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Vanilloid (Capsaicin) Receptors and Mechanisms

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I. Foreword: A Brief History of a Really "Hot" Aspect of Pharmacology

Natural products afford a window of opportunity to study important biology. If the natural product is used or abused by human beings, finding its biological target(s) is all the more significant. Hot pepper is eaten on a daily basis by an estimated one-quarter of the world's population and represents an aspect of pharmacology intimately familiar to most readers of this review. Recall your culinary experiences in, let us say, a Mexican restaurant. Food flavored with jalapeno pepper bites, it induces profuse perspiration, and a subsequent diarrhea is not uncommon (when pepper bites again). However, these symptoms become less intense in regular patrons of the restaurant. In more scientific terms, hot pepper is pungent, affects thermoregulation, activates autonomic reflexes, and is poorly absorbed. However, all these acute effects undergo tachyphylaxis upon repeated applications.

Hot pepper is a native of the Americas (cf. Andrews, 1984; Naj, 1992). Aztecs called it chili (this is how hot pepper is mentioned in the pre-Columbian Aztec manuscripts, known as tlacuilos), a name that has stuck in Latin America (information from the Internet: Encyclo'Pepr'edia, http://thepeppershop.com/index.html, and Chili Gazette, http://mexicanfood.tqn.com/msubhis.html). But the Old World adopted a different name, red pepper, instead, erroneously linking chili pepper (*Capsicum annuum*) to a similarly hot-tasting plant, black pepper (*Piper nigrum*) (Garrison, 1929). Following the discovery of the New World, the habit of the natives to eat their food hot was first noted by Diego Alvarez Chanca, a physician to the fleet of Columbus (cf. De Ybarra, 1906). In Europe, a depiction of the chili pepper plant appeared for the first time in the magnificent book of Fuchs (1542). Hot pepper was cultivated in monastery gardens in Moravia as early as 1566 (cf. Köhler, 1883). The Latin name of the plant, Capsicum, was given by the French botanist de Tournefort for unclear reasons (cf. Naj, 1992). A popular theory holds that the name Capsicum was derived from the Greek kapto, meaning "to bite" (cf. Maga, 1975), which appropriately describes the main characteristic of the fruit. Others argue that the name Capsicum is derived from capsa, the Latin word for box, referring to the fact that the pepper pod is hollow, divided into compartments containing the seeds (cf. Naj, 1992).

The active ingredient in hot pepper was first isolated by Thresh (1846) more than a century ago. Thresh named this compound capsaicin and predicted that the structures of capsaicin and vanillin were closely related. Despite this early discovery, it was not until 1919 that the exact chemical structure of capsaicin (Fig. 1) was determined (Nelson, 1919). The complete synthesis of capsaicin took another

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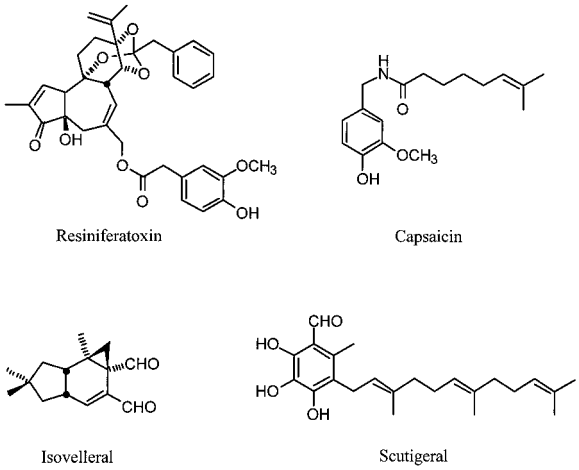

FIG 1. Typical vanilloid structures: RTX, capsaicin, isovelleral, and scutigeral, representing the four known chemical classes of naturally occurring vanilloids, resiniferanoids, capsaicinoids, unsaturated dialdehydes, and triprenyl phenols, respectively.

decade (Spath and Darling, 1930). In 1912, Wilbur Scoville (Scoville, 1912) introduced his Organoleptic Test to quantitate the pungency of hot peppers (that is, their capsaicin content) and, although HPLC has long supplanted the use of the human tongue (cf. Suzuki and Iwai, 1984), for quantitation the Scoville Unit remains the measure of hotness. If a pepper has 50,000 Scoville Heat Units, this means that its alcoholic extract needs to be diluted 1:50,000 for it to cease to be hot-tasting on the human tongue. The hottest pepper is the Mexican habanero, which boasts 350,000 Scoville Units (cf. Naj, 1992). The oral test of Scoville, abandoned by the food industry, survives in laboratories, where it is still used to detect capsaicin-like compounds in plant extracts.

With regard to the varied, nonculinary uses of hot pepper, there is apparently little new under the sun. For example, Incas burned dried chili peppers to combat the invading Spaniards by temporarily blinding them (cf. Naj, 1992). Four centuries later, the first U.S. patent was issued for the use of capsaicin for martial (tear gas) purposes (U.S. patent 1,659,158, 1928). Nowadays, capsaicin-containing sprays, fondly called Sergeant Pepper, are used by law-enforcement officials in the United States to subdue violent criminals, and in California are marketed as a "cop-in-a-can" for self-defense (Hyder, 1996).

The analgesic use of capsaicin is not novel either (cf. Whittet, 1968; Lembeck, 1987; Dasgupta and Fowler, 1997). Native Americans rubbed their gums with pepper pods to relieve toothache (cf. Naj, 1992). This practice later also gained popularity in European folk medicine, as was noted by the Hungarian botanist-turned-clergyman, Hangay (1887). As early as 1850, the Dublin Free Press recommended the use of alcoholic hot pepper extract on sore teeth for instant relief, recognizing for the first time the therapeutic potential of capsaicin (Turnbull, 1850). A fascinating aspect of the rich history of the analgesic use of capsaicin is that eunuchs serving the Chinese Emperors were castrated after their scrotums had been repeatedly rubbed with hot pepper extracts (cf. Anderson, 1990). As a curiosity, it is also notable that Native Americans used hot pepper extracts topically as an aphrodisiac, a practice adopted by early settlers to the dismay of their priests (Chili Gazette, http://mexicanfood.tqn.com/msubhis.html).

In 1640, Sir John Parkinson noted in his famous Theatricum Botanica that dogs detest hot peppers (cf. Naj, 1992). Chickens, by contrast, can be fed dried pepper powder to turn the yolks of their eggs orange-red. These observations anticipate the modern use of capsaicin to make bird food squirrel-free (cf. Rouhi, 1996). It remains a mystery why is it that the same hot taste, which repels a variety of mammals from rats to squirrels to dogs, is found pleasurable by so many humans. Psychologists speculated that eating hot peppers may be a form of masochism (Rozin and Schiller, 1980). This theory, however, is at variance with the well known geographic pattern of hot pepper consumption, namely, people living in tropical climates prefer their food hotter than those residing in temperate climates (Moore, 1970; Rozin, 1978). This pattern gave rise to the theory that eating hot, spicy food helps combat the warm climate via gustatory sweating (Haxton, 1948; Lee, 1954). This model has gained recent reinforcement by the cloning of a capsaicin receptor, which seems to be operated by both heat and capsaicin (Caterina et al., 1997). Nonetheless, the human liking of, or aversion toward, the taste of hot pepper is probably far more complex than this relatively simple physiological model implies. For instance, Russians like their vodka hot (vodka peperovka), whereas efforts by the Pabst Brewing Company to introduce a pepper-flavored beer were unsuccessful (cf. Naj, 1992).

II. Introduction

A. Overview

If one compares the two previous capsaicin reviews in this Journal (Buck and Burks, 1986; Holzer in 1991), one can notice the steady evolution of ideas, for instance, the identification of ultrapotent capsaicin analogs (cf. Szallasi and Blumberg, 1990a) or the development of the first competitive capsaicin antagonist, capsazepine (Bevan et al., 1991). The past years, however, have witnessed unprecedented advances that have revolutionized this field. Holzer (1991) described the vanilloid receptor (VR)[2] as a capsaicin-operated conductance, the expression of which is virtually restricted to a distinct

[2] Abbreviations: ASIC, acid-sensitive ion channel; CGRP, calcitonin gene-related peptide; CNS, central nervous system; DRASIC, dorsal root ganglion-specific acid-sensitive ion channel; DRG, dorsal root ganglion; GDNF, glia-derived neurotrophic factor; NGF, nerve growth factor; NK-1, neurokinin-1; NK-1R, neurokinin-1 receptor; NKA, neurokinin A; NMDA, *N*-methyl-D-aspartate; NOS, nitric oxide synthase; PKC, protein kinase C; PDDHV, phorbol 12,13-didecanoate 20-homovanillate; PPAHV, phorbol 12-phenylacetate 13-acetate 20-homovanillate; RTX, resiniferatoxin; SP, Substance P; TRP, transient release potential; TTX, tetrodotoxin; VR, vanilloid receptor; VR1, vanilloid receptor type 1.

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subpopulation of primary sensory neurons. This is apparently no longer true. In 1997, a VR, termed VR1, was cloned (Caterina et al., 1997). The emerging concept is that this VR1 functions as a molecular integrator of painful chemical and physical stimuli including noxious heat (>48°C) and low pH (Tominaga et al., 1998). Apparently, it is not capsaicin but heat that has the capability of opening the channel pore of VR1, whereas capsaicin and protons only serve to lower the heat threshold of the receptor. Consequently, even room temperature is able to gate VR1 in the presence of mildly acidic conditions and/or capsaicin (Tominaga et al., 1998). Moreover, the expression of VRs is not restricted to sensory neurons. In addition to several brain nuclei (Ács et al., 1996a; Sasamura et al., 1998), nonneuronal tissues, such as mast cells (Bíró et al., 1998a) and glial cells (Bíró et al., 1998b), may express VRs. There is mounting evidence, both biological and electrophysiological, to suggest heterogeneity within VRs (cf. Szallasi and Blumberg, 1996; Szallasi, 1997). As yet, it is not known whether or not all VR subtypes recognize capsaicin. Therefore, we chose to use in this review the broader term vanilloid-sensitive neuron over the traditional term capsaicin-sensitive neuron.

It has also been found that ligands with little resemblance to the vanillyl group in capsaicin can also act as vanilloids (Szallasi et al., 1996a, 1998a, 1999a), implying that the term VR is somewhat of a misnomer. Terpenoid unsaturated dialdehydes (Szallasi et al., 1996a, 1998a) and triprenyl phenols (Szallasi et al., 1999a) (Fig. 1) are two chemical classes of such newly discovered "vanilloids". At present, resiniferatoxin (RTX; see Fig. 1 for structure), an ultrapotent capsacin analog (Szallasi and Blumberg, 1989a, 1990a), is undergoing clinical trials, where it proves clearly superior to capsaicin (Chancellor, 1997; Cruz et al., 1997a; Lazzeri et al., 1997; Cruz, 1998). At a recent meeting on urinary incontinence (1st International Consultation on Incontinence; Monaco, 1998), a proposal was accepted that a Vanilloid Club should be formed. From cloning to clinic, the vanilloid field is now vibrating with a frenzy of activity, which makes the life of review writers most complicated. For instance, rumor has it that additional VR isoforms have been cloned and an endogenous vanilloid has been isolated.

In this review, we are deliberately focusing on recent developments in the field. The reader can refer to either the above review by Holzer (1991) or the subsequently published book on capsaicin edited by John N. Wood (1993) for detailed background information. Excellent, short reviews are also available from several authors, e.g., Andy Dray (1992), Gábor Jancsó and colleagues (1994), Carlo A. Maggi (1991), or János Szolcsányi (1996), among others. In any case, we have tried to provide limited background information to the degree that it is necessary to provide the context for understanding recent developments.

The literature on vanilloids is vast. Using the subject word capsaicin, a Medline search yielded 2892 publications that have appeared since 1991, the year when the comprehensive capsaicin review by Holzer was published. On average, one paper dealing with capsaicin actions has thus been published every day over the past 7 years. Approximately one tenth of these papers (245 publications since 1991) deal with some aspect of the therapeutic application of capsaicin in humans. Because a complete overview of this vanilloid literature would be overwhelming, this review tries to provide a selective coverage of the literature, with the focus on breakthrough discoveries, emerging concepts, and provocative new ideas.

B. Capsaicin: Targets and Actions

Capsaicin excites a subset of primary sensory neurons with somata in dorsal root ganglion (DRG) or trigeminal ganglion (Table 1). As a general rule, these vanilloid-sensitive neurons are peptidergic, small diameter (>50 μm) neurons, giving rise to thin, unmyelinated (C) fibers (cf. Holzer, 1991). Among sensory neuropeptides, the tachykinin Substance P (SP) shows the best correlation with vanilloid sensitivity (cf. Buck and Burks, 1986; Holzer, 1991). However, on the one hand, not all small diameter DRG neurons respond to capsaicin, and, on the other hand, large diameter vanilloid-sensitive neurons, predominantly of the Aδ-type, are also known to exist (Table 1). Indeed, in certain tissues such as the tooth pulp, Aδ-fibers predominate among the vanilloid-sensitive nerve population (Ikeda et al., 1997). Some small diameter sensory neurons are polymodal nociceptors, whereas neurons with Aδ-fibers may function as mechanoheat-sensitive nociceptors (cf. Meyer et al., 1994). In other words, vanilloid-sensitive neurons are heterogeneous morphologically, neurochemically, and functionally, and they encompass several subclasses of DRG neurons (Table 1) (cf. Holzer, 1988, 1991; Szolcsányi, 1996; Holzer and Maggi, 1998). Because sensitivity to vanilloids is the only known trait that all of these neurons seem to share, they are best described as vanilloid-sensitive neurons (cf. Szallasi and Blumberg, 1990a,b; Szallasi, 1996).

According to a recent exciting finding by Seybold and colleagues (Stucky et al., 1998), vanilloid sensitivity is a plastic property of DRG neurons. For instance, the algogenic substance bradykinin is able to recruit intermediate-size neurons (240–320 μm^2), normally unresponsive to capsaicin, to respond to vanilloids (Stucky et al., 1998). Consequently, the number of nociceptors that innervate inflamed tissues increases. As we will see later, this novel mechanism may play an important role in the development of inflammatory hyperalgesia via spatial summation on spinal neurons.

Central fibers of vanilloid-sensitive neurons enter the central nervous system (CNS), where they synapse on second-order neurons of the dorsal horn of the spinal

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TABLE 1
Characteristics of vanilloid-sensitive primary sensory neurons

Main divisions	1. Dorsal root ganglion neurons projecting to spinal dorsal horn
	2. Trigeminal ganglion neurons projecting to spinal nucleus of trigeminal tract
Subdivisions	Small neurons with unmyelinated fibers (C-fiber neurons)
	Large neurons with thin-myelinated fibers (Aδ-neurons)
	Peptidergic neurons expressing neurotrophin receptors trkA and p75
	Nonpeptidergic neurons that bind the lectin B4 and express $P2X_3$ receptors
Sensory modalities evoked by capsaicin	Pain and itch
	C-afferents: polymodal nociceptors; heat nociceptors and warm receptors; mechano-heat insensitive chemonociceptors (mechano-cold receptors?)
	Aδ-afferents: polymodal receptors
Selected neuropeptides	Arginine vasopressin
	Bombesin/gastrin-releasing peptide
	CGRP
	CCK
	Dynorphin
	Eledoisin
	Galanin
	NKA
	Somatostatin
	SP
	VIP
Selected enzymes	Fluoride-resistant acid phosphatase
	NOS
Selected receptors	ASICs
	α_2-Adrenoceptors
	Bradykinin receptors
	CCK receptors
	γ-Aminobutyric acid receptors
	Histamine receptors
	NK-1R
	NMDA receptors
	Opiate receptors
	Prostaglandin receptors
	VRs
Selected activators	a. Endogenous: inflammatory mediators (e.g. bradykinin and histamine)
	protons (low pH)
	b. Exogenous: noxious heat
	protons (low pH)
	toluene diisocyanate
	vanilloids

cord (for DRG neurons) or the spinal nucleus of the trigeminal tract (for trigeminal ganglion neurons), respectively (cf. Yaksh and Malmberg, 1994). Generally speaking, vanilloid-sensitive neurons transmit noxious information (usually perceived as itching or pain) to the CNS (afferent function), whereas peripherial terminals of these neurons are sites of release for a variety of proinflammatory neuropeptides (efferent function; these neuropeptides are summarized in Table 1). These neuropeptides are believed to play an important role in initiating the cascade of neurogenic inflammation (Fig. 2) (cf. Foreman, 1987; Geppetti and Holzer, 1996). In most experimental paradigms, capsaicin was found to activate both afferent and efferent functions, leading to the adaptation of the "axon reflex" model (cf. Bayliss, 1901; Bruce, 1910; Lewis, 1927; Celander and Folkow, 1953; Lisney and Bharali, 1989) by capsaicin researchers (cf. Holzer, 1988; Maggi and Meli, 1988; Burnstock, 1990; Lynn and Cotsell, 1991). However, it has recently been shown that capsaicin is capable of releasing sensory neuropeptides such as SP, somatostatin, and calcitonin gene-related peptide (CGRP) from the peripheral endings of sensitive nerves in the presence of lignocaine, tetrodotoxin, ω-conotoxin, or agatoxin, suggesting a direct mechanism for peptide release not mediated by axon reflex (Szolcsányi et al., 1998a).

In addition to capsaicin, vanilloid-sensitive neurons are also activated by a variety of chemical and physical (both heat and pressure) stimuli (cf. Maggi, 1991; Lundberg, 1993). Some of these compounds, like histamine and bradykinin, have their own receptors. Others (for example, xylene and mustard oil) are believed to work in a non-receptor-mediated fashion (N. Jancsó et al., 1968), possibly by perturbing membranes. Protons (low pH) are unique in that they have their own receptors (known as acid-sensitive ion channels or ASICs) (Waldmann et al., 1997), but, at the same time, they also act on VRs (capsaicin) (Bevan and Yeats, 1991; Petersen and LaMotte, 1993; Liu and Simon, 1994; Martenson et al., 1994; Kress et al., 1996a; Caterina et al., 1997;

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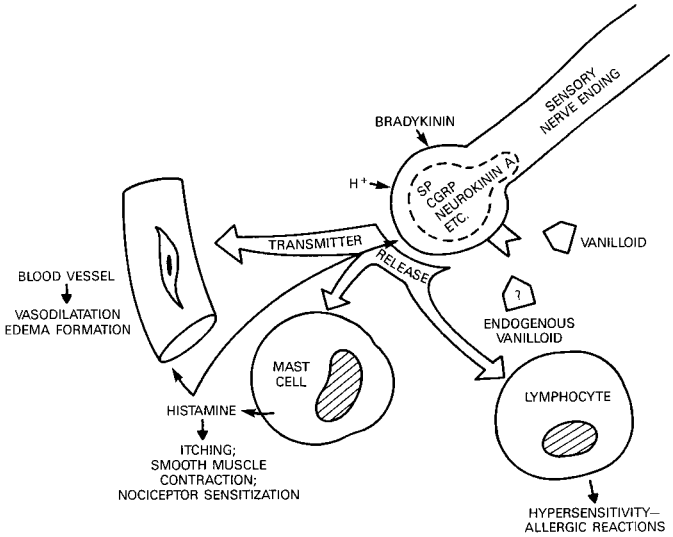

FIG. 2. Schematic illustration of the role of peripheral vanilloid-sensitive nerve endings in evoking neurogenic inflammatory and allergic-hypersensitivity reactions. Reproduced with permission from Szallasi and Blumberg, 1993b. Vanilloid-sensitive nerves may be stimulated to release prestored proinflammatory neuropeptides by both exogenous and endogenous stimuli. Some of these agents, like bradykinin, have their own receptors; others may act on VRs. Protons are unique in that they have their own receptors (called acid-sensitive ion channels or ASICs) but they act also on VRs. The competitive VR antagonist capsazepine ameliorates carrageenan-induced inflammation in vivo, implying a role for an endogenous vanilloid in initiating the inflammatory cascade. Generally speaking, the tachykinin SP released from vanilloid-sensitive nerves causes smooth muscle cells to contract (e.g., bronchospasm) and opens endothelial gaps (plasma extravasation) by interacting at NK-1Rs. Also, SP can stimulate mucus secretion and activate various inflammatory cells. The predominant effect of CGRP is vasodilation. There are several important positive feedback mechanisms involved in neurogenic inflammation. For example, SP released from vanilloid-sensitive nerves activates mast cells. Mast cells liberate histamine, which, in turn, stimulates vanilloid-sensitive nerves to release more SP. It is easy to visualize how the defunctionalization of sensory nerves by vanilloids may prevent, or at least ameliorate, neurogenic inflammatory symptoms. For further details, see text.

Tominaga et al., 1998). Finally, there are compounds (e.g., sesquiterpene unsaturated dialdehydes; Szallasi et al., 1996a) and, more surprisingly, physical stimuli (e.g., noxious heat; Caterina et al., 1997) that appear to stimulate sensory neurons in a VR-mediated fashion. Again, it needs to be emphasized that VR is "a target" but not "the target" for noxious heat, because the overlap between heat- and capsaicin-sensitive DRG neurons is only partial (Cesare and McNaughton, 1996; Kirschstein et al., 1997; Reichling and Levine, 1997).

Among irritant compounds acting on primary sensory neurons, capsaicin and related vanilloids are unique in that the initial stimulation by vanilloids is followed by a lasting refractory state, traditionally termed desensitization (N. Jancsó and A. Jancsó, 1949; N. Jancsó, 1955; 1968; cf. Szolcsányi, 1984; G. Jancsó, 1994). As discussed later, this desensitization is a complex process and has a clear therapeutic potential.

Although capsaicin was regarded as a "remarkably selective tool for primary sensory neurons" (Holzer, 1991), it was clear from the beginning that not all capsaicin actions can be attributed to the activation of primary sensory neurons. The lack of probes to detect VRs has led to considerable confusion as to what constitutes vanilloid-sensitive targets. This confusion persists to the present. There was little debate that there are vanilloid-sensitive neurons in nodose ganglia (Szolcsányi and Barthó, 1978, 1982; Marsh et al., 1987; Raybould and Taché, 1989; Waddell and Lawson, 1989; Sharkey et al., 1991; Carobi, 1996). The actual existence of these neurons was recently confirmed by both [^{3}H]RTX binding (Fig. 3) (Szallasi et al., 1995a) and VR1 mRNA in situ hybridization studies (Helliwell et al., 1998a). It was also generally accepted that intrinsic sensitive neurons located in the hypothalamus can mediate the well known effects

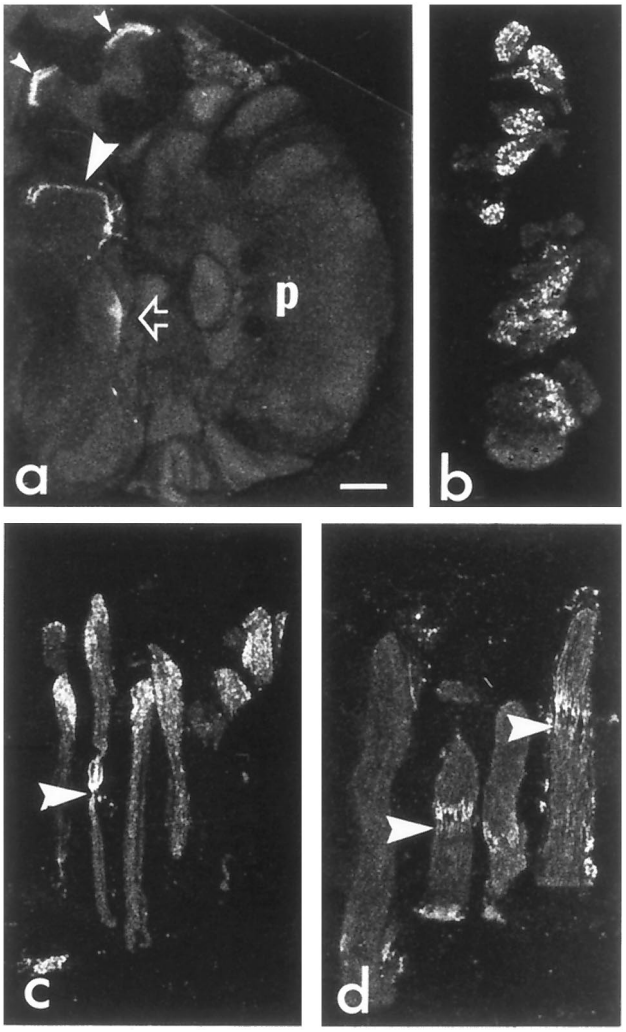

FIG. 3. Visualization by [^{3}H]RTX autoradiography of vanilloid-sensitive neurons in the rat. Note the intense labeling in dorsal root (b, upper five samples), trigeminal (b, lower two samples), and nodose (c) ganglia, containing cell bodies of vanilloid-sensitive neurons. Central fibers of DRG, trigeminal ganglion, and nodose ganglion neurons terminate in the dorsal horn of the spinal cord (a, small arrowheads), the spinal trigeminal nucleus of the medulla oblongata (a, big arrowhead), and the area postrema/nucleus of the solitary tract (a, open arrow), respectively. Peripheral axons of DRG and nodose ganglion neurons traverse in the sciatic (d) and vagus (c) nerves, respectively. Following ligation, there is a marked accumulation of specific binding sites proximal to the ligature in the vagus (c) or sciatic (d) nerves, suggesting that VRs are transported intraaxonally to the periphery in a form capable of ligand binding. Reprinted with permission from Szallasi, 1995.

