conduction block caused by capsaicin on rat vagus nerve *in vitro*. Pain **39:** 237–242, 1989.

WALL, P. D.: The effect of peripheral nerve lesions and of neonatal capsaicin in the rat on primary afferent depolarization. J. Physiol. (Lond.) **329:** 21–35, 1982.

WALL, P. D., AND FITZGERALD, M.: Effects of capsaicin applied locally to adult peripheral nerve. I. Physiology of peripheral nerve and spinal cord. Pain **11:** 363–377, 1981.

WALL, P. D., FITZGERALD, M., NUSSBAUMER, J. C., VAN DER LOOS, H., AND DEVOR, M.: Somatotopic maps are disorganized in adult rodents treated neonatally with capsaicin. Nature **295:** 691–693, 1982a.

WALL, P. D., FITZGERALD, M., AND WOOLF, C. J.: Effects of capsaicin on receptive fields and on inhibitions in rat spinal cord. Exp. Neurol. **78:** 425–436, 1982b.

WANG, J.-P., HSU, M.-F., HSU, T.-P., AND TENG, C.-M.: Antihemostatic and antithrombotic effects of capsaicin in comparison with aspirin and indomethacin. Thromb. Res. **37:** 669–679, 1985.

WANG, J.-P., HSU, M.-F., AND TENG, C.-M.: Antiplatelet effect of capsaicin. Thromb. Res. **36:** 497–507, 1984.

WATANABE, T., KAWADA, T., AND IWAI, K.: Effect of capsaicin pretreatment on capsaicin-induced catecholamine secretion from the adrenal medulla in rats. Proc. Soc. Exp. Biol. Med. **187:** 370–374, 1988a.

WATANABE, T., KAWADA, T., KUROSAWA, M., SATO, A., AND IWAI, K.: Adrenal sympathetic efferent nerve and catecholamine secretion excitation caused by capsaicin in rats. Am. J. Physiol. **255:** E23-E27, 1988b.
WATSON, C. P. N., EVANS, R. J., AND WATT, V. R.: Post-herpetic neuralgia and topical capsaicin. Pain **33:** 333–340, 1988.
WATSON, C. P. N., EVANS, R. J., AND WATT, V. R.: The post-mastectomy pain syndrome and the effect of topical capsaicin. Pain **38:** 177–186, 1989.
WEIHE, E.: Neuropeptides in primary afferent neurons. *In* The Primary Afferent Neuron, ed. by W. Zenker and W. L. Neuhuber, pp. 127–159, Plenum Press, New York, 1990.
WELK, E., FLEISCHER, E., PETSCHE, U., AND HANDWERKER, H. O.: Afferent C-fibres in rats after neonatal capsaicin treatment. Pflügers Arch. **400:** 66–71, 1984.
WELK, E., PETSCHE, U., FLEISCHER, E., AND HANDWERKER, H. O.: Altered excitability of afferent C-fibres of the rat distal to a nerve site exposed to capsaicin. Neurosci. Lett. **38:** 245–250, 1983.
WILLIAMS, J. T., AND ZIEGLGÄNSBERGER, W.: The acute effects of capsaicin on rat primary afferents and spinal neurons. Brain Res. **253:** 125–131, 1982.
WINTER, J.: Characterization of capsaicin-sensitive neurones in adult rat dorsal root ganglion cultures. Neurosci. Lett. **80:** 134–140, 1987.
WINTER, J., DRAY, A., WOOD, J. N., YEATS, J. C., AND BEVAN, S.: Cellular mechanism of action of resiniferatoxin: a potent sensory neuron excitotoxin. Brain Res. **520:** 131–140, 1990.
WINTER, J., FORBES, C. A., STERNBERG, J., AND LINDSAY, R. M.: Nerve growth factor (NGF) regulates adult rat cultured dorsal root ganglion neuron responses to the excitotoxin capsaicin. Neuron **1:** 973–981, 1988.
WOOD, J. N.: Reversible desensitization to capsaicin induced calcium uptake in cultured rat sensory neurones. Neuroscience **22:** S247, 1987.
WOOD, J. N., COOTE, P. R., MINHAS, A., MULLANEY, I., MCNEILL, M., AND BURGESS, G. M.: Capsaicin-induced ion fluxes increase cyclic GMP but not cyclic AMP levels in rat sensory neurones in culture. J. Neurochem. **53:** 1203–1211, 1989.
WOOD, J. N., WALPOLE, C., JAMES, I. F., DRAY, A., AND COOTE, P. R.: Immunochemical detection of photoaffinity-labelled capsaicin-binding proteins from sensory neurons. FEBS Lett. **269:** 381–385, 1990.
WOOD, J. N., WINTER, J., JAMES, I. F., RANG, H. P., YEATS, J., AND BEVAN, S.: Capsaicin-induced ion fluxes in dorsal root ganglion cells in culture. J. Neurosci. **8:** 3208–3220, 1988.
YAGI, T.: Inhibition by capsaicin of NADH-quinone oxidoreductase is correlated with the presence of energy-coupling site-1 in various organisms. Arch. Biochem. Biophys. **281:** 305–311, 1990.
YAKSH, T. L., ABAY, E. O., AND GO, V. L. W.: Studies on the location and release of cholecystokinin and vasoactive intestinal peptide in rat and cat spinal cord. Brain Res. **242:** 279–290, 1982.
YAKSH, T. L., FARB, D. H., LEEMAN, S. E., AND JESSELL, T. M.: Intrathecal capsaicin depletes substance P in the rat spinal cord and produces prolonged thermal analgesia. Science **206:** 481–483, 1979.
YAKSH, T. L., JESSELL, T. M., GAMSE, R., MUDGE, A. W., AND LEEMAN, S. E.: Intrathecal morphine inhibits substance P release from mammalian spinal cord *in vivo*. Nature **286:** 155–157, 1980.
YAMANAKA, K., KIGOSHI, S., AND MURAMATSU, I.: Conduction-block induced by capsaicin in crayfish giant axon. Brain Res. **300:** 113–119, 1984.
YANAGISAWA, M., NAKANO, S., AND OTSUKA, M.: Capsaicin-induced depolarization of primary afferent fibers and the release of substance P from isolated rat spinal cord. Biomed. Res. **1** (suppl.)**:** 88–90, 1980.
YOX, D. P., AND RITTER, R. C.: Capsaicin attenuates suppression of sham feeding induced by intestinal nutrients. Am. J. Physiol. **255:** R569-R574, 1988.
ZAGAMI, A. S., AND LAMBERT, G. A.: Craniovascular application of capsaicin activates nociceptive thalamic neurones in the cat. Neurosci. Lett. **121:** 187–190, 1991.
ZERNIG, G., HOLZER, P., AND LEMBECK, F.: A study of the mode and site of action of capsaicin in guinea-pig heart and uterus. Naunyn Schmiedebergs Arch. Pharmacol. **326:** 58–63, 1984.