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of vanilloid administration on temperature regulation (Jancsó-Gábor et al. 1970; Szolcsányi et al., 1971). Recent experiments, with both RTX binding (Fig. 4) (Ács et al., 1996a) and reverse transcription-polymerase chain reaction using primers based on the VR1 sequence (Sasamura et al., 1998), have confirmed the existence of these VR-expressing hypothalamic neurons. Other capsaicin actions turned out to be mediated by targets unrelated to VRs (cf. Holzer, 1991; Szallasi, 1994). Nonetheless, the recognition that nonneuronal tissues may express VRs (Bíró et al. 1998a,b) implies that capsaicin effects previously considered to be nonspecific may, in fact, be mediated by VRs.

C. Early, Indirect Evidence for and against a Vanilloid (Capsaicin) Receptor VR

From the beginning, three lines of strong evidence pointed to the existence of a specific capsaicin recognition site. First, capsaicin-like activity required fairly strict structure-activity relations (Szolcsányi and Jancsó-Gábor, 1975, 1976; Szolcsányi, 1982; Hayes et al., 1984; Walpole and Wrigglesworth, 1993). Second, capsaicin sensitivity seemed to be restricted to well-defined neuronal tissues (cf. Buck and Burks, 1986; Holzer, 1991). Third, susceptibility to capsaicin showed striking species-related differences. Most authorities agreed that capsaicin sensitivity occurred only in mammals, and even mammalian species differed considerably in their responsiveness to capsaicin (cf. Buck and Burks, 1986; Holzer, 1991). Furthermore, corroborative evidence for the existence of a capsaicin receptor was the finding that the inorganic dye ruthenium red was able to block the capsaicin-induced responses (cf. Amann and Maggi, 1991).

If sufficiently high capsaicin doses are used, the above tissue and species specificities of capsaicin actions are, however, lost. For example, Ritter and Dinh (1993) demonstrated capsaicin-evoked silver staining along almost the entire neural axis of the rat, including the retina. In keeping with this, Szikszay and London (1988) described enhanced glucose use by capsaicin in a wide variety of CNS structures in the rat. Capsaicin was found to inhibit various enzymes (Shimomura et al., 1989; Yagi, 1990; Teel, 1991; Wolvetang et al., 1996), induce pseudochannel formation in lipid bilayers (Feigin et al., 1995), alter membrane fluidity (Aranda et al., 1995), and block K^+ channels (Dubois, 1982; Petersen et al., 1987; Bleakman et al., 1990; Castle, 1992; Baker and Ritchie, 1994; Kuenzi and Dale, 1996), just to name a few characteristic, non-VR-mediated capsaicin actions.

Early efforts to demonstrate specific binding sites for [^{3}H]dihydrocapsaicin (Szebeni et al., 1978) or photoaffinity-labeled capsaicin-like molecules (James et al., 1988) failed due to a combination of the high lipophilicity and relatively low affinity of these molecules.

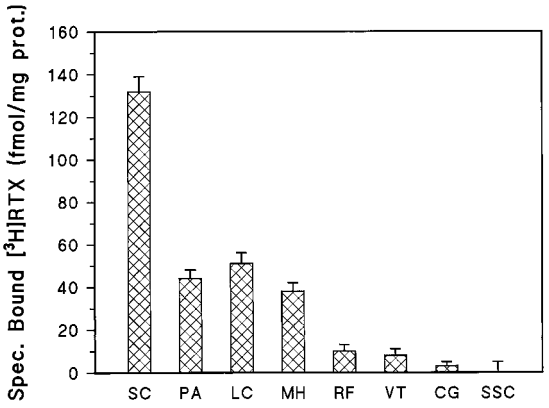

FIG. 4. VRs in the human brain, as detected by specific [^{3}H]RTX binding. For comparison, in the first column we show the density of specific RTX binding sites in the dorsal horn of the human spinal cord (SC), the central termination site for vanilloid-sensitive neurons. In three nuclei (PA, preoptic area; LC, locus ceruleus; MH, medial hypothalamus) the density of RTX binding sites approaches one-third of that in the dorsal horn. The presence of VRs in these nuclei is expected, as they were reported to respond to capsaicin in vivo. A low density (or affinity) but reproducible RTX binding was detected in additional two areas, the reticular formation (RF) and the ventral nucleus of the thalamus (VT). A very low level of binding was found in the midbrain central gray matter (CG). Finally, no specific binding could be detected in the somatosensory cortex (SSC). Reproduced with permission from Ács et al., 1996a.

III. Direct Evidence for a Vanilloid (Capsaicin) Receptor

A. Specific Binding of resiniferatoxin (RTX), a Naturally Occurring Ultrapotent Agonist

It has been known since the dawn of recorded history that the latex of the Moroccan cactus-like plant, *Euphorbia resinifera*, contains an extremely irritant component (Fig. 5) (cf. Appendino and Szallasi, 1997). But it was not until 1975 that the compound responsible for this irritancy was isolated and named RTX (Hergenhahn et al., 1975). RTX (see structure in Fig. 1) combines the structural features of two classes of natural irritants, phorbol esters and capsaicinoids. Although RTX did bind to the phorbol ester receptor, protein kinase C (PKC) (see in *Section XIII.B*), this low-affinity interaction could not explain its extreme pungency. In a series of experiments beginning in 1989 and continuing to date, we have identified RTX as an ultrapotent capsaicin analog with a unique spectrum of biological activities (Table 2). Because capsaicin and RTX analogs share a (homo)vanillyl group as a structural motif essential for bioactivity but differ dramatically in the rest of the molecule (Fig. 1), they are collectively termed vanilloids. Consequently, the primary target for these compounds appears to be best described as the VR.

In several assays, RTX is several thousandfold more potent than capsaicin (Table 2) (cf. Szallasi and Blumberg, 1990a, 1993b, 1996). This ultrapotency predicted the existence of high-affinity specific RTX binding sites (VRs). Despite multiple obstacles, in 1990 we finally managed to demonstrate specific [^{3}H]RTX binding by rat DRG membranes (Szallasi and Blumberg, 1990b),

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FIG. 5. The collection of the latex of an euphorbia plant as depicted in Codex Ayasofia (3103, f. 136.1.3, Freer Gallery of Art, Smithsonian Institution, Washington, DC), an Arabic version of *De Materia Medica* by Dioscorides. During antiquity, the dried latex, called euphorbium, was used as a vesicant (skin irritation) and sternutative (nose irritation) agent. It was also given as a general remedy for various snakebites and poisons.

providing the first unequivocal evidence for the existence of VRs (Fig. 6). This initial binding assay was, however, plagued by a very high nonspecific binding, which prevented the detection of VRs in the spinal cord or peripheral tissues.

The methodological means to overcome the problems created by the high nonspecific RTX binding was furnished by an accidental observation of ours. In search of endogenous ligands for VRs, we assayed a variety of body fluids and tissue extracts and found that the acute phase serum protein, α_1-acid glycoprotein (also known as orosomucoid), binds RTX (Szallasi et al., 1992). α_1-Acid glycoprotein is an important drug-binding protein (Paxton, 1983; Kremer et al., 1988; Maruyama et al., 1990). We showed that RTX binds to the well known drug-recognition domain on α_1-acid glycoprotein that it shares with warfarin (Szallasi et al., 1992). Plasma binding of RTX to α_1-acid glycoprotein may have clear consequences on pharmacokinetics upon systemic administration that we will discuss later. RTX binding to α_1-acid glycoprotein, however, differs in two very important aspects from VR binding. First, it is not markedly

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TABLE 2
Potency of RTX relative to capsaicin in selected assays in the rat

Assay	Relative Potency
In vitro experiments	
Inhibition of [^{3}H]RTX binding to DRG neurons (1)	100,000
Induction of $^{45}Ca^{2+}$ uptake by DRG neurons (1)	300
Activation of single channels in patch-clamped DRG neurons (2)	500
Evoking currents via cloned VR1 expressed in oocytes (3)	20
Twitch inhibition in vas deferens (4)	10,000
Desensitization of urinary bladder to subsequent challange (4)	1,000
Induction of cGMP in DRG neurons (5)	660
Contractions of isolated urinary bladder (4)	1
In vivo experiments	
Inhibition of xylene-induced Evans' blue extravasation (6)	20,000
Induction of hypothermia (6)	7,000
Pungency (eye-wipings) (6)	10
Activation of pulmonary chemoreflex (7, 8)	RTX inactive
Desensitization of pulmonary chemoreflex (7)	Capsaicin inactive

References indicated in parentheses: (1) Ács et al., 1996b; (2) Oh et al., 1996; (3) Caterina et al., 1997; (4) Maggi et al., 1990; (5) Winter et al., 1990; (6) Szallasi and Blumberg, 1989a; (7) Szolcsányi et al., 1990; and (8) Szolcsányi et al., 1991.

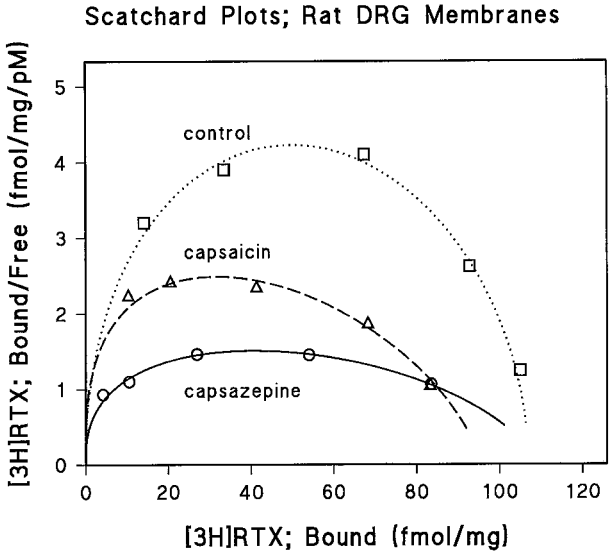

FIG. 6. Scatchard plots of specific [^{3}H]RTX binding to rat DRG membranes. This binding is of high affinity (K_d is 20 pM) and follows positive cooperative behavior, as reflected in the convex Scatchard plot. The cooperativity index is 2, indicating the existence of at least two interacting binding sites. Capsaicin and capsazepine, both at a concentration of 3 μM, reduce the apparent affinity of RTX binding, having little or no effects on cooperativity or maximal receptor density. This behavior is consistent with a competitive binding mechanism.

influenced by temperature, and second, it is of an at least 1000-fold lower affinity (Szallasi et al., 1992). At 0°C, the dissociation of receptor-bound RTX is unmeasurably slow (Szallasi and Blumberg, 1993a). By contrast, α_1-acid glycoprotein readily binds unbound RTX in the aqueous phase (Szallasi et al., 1992). Because nonspecifically bound RTX in the membrane lipids is in equilibrium with the unbound RTX in the aqueous phase, α_1-acid glycoprotein is able to extract nonspecifically bound RTX from the membranes without compromising specific binding (Szallasi et al., 1992). By centrifuging the membranes, it is then easy to separate the membrane-, mostly receptor-bound RTX from the α_1-acid glycoprotein-bound form that remains in the supernatant.

The introduction of α_1-acid glycoprotein to the VR binding assay resulted in a dramatic improvement in the ratio of specific binding from 50% of the total at concentrations close to the K_d to 90% of the total binding or even higher. Using this improved assay, we are now able to detect specific RTX binding sites in the dorsal horn of the spinal cord (Szallasi et al., 1993a,b; Ács and Blumberg, 1994; Ács et al., 1994a,b; Szallasi and Goso, 1994), in various peripheral tissues [e.g., urinary bladder (Szallasi et al., 1993c; Ács et al., 1994a), urethra (Parlani et al., 1993), nasal mucosa (Rinder et al., 1996), airways (Szallasi et al., 1993b, 1995b), colon (Goso et al., 1993a)] as well as in several brain nuclei (Fig. 4 and Table 3) (Ács et al., 1996a). Furthermore, we developed an autoradiographic approach to visualize VRs in several species, including human (Figs. 3 and 7) (cf. Szallasi, 1995).

B. Development of Capsazepine, a Competitive Vanilloid Antagonist

Capsazepine (Fig. 8), the first and, as yet, only commercially available competitive VR antagonist comes from an extensive program at the former Sandoz, now Novartis, Institute for Medical Research, London, to explore structure-activity requirements for vanilloid-like activity (cf. Walpole and Wrigglesworth, 1993). Capsazepine inhibits vanilloid responses in vitro with Schild plots consistent with a competitive mechanism (Bevan et al., 1991, 1992). Moreover, capsazepine competes for specific RTX binding sites in a competitive manner (Szallasi et al., 1993b). In rat trigeminal ganglion neurons, capsazepine inhibits vanilloid-evoked currents (Liu and Simon, 1994). In a variety of bioassays, capsazepine is effective against both capsaicin (Dickenson and Dray, 1991; Urbán and Dray, 1991; Belvisi et al., 1992; Perkins and Campbell, 1992; Maggi et al., 1993; Santicioli et al., 1993; Seno and Dray, 1993; Ueda et al., 1993; Lee and Lundberg, 1994; Lalloo et al., 1995; Fox et al., 1995) and RTX (Ellis and Undem, 1994; Walpole et al., 1994; Ács et al., 1996b, 1997; Wardle et al., 1996, 1997) but, surprisingly, not against olvanil (Davey et al., 1994). The utility of capsazepine is, however, limited by its moderate potency. At micromolar concentrations, which are necessary to inhibit capsaicin-evoked responses in most tissues, capsazepine also blocks voltage-gated calcium channels (Docherty et al., 1997) as well as nicotinic acetylcholine receptors (Liu and Simon, 1997). Furthermore, recent evidence suggests that capsazepine-insensitive VRs also exist (Liu et al., 1998). Therefore one should be very careful when interpreting results obtained with capsazepine, because positive effects are not necessarily mediated by VRs, nor do negative data rule out the involvement of VRs. One more reason for being cautious when working with capsazepine was fur-

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TABLE 3
Parameters of [^{3}H]RTX binding to VRs in the rat

Tissue	RTX	Cooperativity Index	Capsaicin	Capsazepine
	K_d; *pM*		K_i, *nM*	K_i, *nM*
DRG (membranes) (1–3)	18–46	1.7–1.8	600–4,900	3,500–3,900
DRG (isolated neurons) (4)	48	1.8	2,100	3,200
Spinal cord (1, 3, 5)	13–31	1.9–2.3	300–5,400	3,300–4,000
Sciatic nerve (1)	46	2.0	4,700	3,400
Urinary bladder (1, 4, 6, 7)	30–87	1.0–1.9	500–3,700	4,800–5,000
Urethra (8)	105	1.0	N.D.	N.D.
Trachea and main bronchi (5)	250	1.1	100	100
Colon (9)	3,000	1.0	3,000	100
Vagal nerve (1)	45	2.3	N.D.	N.D.
Dorsal vagal complex (1)	28	2.6	N.D.	N.D.

References indicated in parentheses: (1) Ács et al., 1994a; (2) Szallasi and Blumberg, 1993a; (3) Szallasi et al., 1993a; (4) Ács et al., 1996b; (5) Szallasi et al., 1993b; (6) Szallasi et al., 1993c; (7) Szallasi et al., 1993d; (8) Parlani et al., 1993; (9) Goso et al., 1993a.

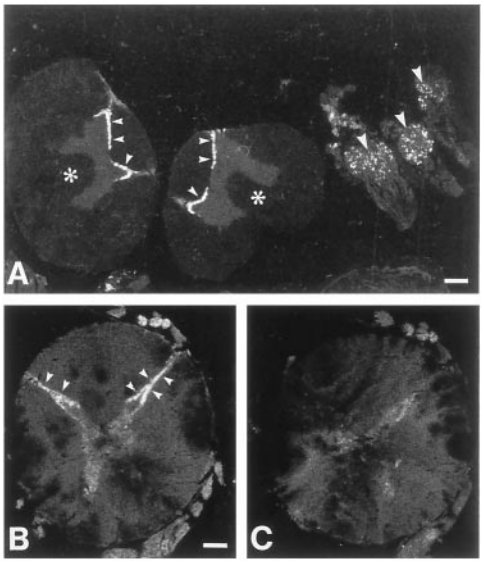

FIG. 7. Autoradiographic visualization by [^{3}H]RTX binding of VRs in porcine (A, small arrowheads) and human (B) dorsal horn of the spinal cord, the central termination site for vanilloid-sensitive neurons. The labeling is highly specific, because it is completely missing in the presence of nonradioactive RTX (C). Reprinted with permission from Szallasi et al., 1994a.

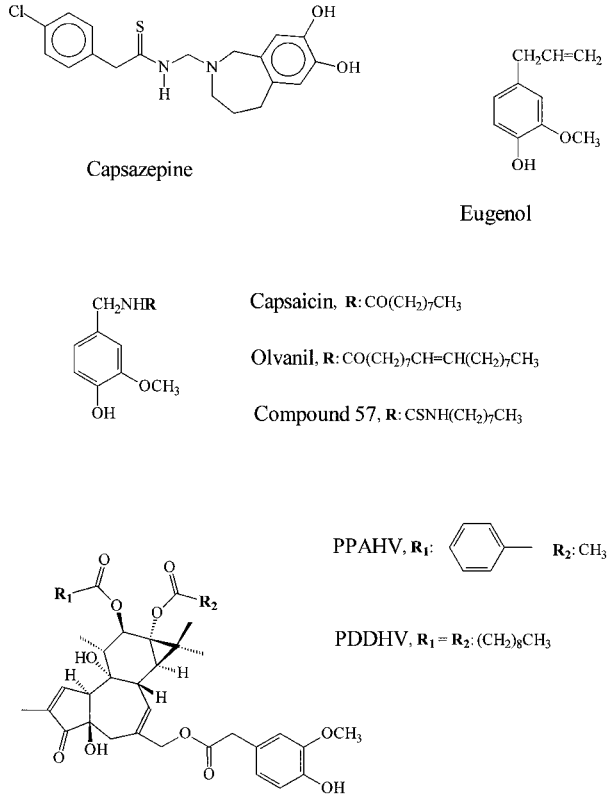

FIG. 8. Selected vanilloid structures. Capsazepine is a competitive VR antagonist. Eugenol in an analgesic used in dental practice. Olvanil is a nonpungent, orally active capsaicin analog. Compound 57 is the most potent capsaicinoid for inducing Ca^{2+}-uptake by DRG neurons in culture. PPAHV binds to VRs in a noncooperative fashion. Also, it gates two pharmacologically distinct conductances in rat DRG neurons, one that is inhibited by capsazepine and another that does not recognize this antagonist. PDDHV induces Ca^{2+}-uptake by DRG neurons with a potency of 15 nM; however, it fails to inhibit [^{3}H]RTX binding to these cells up to a concentration of 10 μM. This finding implies that the RTX binding domain on VRs is distinct from the site mediating calcium influx.

nished by the observation that, at least in the rabbit, it may act as a weak vanilloid agonist (Wang and Håkanson, 1993).

C. Cloning of the First VR, Termed VR1

Repeated efforts to clone a VR using RTX-like photoaffinity probes resulted in the identification of several, relatively low-affinity RTX-binding proteins, none of which showed the expected tissue distribution for VRs, nor did they show a VR-like activity in functional assays (Ninkina et al., 1994; Davies et al., 1997). David Julius' group at the University of California in San Francisco chose, therefore, a different approach. They transfected eukaryotic cells with pools of a rat cDNA library and used calcium imaging to identify those cells that responded to capsaicin (Caterina et al., 1997). Once a positive pool was found, it was divided into smaller pools (a procedure known as sib-selection) until they had isolated a single cDNA encoding the capsaicin-gated channel. They named this receptor VR1.

The rat VR1 cDNA contains an open reading frame of 2514 nucleotides. This cDNA encodes a protein of 838 amino acids with a molecular mass of 95 kDa. At the N terminus, VR1 has three ankyrin repeat domains (Fig. 9A). The carboxy terminus has no recognizable motifs. Predicted membrane topology of VR1 features six trans-

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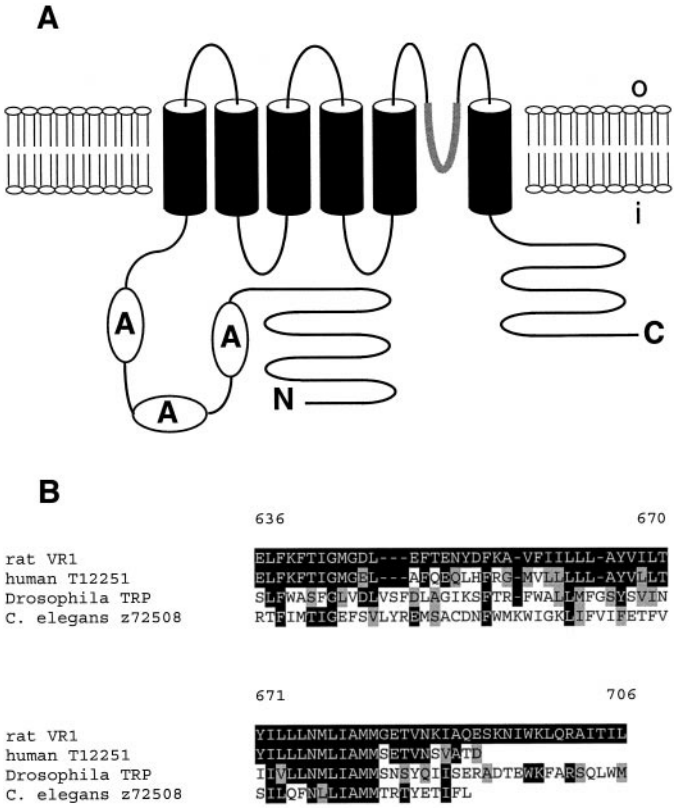

FIG. 9. A, predicted membrane topology and domain structure of the first cloned VR, called VR1. VR1 has six complete transmembrane segments and a partial one, which is believed to be associated with the channel pore. As indicate ankyrin repeat domains. Outer (o) and inner (i) plasma membrane leaflets are also indicated. B, rat VR1 shows homology to the *Drosophila* TRP channel and a related protein in the nematode *C. elegans*. Several human ESTs, such as T12251 in heart, are also similar to VR1. Identical residues are in black boxes and conservative substitutions are in gray. Reprinted by permission from *Nature* (Caterina et al., 1997). Copyright (1997) Macmillan Magazines Ltd.

membrane domains and a possible pore-loop between the fifth and sixth membrane-spanning regions (Fig. 9A). There are three possible protein kinase A phosphorylation sites on the VR1 that might play a role in receptor desensitization.

VR1 is a distant relative of the transient release potential (TRP) family of store-operated calcium channels (Montell and Rubin, 1989; Hardie and Minke, 1993; Wes et al., 1995; Clapham, 1996; Colbert et al., 1997; Roayaie et al., 1998). There is considerable homology between VR1 and the drosophila TRP protein in retina (Fig. 9B). This sequence similarity seems to be restricted to the pore-loop and the adjacent sixth transmembrane segment in VR1. Interestingly, VR1 also shows similarity to a Soares human retina cDNA (L. Hillier, N. Clark, T. Dubuque, K. Elliston, M. Hawkins, M. Holman, M. Hultman, T. Kucaba, M. Le, G. Lennon, M. Marra, J. Parsons, L. Rifkin, T. Rohlfing, F. Tan, E. Trevaskis, R. Waterston, A. Williamson, P. Wohldman and R. Wilson, unpublished observations, Washington University-Merck expressed sequence tags (EST) Project; Accession: AA047763). Because capsaicin causes a marked calcium accumulation in rat retina (Ritter and Dinh, 1993), it might be speculated that the retina has a site, related to VR1, that recognizes vanilloids. OSM-9, a novel protein with similarity to rat VR1, plays a role in olfaction, mechanosensation, and olfactory adaptation in *Caenorhabditis elegans* (Colbert et al., 1997). OSM-9, however, does not recognize capsaicin (Cornelia Bargmann, personal communication). These findings imply that 1) in contrast to previous beliefs, VR isoforms did occur early during evolution, but 2) the capsaicin recognition site is a recent addition to VR1.

It should be noted that a human EST in heart (T12251) displays striking similarity (68% amino acid identity) to the pore-loop and the adjacent sixth transmembrane segment in rat VR1 (Fig. 9B). Additional human EST clones are similar to other regions of the VR1 and could represent fragments of the human transcript. The presence of a VR1-like EST clone in heart is surprising but it is entirely in accord with the recent recognition of nonneuronal VRs (Bíró et al., 1998a,b). Capsaicin has long been known to influence cardiac functions (Fukuda and Fujiwara, 1969; Molnár et al., 1969). Some capsaicin actions on heart were attributed to an interaction at K^+ channels (Castle, 1992), whereas others were explained by the liberation of neuropeptides, most notably CGRP, from the vanilloid-sensitive innervation of the heart (Franco-Cereceda et al. 1988, 1991; Ono et al., 1989). It is not impossible that capsaicin can also act directly on the heart via a cardiac VR.

When expressed in *Xenopus* oocytes, VR1 is similar in its electrophysiological properties to native vanilloid-operated channels in sensory ganglia (Caterina et al., 1997). As observed in cultured DRG neurons (Baccaglini and Hogan, 1983; Heyman and Rang, 1985; Forbes and Bevan, 1988; Winter et al., 1990; Vlachová and Vyklicky, 1993; Liu and Simon, 1994; Petersen et al., 1996), capsaicin-evoked currents readily disappear after agonist removal, whereas RTX-induced currents are much longer in duration and often persist even in the absence of the agonist. Hill coefficients (approximately 2) derived from the analysis of capsaicin-induced currents in oocytes injected with mRNAs encoding VR1 indicate the existence of more than one agonist binding site (Caterina et al., 1997). Again, this is in accord with the properties of native VRs in sensory neurons (Szallasi et al., 1993a; Oh et al., 1996). The implications of this finding are discussed in *Section VI.J.*

RTX is, however, only 20-fold more potent than capsaicin to activate the cloned VR1 (Caterina et al., 1997), which is at variance with the several thousandfold higher affinity of RTX in the binding assay (cf. Szallasi and Blumberg, 1990a, 1996; Szallasi, 1994). This apparent contradiction was first explained by postulating the existence of two distinct classes of VRs, the channel, which represents a low-affinity site for RTX, and a yet-to-be cloned high-affinity site, seen in the binding assay. We referred to these

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receptors as C-type and R-type VRs, respectively (cf. Ács et al., 1997; Bíró et al. 1997; Szallasi, 1997). However, preliminary binding experiments with human embryonic kidney (HEK)293 cells stably transfected with VR1 cDNA suggests that this is not the case: the same receptor protein seems to mediate both the high-affinity RTX binding and the lower affinity calcium uptake response (A. Szallasi, D. N. Cortright, P. M. Blumberg, and J. E. Krause, manuscript in preparation).

IV. Anatomical Localization and Tissue Specificity of VRs

A. VRs in Primary Sensory Neurons; Colocalization with Other Receptors, Neuropeptides, and the Isolectin B4

As first shown by RTX autoradiography (cf. Szallasi, 1995), VRs are expressed along the entire length of vanilloid-sensitive sensory neurons, from the peripheral terminals to the axons to the somata to the central

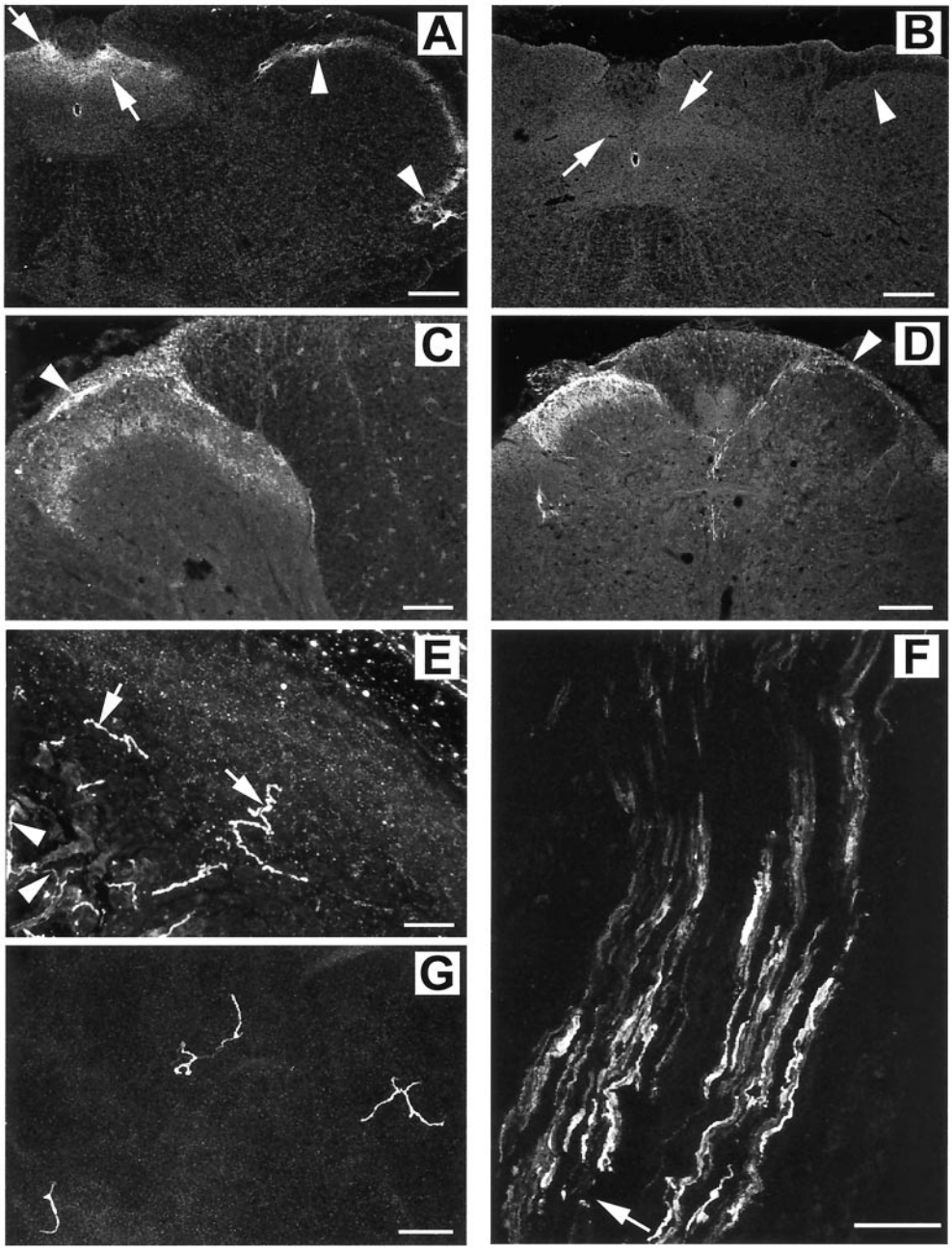

FIG. 10. Visualization by immunohistochemistry of VR1 receptor-like immunoreactivity in central (A and C) and peripheral (E and G) terminals of vanilloid-sensitive neurons in the rat. Compare this figure to Fig. 3 and notice the colocalization of specific RTX binding sites and VR1 in the nucleus of the solitary tract (A, arrows), the spinal trigeminal nucleus (A, arrow heads), and the superficial dorsal horn of the spinal cord (C). VR1-like immunoreactivity is also present in the sciatic nerve (F), the paw skin (E), and the cornea (G). The specificity of the immunostaining is demonstrated by peptide blocking (B), and by the absence of labeling following unilateral dorsal rhizotomy (D). Reprinted with permission from the *European Journal of Neuroscience* (Guo et al., 1999).

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endings (Fig. 3 and Table 3). In corresponding areas (compare Figs. 3 and 10) the presence of VR1-like immunoreactivity has also been demonstrated (Guo et al., 1999; Tominaga et al., 1998). Nerve ligation studies suggest that VRs are transported from the cell bodies to the periphery in a form capable of ligand binding (Fig. 3) (Szallasi et al., 1995a). Peripheral tissues in which VRs were demonstrated by RTX binding to be present are listed in *Section III.A* and in Table 3. In the urinary bladder, VRs seem to be expressed exclusively on nerve endings rather than in bladder epithelium or the muscle layer, for denervation of the bladder leads to a complete depletion of specific RTX binding sites (Szallasi et al., 1993d). The majority (approximately 90%) of the RTX sites appear to be present on the pudendal nerve, with a relatively minor fraction on the hypogastric nerve (Szallasi et al., 1993d).

Northern blot analysis confirmed the presence of VR1 transcripts in dorsal root and trigeminal ganglia (Caterina et al., 1997; Helliwell et al., 1998a). VR1-like immunoreactivity was detected in more than 50% of DRG neurons (Fig. 11), with the expression being most prevalent in small to medium sized neurons (Guo et al., 1999). VR1-like immunoreactivity was also observed in both the central (e.g., dorsal horn of the spinal cord and the caudal nucleus of the spinal trigeminal complex) and peripheral (e.g., skin and cornea) processes of primary afferent neurons (Fig. 10) (Guo et al., 1998). In DRG neurons, VR1-like immunoreactivity was associated with the Golgi complex and the plasma membrane (Guo et al., 1999). In the dorsal horn of the spinal cord, the VR1 protein was associated with "small clear vesicles" in preterminal axons and with the plasma membrane of nerve terminals (Guo et al., 1999). The latter finding is in line with an earlier observation of Szolcsányi (Szolcsányi et al., 1975), according to which capsaicin depletes "small clear vesicles" from nerve endings.

The distribution of VR1-like immunoreactivity in the spinal dorsal horn deserves particular attention. The labeling is strongest in the Lissauer zone (lamina I; Fig. 10C). VR1 protein is also abundant in the inner, but not in the outer, layer of lamina II (Fig. 10C) (Guo et al., 1999). [There is an apparent discrepancy between this finding and the even distribution of VR1-like immunoreactivity in the substantia gelatinosa reported by Julius and colleagues (Tominaga et al., 1998), the reason for which is not clear.] It should be noted that several important proteins involved in pain transmission are also enriched in the inner layer of lamina II (i.e., in close proximity to VR1). Notable examples include PKC isozyme γ (PKCγ) (Malmberg et al., 1997a) as well as ATP-sensitive $P2X_3$ receptors (Vulchanova et al., 1998). $P2X_3$ receptors colocalize with VR1 both in DRGs, where 75% of the $P2X_3$ positive neurons also express VR1 (Fig. 11F), and in the inner layer of lamina II (Fig. 11, D and E) (Guo et al., 1999). By contrast, PKCγ is confined to a population of interneurons that reside in lamina II of the dorsal horn (Malmberg et al., 1997a). The possible role of PKCγ and $P2X_3$ receptors in nociception is discussed in *Section VIII*.

Surprisingly, the colocalization of VR1 with SP in DRG neurons is limited: for example, only 33% of L5 DRG neurons positive for VR1 contain SP-like immunoreactivity as well (Fig. 11I) (Guo et al., 1999). Moreover, much of the VR1 staining in the dorsal horn is concentrated in the inner layer of lamina II where both SP- (Fig. 11, G and H) and CGRP-like immunoreactivities are sparse (Fig. 11, J and K) (Guo et al., 1999). This limited colocalization between VR1 and SP is at variance with the profound effects that vanilloids have on SP expression (see in *Section VIII.B.5*).

The colocalization of VR1 with the lectin IB4 (Fig. 11, A–C) (Guo et al., 1999; Tominaga et al., 1998) may shed new light on the regulation of VR expression by trophic factors. Vanilloid-sensitive neurons require nerve growth factor (NGF) for survival during embryogenesis (Ruit et al., 1992), as evidenced by a severe deficit in nociception and thermoreception in mice with a null mutation in the gene encoding trkA, the signal transduction receptor for NGF (Smeyne et al., 1994). Mature DRG neurons, however, are able to survive in the absence of NGF (Yip et al., 1984; Lindsay, 1988). There is evidence that those neurons that bind IB4 also express receptors for glial cell-derived neurotrophic factor, abbreviated GDNF (Bennett et al., 1998). In 1997, Snider and coworkers (Molliver et al., 1997) showed in elegant experiments that IB4-positive DRG neurons switch from dependence on NGF to dependence upon GDNF during development. Subsequently, McMahon and colleagues (Bennett et al., 1998) demonstrated that IB4-positive neurons may be rescued by exogenous GDNF following nerve injury. Clearly, it would be interesting to explore the effects of GDNF on VR expression.

B. VRs in Vagal (Nodose Ganglion) Neurons

In addition to DRG (Figs. 3 and 7) and trigeminal ganglion (Fig. 3) neurons (Szallasi and Blumberg, 1990b; Szallasi et al., 1993a,b, 1994a; Ács et al., 1994a), a subset of nodose ganglion neurons also expresses VRs (Fig. 3 and Table 3) (Ács et al., 1994a; Szallasi et al., 1995a). A strong autoradiographic signal is present in the area postrema/nucleus of the solitary tract region (Fig. 3), representing the central termination area for sensory neurons of the vagus nerve (Szallasi et al., 1995a). This area is also stained by an anti-VR1 antibody (Fig. 10A; Guo et al., 1999).

An unexpected result in the VR1 cloning article by Julius and colleagues (Caterina et al., 1997) is the failure of Northern blot hybridization to detect mRNAs encoding VR1 in nodose ganglia. However, using a probe corresponding to nucleotides 1513-2482 of the rat VR1 sequence, a strong in situ hybridization signal was detected in nodose ganglion neurons (Helliwell et al., 1998). To resolve the apparent contradiction between these studies, it should be noted that whereas Julius and coworkers (Caterina et al., 1997) used the entire VR1

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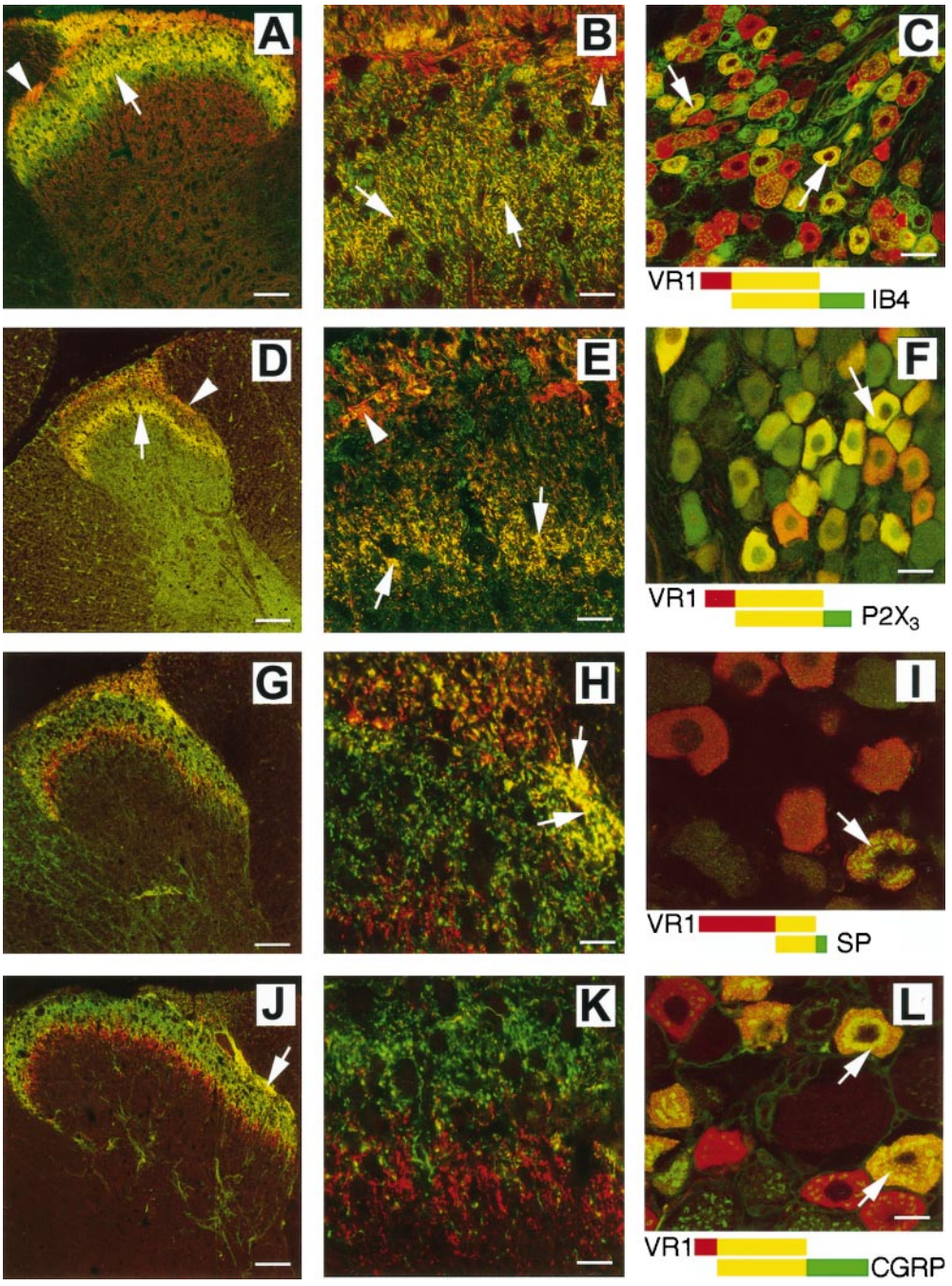

FIG. 11. Colocalization of VR1-like immunoreactivity in the spinal dorsal horn (A, B, D, E, G, H, J, and K) and in DRG neurons (C, F, I, and L) with the lectin IB4 (A, B, and C), the $P2X_3$ receptor (D, E, and F), and the neuropeptides SP (G, H, and I) and CGRP (J, K, and L). This figure suggests that vanilloid-sensitivity encompasses several subpopulations of DRG neurons, both peptidergic and nonpeptidergic. However, none of the markers tested (IB4, $P2X_3$, SP, and CGRP) is present exclusively in VR1-expressing neurons. Note that the intensity of VR1 receptor-like immunostaining is strongest in the substantia gelatinosa (lamina I) and the inner layer of Rexed lamina II. Reproduced with permission from the *European Journal of Neuroscience* (Guo et al., 1999).

sequence for Northern blot hybridization, Bevan and colleagues (Helliwell et al., 1998) generated by polymerase chain reaction a partial VR1 sequence only. Therefore, it is entirely possible that nodose ganglion neurons express a VR isoform that differs from VR1 in nucleotides not included in the probe used for in situ hybridization histochemistry. The existence of distinct VR isoforms in DRGs and nodose ganglia would be consistent with the different embryonic origin of these tissues (cf. Vogel, 1992). It would also be in accord with the observations that different neurotrophic factors regulate capsaicin sensitivity in DRG (NGF and possibly also GDNF) and nodose ganglion neurons (brain-derived neurotrophic factor), respectively (Winter et al., 1988; Winter, 1998).

C. VRs in Brain

A low to moderate level of specific RTX binding can be detected in various CNS areas not associated with primary sensory neurons (Ács et al., 1996a), suggesting the

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existence of intrinsic brain neurons with VRs (Fig. 4). Among these brain areas, the presence of VRs in the hypothalamus was expected based on the proposed role of this structure in the hypothermic action of vanilloids (Jancsó-Gábor et al., 1970; Szolcsányi et al., 1971). The presence of VR1 mRNA in the hypothalamus has recently been confirmed by reverse transcription-polymerase chain reaction (Sasamura et al., 1998). These vanilloid-sensitive terminals in the hypothalamus are probably glutaminergic because capsaicin evokes glutamate release from slices of hypothalamus (Sasamura et al., 1998). It is also easy to visualize how VRs in the reticular formation (Ács et al., 1996a) may mediate some vanilloid actions on autonomic regulation (Jancsó and Such, 1985; Koulchitsky et al., 1994; Seller et al., 1997). The biological role of specific RTX binding sites in other brain areas is unclear.

A link between the SP content of sensory neurons and their sensitivity to capsaicin was postulated as early as the mid-1960s (Gašparovic et al., 1964). In 1976, the high concentration of SP in the substantia nigra was first reported (Brownstein et al., 1976) and the search for capsaicin-sensitive neurons in the basal ganglia began. Five years later, an enhanced locomotor activity in rats following bilateral intranigral capsaicin injection and a concomittant decrease in the cataleptic action of fluphenazine was described (Dawbarn et al., 1981). In 1988, capsaicin microinjected into the substantia nigra or caudatus putamen was reported to induce peripheral vasodilatation and a subsequent hypothermic response (Hajós et al., 1988). These biological actions suggested the existence of intrinsic vanilloid-sensitive cells in the basal ganglia. The recent detection of VR1 mRNA in the striatum now firmly establishes the existence of such neurons (Sasamura et al., 1998). As yet, it is not known which subpopulation of nigral neurons express VRs. Neonatal capsaicin treatment does not deplete SP from the striatum, nor does it alter the expression of the opioid peptides dynorphin and met^5-enkephalin (Sivam and Krause, 1992). Repeated attempts to elucidate the effects of capsaicin treatment on monoaminergic systems in the brain have yielded controversial results. For example, increased (Holzer et al., 1981) or decreased (Dawbarn et al., 1981) 5-hydroxytryptamine levels, or no change at all (G. Jancsó et al., 1981), were reported in the very same year. With the recent availability of VR1 detecting antibodies as well as probes for in situ hybridization histochemistry, the identity of the VR1-expressing subpopulation of basal ganglion neurons will be revealed shortly.

VR1 mRNA seems to be widely present in the brain. Relative levels are as follows: hypothalamus and cerebellum > cortex, striatum, and midbrain > olfactory bulb, pons, hippocampus, and thalamus (Sasamura et al., 1998). Although Northern blot analysis failed to show VR1 mRNA in the brain (Caterina et al., 1997), this discrepancy may be due to the difference in sensitivity between polymerase chain reaction and Northern blot hybridization. In support of this explanation is the detection of VR1 mRNA in the rat cortex in solution hybridization-nuclease protection experiments (D. N. Cortright and J. E. Krause, personal communication). VR1-positive cells may also be visualized in the brain by both in situ hybridization and immunostaining experiments (E. Mezey, A. Guo, D. N. Cortright, R. Elde, J. E. Krause, P. M. Blumberg, and A. Szallasi, manuscript in preparation).

D. Possible Presence of VRs in Nonneuronal Tissues Such as Mast Cells and Glia

It has been long noted that capsaicin exerts a variety of effects on nonneuronal tissues (cf. Buck and Burks, 1986; Holzer, 1991). These actions were considered nonspecific because they were at variance with the prevailing concept of capsaicin being selective for sensory neurons. In 1998, we showed that vanilloids induce calcium uptake by mast cells (Bíró et al., 1998a) and in a glioma cell line (Bíró et al., 1998b). The pharmacological characteristics of this calcium influx response by mast cells and glioma cells were very similar to those described in DRG neurons (Table 4), with the exception of the magnitude of the uptake, which was much smaller, implying a VR density lower than in sensory neurons. In keeping with this, no specific [^{3}H]RTX binding by mast cells or glioma cells could be detected (Table 4). We will return to the biological relevance of such nonneuronal vanilloid interactions later. The novel concept of nonneuronal VRs has received support from the presence of EST clones homologous to VR1 in the heart and other nonneuronal tissues (Caterina et al., 1997).

Capsaicin stimulates the migration of human polymorphonuclear cells (Partsch and Matucci-Cerinic, 1993), blocks melanotroph cells in rat pituitary (Kehl, 1994), and inhibits the activation of pheochromocytoma

TABLE 4
Comparison of VR binding and vanilloid-induced $^{45}Ca^{2+}$ uptake in sensory (DRG) neurons, mast cells, and glioma cells

	Neurons	Mast Cells	Glioma Cells
Calcium influx	Yes	Yes	Yes
$^{45}Ca^{2+}$ uptake by capsaicin (potency, nM)			
Induction	320	660	380
Desensitization	440	525	360
Inhibition by capsazepine	290	310	40
Block by ruthenium red	800	980	690
$^{45}Ca^{2+}$ uptake by resiniferatoxin (potency, nM)			
Induction	1.2	2.1	0.5
Desensitization	0.08	2.3	0.4
Inhibition by capsazepine	325	290	N.D.
Block by ruthenium red	790	910	N.D.
[^{3}H]RTX binding	Yes	No	No
Affinity (K_d, nM)	0.04	N/A	N/A
Inhibition by capsaicin (K_i, nM)	4,900	N/A	N/A
Inhibition by capsazepine (K_i, nM)	4,000	N/A	N/A
Inhibition by ruthenium red (IC_{50}, nM)	14	N/A	N/A

N.D., not determined; N/A; not available. Data are from Ács et al., 1996b, 1997 (DRG neurons), Bíró et al., 1998a (mast cells) and b (glial cells), respectively.

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cells by acetylcholine (Nakazawa et al., 1994), just to name a few nonneuronal actions. The biological relevance of these findings is unclear. On isolated human bronchi, capsaicin may exert a contractile (Lundberg et al., 1983), relaxant (Chitano et al., 1994), or biphasic (bronchoconstriction at low and relaxation at high capsaicin concentrations) action (Ellis et al., 1997). Unlike in the mouse (Manzini, 1992), the relaxant effect of 2capsaicin on human bronchi does not appear to be mediated by nitric oxide but may reflect a direct action on airway smooth muscle instead (Ellis et al., 1997). Indeed, capsaicin was shown to evoke an outward current in human airway smooth muscle cells in culture via charybdotoxin-sensitive Ca^{2+}-activated K^+ channels (Ellis et al., 1997). At present, the possibility cannot be ruled out that capsaicin activates these channels directly; however, we prefer the alternative explanation, that capsaicin increases intracellular calcium levels in smooth muscle cells by opening a VR1-like conductance, which, in turn, activates the charybdotoxin-sensitive channels. With the availability of sensitive molecular probes for VR1 message, a systemic evaluation of RNA samples obtained from various tissues might identify further nonneuronal cells expressing VRs.

The most recent addition to the list of candidate tissues to express VRs is human lymphocytes (Lai et al., 1998). Human lymphocytes, like sensory neurons (cf. Hökfelt et al., 1975; Pernow, 1983; Nakanishi, 1987), apparently store preformed SP (Lai et al., 1998). As we already discussed, capsaicin releases SP from sensory neurons (Gašparovic et al., 1964; Jessell et al., 1978; Yaksh et al., 1979; Gamse et al., 1980, 1981). Capsaicin also seems to release SP from human lymphocytes (Lai et al., 1998). Unfortunately, it is not known whether the release of SP by capsaicin from human lymphocytes is blocked by VR antagonists. Nevertheless, the similarity between capsaicin actions in sensory neurons and lymphocytes is striking.

V. Evidence for Multiple VRs

The spectrum of biological activities of vanilloid compounds show marked differences, which are difficult, if not impossible, to reconcile with the concept of a single VR but which are entirely consistent with the existence of multiple VRs. Szolcsányi and coworkers (Szolcsányi et al., 1990, 1991) compared the effects of capsaicin and RTX on the pulmonary chemoreflex in the rat. This reflex response involves three components, namely bradycardia, a slowing of respiration, and a drop in the systemic blood pressure, and is also known as the Bezold-Jarisch reflex (cf. Coleridge and Coleridge, 1986). Capsaicin evokes this reflex (Pórszász et al., 1955; Toh et al., 1955; Makara et al., 1967), which, unlike other capsaicin-induced responses, shows little or no desensitization upon repeated challenge (Szolcsányi et al., 1990). Interestingly, RTX shows the opposite behavior: it desensitizes the pulmonary chemoreflex with no prior activation (Szolcsányi et al., 1990). A likely explanation is that two receptors are involved, a receptor that is selective for capsaicin and mediates excitation, and another one that is selective for RTX and causes desensitization.

Another interesting observation was made by Appendino and coworkers (1996) when exploring the biological activities of a phorbol-base vanilloid, phorbol 12-phenylacetate 13-acetate 20-homovanillate (PPAHV; Fig. 8). PPAHV mimics most characteristic vanilloid actions in the rat (such as the induction of protective eye-wiping movements upon intraocular challenge or the down-regulation of specific RTX binding sites following systemic administration); however, it fails to induce hypothermia (Appendino et al., 1996). It was speculated that VRs expressed by neurons mediating vanilloid effects on temperature regulation are pharmacologically distinct and do not recognize PPAHV. [As we will see below, PPAHV is also interesting in that 1) it distinguishes pharmacologically distinct vanilloid-activated conductances in trigeminal ganglion neurons (Fig. 12; Liu et al., 1998) and 2) it binds to VRs in a noncooperative manner (Fig. 13; Szallasi et al., 1996b).]

Colquhoun and colleagues (Colquhoun et al., 1995; Griffiths et al., 1996) measured oxygen uptake and vasoconstriction in the isolated rat hindpaw and came to the conclusion that these responses were mediated by distinct peripheral VR subtypes, which they termed VN_1 and VN_2, respectively. Activation of VN_1 receptors stimulates oxygen uptake; this response is selectively inhibited by capsazepine (Griffiths et al., 1996). If agonist concentrations are further increased, VN_2 receptors are also occupied (Colquhoun et al., 1995). It leads to vasoconstriction and an inhibition of the initial oxygen uptake response. These responses are blocked by ruthenium red (Griffiths et al., 1996).

As yet, the strongest evidence for VR heterogeneity is furnished by electrophysiology, which gives a complicated picture of multiple targets, activated selectively by different vanilloids (Liu and Simon, 1996a,b; Liu et al., 1996, 1997, 1998; Petersen et al., 1996). Under voltage-clamp conditions, capsaicin and RTX evoke a number of currents that differ both in peak amplitude and duration (Fig. 14, A and B). At present, it is unclear how these conductances translate into VR subtypes. Nonetheless, the tendency of RTX to provoke slow, sustained currents (Fig. 14B) as opposed to the rapidly activating and dissipating capsaicin-induced fluxes (Fig. 14A) may give a rationale to explain why RTX, in general, is more potent for desensitization than activation of biological responses (Table 2).

PPAHV (see Fig. 8 for structure) operates two kinetically distinct conductances in cultured trigeminal ganglion neurons (Fig. 12) (Liu et al., 1998). The rapidly activating inward current disappears completely upon repeated PPAHV application, leaving a reduced slowly activating current (Fig. 12) (Liu et al., 1998). Interest-

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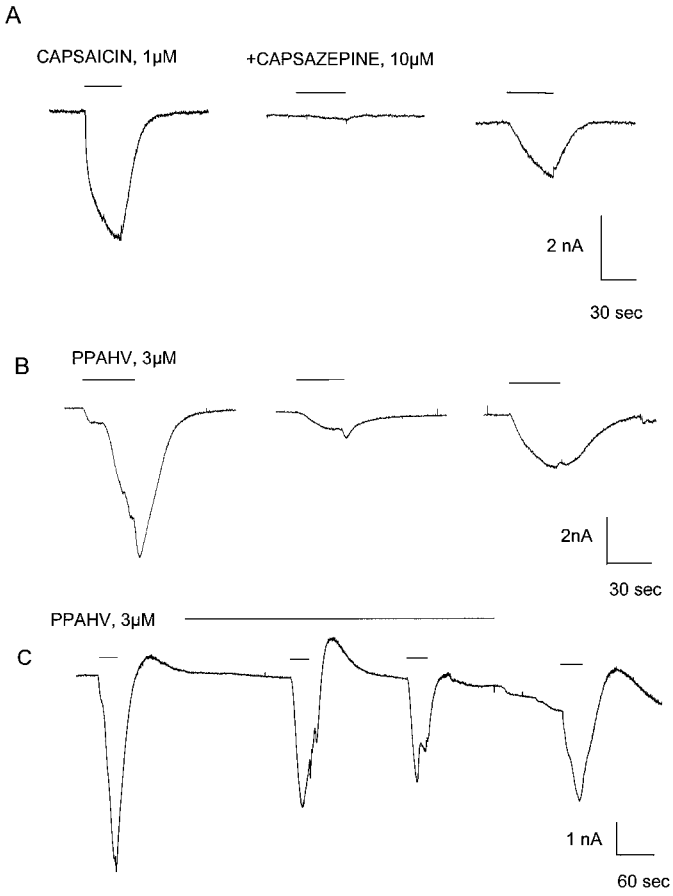

FIG. 12. Typical capsaicin- and PPAHV-induced currents in the absence or presence of capsazepine, a competitive VR antagonist. Capsazepine prevents capsaicin-evoked currents. PPAHV, however, elicits both capsazepine-sensitive and -insensitive currents, implying the existence of pharmacologically distinct VR subclasses. A modification of Fig. 4 in Liu et al., 1998. Reproduced with permission.

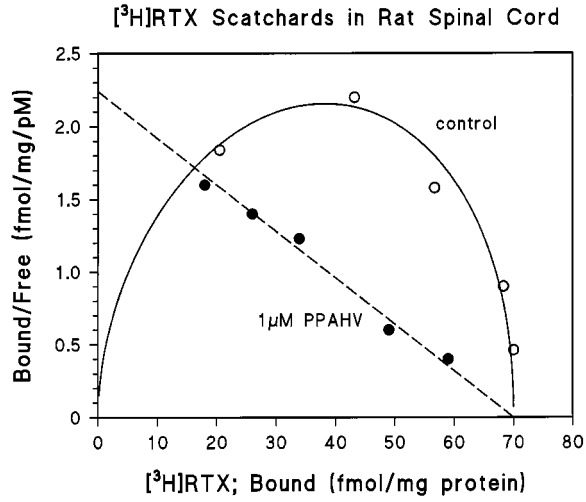

FIG. 13. PPAHV not only binds to VRs in a noncooperative manner but also abolishes positive cooperativity of [^{3}H]RTX binding (compare the convex Scatchard plot in the absence of PPAHV and the linear plot in the presence of PPAHV, respectively), implying that positive cooperativity is a ligand-induced property of VRs. Reprinted with permission from Szallasi et al., 1996b.

ingly, scutigeral, a novel VR agonist belonging to the triprenyl phenol class of vanilloids (see below), likewise abolishes the rapidly activating component of the capsaicin-evoked response, lending further support to the concept that rapidly and slowly activating conductances are pharmacologically different (Szallasi et al., 1999a). The rapidly and slowly activating channels also differ in reversal potentials (−5.8 mV and 10.4 mV) and have ED_{50}s for PPAHV of 2.7 μM and 0.9 μM, respectively. Even more interesting, capsazepine fails to inhibit a subset of PPAHV-evoked inward currents, especially of the slowly activating type (Fig. 12) (Liu et al., 1998). This latter finding may be interpreted as an indication of the existence of capsazepine-sensitive and -insensitive VRs.

The cloned VR1 when expressed in *Xenopus* oocytes has a reversal potential of −4 mV and is inhibited by capsazepine (Caterina et al., 1997). The similarity between VR1 and the PPAHV-gated rapidly activating current is obvious. It is tempting to speculate that the slowly activating current evoked by PPAHV represents another, yet-to-be cloned, VR1 isoform.

Whether these pharmacologically distinct vanilloid-activated conductances represent real isoforms, or, alternatively, reflect interaction with putative regulatory factors, remains to be seen. For instance, the DRG-specific form of proton-gated channels (called DRASICs) has been shown to form oligomers with other members of the degenerin/epithelial Na^+ channel superfamily, most notably with the m-degenerins MDEG1 and MDEG2 (Lingueglia et al., 1997). These DRASIC/MDEG1/MDEG2 heteropolymers display different kinetics, pH dependences, and ion selectivities (Lingueglia et al., 1997). As opposed to VR1 heterogeneity, a similar association of VR1 with other members of the TRP family of store-operated calcium channels (or other regulatory proteins) might provide an attractive alternative model to explain the diversity of vanilloid-evoked currents. Also, the existence of such oligomeric VRs would entirely be in accord with the radiation inactivation size (approximately 300 kDa) of VRs (Szallasi and Blumberg, 1991). At the time when this review was written, several groups were searching for VR1 isoforms, thus a satisfactory answer to the above question is anticipated in the foreseeable future. Finally, it has to be emphasized that VR1 homologs do not necessarily mediate heat sensitivity, pungent chemogenic activation, or acid sensitivity. The recognition domains for these types of activation are not well understood, and they may not be at all conserved. There may be an extended gene family, but there may be a very diverse biology associated.

VI. Biochemical Pharmacology of VRs

VRs show a variety of unusual features (Table 5) that should be taken into account when interpreting the differences among vanilloid actions. Some of these properties may even be of relevance for future drug development.

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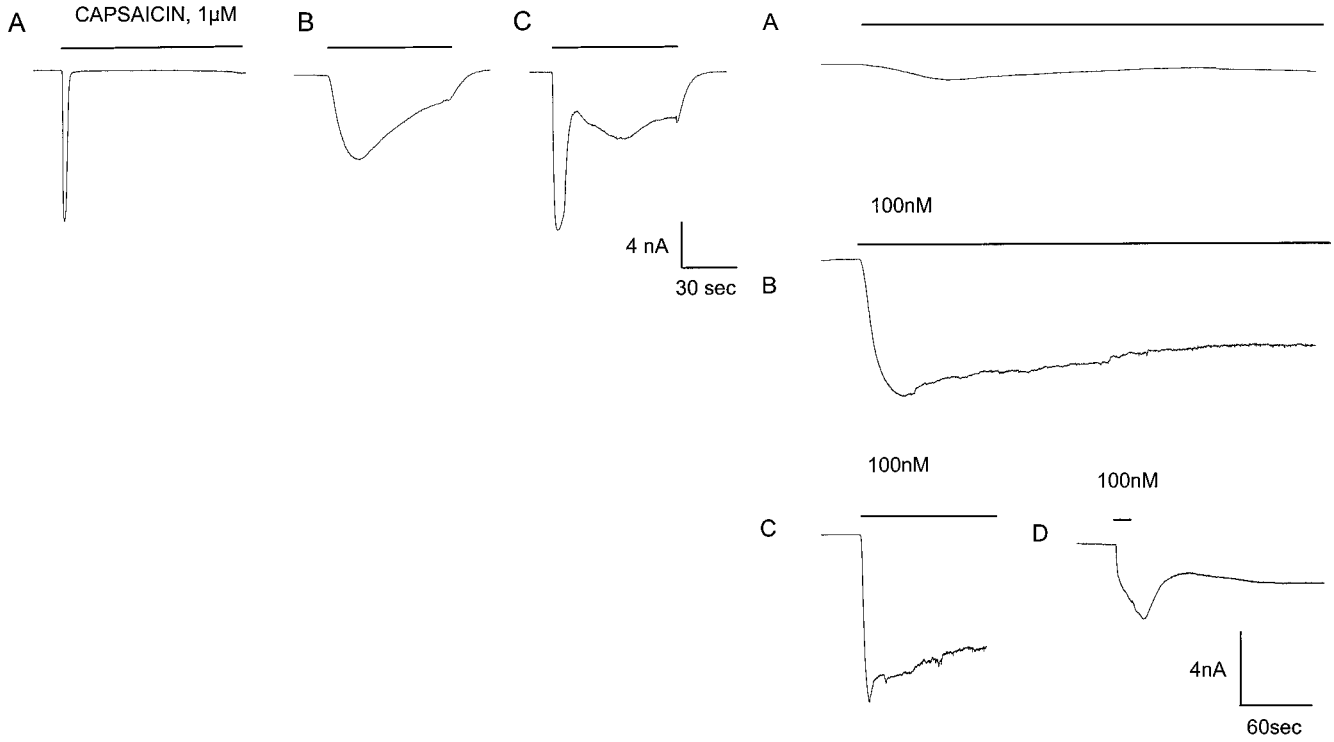

FIG. 14. Typical capsaicin- (A) and RTX-induced (B) currents in rat trigeminal ganglion neurons in culture. Bars represent the duration of vanilloid application. A, observe that capsaicin may elicit a rapidly activating current (A), a slowly activating current (B), or a combination of rapidly and slowly activating currents (C). B, a 2.5-nM concentration of RTX evokes a sustained current (A). An increase in the concentration of RTX to 100 nM enhances the amplitude of the current (B–D) but it remains persistent with a very slow return to baseline. Reprinted with permission from Liu and Simon, 1996a.

A. The Cloned VR Is a Nonselective Cation Channel with a Limited Selectivity for Calcium

The cloned VR1 does not discriminate among monovalent cations, however, it exhibits a notable preference for divalent cations (Caterina et al., 1997). The reported permeability sequence is as follows: $Ca^{2+} > Mg^{2+} > Na^{+} \approx K^{+} \approx Cs^{+}$. This finding is in agreement with previous observations in cultured DRG neurons (Wood et al., 1988) and thus lends further support to the long-held concept of the "capsaicin receptor" being a nonselective cation channel with a preference for calcium (cf. Bevan and Szolcsányi, 1990). The relative permeability of VR1 to calcium is high (the P_{Ca}/P_{Na} ratio is approaching 10) for a nonselective cation channel (Caterina et al., 1997). This calcium permeability is very similar to the value reported (P_{Ca}/P_{Na} = 10.6) for *N*-methyl-D-aspartate (NMDA)-type glutamate receptors (Mayer and Westbrook, 1987).

TABLE 5
Biochemical pharmacology of VRs

Receptor family	TRP family of store-operated calcium channels
Cloned member	VR1 (rat)
Signal transduction	non-selective cation channel
Size	95 kDa (cloned VR1); 300 kDa (radiation inactivation size)
Typical natural agonists	RTX, capsaicin, isovelleral, scutigeral
Other activators	low pH, noxious heat
Endogenous activators	low pH (?)
Antagonists	capsazepine (competitive); ruthenium red (functional)

B. Role of Calcium in Modulating VR Functions

For capsaicin, desensitization has been shown to depend on a variety of factors, including concentration, the duration of application, and the presence or absence of extracellular calcium (cf. Holzer, 1991; Szolcsányi, 1993). Mechanisms underlying desensitization will be discussed later; here we concentrate on the role of calcium only. Numerous studies have shown that the removal of extracellular calcium diminished desensitization to capsaicin (Santicioli et al., 1987; Amann, 1990; Craft and Porreca, 1992; Cholewinski et al., 1993; Garcia-Hirschfeld et al., 1995; Liu and Simon, 1996a). It was speculated that a rise in intracellular calcium served as an inital step only to activate biochemical pathways ultimately leading to VR desensitization. This model was reinforced by the findings that 1) specific inhibitors of protein phosphatase 2B (also known as calcineurin) reduced desensitization (Docherty et al., 1996), and 2) removal of ATP or GTP from the internal solution resulted in a nearly complete tachyphylaxis even in the presence of calcium (Koplas et al., 1997). Recent evidence implies an even more complex situation. In addition to the above calcium-activated indirect pathways of tachyphylaxis, a direct action of calcium on VRs leading to desensitization is also likely to exist. For example, the

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electrophysiological desensitization of VR1 expressed in *Xenopus* oocytes requires the presence of extracellular calcium (Caterina et al., 1997). In the absence of extracellular calcium, VR1 shows little or no tachyphylaxis in response to repeated capsaicin challenges. In the presence of calcium, capsaicin-evoked currents via VR1 have two distinct components, one desensitizing and one relatively constant upon repeated agonist applications (Caterina et al., 1997). Thus, the calcium dependence of vanilloid desensitization can be reproduced without a neuronal context.

To complicate matters even further, the role of calcium in modulating desensitization to vanilloids is also dependent on the agonist used. In contrast to observations by capsaicin (Fig. 15A), desensitization to olvanil (see Fig. 8 for structure) is apparently not influenced by the removal of extracellular calcium (Fig. 15B) (Liu and Simon, 1998). Under resting conditions, the channel pore of C-type receptors is closed. Agonist binding is likely to induce a conformational change in receptor protein leading to an opening of the conductance. According to a recent model by Sidney A. Simon, vanilloid-gated conductances cycle between open and closed states via various transitional states reflecting desensitization (Liu and Simon, 1996a). Tachyphylaxis can be viewed as the time required for the receptors to recover from these transitional states to the closed state in which the receptor is capable of ligand binding again. For capsaicin, Simon argues that calcium may increase the probability of a transition from the open state into a transitional, desensitized state. Alternatively, calcium may inhibit the recovery of the receptor from the desensitized states to the closed state. Either mechanism may explain how the removal of extracellular calcium can reduce desensitization to capsaicin (Fig. 15A). Simon speculates (Liu and Simon, 1998) that olvanil utilizes a different mechanism (maybe receptor internalization) to achieve desensitization, hence the indifference of desensitization to olvanil for calcium (Fig. 15B).

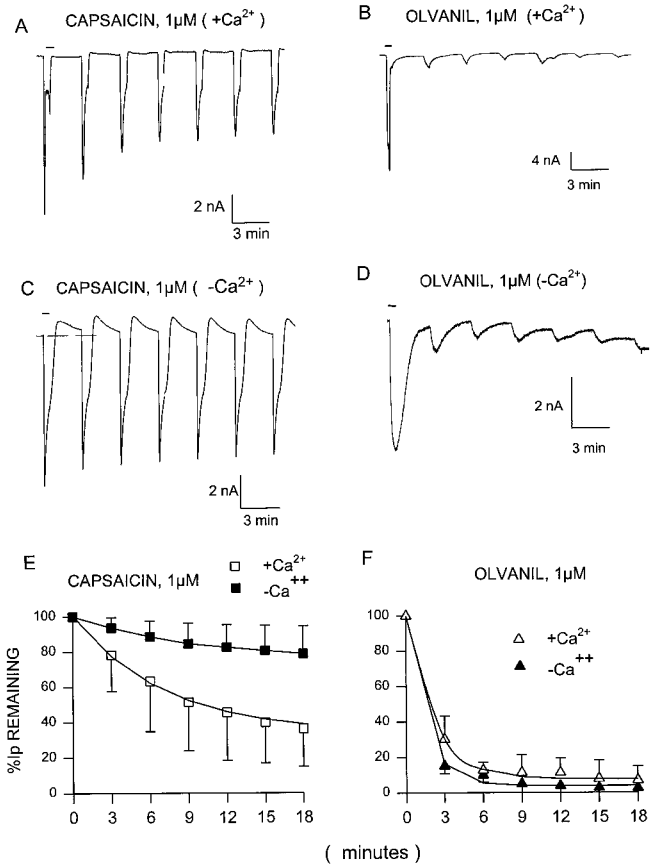

FIG. 15. Tachyphylaxis of capsaicin- and olvanil-induced currents in rat trigeminal ganglion neurons in culture. Tachyphylaxis to olvanil is rapid (the second application evokes less than 20% of the control current) and does not require the presence of extracellular Ca^{2+}. By contrast, tachyphylaxis to capsaicin develops gradually (the 4th challenge still elicits a half-maximal response) and occurs in the presence of extracellular Ca^{2+} only. A possible interpretation of these findings is that capsaicin and olvanil employ different biochemical mechanisms to achieve tachyphylaxis. Recordings are from Figs. 1, 3, and 4 in Liu and Simon, 1998. Reproduced with permission.

C. Where Is the Vanilloid Binding Site on VRs?

At present, the ligand recognition domain of VRs is not known, although site-directed mutagenesis studies with the VR1 clone will, no doubt, soon identify structures involved in agonist binding. Humphrey H. Rang suggested (Spring Pain Conference, Grand Cayman, BWI, 1998) that the capsaicin binding site is, in fact, intracellular. They added capsaicin to the bathing solution of voltage-clamped sensory neurons and to the buffer in the intracellular electrode, respectively, and found that lower capsaicin concentrations are required to activate the cells from the inside. Surprisingly, Julius and coworkers (Caterina et al., 1997) reported identical capsaicin responses from either side of a patch excised from a cell expressing VR1. Julius speculates that either capsaicin permeates the lipid bilayer freely or that there are functionally equivalent capsaicin binding sites on both sides of the plasma membrane (Caterina et al., 1997). The reason for the apparent contradiction between these two studies is unclear.

D. VR1 Is Activated by Noxious Heat and Low pH (Protons): the VR as an Integrator of Painful Chemical and Physical Stimuli

Protons have long been regarded as "small stimulants of capsaicin-sensitive sensory nerves" (Bevan and Geppetti, 1994). The similarity between heat-induced and capsaicin-evoked inward currents in isolated DRG neurons was also noted before (Baumann and Martenson, 1994; Cesare and McNaughton, 1996; Kirschstein et al., 1997). As discussed in *Section III.C*, an exciting aspect of the cloning of VR1 is the finding that both noxious heat (a rapid increase in temperature from 22 to 48°C) and low pH are able to activate the capsaicin-gated channel (Caterina et al., 1997; Tominaga et al., 1998). In fact, this may be the very reason why capsaicin is "hot-tasting" to humans. Thus, VR1 can be viewed as an integrator of painful chemical and physical stimuli. Probably it

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is heat only that has the power to open VR1—capsaicin and low pH merely serve to reduce the heat threshold of the receptor (Tominaga et al., 1998). Consequently, even room temperature is able to open VR1 under mildly acidic conditions.

Protons, however, exert a complex action on the cloned VR1. For instance, a reduction from 7.6 to 6.3 in the pH of the bath solution does not open VR1 expressed in oocytes; nonetheless, it results in a 5-fold increase in the amplitude of the current evoked by 300 nM of capsaicin (Caterina et al., 1997). This is consistent with the earlier observations that 1) low pH potentiated responses to low concentrations of capsaicin in rat (Petersen and LaMotte, 1993; Kress et al., 1996a), rabbit (Martenson et al., 1994), or human (Baumann et al., 1996) sensory neurons in culture (Fig. 16), and 2) low pH evoked a current in cultured trigeminal neurons that could be prevented by the VR antagonist capsazepine (Liu and Simon, 1994). A further reduction in the pH to 5.0, however, evokes a current through VR1 (Tominaga et al., 1998). Moreover, hydrogen ions can increase the response of VR1 to noxious heat (Caterina et al., 1997). Protons also inhibit RTX binding to DRG neuron or spinal cord membranes (Szallasi et al., 1995c). It is important to remember, however, that VR1 represents only one target—and not "the target"—for noxious heat or acids in sensory neurons.

E. Ruthenium Red Blocks VRs by an Unknown Mechanism

Ruthenium red is an inorganic dye that was introduced into sensory pharmacology in the late 1980s as a "functional capsaicin antagonist" (cf. Amann and Maggi, 1991). The molecular mechanism(s) by which ruthenium red blocks vanilloid actions is(are) unknown. Loris Chahl (1989) postulated two sites for ruthenium red, a reversible site inhibiting excitation by vanilloids and a second site that binds ruthenium red irreversibly and is involved in desensitization. By the mid-1990s, ruthenium red had largely been replaced by capsazepine, a competitive antagonist (see above). The interest in ruthenium red has been rekindled by a recent report (Ács et al., 1997) that this compound may be fairly selective for the RTX site on VRs. In addition, ruthenium red seems to inhibit VN_2 receptors preferentially, mediating an increase in perfusion pressure in the isolated rat hindpaw model (Griffiths et al., 1996).

F. VRs Are Sensitized by Inflammatory Mediators and Proinflammatory Cytokines

According to an emerging concept, hydrogen ions, heat, and capsaicin (or putative endogenous capsaicinoids) may act synergistically to activate VR1 (Caterina et al., 1997; Tominaga et al., 1998). Thus, a combination

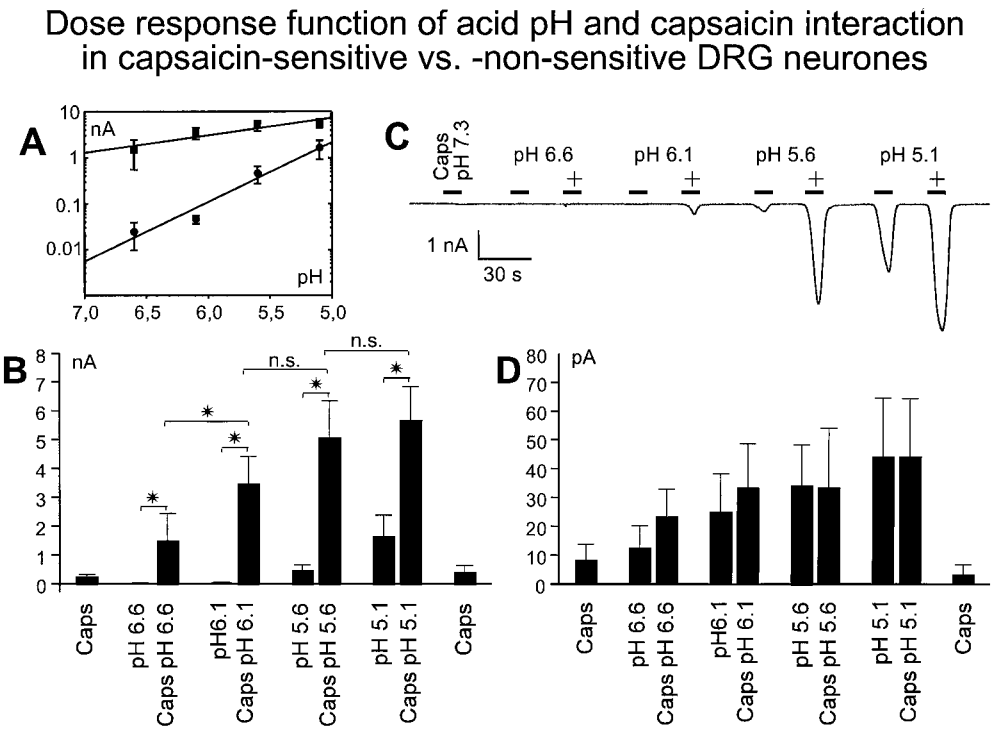

FIG. 16. Low pH faciliates the capsaicin-evoked inward current in rat DRG neurons in culture. A, dose-response curves for proton-activated currents in the absence (■) or presence (●) of 3 μM capsaicin. Note that at pH 7.3 capsaicin (3 μM) evokes a current with a mean amplitude of 365 pA (B). Mild acidification of the buffer (to pH 6.6) induces a current of 46 pA, which is enhanced to 1700 pA in the presence of capsaicin. Increasing the proton concentration together with 3 μM capsaicin results in a potentiation of the inward current that is more than additive for each pH value examined (B). C, an original recording: observe that the inward current in the presence of pH 5.6 and capsaicin greatly exceeds the sum of the currents evoked by pH 5.6 and capsaicin (at pH 7.3) alone. D, capsaicin (3 μM) evokes a negligible (smaller than 10 pA) current in large DRG neurons; this current is not facilitated by protons. Reproduced with permission from Kress et al., 1996a.

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of otherwise harmless heat and tissue acidosis may become very painful (Steen et al., 1995, 1996). In addition to protons, other ingredients in inflammatory exudates may also target VRs. Reeh and coworkers (Kress et al., 1997; Vyklicky et al., 1998) demonstrated that a combination of at least four inflammatory mediators, namely, bradykinin, histamine, serotonin, and prostaglandin E_2, act together to activate a conductance also operated by capsaicin. This action is prevented by capsazepine (Vyklicky et al., 1998). Peter W. Reeh speculates that these inflammatory mediators do not interact directly at VRs but rather converge on a secondary messenger pathway, which, in turn, sensitizes VR1 to heat or protons. Some of these inflammatory mediators like bradykinin can activate vanilloid-sensitive sensory neurons on their own (cf. Dray and Perkins, 1993); however, their action is more powerful in the presence of other mediators with which they act synergistically.

It has long been known that proinflammatory prostaglandins enhance the sensitivity of primary sensory neurons to noxious stimuli (Higgs et al., 1984; Salmon and Higgs, 1987). Prostaglandin E_2 increases the intracellular levels of cAMP in sensory neurons (Hingtgen et al., 1995). Moreover, sensitizing effects of prostaglandin E_2 on sensory neurons are mimicked by membrane-permeant cAMP analogs (Cui and Nicol, 1995). These observations imply a major role for the cAMP transduction cascade in the sensitization of vanilloid-sensitive neurons. In keeping with this, Lopshire and Nicol (1997, 1998) recently demonstrated the enhancement by prostaglandin E_2 of the capsaicin-elicited current in rat DRG neurons in culture (Fig. 17A), which was mimicked by forskolin (Fig. 17B).

Proinflammatory cytokines like tumor necrosis factor-α (TNFα) and interleukin-1β can also enhance the capsaicin sensitivity of rat DRG neurons in culture (Nicol et al., 1997). Following pretreatment with 10 ng/ml TNFα, a greater than 2-fold increase in the peak amplitude of the inward current evoked by 100 nM capsaicin was described (Nicol et al., 1997). This increase was prevented by either indomethacin or the specific cyclooxygenase-2 inhibitor, SC-236, implying a central role for neuronal prostaglandin production in sensitization to capsaicin (Nicol et al., 1997). Of relevance is the finding that intradermal injection of TNFα or interleukin-1β lowers the response threshold to noxious stimulation (Ferreira et al., 1988; Scheizer et al., 1988; Follenfant et al., 1989). The exact mechanism(s) by which cyclooxygenase-2 products potentiate capsaicin responses via VRs is(are) unknown.

A direct interaction at VRs has been suggested for a variety of irritant compounds, ranging from environmental pollutants to chemicals causing occupational asthma (cf. Maggi, 1991; Lundberg, 1993, 1995). These xenobiotics may induce conformational changes in VRs similar to those proposed for hydrogen ions and heat. Other compounds probably act on separate targets on sensory nerves but then sensitize VRs in a way similar to that suggested for inflammatory mediators.

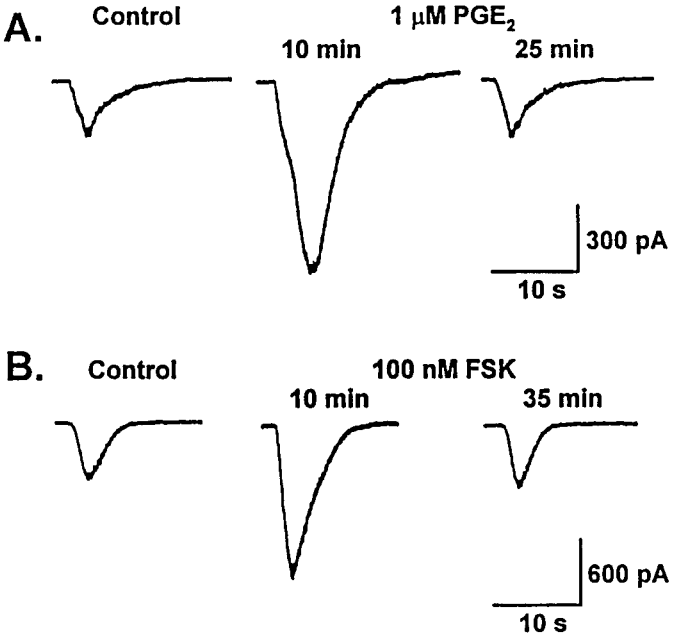

FIG. 17. Prostaglandin E_2 (PGE_2, 1 μM) enhances the amplitude of capsaicin-induced (control = 100 nM capsaicin) currents in rat embryonal DRG neurons in culture (A). The effect of PGE_2 is mimicked by forskolin (FSK), implying a central role for the cAMP transduction cascade in sensitization. A modification of Fig. 1 in Lopshire and Nicol, 1998. Reprinted with the permission of the Society for Neuroscience.

G. Aspirin and Related Drugs May in Part Exert Their Analgesic Actions by Blocking VRs

Reeh (Kress et al., 1996b) had another intriguing observation according to which aspirin and diclofenac, along with other commonly used nonsteroid anti-inflammatory drugs, can block VRs. This observation raises the possibility that the well known analgesic-anti-inflammatory actions of nonsteroid anti-inflammatory drugs are, at least in part, mediated by VRs. In human volunteers, topically applied acetylsalicylic acid was shown to attenuate capsaicin-induced pain and allodynia, probably by blocking cutaneous nociceptors (Schmelz and Kress, 1996).

H. Proposed Role of Phosphorylation Sites in Modulating VR1 Activity

Removal of ATP and GTP from the intracellular solution resulted in a state of nearly complete insensitivity to capsaicin even with intracellular calcium buffered to low levels (Koplas et al., 1997). One might argue, therefore, that sustained capsaicin-sensitivity requires VR1 to be phosphorylated via an ATP-dependent mechanism (please recall that VR1 has three predicted phosphorylation sites for protein kinase A). As a matter of fact, it has recently been demonstrated that the activity of VRs is reduced by dephosphorylation (Oh et al., 1998). By contrast, dephosphorylation by Ca^{2+}-dependent phosphatases such as calcineurin might be an important

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mechanism of receptor tachyphylaxis once VRs are opened and intracellular calcium begins to rise (Docherty et al., 1996).

As mentioned above, PKCγ is abundant in the inner layer of lamina II of the spinal dorsal horn, an area also rich in VR1-like immunoreactivity (Guo et al., 1999). As demonstrated by Basbaum and coworkers (Malmberg et al., 1997a), PKCγ knockout mice show an elevated threshold for chronic, neuropathic pain. Consequently, it was suggested that PKCγ-mediated phosphorylation of proteins in lamina II played a central role in the development of neuropathic pain. Although PKCγ is predominantly present in interneurons, it is not impossible that VR1-expressing nerve terminals also express PKCγ. If this assumption holds true, VR1 may be an attractive target to explore for phosphorylation by PKCγ.

I. Regulation of VR Expression

NGF is produced in the periphery from where it is transported intra-axonally to the cell bodies of DRG neurons (cf. Lewin and Mendell, 1993). In DRG neurons, NGF plays a central role in the regulation of gene expression. DRG neurons obtained from neonatal rats and cultured in the absence of NGF lose their sensitivity to capsaicin (Winter et al., 1988). In keeping with this, NGF has recently been shown to regulate the expression of VR1 mRNA in adult rat DRG neurons in culture (Helliwell et al., 1998). Interestingly, capsaicin sensitivity of nodose ganglion neurons is regulated by brain-derived neurotrophic factor and not by NGF (Winter, 1998). NGF production is enhanced during inflammation (cf. Lewin and Mendell, 1993). Neurons isolated from DRGs of rats with inflammation, however, do not show increased sensitivity to capsaicin (Hu-Tsai et al., 1996). Nor did we observe an increase in autoradiographic labeling of VRs with [^{3}H]RTX in lumbar DRGs of the rat with hindpaw skin inflammation (A. Szallasi, T. Farkas-Szallasi, and T. Hökfelt, unpublished observation). Taken together, these findings imply the existence of a NGF-response element in the gene(s) encoding VRs. VR expression, however, appears to be maximal in the presence of physiological NGF concentrations and cannot be enchanced by extra NGF.

J. VRs Are Thiol Proteins Displaying Positive Cooperativity

RTX binding to rat DRG neurons follows sigmoidal saturation kinetics (Szallasi et al., 1993a), resulting in convex Scatchard plots (Fig. 6). This binding behavior is consistent with the existence of multiple binding sites that cooperate: i.e., occupation of one site by a given ligand helps one or more additional molecules bind to additional site(s) (positive cooperativity). Dose-response relations for capsaicin-evoked calcium uptake by cultured sensory neurons (Ács et al., 1996b) or capsaicin-evoked currents detected under voltage-clamp conditions (Oh et al., 1996; Koplas et al., 1997) can also be fitted to the Hill equation with a cooperativity index of approximately 2. In keeping with this, capsaicin and RTX gate VR1 when expressed in *Xenopus* oocytes with Hill numbers of 2.08 and 1.95, respectively (Caterina et al., 1997), implying that the full activation of VR1 involves the binding of more than one agonist molecule. As mentioned above, PPAHV acts on two kinetically distinct conductances in sensory neurons under voltage-clamp conditions (Liu et al., 1998). PPAHV, however, activates both conductances in a noncooperative manner (Liu et al., 1998). Moreover, PPAHV not only binds to DRG membranes noncooperatively but also abolishes the positive cooperativity of RTX binding (Fig. 13; Szallasi et al., 1996b). These latter findings imply that positive cooperativity, or the lack of it, may be a ligand-induced property of VRs.

VRs appear to be thiol-proteins, inasmuch as heavy metals and other sulfhydryl reactive agents inhibit RTX binding (Szallasi and Blumberg, 1993a; Szallasi et al., 1993a) and block capsaicin-evoked ion fluxes (Wood et al., 1988). An unexplained finding is that cadmium, like vanilloids, produces a concentration-dependent contraction of the rat isolated urinary bladder, which is in cross-tachyphylaxis with the contractile response to capsaicin (Patacchini et al., 1988). Both reducing and oxidizing agents reduce the affinity as well as the positive cooperativity of RTX binding to DRG and spinal cord membranes (Szallasi et al., 1993a). This observation implies that maximal ligand binding is dependent on an optimal redox state of VRs.

VII. Requirements for Ligand Recognition by VRs: Typical and Novel Vanilloids

A. Structure-Activity Relations for Capsaicinoids

In the mid-1970s, Szolcsányi and Jancsó-Gábor (1975, 1976) attempted a systematic exploration of structure-activity relations for capsaicin-like activity. Based on the fairly strict structure-activity relations, they came to the astute and to-date valid conclusion that capsaicin effects are most likely mediated by a receptor (Fig. 18, upper panel). The authors used a test in which the protective wiping movements upon intraocular instillation provided a biological measure of capsaicin-like activity. This assay is easy to perform, but, according to our present knowledge, is difficult to interpret because pain is not a direct consequence of capsaicin binding to its receptor. Ion flux through the capsaicin-operated conductance needs to cause membrane depolarization sufficient to result in impulse (action potential) generation (cf. Bevan and Szolcsányi, 1990). Now we know that capsaicinoids differ not only in affinity for receptor binding (cf. Szallasi, 1994) but also in channel-gating kinetics (Figs. 12 and 14) (Winter et al., 1990; Liu and Simon, 1996a; Caterina et al., 1997; Liu et al., 1998). Current kinetics, in turn, has a marked effect on membrane depolarization and thus on pain perception. Therefore,

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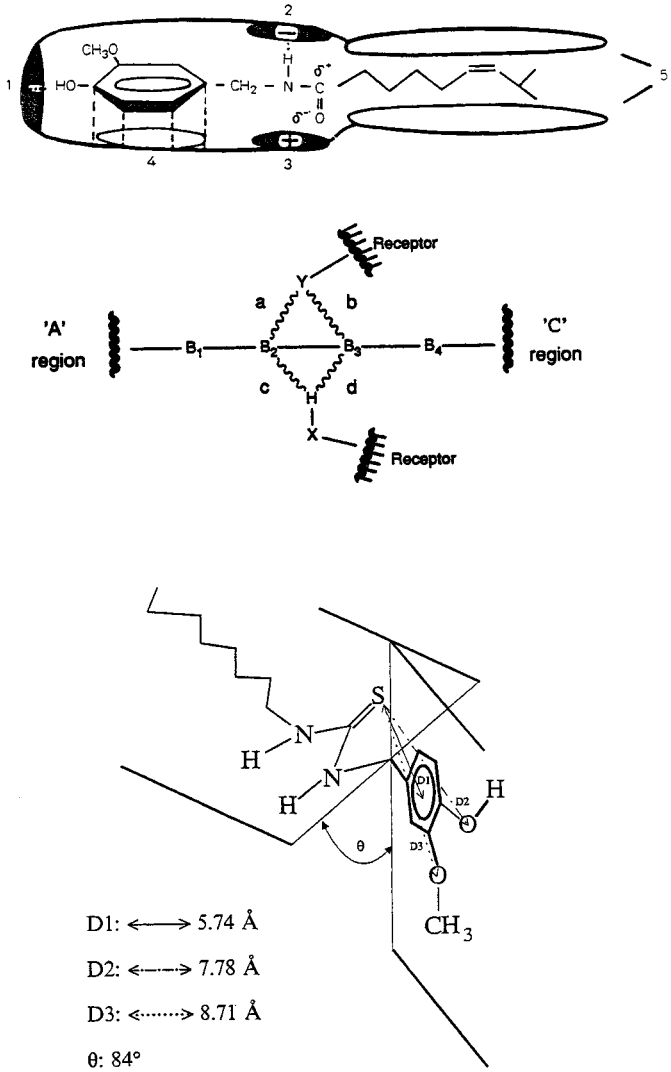

FIG. 18. VR models. The first model (upper drawing) is based on structure-activity relationships for the pungency of capsaicin congeners, as determined in the eye-wiping assay (Szolcsányi and Jancsó-Gábor, 1975). The second model (middle drawing) was constructed based on structure-activity relationships for capsaicinoid-induced Ca^{2+}-uptake by rat DRG neurons in culture (Walpole and Wrigglesworth, 1993). The three-dimensional model of the capsaicinoid pharmacophore was worked out by Klopman and Li (1995) using the measured Ca^{2+}-uptake values of Walpole and Wrigglesworth. Reproduced with permission.

there is no direct relationship between the affinity of a given capsaicinoid for VRs and the resulting pungency. A well known example of this phenomenon is olvanil (Fig. 8) (Brand et al., 1987), which induces calcium uptake by DRG neurons with a potency similar to that of capsaicin (Winter et al., 1993) but is nonpungent (Dray et al., 1990).

Important natural capsaicin congeners include piperine, zingerone, eugenol (Fig. 8), and guaiacol. Piperine and zingerone were identified as vanilloids decades ago (cf. Szolcsányi, 1982). Both piperine and zingerone show important dissimilarities in action to capsaicin (see below) and therefore they are frequently used tools to study the biology of VRs. Eugenol and guaiacol are obtained from oil of cloves, have a pungent taste, and are used as dental analgesics. Recently, Ohkubo and colleagues (Ohkubo and Kitamura, 1997; Ohkubo and Shibata, 1997) demonstrated the existence of two separate targets for eugenol in DRG neurons. One receptor is a Ca^{2+}-permeable channel blocked by capsazepine (that is, probably a VR), whereas the other one seems to be a chloride channel (Ohkubo and Kitamura, 1997). When given intrathecally, both eugenol and guaiacol exert antinociceptive activity, as demonstrated by the inhibition of acetic acid-induced writhings in the mouse (Ohkubo and Shibata, 1997). This antinociceptive effect does not occur in the presence of capsazepine. These results suggest that eugenol and guaiacol are analgesic by desensitizing sensory nerve endings in the tooth pulp in a VR-mediated fashion.

Olvanil (Fig. 8) and nuvanil are synthetic vanilloids coming from a program at Procter & Gamble (discontinued in the late 1980s) aimed at exploring structural requirements for capsaicin-like activity (Brand et al., 1987). Another major effort to understand capsaicin mechanisms and to synthesize novel capsaicinoids devoid of the irritancy of capsaicin was launched at the Sandoz (now Novartis) Institute for Medical Research, London. Christopher Walpole, Roger Wrigglesworth, and coworkers established comprehensive structure-activity relationships for capsaicin analogs (cf. Walpole and Wrigglesworth, 1993). As a biological assay, they used calcium uptake by cultured DRG neurons, which is thought to be a direct consequence of VR activation. They analyzed capsaicin structure-activity requirements in terms of three functional regions, an aromatic A region (Walpole et al., 1993a), an aliphatic C region (Walpole et al., 1993c), and the ester or amide linker, referred to as the B region (Walpole et al., 1993b), between the A and C regions (Fig. 19). Their most important findings may be summarized as follows: 1) a parent homovanillyl (3-methoxy 4-hydroxybenzyl) group is optimal in the A region; 2) a dipolar amide or thiourea in the B region is beneficial (but an ester is also adequately tolerated); and 3) a lipophilic octanyl or *p*-chlorophenethyl moiety in the C region is associated with the highest potency. A two-dimensional model was proposed to rationalize the profile of compounds that differ in the B region, based on consideration of multiple hydrogen bonding interactions (Fig. 19, middle panel) (Walpole and Wrigglesworth, 1993).

In 1995, Klopman and Li (1995) used a MULTICASE (Multiple Computer Automated Structure Evaluation) method to delineate structural features essential for the activation of VRs using a database of 123 capsaicin analogs compiled from the publications of Walpole and coworkers (Walpole et al., 1993a,b,c; Walpole and Wrigglesworth, 1993). After the cluster analysis, MULTICASE identified three structural motifs (biophores I–III) with high probability of relevance (Fig. 19). Biophore I (present in the A region; see Fig. 19) seems to be the most significant fragment, which alone could ac-

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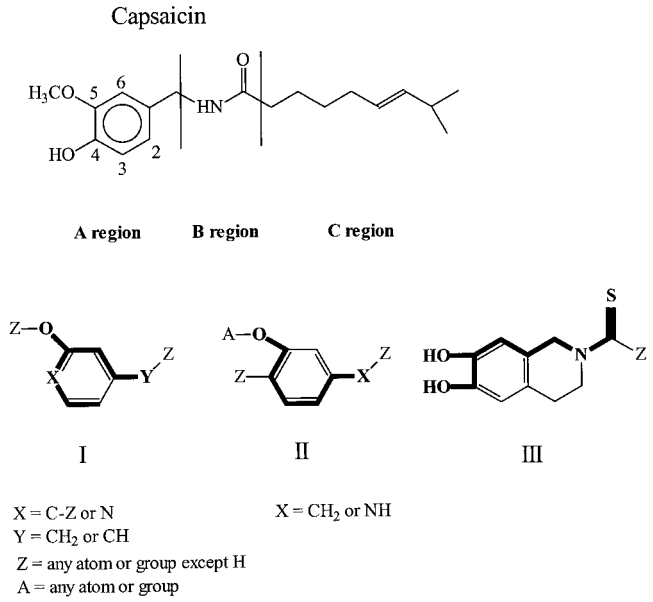

FIG. 19. Functional regions of the capsaicin pharmacophore: an aromatic A region, an aliphatic C region, and the ester or amide linker, referred to as the B region, between A and C regions. Biophores identified by the Multiple Computer Automated Structure Evaluation program (Klopman and Li, 1995) for capsaicinoid-induced Ca^{2+}-influx response. See *Section VII.A.* for details. Reproduced by permission.

count for 76% of the active compounds in the database. Compounds with a 3-alkoxy-4-substituted benzyl ring have the highest probability of being active. Although biophore I does not specify the substituent requirement at the 4 position, it was noted that 66 of the 70 ligands containing this biophore have a hydroxyl group at this position. This is entirely consistent with the observation that the removal of the phenolic OH group at the 4 position of the benzene ring leads to a loss of capsaicin-like activity. Substitution of the hydrogens at positions 2, 5, or 6 also results in an abolition of agonist activity. Biophore II (Fig. 19) is similar to biophore I, because it contains a hydroxyl group at the 3 position and a NH group next to the phenyl ring. Biophore III (Fig. 19) constitutes an α,α-catechol moiety in the A region. In this case, A and B regions are constrained by a fusion ring. Based on the MULTICASE analysis, the authors have worked out a three-dimensional model of capsaicin-receptor interactions (Fig. 18, lower panel). For further details, interested readers are referred to the article.

Capsaicin induces $^{45}Ca^{2+}$ uptake by cultured DRG neurons with an EC_{50} value in the range of 200 to 300 nM (Wood et al., 1988; Ács et al., 1996b). Although several synthetic capsaicin analogs approach the potency of capsaicin, only a few are more active (cf. Walpole and Wrigglesworth, 1993). The improvement in the potency of capsaicinoids in the calcium uptake assay is very moderate, at best 5-fold (see compound 57 in Fig. 8).

A systematic comparison of the activities of capsaicin analogs in the calcium assay versus their potencies for inhibiting RTX binding is yet to be performed. Winter and coworkers (Winter et al., 1993) examined five capsaicin analogs and found them from 13- to 60-fold less potent for inhibiting binding than for inducing calcium uptake (Table 6). The finding that capsaicin is at least 10-fold less potent in the RTX binding assay than in the calcium uptake measurements (Winter et al., 1993) has been confirmed repeatedly (Ács et al., 1996b, 1997).

B. Structure-Activity Relations for Resiniferanoids for Inducing Calcium Uptake by Sensory Neurons

For comparison with capsaicinoids, RTX (Fig. 1) may likewise be dissected into three regions (Walpole et al., 1996). Regions α and β are similar to the corresponding regions (A and B) in capsaicin; nevertheless, structure-activity relations for these two regions in capsaicinoids and resiniferanoids show important differences. The diterpene skeleton (χ region) in resiniferanoids is much more complex than the relatively simple aliphatic C region of capsaicin and appears to play a far more important role in receptor recognition as well.

As we saw above, all of the three biophores identified by the MULTICASE analysis in capsaicinoids represent the A region; the B region modifies the activity set by the A region biophore, and the importance of the C region seems to lie mostly in determining lipophilicity (Klopman and Li, 1995). Generally speaking, resiniferanoids tolerate substitutions in the phenolic ring (α region) better than capsaicinoids but are very sensitive to modifications of the diterpene pharmacophore (χ region). The phenolic OH group, which is critical for the activity of capsaicin analogs (A region) (cf. Walpole and Wrigglesworth, 1993), is of little importance in RTX

TABLE 6
Distinct structure activity relations for VR binding and subsequent calcium uptake

Ligand	Binding	Ca Uptake	Relative Potency
	K_i, nM	*EC_{50}, nM*	*binding vs. Ca uptake*
Resiniferanoids (1–4)			
Orthobenzoyl-RTX	0.17	7.6	44.00
RTX	0.04	0.9	22.00
ROPA-20-dimethoxyphenylacetate	1.00	15.0	15.00
3β-Hydroxy-RTX	4.40	57.5	13.00
Tinyatoxin	0.17	1.5	9.00
ROPA-20-[4-aminoethoxy-3-methoxyphenylacetate]	4.30	13.0	3.00
RTX-thiourea	69.00	150.0	2.00
ROPA-20-phenylacetate	8.30	10.2	1.20
RTX-amide	26.00	26.5	1.00
Capsaicinoids (2, 5)			
Capsaicin	4,900	340	0.07
Vanillyl-octylthiourea	8,500	150	0.02
Olvanil	340	180	0.02
Phorboid vanilloids (6, 7)			
PPAHV	3,100	1,800	0.60
PDDHV	>10,000	60	<0.002
Terpenoid 1,4-unsaturated dialdehydes (8)			
Isovelleral	5,200	95	0.02

References indicated in parentheses: (1) Ács et al., 1995; (2) Ács et al., 1996b; (3) Lee et al., 1995; (4) Walpole et al., 1996; (5) Winter et al., 1993; (6) Szallasi et al., 1996b; (7) Szallasi et al., 1998c; (8) Szallasi et al., 1996a.

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analogs (α region) for Ca^{2+} uptake (Walpole et al., 1996). Changing the ester link to amide, which is adequately tolerated in capsaicin (B region), results in a 30- to 100-fold drop in activity in RTX analogs, implying the importance of the β region (Ács et al., 1996b; Walpole et al., 1996). With regard to the diterpene moiety (χ region), reduction of the 3-keto group in RTX leads to a significant loss of activity (Walpole et al., 1996). The inactivity of the simplified RTX analog described by Bloomfield and coworkers (Bloomfield et al., 1992), which contains the phenylacetyl ortho ester moiety but not the fused 5- and 7-member rings, is consistent with the notion that the diterpene moiety in resiniferanoids plays a far more important role than being a simple hydrophobic anchor.

C. Differences in Structure-Activity Relationships of Vanilloids for Receptor Binding and Calcium Uptake

Whereas capsaicinoids seem to be consistently less potent for inhibiting RTX binding than for inducing $^{45}Ca^{2+}$ (Winter et al., 1993; Ács et al., 1996b), RTX structural analogs differ greatly in relative potency for these responses (Table 6). One extreme is RTX (Fig. 1), which is approximately 25-fold more potent for binding than in the calcium uptake assay (Ács et al., 1996b). The other extreme is phorbol 12,13-didecanoate 20-homovanillate (PDDHV; Fig. 8), which evokes calcium uptake by adult rat DRG neurons in culture with an affinity of 15 nM (that is, it is 20-fold more active in this assay than capsaicin), but fails to inhibit [^{3}H]RTX binding by these cells up to the concentration of 10,000 nM (Table 6) (Szallasi et al., 1998a). In the middle, we have RTX-amide (Ács et al., 1995), RTX-thiourea (Lee et al., 1995), and PPAHV (Fig. 18) (Appendino et al., 1996): these compounds display similar potencies in the binding and calcium uptake assays (Table 6) (Ács et al., 1996b; Szallasi et al., 1996b). These findings were originally interpreted in terms of separate VR subtypes mediating binding and calcium uptake (cf. Szallasi and Blumberg, 1996; Bíró et al., 1998). Recent evidence, however, suggests that it is not the case: HEK293 cells transfected with VR1 cDNA bind vanilloids with parameters similar to those described in native rat DRG neurons (A. Szallasi, D. N. Cortright, P. M. Blumberg, and J. E. Krause, manuscript in preparation). One explanation is that VR1 has two separate, although overlapping, binding domains for resiniferanoids and capsaicinoids, respectively. PDDHV is unique in that it binds exclusively to the portion of the capsaicin site that does not overlap with the RTX recognition domain. In a much simplified manner, the capsaicin domain is more efficient in opening the channel pore, whereas activation of the RTX site predominantly leads to tachyphylaxis. Alternatively, the distinct pharmacology for binding and calcium uptake could reflect different receptor conformations.

D. Why Is RTX Ultrapotent as a Vanilloid?

RTX has a relatively rigid diterpene skeleton, to which two flexible aromatic moieties are bound. Although the presence of the aryl ring is essential in RTX (but, as we will see later, not in novel "vanilloid" classes of terpenoid unsaturated dialdehydes and triprenyl phenols) for vanilloid-like activity, it tolerates chemical substitutions unexpectedly well. Recent evidence suggests an equally important role for the orthoester functionality in RTX. PPAHV is very similar to RTX (cf. Figs. 1 and 8) but possesses a phenylacetate group at the 12 position. In its pharmacological properties, PPAHV differs from RTX in four important aspects: 1) PPAHV is 60,000-fold less potent than RTX for binding; 2) PPAHV binds to cultured DRG neurons and induces calcium uptake by them with similar affinities; 3) PPAHV binds in a noncooperative manner (Fig. 13); and 4) PPAHV is devoid of the characteristic hypothermic action of RTX (Appendino et al., 1996; Szallasi et al., 1996b). At the electrophysiological level, RTX- and PPAHV-evoked currents in DRG neurons in culture differ both in onset and duration (cf. Figs. 12 and 14) (Liu et al., 1998). Furthermore, unlike RTX, PPAHV elicits both capsazepine-sensitive and -insensitive currents (Fig. 12) (Liu et al., 1998).

In polar solution, the aromatic moieties of RTX show a pronounced clustering (Victory et al., 1998). This phenomenon is known as hydrophobic collapse. Vander Velde (Victory et al., 1998) and colleagues reasoned that the clustering of the orthophenylacetate group may facilitate the attainment of an optimal aligment between the vanillyl moiety and the diterpene core, necessary for high-affinity receptor binding. On the other hand, the necessarily different alignment of the orthoester phenyl ring in resiniferonol 9,13,14-orthobenzoate 20-homovanillate, which retains binding affinity and shows only modest loss of potency for Ca^{2+} uptake, argues against this model.

E. Novel Vanilloids Lacking 3-Hydroxy 4-methoxyphenyl (Vanillyl) Functionality

1. Sesquiterpene Unsaturated 1,4-Dialdehydes and Related Bioactive Terpenoids. To date, approximately 80 terpenoids containing an α,β-unsaturated 1,4-dialdehyde (3-formyl 3-butenal) functionality have been isolated from natural sources (cf. Jonassohn and Sterner, 1997). The majority of these compounds are present in terrestrial plants and fungi. However, algae, liverworts, arthropods, sponges, and molluscs are included among the natural sources of unsaturated 1,4-dialdehydes. In general, these compounds are believed to form a multifaceted chemical defense system that protects the producing organism from parasites and predators (Kubo and Nakanishi, 1979; Kubo and Ganjian, 1981; Camazine et al., 1983; Cimino et al., 1983; Caprioli et al., 1987; Vidari et al., 1997). Because these attacking or-

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ganisms may range from bacteria to mammals, it is hardly surprising that most of these unsaturated dialdehydes exert a very broad spectrum of bioactivities (cf. Jonassohn and Sterner, 1997).

A prominent representative of unsaturated dialdehydes is warburganal, isolated from the bark of *Warburgia ugandensis* and *W. stuhlmannii*, two tropical trees growing in East Africa (Kubo and Nakanishi, 1979). Warburganal has antifungal, antibacterial, and phytotoxic activities (cf. Anke and Sterner, 1991; Jonassohn, 1996). Moreover, it is antifeedant to nematodes (Kubo et al., 1976) and is hot-tasting to humans (Kubo and Ganjian, 1981). Native tribes use the bark of warburgia trees as a spice to flavor food (Watt and Breyer-Brandwijk, 1962). Along with warburganal, another unsaturated dialdehyde, polygodial, is also present in water pepper (*Polygonum hydropiper*) (Fukuyama et al., 1982). At one time water pepper was used as a pepper substitute in Europe and its sprout, called "mejiso" or "benitade" in Japanese, is still a popular relish for "sashimi" (raw fish) (Fukuyama et al., 1982). The extract of *Cinnamosma fragrans*, a native plant of Madagascar, which contains several sesquiterpenes (e.g., cinnamolide, cinnamodial, and cinnamosmolide), was described as having a "distinct pepper-like taste" (Canonica et al., 1969). The similarity between the pungent sensation evoked in the human tongue by capsaicin and isovelleral (compare structures in Fig. 1), isolated from the hot mushroom *Lactarius vellereus*, was also noted (List and Hackenberger, 1973). Despite these telling observations, it was not until 1996 that the possibility that unsaturated dialdehydes may be pungent by activating VRs was investigated.

Like capsaicin, the fungal terpenoid isovelleral causes protective eye-wiping movements in the rat upon intraocular instillation (Szallasi et al., 1996a) There is cross-tachyphylaxis between capsaicin and isovelleral actions both in the rat eye and the human tongue (Szallasi et al., 1996a). Isovelleral induces calcium uptake by rat DRG neurons in culture (Table 6), which is fully inhibited by the competitive VR antagonist capsazepine (Szallasi et al., 1996a). Furthermore, isovelleral inhibits [^{3}H]RTX binding by rat trigeminal ganglion or spinal cord membranes (Table 6) consistent with a competitive mechanism (Szallasi et al., 1996a). Taken together, these findings strongly suggest that isovelleral is pungent by activating VRs on sensory neurons. For a series of 14 terpenoids with an unsaturated 1,4-dialdehyde moiety, a good correlation was found between pungency on the human tongue and affinity for VRs in the rat spinal cord (Szallasi et al., 1996a).

However, as expected from their reactive nature, dialdehyde sesquiterpenes and other terpenoids possess additional sites of action, as reflected in the complex behavior of the calcium uptake responses induced by cinnamodial and cinnamosmolide (Szallasi et al., 1998a). At low concentrations, cinnamodial and cinnamosmolide evoke calcium uptake in a dose-dependent manner, which is superceded by a blockade of the response at higher concentrations. The separation between cinnamodial concentrations causing stimulation and block of the calcium influx, respectively, is incomplete, which makes cinnamodial only a partial agonist (Szallasi et al., 1998a). This observation may also explain the unexpectedly weak membrane depolarization by cinnamodial compared with capsaicin under current-clamp conditions (Szallasi et al., 1998a).

At the whole animal level, polygodial inhibits the pain response evoked by intradermal formalin or capsaicin injection in the mouse and it also blocks acetic acid-induced writhings (Mendes et al., 1998). Moreover, polygodial has antiallergic and anti-inflammatory activities (Tratsk et al., 1997). At present, the role played by vanilloid-sensitive neurons in these beneficial effects of polygodial is unclear. Polygodial has a supraspinal antinociceptive action mediated by opioid and/or serotoninergic mechanisms (J. B. Calixto, personal communication). Furthermore, polygodial blocks tachykinin NK-2 receptors (El Sayah et al., 1998), which might play a role in its antiallergic and anti-inflammatory actions. Polygodial appears to be an interesting new lead for drug development, inasmuch as it targets a variety of pathways involved in pain perception and inflammation.

2. Triprenyl Phenols as Vanilloids. The archetypal triprenyl phenol is scutigeral (Fig. 1), isolated from *Albatrellus ovinus* (Dekermendjian et al., 1997). Unlike terpenoid unsaturated dialdehydes, scutigeral is not pungent (Szallasi et al., 1998b). As a matter of fact, *A. ovinus* is a delicious mushroom often used by the food industry as a substitute for truffles. Scutigeral and related compounds were first isolated based on their affinity for dopamine D1 receptors (Dekermendjian et al., 1997). Scutigeral induces calcium uptake by rat DRG neurons in culture and blocks RTX binding to rat spinal cord membranes (Table 6). Calcium uptake by scutigeral is prevented by both capsazepine and ruthenium red (Szallasi et al., 1999a). Taken together, these observations are consistent with scutigeral being a vanilloid. The finding that scutigeral is nonpungent is surprising but hardly unprecedented. Olvanil is also considered nonpungent (Brand et al., 1987; Dray et al., 1990), although it mimics most capsaicin responses (Brand et al., 1987; Dray et al., 1990; Wrigglesworth et al., 1996). Interestingly, pretreatment with scutigeral abolishes the first, rapidly activating current elicited by a subsequent capsaicin challenge, leaving the second, slowly activating current relatively intact (Szallasi et al., 1999a). This latter finding implies that scutigeral should be able to selectively block capsaicin responses mediated by the rapidly activating conductance. This hypothesis is currently being investigated.

3. Implications of the Discovery of Novel Vanilloids Lacking a Vanillyl-Like Functionality. Terpenoid unsaturated dialdehydes and triprenyl phenols are not real

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vanilloids from a chemical point of view (compare structures in Fig. 1), implying that the term VR is somewhat of a misnomer. Receptors are preferentially named after their endogenous activators. Identification of such endogenous activator(s) of "vanilloid" receptors will ultimately give these receptors a rational name. From the perspective of drug discovery, the demonstration that the presence of a vanillyl functionality is not essential for vanilloid-like activity opens up new possibilities. In collections of natural products and/or compound libraries of pharmaceutical companies, many interesting vanilloids may be hidden.

VIII. Vanilloid Mechanisms

Binding of vanilloids to their receptors initiates a complex, and, as yet, poorly understood cascade of intracellular events, which, for practical purposes, can be divided into three separate (but not independent) phenomena, namely 1) excitation, 2) desensitization, and 3) neurotoxicity (cf. Nagy, 1982; Buck and Burks, 1986; Holzer, 1991; Wood, 1993).

A. Excitation by Vanilloids

1. Stimulation of Vanilloid-Sensitive Neurons and Its Consequences. Agonist binding to the vanilloid-operated nonspecific cation channel opens the channel pore and leads to cation, predominantly calcium influx (Marsh et al., 1987; Wood et al., 1988). This cation influx may cause membrane depolarization (cf. Bevan and Szolcsányi, 1990). When membrane depolarization reaches the threshold level, an action potential is generated (cf. Bevan and Docherty, 1993). The action potential is propagated along the entire length of the vanilloid-sensitive neuron and may be perceived as itch or pain in the CNS (cf. Holzer, 1991). It is not known exactly how the painful information is transmitted from the central terminals of the vanilloid-sensitive neurons to second-order neurons of the dorsal horn. Vanilloid-sensitive neurons use glutamate, ATP, and a variety of neuropeptides as transmitters (cf. Holzer, 1991; Yaksh and Malmberg, 1994; Lundberg, 1996). It is likely that not a single transmitter, but rather a combination of them, play roles in pain transduction, because none of the tested receptor antagonists alone could achieve complete analgesia (cf. Dray and Urbán, 1996).

It should be noted, however, that the selective and competitive NMDA receptor antagonists D(−)-2-amino-5-phosphono-valeric acid and (±)-3-(2-carboxypiperazine-4-yl) propyl-1-phosphoric acid are very effective at inhibiting the acute nociceptive response to intraplantar injection of capsaicin in the mouse (Sakurada et al., 1998). The noncompetitive NMDA receptor antagonist MK-801 is likewise effective (Sakurada et al., 1998). Even more important, injection of NMDA into the cerebrospinal fluid of the rat mimics both acute pain and the subsequent hyperalgesia and allodynia that develop following intradermal capsaicin injection (also see below) (Aanonsen and Wilcox, 1987). It is known that both capsaicin and RTX can release glutamate from the rat spinal dorsal horn (Kangrga and Randic, 1991), which, in turn, may excite about 85% of spinal dorsal horn neurons, those that possess NMDA receptors (Murase et al., 1989; Tölle et al., 1993). These findings present a strong case for presynaptic NMDA receptors (Liu et al., 1994) on central vanilloid-sensitive nerve terminals being centrally involved in the facilitation of pain transmission.

Initial reports described a single vanilloid-evoked current in sensory neurons (Heyman and Rang, 1985; Forbes and Bevan, 1988). Recent studies, however, have found multiple currents (Liu and Simon, 1994; Liu et al., 1996; Petersen et al., 1996) that seem to differ not only in kinetics (Figs. 12 and 14) but also in affinity for agonists and in sensitivity to the antagonist capsazepine (Fig. 12) (Liu et al., 1998). A likely explanation for this phenomenon is the existence of VR1 isoforms and/or VR subtypes.

Agonist binding to VRs, however, does not necessarily lead to neuronal excitation. Several vanilloids [for example, olvanil (Dray et al., 1990) and scutigeral (Szallasi et al., 1999a)] have been noted for their nonpungent nature. It is thought that pungent and nonpungent vanilloids differ in their channel-gating properties (Liu et al., 1997). Pungent vanilloids, like capsaicin, open the conductance promptly (Figs. 12 and 14) and cause a massive cation influx. Nonpungent vanilloids, by contrast, open the channel pore slowly (Figs. 12 and 14) and the resulting, subdued cation influx is not sufficient to generate action potentials.

The mechanisms by which vanilloids liberate neurotransmitters are not completely clear, either. Originally, it was postulated by Maggi and colleagues (Maggi et al., 1988) that capsaicin releases neuropeptides via two independent mechanisms, one of which is sensitive to both tetrodotoxin (TTX) and ω-conotoxin (CTX), and another that is resistant to these toxins. TTX and CTX block voltage-sensitive Na^+ channels and voltage-dependent N-type Ca^{2+} channels, respectively. The observation that both TTX and CTX inhibit sensory neuropeptide release by capsaicin implies a central role for action potential generation and is in accord with the classical axon reflex theory (see above). Furthermore, the finding that TTX and CTX achieve only a partial blockade of the neuropeptide release implies a direct role for VRs in this response. The TTX- and CTX-resistant neuropeptide release by capsaicin requires the presence of extracellular calcium (Gamse et al., 1981). It is easy to visualize how rising intracellular calcium concentrations may lead to a fusion of vesicles, in which neuropeptides are stored, with the plasma membrane, resulting in exocytosis.

In 1996, Lundberg (1996) reevaluated this model and came to the conclusion that these mechanisms are not really independent but are related to the capsaicin concentration used. At low (10^{-8} M) concentration, capsa-

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icin occupies only a fraction of VRs and thus the resulting calcium influx is moderate. The increase in intracellular calcium is sufficient to generate action potential formation but not massive exocytosis. Neuropeptide release occurs via TTX- and CTX-sensitive mechanisms (Kröll et al., 1990; Lou et al., 1991, 1992. At high (10^{-6} M) concentration, capsaicin occupies most the receptors and causes a massive calcium influx via the channel pore. At this stage, the axon reflex mechanism is of minor importance (because N-type Ca^{2+} channels are already blocked by the high intracellular calcium levels) and the calcium-mediated exocytosis plays the major role in neuropeptide release (Lou et al., 1992).

Most recently, Szolcsányi (Szolcsányi et al., 1998a) questioned the role of the axon reflex in capsaicin-evoked neuropeptide release. His colleagues examined in parallel the release of three neuropeptides, SP, CGRP, and somatostatin, from rat trachea induced by capsaicin in the concentration range of 10^{-8} to 10^{-5} M. They found that none of the channel blockers, TTX, CTX, agatoxin, and lidocaine, inhibited release at any of the capsaicin concentrations tested. Consequently, Szolcsányi postulated that "the release sites of sensory neuropeptides serve also as sensory receptors". This hypothesis has gained recent support by the demonstration in the laboratory of Robert Elde (Guo et al., 1999) that VR1 is associated with small clear vesicles in nerve endings. The cause of the contradiction between studies by the groups of Maggi (Maggi et al., 1988) and Lundberg (Kröll et al., 1990; Lou et al., 1991, 1992), on the one hand, and Szolcsányi and coworkers (1998a), on the other hand, is unknown.

Vanilloids release a variety of proinflammatory neuropeptides from sensitive nerve endings (Table 1) (cf. Holzer, 1988). These neuropeptides initiate the cascade of neurogenic inflammation (Fig. 2) (cf. Geppetti and Holzer, 1996). Among these neuropeptides, SP and CGRP are the best studied. SP preferentially interacts at NK-1 receptors (NK-1R) (cf. Regoli et al., 1994). Stimulation of NK-1Rs in endothelial cells leads to plasma extravasation (edema formation) by opening gaps at postcapillary venules (Fig. 2) (McDonald et al., 1988; Rogers et al., 1988). In the airways, NK-1Rs can mediate both bronchoconstriction and bronchodilation (Devillier et al., 1988; Maggi et al., 1991; Manzini, 1992), depending on their localization (smooth muscle versus epithelium; Fig. 2). Bronchodilation is believed to be mediated by cyclooxygenase products (Manzini, 1992). In addition to the bronchomotor and vasoregulatory actions, SP can stimulate myoepithelial cells in submucosal glands to produce mucus and activate alvelolar macrophages (Coles et al., 1984; Barnes et al., 1991). Finally, SP was shown to stimulate human neutrophils and T lymphocytes, although these actions are probably not mediated by tachykinin receptors (Repke and Bienert, 1987).

Interestingly, not all sensory neuropeptides are proinflammatory. For instance, somatostatin seems to block neurogenic plasma extravasation by mustard oil (Szolcsányi et al., 1998b). If mustard oil is applied to the rat hindpaw skin repeatedly, the inflammatory response for the latter applications is diminished. This preventive action is abolished by pretreatment with a somatostatin antiserum (Szolcsányi et al., 1998c). It is concluded that somatostatin might play an important role in the beneficial effects of counterirritation.

An exciting new advance in the field is the concept that vanilloids may activate mast cells directly in a VR-mediated fashion (Table 4) (Bíró et al., 1998a). Mast cells are known to exist in close proximity to sensory nerves in several tissues such as the dura (Dimitriadou et al., 1987), airways (Kiernan, 1990; Alving et al., 1991), and small intestines (Bienenstock et al., 1991). Furthermore, there appears to be important cross talk between sensory nerves and mast cells, inasmuch as SP released from the nerve endings can activate mast cells (Repke and Bienert, 1987) and histamine liberated from mast cells can stimulate sensory nerves (cf. Lembeck, 1983). The finding that vanilloids activate sensory neurons and mast cells at similar concentrations (Bíró et al., 1998a) raises the interesting possibility that neuron-mast cell interactions may greatly facilitate the actions of low vanilloid concentrations. Moreover, it forms another bridge between vanilloid-sensitive sensory nerves and neuroimmune regulation. Interestingly, nociceptin, a novel neuropeptide related to opioids (cf. Darland et al., 1998), blocks capsaicin-evoked release of sensory neuropeptides from sensory nerves and, at the same time, prevents the actions of mast cell degranulating peptide (Helyes et al., 1997; Németh et al., 1998).

2. Hyperalgesia and Allodynia Following Vanilloid Administration. Intradermal injection of capsaicin in humans results in primary hyperalgesia to heat and mechanical stimuli in the vicinity of the injection site (Simone et al., 1987, 1989; LaMotte et al., 1991). This is followed by the development of secondary mechanical hyperalgesia and allodynia in an area surrounding the site of primary hyperalgesia (Simone et al., 1991; Torebjörk et al., 1992). In the rat, surgical removal of the sympathetic postganglionic neurons innervating the paw skin or treatment with the α-adrenoreceptor blocker phentolamine or with prazosin, a selective α_1 receptor antagonist, prevents the development of secondary hyperalgesia by capsaicin (Kinnman and Levine, 1995), implying a role for the autonomic nervous system. Subcutaneous injection of phentolamine is also effective in humans in the prevention of hyperlagesia that develops after intradermal capsaicin injection (Kinnman et al., 1997).

Mounting evidence suggests a central role for nitric oxide in nociceptive processing in the spinal cord (cf. Meller and Gebhart, 1993). Intradermal injection of capsaicin in the hindpaw of the rat induces a marked increase in nitric oxide production in the spinal cord (Wu et al., 1998). Nitric oxide synthase inhibitors block the

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behavioral responses (scratching, biting, and licking) evoked by capsaicin in the mouse (Sakurada et al., 1996). It is now believed that capsaicin stimulates nitric oxide production via ill-defined mechanisms, which, in turn, initiates the release of glutamate from central terminals of vanilloid-sensitive neurons (Sakurada et al., 1996). Glutamate activates NMDA receptors both presynaptically, on central terminals of vanilloid-sensitive neurons, and postsynaptically, on second-order neurons of the dorsal horn of the spinal cord (cf. Yaksh and Malmberg, 1994). Dorsal horn neurons, including spinothalamic tract cells, show enhanced responses (sensitization) to excitatory amino acids during capsaicin-induced hyperalgesia in the monkey (Dougherty and Willis, 1992). This sensitization is predominantly mediated by NMDA receptors (Dougherty et al., 1992). The development of NMDA receptor-mediated hyperalgesia can be prevented by both NMDA receptor antagonists (Dougherty et al., 1992) and nitric oxide inhibitors (Kitto et al., 1992; Meller et al., 1992). It is notable that spinal glutamate receptors of the non-NMDA-type (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors) are up-regulated in chronic pain (Harris et al., 1996). In addition to the effects of glutamate released from vanilloid-sensitive nerve terminals on NMDA- and non-NMDA-type glutamate receptors on dorsal horn neurons, the positive feedback by glutamate on vanilloid-sensitive nerve endings is also very important in that it facilitates the release of SP (Liu et al., 1997). Spinal cord neurons involved in pain transmission express receptors (NK-1Rs) for SP whose expression is remarkably up-regulated during inflammatory hyperalgesia (Schäfer et al., 1993; McCarson and Krause, 1994). SP binding initiates the internalization of NK-1Rs into dorsal horn neurons and causes structural changes in their dendrites (Mantyh et al., 1995). Furthermore, ablation of dorsal horn neurons by a cytotoxic conjugate of SP and saporin attenuates the mechanical and thermal hyperalgesia following capsaicin administration (Mantyh et al., 1997). NK-1R antagonists prevent the sensitization of spinothalamic tract neurons after intradermal capsaicin injection (Dougherty et al., 1994). Finally, tachykinins given intrathecally, like capsaicin, evoke scratching, biting, and licking responses (Hylden and Wilcox, 1981), followed by hyperalgesia (Yashpal et al., 1982). These observations suggest a major role for SP in the development of hyperalgesia. However, unlike capsaicin-induced pain behavior, nociceptive responses to tachykinins are not antagonized by nitric oxide synthase inhibitors (Sakurada et al., 1996). Therefore, it may be concluded that although SP is important, it is NMDA that plays the pivotal role in the development of capsaicin-induced hyperalgesia.

Zinc is concentrated in the dorsal horn of the spinal cord (Donaldson et al., 1973) and has been proposed to alter the excitability of afferent C-fibers (Larson and Kitto, 1997). Recently, zinc injected intrathecally has been shown to block the behavioral responses (biting, scratching, etc.) induced by capsaicin given via the same route in the mouse (Larson and Kitto, 1997). Zinc is known to inhibit a variety of ion channels including NMDA receptors (Westbrook and Mayer, 1987). Furthermore, zinc inhibits RTX binding to spinal cord membranes (A. Szallasi, unpublished observation), suggesting a possible interaction at VRs, as well. Thus, endogenous zinc may exert an important inhibitory control over spinal pain transmission, possibly by blocking several receptors simultaneously.

Another important player in capsaicin-evoked hyperalgesia is the cAMP transduction cascade. The adenylate cyclase inhibitor, tetrahydrofuryl adenine, reduces mechanical hyperalgesia and allodynia produced by capsaicin injection in a dose-dependent manner (Sluka, 1997a). Furthermore, targeted disruption of the gene encoding the neuronal-specific isoform of the type I regulatory subunit (RIβ) of the cAMP-dependent protein kinase A significantly reduces pain behavior and hyperalgesia by intradermal capsaicin (Malmberg et al., 1997b). As we already discussed, cAMP is believed to mediate the facilitatory action of prostaglandin E_2 on capsaicin-evoked neuropeptide release (Lopshire and Nicol, 1998). It is easy to visualize how rising intracellular cAMP levels can prolong the transmission of nociceptive information by facilitating SP release from central terminals of vanilloid-sensitive neurons.

As discussed in *Section IV.A*, the majority (approximately 75%) of VR1-expressing DRG neurons also possess ATP-sensitive $P2X_3$ receptors (Fig. 11) (Guo et al. 1999). To date, six ATP (P2X) receptors have been cloned, all of which are expressed in sensory neurons in dorsal root, trigeminal, and nodose ganglia (cf. Wood and Docherty, 1997). $P2X_3$ receptors are, however, unique in that their expression is confined to small, nociceptive neurons (Chen et al., 1995; Lewis et al., 1995). ATP is painful when injected on to human blister base (Bleehen and Keele, 1977), suggesting a pain transmitter role for ATP released from injured tissues. Moreover, spinal $P2X_3$ receptors are believed to play a role in the processing of nociceptive information (Kennedy and Leff, 1995). Interestingly, VRs (Docherty et al., 1996) and $P2X_3$ receptors (King et al., 1997) share a biochemical mechanism of desensitization (i.e., dephosphorylation by Ca^{2+}-dependent calcineurin), implying a coregulation of these two receptor classes.

The ASIC also has a DRG-specific isoform, called DRASIC (Lingueglia et al., 1997). Moreover, DRG neurons express at least three types of voltage-gated Na^+-channels that differ in sensitivity to TTX (cf. Nowycky, 1992; Rang et al., 1994). In the context of persistent pain, the slowly activating, TTX-resistant voltage-gated Na^+ channel (Akopian et al., 1996) seems to be of particular importance for the following reasons: 1) persistent activation of nociceptors is associated with development of chronic pain, 2) increased Na^+ channel activity may underlie such persistent activation, and 3) TTX is frequently ineffective in blocking this enhanced channel

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activity (cf. Rang et al., 1994; Akopian et al., 1996; Dray and Urbán, 1996).

We also mentioned in *Section IV.A* that VR1 is abundant in the inner layer of lamina II of the spinal dorsal horn, an area rich in PKCγ (Guo et al., 1999). Mice with a disrupted PKCγ gene respond normally to acute painful stimuli but show an elevated threshold for chronic, neuropathic pain (Malmberg et al., 1997a). Changes in gene expression induced by persistent pain are virtually absent in these mutant mice (Malmberg et al., 1997a). These findings suggest that a PKCγ-mediated phosphorylation of proteins in lamina II is involved in the development of neuropathic pain. Although PKCγ is predominantly present in interneurons, it is not unlikely that VR1-possessing nerve terminals in lamina II also express PKCγ. VR1 has predicted phosphorylation sites and its dephosphorylation by calcineurin is thought to represent a biochemical mechanism for receptor desensitization. If dephosphorylation of VR1 causes desensitization, phosphorylation, by contrast, might lead to sensitization. Therefore, it might be speculated that phosphorylation of VR1 by PKCγ can play a role in the development of persistent pain states.

Finally, a blockade of voltage-gated Ca^{2+}-channels can also prevent the onset of secondary hyperalgesia and allodynia that may follow intradermal capsaicin injection in the rat (Sluka, 1997b).

In conclusion, mechanisms of capsaicin-induced hyperalgesia and allodynia are very complex and are only beginning to be understood (cf. Meyer et al., 1994). An added complication is the finding that bradykinin (and maybe also other algogenic-proinflammatory mediators) recruit normally vanilloid-insensitive sensory neurons to respond to capsaicin and/or low pH (Stucky et al., 1998). As a consequence, inflamed tissues may contain an increased number of nociceptors that could contribute to hyperalgesia via spatial summation on spinal neurons. During inflammation, so-called "silent nociceptors" (cf. McMahon and Koltzenburg, 1990) may also be activated. It is not known whether or not such silent nociceptors may respond to vanilloids.

B. Desensitization to Vanilloids

Excitation of sensory neurons by vanilloids is followed by a refractory state in which 1) neurons do not respond to a subsequent vanilloid challenge, or 2) neurons are resistant to various stimuli, ranging from noxious heat to mechanical pressure to endogenous (e.g., histamine and bradykinin) or exogenous (e.g., xylene and mustard oil) algesic-proinflammatory agents. The late Nicholas Jancsó, who was the first to describe this phenomenon in 1949, did not distinguish between these two forms of neuronal insensitivity: he termed them collectively desensitization [Note: In 1949, Jancsó originally described desensitization of sensory nerves by capsaicin in Hungarian. Therefore, as a reference for desensitization to capsaicin, most authors cite a later review of his in English. However, this review was written not by Jancsó himself but by his wife and coworker Aurelia (Aranka) Jancsó-Gábor and his student János Szolcsányi. To make the first literature on capsaicin desensitization broadly available, as an Appendix to this review we provide its English translation.] For this historical reason, the refractory state that follows vanilloid treatment is still generally called desensitization, despite several efforts to introduce a more accurate terminology (cf. Szolcsányi, 1989, 1991; Holzer, 1991; Wood, 1993; Szolcsányi et al., 1994).

Now it is clear that desensitization to vanilloids is not a single, well-defined biochemical process but rather a cascade of events, the relative contributions of which vary depending on the vanilloid dose used for the challenge and the time elapsed since then. For didactic reasons, we will distinguish below receptor "desensitization" and "tachyphylaxis" from "impairment of neuronal functions". Both desensitization and tachyphylaxis occur at the receptor level. By desensitization, we mean a rapid loss of activity of the receptor occupied by an agonist. For example, in the continuous presence of capsaicin, capsaicin-elicited currents quickly fade. Tachyphylaxis represents gradually diminishing response to repeated agonist administrations.

As follows from the above definition, tachyphylaxis is selective to a subsequent vanilloid challenge and does not prevent neurons from responding to other stimuli. Impaired neurons do not respond to various stimuli regardless of whether or not those stimuli target VRs. Impairment of neuronal functions by vanilloids is often referred to as defunctionalization of vanilloid-sensitive neurons (cf. Holzer, 1991). It is imperative to understand that both tachyphylaxis and impairment are reversible and thus should be clearly distinguished from gross neurotoxicity, an irreversible process.

1. Desensitization. Desensitization of VRs probably reflects an agonist-induced conformational change in receptor protein, which ultimately leads to the closing of the channel pore. It is notable, however, that capsaicin may elicit not one but multiple currents that differ in desensitization kinetics (Liu and Simon, 1996a, 1998). It might also be relevant to desensitization that [^{3}H]RTX binding shows an unusual dissociation kinetics that depends on fractional receptor occupancy (Szallasi and Blumberg, 1993a). If only a small percentage (10% or less) of specific RTX binding sites are occupied, dissociation follows first-order kinetics (Szallasi and Blumberg, 1993a). With increasing receptor occupancy, the release becomes multiphasic and progressively more receptors bind RTX in an irreversible manner (Szallasi and Blumberg, 1993a).

2. Tachyphylaxis. According to a current electrophysiological model, VRs cycle between closed (resting) and open (active) states via numerous nonconducting intermediate states (Liu and Simon, 1996a). Consequently, tachyphylaxis can be viewed as the rate of recovery of

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VRs from the intermediate states to the resting state, when receptors can be activated again by agonist binding. This cycle probably occurs via conformational changes in the receptor protein. As we saw above, extracellular calcium may play a crucial role in regulating such conformational changes leading to tachyphylaxis or the lack of it (Koplas et al., 1997; Liu and Simon, 1998).

Piperine (the active principle in black pepper) and zingerone (the pyrolytic product of ginger oleoresin) evoke similar, rapidly activating currents. Both piperine and zingerone are pungent (cf. Szolcsányi, 1982). Piperine and zingerone differ, however, in tachyphylaxis. Piperine-evoked currents show little tachyphylaxis upon repeated applications (Liu and Simon, 1996b). By contrast, zingerone-induced ion fluxes dissipate rapidly (Liu and Simon, 1996b). Thus, vanilloids differ not only in their relative activation of rapid versus slow currents, but also in their ability to shift the activated conductances into a state of tachyphylaxis.

3. Is Lasting Tachyphylaxis Possible Without Prior Excitation? Of great importance is the question whether it is possible to synthesize nonirritant vanilloids capable of lasting "desensitization" in the sense originally used by Nicholas Jancsó. Generally speaking, to find such a vanilloid (that is a ligand that does not evoke action potentials but desensitizes VRs) one requires a compound that will slowly activate VRs while relatively rapidly inactivating (via increasing intracellular calcium levels) voltage-dependent Na^+ and Ca^{2+} channels. Olvanil (Brand et al., 1987; Dray et al., 1990), SDZ 249-482 (Bevan et al., 1995; Wrigglesworth et al., 1996), and low-dose RTX (Szallasi and Blumberg, 1989; Cruz et al., 1997a) represent prototypes.

4. Impairment of Neuronal Functions after Vanilloid Treatment. Vanilloid-sensitive nerves include polymodal nociceptors detecting noxious heat and pressure (cf. Szolcsányi, 1989; Meyer et al., 1994) and express receptors for algesic and proinflammatory agents such as hydrogen ions, bradykinin, histamine, and serotonin, just to name a few (cf. Maggi, 1991; Lundberg, 1993; Rang et al., 1994). It is easy to visualize how the well known depletion by vanilloid treatment of neurotransmitters (cf. Buck and Burks, 1986; Holzer, 1991) prevents the actions of agents stimulating sensory nerves.

As we saw in the section dealing with excitation, vanilloids release sensory neuropeptides via exocytosis and maybe also via the axon reflex (cf. Lundberg, 1996). What is the mechanism by which vanilloids prevent the restoration of neuropeptides? Capsaicin was shown to block the intra-axonal transport of macromolecules, including NGF (Gamse et al., 1982; Miller et al., 1982; Taylor et al., 1984). There is an NGF-responsive element in the preprotachykinin gene encoding SP and neurokinin A (NKA) (Gilchrist et al., 1991). Thus, it is easy to visualize how capsaicin treatment can down-regulate SP and NKA expression by depleting NGF from the perykarya of sensory neurons. Less clear is the mechanism by which vanilloid treatment depletes neuropeptides whose expression does not depend on the presence of NGF.

4. Down-Regulation of VRs as a Mechanism of Long-Term Desensitization to Vanilloids. There is a complete, dose-dependent loss of specific RTX binding sites in trigeminal and dorsal root ganglia, spinal cord, as well as urinary bladder of the rat following systemic RTX treatment (Szallasi and Blumberg, 1992b; Goso et al., 1993b; Szallasi et al., 1995a). This receptor loss occurred later (24 h) than the loss of the biological responses (protective eye-wiping and xylene-induced neurogenic inflammation responses disappeared by 6 h after treatment) and required higher RTX doses (Szallasi and Blumberg, 1992b). The receptor loss in the spinal cord was entirely due to a reduction in the B_{max}. In the bladder of rats pretreated with 30 μg/kg RTX, approximately at a concentration of the EC_{50} for the loss of binding sites in the spinal cord, both receptor binding and the neurogenic inflammatory responses recovered almost completely within 2 months after treatment (Fig. 20) (Goso et al., 1993b). By contrast, no recovery of specific [^{3}H]RTX binding to spinal cord membranes was observed (Goso et al., 1993b). These finding suggest that VR loss after RTX treatment can be either reversible (reflecting desensitization) or irreversible (indicating neurotoxicity), and that peripheral and central terminals of vanilloid-sensitive neurons have a differential sensitivity to these long-term vanilloid actions.

6. Messenger Plasticity by Vanilloids as a Novel Mechanism of Analgesia. Early reports indicated a nondiscriminative depletion of sensory neuropeptides in the rat following systemic capsaicin treatment (cf. Buck and Burks, 1986). Most authorities agreed that this loss

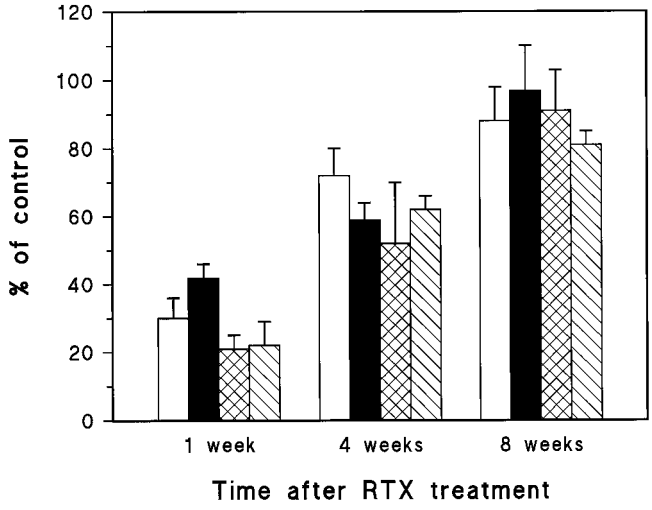

FIG. 20. Loss and recovery of the xylene-induced Evans' blue extravasation response (open columns), specific [^{3}H]RTX binding (closed columns), SP (cross-hatched columns) and CGRP (hatched columns) content in the urinary bladder of the rat following RTX treatment. Evans' blue extravasation and RTX binding values are from Goso et al., 1993b. Neuropeptide measurements are a courtesy of Dr. Francisco Cruz, University of Porto, Porto, Potugal.

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might play a central role in desensitization to capsaicin (cf. Buck and Burks, 1986; Holzer, 1991). Nevertheless, there has always been a lingering doubt as to what degree this depletion of neuropeptides reflects desensitization as opposed to possible neurotoxicity. Capsaicin activates a variety of autonomic reflexes (cf. Monsereenusorn et al., 1982; Buck and Burks, 1986). Consequences include, but are not limited to, a severe depression of respiration (Fig. 21), which was first noted by Toh and coworkers in 1955 (Toh et al., 1955). These acute responses severely limit the initial dose of capsaicin that can be given for desensitization. For instance, Gamse and coworkers noted in 1980 that rats given 50 mg/kg capsaicin s.c needed manually assisted respiration for up to 5 min to survive the severe impairment in respiration. To circumvent this problem (and to achieve lasting desensitization), capsaicin needs to be given repeatedly in increasing doses, taking advantage of the tachyphylaxis as it develops. Although N. Jancsó and colleagues showed as early as 1961 that 4, 8, and finally 15 mg of capsaicin administered to adult rats (approximately 80 mg/kg s.c.) over a period of 1 to 3 days is sufficient to render the animals fully insensitive to chemically evoked pain for 1 to 3 months, later studies adopted a more aggressive treatment protocol, which included 950 mg/kg capsaicin given s.c. over a period of 5 days (G. Jancsó and Khinyár, 1975; Jessell et al., 1978). Such high doses, of course, enhance the possibility for toxicity. In fact, using this protocol, a loss of DRG neurons in adult rats was demonstrated (G. Jancsó et al., 1985).

Severing the peripheral fibers, for example by axotomy, leads to dramatic changes in the expression of neuropeptides and their receptors in primary sensory neurons, including those sensitive to vanilloids (cf. G. Jancsó, 1992; Hökfelt et al., 1994). Certain neuropeptides are up-regulated, whereas other peptides, by contrast, are down-regulated. It was suggested that those neuropeptides that are up-regulated promote the survival and/or regeneration of neurons (cf. Hökfelt et al., 1994). These neuropeptides have been called injury peptides (G. Jancsó, 1992). The best studied of these injury peptides is galanin. Galanin administered intrathecally has a clear analgesic effect in mice in both the tail-flick and hot-plate tests (Post et al., 1988). As first noted by Hökfelt and coworkers in 1987, galanin is up-regulated in DRG neurons after their peripheral axons have been severed. In these axotomized animals, the galanin receptor antagonist M35 potentiates the facilitation of the flexor reflex, a neurophysiological equivalent of pain sensation (Wiesenfeld-Hallin et al., 1992). Taken together, these findings suggest that galanin acts as an endogenous analgesic compound to counteract neuropathic pain evoked by nerve injury. Contrasting to this model, galanin was found to induce membrane depolarizations in DRG neurons in culture (Puttick et al., 1994). DRG neurons express both known classes of galanin receptors, referred to as GAL-R1 (Burgevin et al., 1995; Xu et al., 1996) and GAL-R2 (Ahmad et al., 1996; Shi et al., 1997), respectively. However, the expression of both GAL-R1 and GAL-R2 is down-regulated following axotomy (Xu et al., 1996; Shi et al., 1997), which might protect DRG neurons from an exaggerated feedback response.

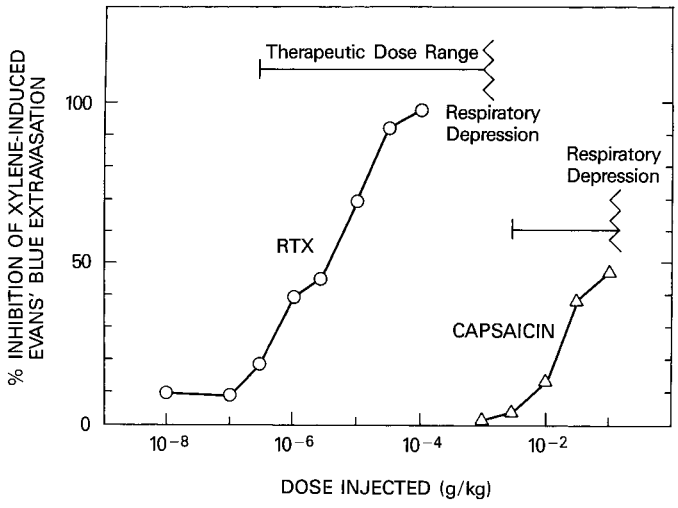

FIG. 21. The therapeutic window of systemic RTX in the rat. By means of a single s.c. injection, RTX can fully desensitize the animals against xylene-induced Evans' blue extravasation without causing any respiratory depression. This is not possible with capsaicin: only a partial densitization can be achieved before a rapidly developing respiratory depression kills the injected animals. Reproduced with permission from Szallasi and Blumberg, 1993b.

Most neuropeptides known to disappear from neurons with axotomy are thought to be involved in chemical neurotransmission. These changes in peptides and receptors are collectively referred to as messenger plasticity (cf. Hökfelt et al., 1994). Messenger plasticity has attracted much publicity lately as a likely mechanism underlying the poor efficacy of opiates to relieve neuropathic pain: cholecystokinin (CCK) up-regulated by nerve injury is believed to act as an endogenous antiopiate (cf. Stanfa et al., 1994). Messenger plasticity also explains why SP antagonists are ineffective in axotomized animals (SP is down-regulated), whereas vasointestinal polypeptide (VIP) antagonists gain therapeutic value (VIP is up-regulated) (Wiesenfeld-Hallin et al., 1990).

Mechanisms underlying the up-regulation of injury peptides are poorly understood; however, there is a striking parallel between the induction of c-Jun and the up-regulation of galanin in rat DRG neurons following axotomy (Herdegen et al., 1993), implying a role for immediate early genes (Jenkins and Hunt, 1991; Leah et al., 1991; Herdegen et al., 1992).

By means of a single, well-tolerated injection of RTX, a complete, long-lasting desensitization against chemogenic pain (Szallasi and Blumberg, 1989a; Szallasi et al., 1989a), noxious heat (Xu et al., 1997), and neurogenic inflammation (Szallasi et al., 1989a; Goso et al., 1993b)

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can be achieved (Fig. 21). Using this treatment protocol, RTX-treated rats show changes in neuropeptide, in neuropeptide receptor, and in nitric oxide synthase (NOS) expression very similar to those described in rats with axotomy (Table 7) (cf. Szallasi, 1996; Szallasi and Blumberg, 1996). For example, the expression of the neuropeptides galanin and VIP (Farkas-Szallasi et al., 1995; Xu et al., 1997), the neuropeptide receptor CCK_B-R (C. Broberger, T. Farkas-Szallasi, A. Szallasi, J. M. Lundberg, T. Hökfelt, Z. Wiesenfeld-Hallin, and X.-J. Xu, submitted for publication), as well as the enzyme NOS (Farkas-Szallasi et al., 1995) are markedly enhanced. These changes are fully reversible. Other neuropeptides, for instance SP, are depleted (Szallasi, 1996). This change in SP expression is also reversible and is due to a decrease in the steady-state levels of total mRNAs encoding SP (Szallasi et al., 1999b). Importantly, the number of DRG neurons showing an in situ hybridization signal for mRNAs encoding SP is not reduced (Szallasi et al., 1999b). Finally, there are neuropeptides that do not show changes in expression following RTX treatment. CGRP and somatostatin are notable examples of this phenomenon (Szallasi, 1996). However, the regulation of neuropeptide expression by RTX seems to be very complex. For example, RTX dramatically up-regulates CGRP expression in DRG neurons obtained from mouse embryos (Jakab et al., 1994).

However, RTX when given repeatedly in a capsaicin-like treatment protocol depletes CGRP-like immunoreactivity from DRG neurons (Szolcsányi et al., 1990). The finding that single, moderate doses of RTX have a differential effect on neuropeptide expression compared with high, cumulative RTX doses represents a powerful argument that depletion of neuropeptides by high vanilloid doses reflects neurotoxicity rather than desensitization.

Despite the striking similarities between vanilloid- and axotomy-induced changes in the expression of neuropeptides, there is an essential difference in the behavior of animals: whereas mechanical nerve injury usually results in the development of neuropathic pain (as most dramatically demonstrated by autotomy behavior), vanilloids, by sharp contrast, have a clear analgesic action (cf. Szallasi and Blumberg, 1996). Spinal cord injury leads to allodynia-like behavior to cold stimuli (Xu et al., 1992). RTX treatment abolishes this behavior (Hao et al., 1996). Moreover, RTX induces a long-lasting analgesic action on the hot-plate test, as well as a transient hypoalgesia to mechanical stimuli (Fig. 22A) (Xu et al., 1997). This is surprising because capsaicin when given to adult rats fails to achieve similar changes (Obál et al.,

TABLE 7

Changes in selected markers (neuropeptides, enzymes, receptors) of rat primary sensory neurons following RTX (single s.c. dose) or capsaicin (cumulative s.c. dose) treatment as well as axotomy

Resiniferatoxin (300–500 μg/kg) was given s.c. into the scruff of the neck of adult Sprague-Dawley rats. Capsaicin was administered in high, cumulative doses. A typical protocol includes one daily treatment with increasing doses on 5 consecutive days to achieve a total dose of 950 mg/kg. See Section *VIII.B.5* and *B.6* for details and references.

	Capsaicin	RTX	Axotomy
Neuropeptides			
VIP	Depleted	Up-regulated	Up-regulated
Galanin	Depleted	Up-regulated	Up-regulated
CGRP	Depleted	No major changes	Depleted
CCK	Depleted	No major changes	Up-regulated
Somatostatin	Depleted	No major changes	Depleted
SP	Depleted	Depleted	Depleted
Neuronal enzyme			
NOS	Up-regulated	Up-regulated	Up-regulated
Receptors			
CCK-B	Depleted	Up-regulated	Up-regulated
C-type VR	Depleted	Depleted	Depleted
R-type VR	Depleted	Depleted	No major changes

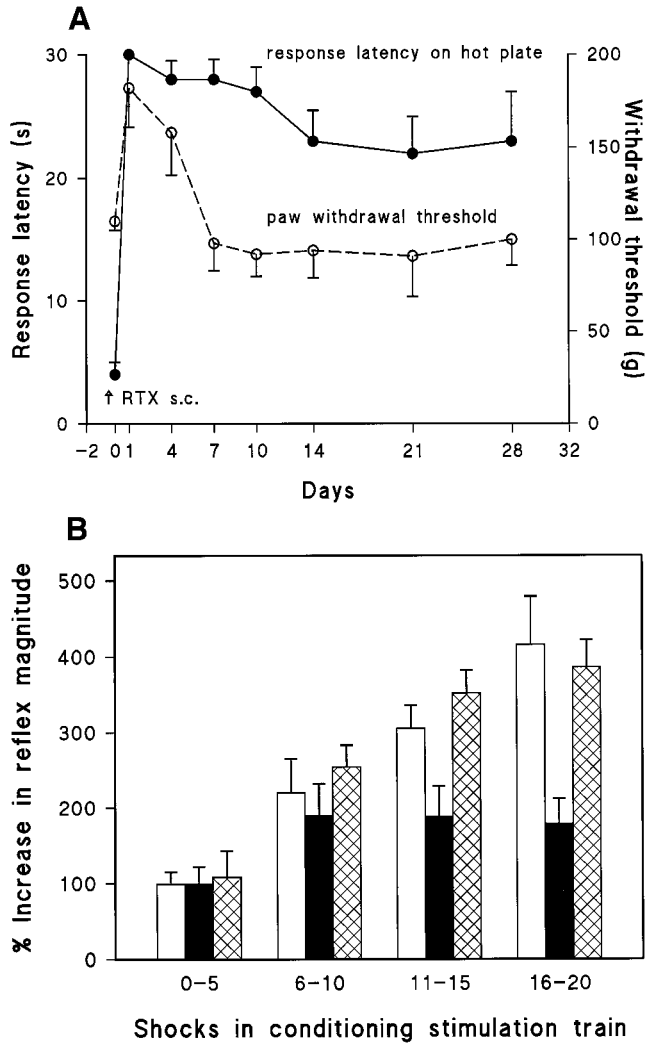

FIG. 22. A, in rats, systemic RTX (500 μg/kg s.c.) causes a profound, long-lasting analgesia response in the hot-plate test. Also, it evokes a transient increase in paw withdrawal threshold to pressure. B, repeated trains of conditioning stimuli applied to the sural nerve of the rat evoke contractions in hamstring muscles of increasing amplitude (open columns). This phenomenon is often referred to as "wind-up" (or facilitation of the flexor reflex) and is thought to reflect sensitization of spinal cord neurons. The facilitation of the flexor reflex is diminished in RTX-treated rats (closed columns). This effect is probably mediated by galanin (which is known to be up-regulated following RTX treatment) since the galanin receptor antagonist M35 is able to restore the "wind-up" phenomenon in RTX-treated animals (crossed-hatched columns). Data are from Xu et al., 1997.

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1979; Hayes and Tyers, 1980; G. Jancsó and Jancsó-Gábor, 1980).

It has long been known that trains of equal stimuli to the sural nerve evoke contractions of increasing strength in the hamstring muscles (cf. Hökfelt et al., 1994). This phenomenon is called facilitation of the flexor reflex (also known as the wind-up phenomenon) and is believed to reflect spinal hyperexcitability as a consequence of C-fiber activation (Wall and Woolf, 1984). A causal relationship between spinal hyperexcitability and neuropathic pain has been postulated (cf. Hökfelt et al., 1994). The wind-up phenomenon is greatly reduced in RTX-treated rats (Fig. 22B) (Xu et al., 1997). These RTX-treated animals show enhanced galanin expression both at the mRNA and the peptide levels (Farkas-Szallasi et al., 1995). The galanin receptor antagonist M35 restored the C-fiber mediated hyperexcitability in the RTX-treated rats (Fig. 22B) (Xu et al., 1997). Furthermore, the decrease in galanin expression with increasing time after RTX administration was accompanied by a gradual restoration of heat sensitivity (Xu et al., 1997). Taken together, these observations imply that up-regulation of the inhibitory neuropeptide, galanin, plays a central role in the prolonged analgesic action of RTX.

As already mentioned, CCK_B receptor expression is markedly up-regulated in RTX-treated animals. The level of mRNAs encoding CCK_A receptors is moderately increased. This is surprising for two reasons. First, enhanced CCK_B receptor expression in rats with nerve injury is believed to contribute to the persistent, morphine-resistant pain that develops in such animals (cf. Stanfa et al., 1994). RTX-treated animals, however, show marked analgesia and not chronic pain (Szallasi and Blumberg, 1989a; Xu et al., 1997). Second, capsaicin treatment results in a marked loss of CCK binding sites, suggesting the down-regulation of CCK receptors (Ghilardi et al., 1992). A potentially important difference between the effects of mechanical nerve injury and RTX treatment is that CCK is elevated in axotomized (Verge et al., 1993) but not in RTX-treated rats (Xu et al., 1998). Thus, probably there is no agonist to occupy the extra CCK_B receptors following RTX treatment. Another vanilloid effect not mimicked by axotomy is the loss of VRs (Farkas-Szallasi et al., 1996).

The differential regulation of CCK_B receptor expression in capsaicin- and RTX-treated animals is not unprecedented. Table 7 lists several additional interesting examples of this phenomenon. The most likely explanation is a dominating nonspecific neurotoxicity by capsaicin.

C. Neurotoxicity by Vanilloids

In 1977, it was reported that capsaicin given to newborn rats sacrificed the majority of small- to medium-seized DRG neurons (G. Jancsó et al., 1977). Since then, neonatal capsaicin treatment has been used routinely to identify capsaicin-sensitive neuronal pathways and to explore their contributions to physiological and pathological regulatory processes (cf. Buck and Burks, 1986; Holzer, 1991). It is known that NGF is required for the survival of immature DRG neurons (Ruit et al., 1992). It is also known that capsaicin treatment stops the intraaxonal transport of NGF from the periphery, where it is produced, to the cell bodies of DRG neurons (Taylor et al., 1985). Based on these observations it was postulated that neonatal capsaicin administration kills neurons by depriving them of NGF (cf. Wood, 1993). Experimental support for this explanation was provided by Otten and colleagues in 1983 (Otten et al., 1983), who showed that DRG neurons doomed to perish following neonatal capsaicin treatment may be rescued by exogenous NGF.

There can be little doubt that capsaicin is able to kill adult sensory neurons in culture in a VR-mediated fashion (Winter, 1987; Wood et al., 1988). This action is mimicked by RTX (Jeftinija et al., 1992) and is most likely mediated by calcium, because removal of extracellular calcium, or block of the calcium influx by ruthenium red, prevents capsaicin-induced cell death (Wood et al., 1988; Winter et al., 1990; Chard et al., 1995). Inhibiting calcium-activated proteases such as calpain by E64 or MDL 28,170 also minimizes capsaicin-mediated cell death (Chard et al., 1995). There are, however, important differences between capsaicin-induced neurodegeneration in animals and in culture. In vitro, capsaicin kills DRG neurons rapidly regardless of the presence of NGF (Wood et al., 1988; Jeftinija et al., 1992). In sharp contrast, capsaicin given to neonatal rats induces no early cell death (Szolcsányi et al., 1998) and even the delayed neuronal loss can be prevented by NGF (Otten et al., 1983; Szolcsányi et al., 1998d).

Although calcium seems to be an important orchestrator of capsaicin-induced neuronal degeneration in vitro, the question remains open whether or not the rise in intracellular calcium by capsaicin can achieve sufficiently high levels in adult sensory neurons in vivo to cause irreversible neuronal damage. In humans, intradermal injection of high capsaicin doses produces degeneration and subsequent reinnervation of cutaneous nerve endings (Simone et al., 1998). It is not known, however, whether any DRG neurons innervating the capsaicin-treated skin area perish. In adult rats, a significant loss of DRG neurons was reported following systemic (s.c.) capsaicin administration (G. Jancsó et al., 1985). The interpretation of this findings is, however, complicated by subsequent studies by Ritter and Dihn (1993) who demonstrated that capsaicin given at similarly high doses may induce argyrophylia (believed to reflect neurotoxicity) along the entire neuroaxis of the rat, including the retina. RTX ablates DRG neurons in newborn (Szallasi et al., 1990), but not in adult (Szallasi and Blumberg, 1992b), rats. Therefore, toxicity by capsaicin in adult rats may reflect a nonspecific toxic action via an RTX-insensitive site (see *Section IX* for details).

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Alternatively, respiratory depression by capsaicin (see above) may lead to tissue hypoxia, which, in turn, may damage neurons regardless of whether or not they possess VRs. Finally, novel VRs may exist that recognize capsaicin but not RTX. As already mentioned, several ESTs show a high degree of similarity to VR1 (Caterina et al., 1997) including an EST in the retina (Washington University, St. Louis, MO-Merck EST Project; unpublished data; accession no. AA047763), a tissue particularly susceptible to toxicity by capsaicin (Ritter and Dihn, 1993).

IX. Diverse Biological Actions of Vanilloids; VR-Mediated and Independent Mechanism

Vanilloid ligands show striking differences in biological actions. Some of these differences can be explained at the level of a single receptor. For instance, the archetypal vanilloid, capsaicin, is both pungent and desensitizing. Piperine is pungent (Szolcsányi, 1982) but does not desensitize (Liu and Simon, 1996b). In the case of olvanil the pattern is the opposite: it is nonpungent (Brand et al., 1987; Dray et al., 1990) but desensitizing (Dray et al., 1990; Liu L. et al., 1997). As we saw above, these differences in biology are likely to reflect kinetic differences in channel gating properties.

Other differences are better explained by postulating VR heterogeneity. For example, vanilloids evoke multiple currents that seem to differ in affinity, kinetics, and sensitivity to antagonists (Figs. 12 and 14) (Liu and Simon, 1996a; Liu et al., 1996, 1998).

Capsaicin actions have traditionally been divided into "specific" (i.e., VR-mediated) and "nonspecific" effects (cf. Holzer, 1991). Specific action was defined as that occurring via interaction of capsaicin at primary sensory neurons. With the discovery of VRs on cells other than primary sensory neurons [such as mast cells and glial cells (Bíró et al., 1998a,b)], it needs to be reevaluated whether certain biological actions of capsaicin, previously considered "nonspecific", may be "specific" after all.

Capsaicin interacts at several targets other than neuronal and nonneuronal VRs. We already commented briefly on some of these targets, including a block of K^+ channels (Dubois, 1982; Petersen et al., 1987; Kehl, 1994; Kuenzi and Dale, 1996), inhibition of NADH-oxidoreductase and other enzymes (Shimomura et al., 1989), altered membrane fluidity (Meddings et al., 1991; Aranda et al., 1995), and formation of so-called pseudochannels (Feigin et al., 1995). For instance, changes in membrane fluidity may underlie the inhibitory effect of capsaicin on thrombocyte aggregation (Hogaboam and Wallace, 1991), whereas ligand-induced pseudochannel formation may contribute to the nondiscriminative neurotoxic action of capsaicin at high doses (Ritter and Dinh, 1993). What we have not mentioned yet, but may be important, is the finding that capsaicin acts as a competitive inhibitor of tyrosyl-tRNA synthetase (Cochereau et al., 1996). Capsaicin inhibits tyrosyl-tRNA synthetase in hippocampal astrocytes with a K_i value of 42 μM and then kills these cells. Astrocytes may be rescued by adding tyrosine to the culture medium (Cochereau et al., 1997). As mentioned above, capsaicin induces degeneration of neurons not supposed to express VRs (Ritter and Dinh, 1993). It is not unlikely that some of the unexpected neurotoxic actions of capsaicin are due to an inhibition of tRNA aminoacylation.

RTX differs from capsaicin in its spectrum of non-VR-mediated actions. For example, RTX does not inhibit K^+ channels (Castle, 1992). However, RTX has its own independent targets. RTX binds to (Szallasi et al., 1989b; Ács et al., 1995) and activates PKC (Ács et al., 1995; Harvey et al., 1995), inhibits nuclear transcription factor κB (NF-κB) (Singh et al., 1996), and induces apoptosis in human B-cells via Bcl-2 and calcineurin (Wolvetang et al., 1996). It should be noted, however, that whereas capsaicin acts on VRs and other targets in overlapping concentration ranges, RTX shows from a hundred- to several thousand-fold separation in favor of VRs. Because of its side effects, capsaicin is often referred to as a double-edged sword (cf. Surh and Lee, 1995). In light of the above findings, it is not surprising that RTX at doses relevant for VRs seems to be devoid of most undesirable, capsaicin-like side effects (cf. Szallasi and Blumberg, 1996).

Previously, we discussed vanilloid binding by α_1-acid glycoprotein in serum as a methodological means to reduce nonspecific RTX binding (Szallasi et al., 1992). In the context of this section, it should be noted that serum binding of vanilloids may be a major pharmacodynamic/pharmacokinetic factor influencing vanilloid actions in vivo. If the concentration of a drug binding plasma protein and its affinity for the drug are known, the fraction of the drug that remains unbound in the plasma (and thus is available for specific receptor binding) can be estimated. By using the reported plasma α_1-acid glycoprotein level in the rat (4 μM) and the affinities of α_1-acid glycoprotein for RTX (0.5 μM) and capsaicin (10.5 μM), respectively, it can be calculated that a much higher (72%) fraction of capsaicin remains free than that of RTX (13%) upon systemic administration (cf. Szallasi et al., 1992). This is in accord with the observation that RTX actions are characteristically subdued and often occur after a delay (Szallasi and Blumberg, 1989a; Maggi et al., 1990). Furthermore, this serum binding may provide a rationale to explain the observation that chloral hydrate (that also binds to α_1-acid glycoprotein) facilitates acute vanilloid actions in the rat (Szallasi et al., 1998d). α_1-Acid glycoprotein is a well known drug binding protein in serum (cf. Paxton, 1983; Kremer et al., 1988). In clinical practice, the possible competition between vanilloids and nonvanilloid drugs (e.g., chlorpromazine and warfarin) for sites on plasma proteins needs to be carefully evaluated.

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X. Species-Related Differences in Vanilloid Actions

It has long been known that vanilloids show striking species-related differences in biological actions (Glinsukon et al.,1980; Buck and Burks, 1986; Holzer, 1991). According to a frequently cited example, the dose of capsaicin that can kill the guinea pig almost instantaneously is well tolerated by the hamster (Glinsukon et al., 1980). In principle, these differences may reflect: 1) species-related differences in VR expression (Table 8); 2) species-related differences in neurotransmitter expression in vanilloid-sensitive neurons; and 3) species-related differences in the expression of receptors for these neurotransmitters. There are several examples for the relevance of all these three mechanisms in the markedly dissimilar vanilloid actions in different species. An important consequence of these differences is that great attention must be paid to the choice of animal models in preclinical studies to evaluate vanilloid toxicity.

A. Species-Related Differences in VR Expression

Birds do not respond to capsaicin (Jancsó, 1968). As expected, no specific RTX binding was found in chicken DRGs (Szallasi and Blumberg, 1990b). Among mammalian species, rabbits are distinguished by their marginal sensitivity to capsaicin (Glinsukon et al., 1980; Tervo, 1981). In keeping with this, the density and/or affinity of RTX binding sites in rabbit trigeminal ganglion membranes is(are) under the detection limit of the binding methodology (Szallasi and Blumberg, 1993a). Hamsters are interesting in that their DRGs are comparable with those of the rat in terms of RTX binding (Szallasi and Blumberg, 1993a); however, in hamster urinary bladder (Szallasi et al., 1993d) or trachea (Szallasi et al., 1995b) no specific RTX binding can be detected. Apparently, hamster sensory neurons are capable of the synthesis of VRs but the receptor protein is not transported to the periphery at a measurable level. Hamsters are noted for their resistance to capsaicin (Glinsukon et al., 1980; Maggi et al., 1987a), which is in accord with the lack of detectable RTX binding sites in their peripheral tissues. In general, insensitivity to vanilloids seems to be associated with the absence (or expression at undetectably low levels) of specific RTX binding sites. However, the reverse statement is not true: a hypersensitivity to vanilloid actions does not necessarily mean the existence of unusually high affinity or density of VRs. For instance, although guinea pigs are very sensitive to vanilloids (cf. Buck and Burks, 1986; Holzer, 1991), neither the affinity nor the density of RTX binding sites in this species exceeds the parameters determined in the rat (Szallasi and Goso, 1994; Szallasi et al., 1995b).

TABLE 8
Parameters of [^{3}H]RTX binding to VR in spinal cord of several species, including humans

Species	K_d	Cooperativity Index	Capsaicin	Capsazepine
	pM		K_i, *μM*	K_i, *μM*
High (pM)-affinity binding				
Rat (1–3)	13–31	1.9–2.3	0.3–5.0	3.3–4.0
Mouse (4)	18	2.1	N.D.	N.D.
Pig (5)	88	2.3	9.7	6.8
Moderate (nM)-affinity binding				
Human (6, 7)	0.9–11.0	1.1–1.4	0.3–1.1	0.06–0.3
Monkey (8)	3,000	1.0	17.0	3.0
Guinea pig (7)	5,000	1.0	0.5	0.1

N.D., not determined. References indicated in parentheses: (1) Ács et al., 1994a; (2) Szallasi and Blumberg, 1993a; (3) Szallasi et al., 1993b; (4) Szallasi, 1994; (5) Ács and Blumberg, 1994; (6) Ács et al., 1994b; (7) Szallasi and Goso, 1994; (8) T. Biró and P.M. Blumberg, unpublished observations.

B. Species-Related Differences in Expression of Sensory Neuropeptides and Their Receptors

Interested readers may find several excellent reviews on this topic. Here we wish to mention one intriguing example only. Vanilloids induce equally powerful edema responses in airways of the guinea pig (Lundberg et al., 1984), mouse (A. Szallasi, unpublished observation), and rat (Saria et al., 1983). However, with regard to bronchomotor responses, these three species show markedly dissimilar reactions to capsaicin treatment. Guinea pig airways are contracted (Szolcsányi and Barthó, 1982; Lundberg and Saria, 1987), whereas mouse airways are dilated (Manzini, 1992), by capsaicin administration. Rat airways show no changes in bronchial tone (Joos et al., 1986). Interestingly, the very same neuropeptide, SP, mediates bronchoconstriction in the guinea pig and bronchodilation in the mouse (cf. Manzini et al., 1994). The difference in biological responses seems to stem from the cellular localization of SP receptors (NK-1Rs), which are present on bronchial smooth muscle in the guinea pig (Devillier et al., 1988; Maggi et al., 1991) and on airway epithelium in the mouse (Manzini, 1992). SP binding to NK-1Rs leads to a direct contraction of bronchial smooth muscle in the guinea pig (Maggi et al., 1991). In the mouse, bronchodilation is an indirect effect, mediated by cylcooxgenase products generated in the epithelium upon NK-1R activation (Manzini, 1992). Interestingly, equine airways are also relaxed by capsaicin (Zhu et al., 1997). This effect, however, is not mimicked by neuropeptides or prostanoids but is prevented by charybdotoxin, a blocker of Ca^{2+}-activated K^+ channels (Zhu et al., 1997). As we will see below, human airways behave in a unique manner in response to vanilloids.

C. Human VRs

The presence of vanilloid-sensitive nerves in humans is well established (cf. Fuller, 1990; Lynn, 1990; Winter et al., 1995). For obvious reasons, it is very difficult to study VRs in freshly obtained human sensory ganglia or spinal cord. Nevertheless, Baumann and colleagues (1996) characterized capsaicin-evoked responses in adult human DRG neurons in culture. For cell culture, they used DRGs removed surgically for chronic intrac-

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table pain, a fortunately uncommon procedure. In neuronal tissues obtained post mortem, a high density of specific RTX binding sites can be demonstrated using membranes (Table 8) (Ács et al., 1994b; Szallasi and Goso, 1994) or an autoradiographic approach (Fig. 7) (Szallasi et al., 1994a). There are conflicting reports about the cooperativity of RTX binding by human spinal cord membranes. Ács and colleagues (1994b) reported positive cooperative binding to spinal cords removed within 4 h after death from victims of traffic accidents in Hungary. Szallasi and Goso (1994), by contrast, found noncooperative binding to spinal cord samples removed from elderly Italians 24 to 48 h after death. This change in cooperativity may reflect differences in the time elapsed after death (4 h versus 24 h) and may also be influenced by other factors such as age, gender, and/or ethnicity of the tissue donors.

Surgically obtained human airway specimens and guinea pig airways bind RTX with similar parameters (Szallasi et al.,1995b). Airways removed from cadavers show marked differences in RTX binding, ranging from no binding at all to binding similar to surgical samples (Szallasi et al., 1995b). Whether this variation reflects post- mortem autolysis or is due to the underlying disease remains to be seen. Interestingly, a similar variability was noted in bronchomotor responses to capsaicin in humans (Lundberg et al., 1983; Honda et al., 1991; Chitano et al., 1994; Molimard et al., 1994; Ellis et al., 1997).

Actually, the high density of specific RTX binding sites in human bronchi is surprising. Vanilloids contract isolated human bronchi only to a minimal degree (Lundberg et al., 1983; Honda et al., 1991) or not at all (Molimard et al., 1994), and the neurogenic plasma extravasation response also seems to be missing in human airways (Bascom et al., 1991; Greiff et al., 1995). However, capsaicin may provoke severe bronchoconstriction in asthmatic patients (Marciniak et al., 1995). The reason why asthmatics are more susceptible to capsaicin inhalation than healthy individuals is not known (cf. Lundberg, 1995). In animals, the bronchocontractile action of capsaicin is mediated by SP acting on NK1-Rs, and the expression of both SP (Ollerenshaw et al., 1991) and its receptor (Adcock et al., 1993) were reported to be increased in patients with asthma. However, unlike in guinea pigs, in humans the bronchoconstrictor action of capsaicin is apparently not mediated by tachykinins (Ellis et al., 1997). The only known response that capsaicin reproducibly provokes in healthy human airways is cough (cf. Karlsson, 1996).

The affinity of specific RTX binding sites and their density seem to be fairly similar in human (Ács et al., 1994b; Szallasi and Goso, 1994), monkey (T. Bíró and P. M. Blumberg, unpublished results), and porcine spinal cord (Ács and Blumberg, 1994; Szallasi et al., 1994b) (Table 8). As regards human VRs, a cell line stably transfected with a human VR1-like receptor c DNA is probably already in the pipeline.

XI. Endogenous Vanilloids: Do They Exist?

The high affinity of VRs strongly argues for the existence of endogenous vanilloids. VRs show positive cooperativity in ligand binding and it has been speculated that this behavior might serve as a relay mechanism to amplify the actions of an endogenous activator produced in low quantity or affinity (Maderspach and Fajszi, 1982).

Endogenous VR activators are yet to be identified. As we saw, VRs are expressed along the entire length of sensory neurons, in several brain nuclei, and also in nonneuronal tissues. This broad expression of VRs hinders the isolation of endogenous ligands, especially if they are produced on demand only. For VR1, noxious heat and/or low pH (Caterina et al., 1997; Tominaga et al., 1998), or a combination of inflammatory mediators (Kress et al., 1997; Vyklicky et al., 1998) have been suggested to act as "natural" activators. Whereas it is easy to visualize how peripheral VRs may be activated by such agents, the relevance of heat or hydrogen ions in stimulating central VRs is rather doubtful. Consequently, it may be postulated that VRs, depending on the tissue in which they are expressed, have distinct endogenous activators.

Capsazepine given to control rats has no perceptible actions (Perkins and Campbell, 1992), which led to the conclusion that endogenous vanilloids either do not exist or are produced on demand only. In accord with the latter model are the findings that capsazepine is beneficial in animal models of pain and inflammation (Santos and Calixto, 1995; Campbell et al., 1996; Kwak et al., 1998). For example, capsazepine can antagonize carrageenan- or formalin-induced hyperalgesia in the rat as well as the corresponding increase in the number of dorsal horn neurons positive for Fos-like immunoreactivity (Kwak et al., 1998). These observations imply that hyperalgesia is mediated at least in part by a substance that is released from inflamed tissues and that acts on VRs. This concept is, however, weakened by a recent report by Reeh and coworkers (Vyklicky et al., 1998). Carrageenan is known to promote the release of inflammatory mediators such as histamine, 5-hydroxytryptamine, prostaglandins, and cytokinins (Watkins et al., 1995). Reeh and colleagues showed that a combination of such inflammatory mediators can activate vanilloid-sensitive neurons in a capsazepine-sensitive manner (Vyklicky et al., 1998). Therefore, now it is unclear whether capsazepine inhibition of carrageenan inflammation-induced hyperalgesic responses really implies the release of a novel, specific endogenous VR activator or rather reflects the interaction of well known mediators at sensory nerves.

There is indirect evidence to imply a role for an endogenous vanilloid in maintaining physiological func-

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tions. A well known long-term sequel of vanilloid treatment is a loss of hair, which may be followed by skin exulcerations (Maggi et al., 1987b; Carrillo et al., 1998). This skin damage might be due to the depletion of SP from dermal sensory nerve endings (Gamse et al., 1980), because SP has been shown to exert trophic actions on fibroblasts and keratinocytes (Sporn and Roberts, 1988). Consequently, a link has been postulated between alopecia areata and malfunctioning of vanilloid-sensitive nerves (Rossi et al., 1997). In fact, SP was shown to facilitate hair growth in mice (Paus et al., 1994). It was also speculated that a sustained release of SP from sensory nerves is important in keeping the skin and its appendices healthy (Sporn and Roberts, 1988). An endogenously produced VR activator might subserve this role. Furthermore, an accelerated release of SP form cutaneous terminals may explain how topical capsaicin can accelerate wound healing in the pig (Watcher and Wheeland, 1989).

XII. Vanilloids in Clinical Practice: Current Uses and Future Perspectives

In principle, all the three characteristic actions of vanilloids (excitation, desensitization, and neurotoxicity) may have therapeutic value (cf. Szolcsányi, 1991; Szallasi and Blumberg, 1993b). Stimulation (counterirritation) and desensitization are already in use in clinical practice. Ablation of C-fibers by perineural capsaicin injection (cf. G. Jancsó and Ambrus, 1994) may be attempted in cancer patients with otherwise untractable pain.

A. Counterirritation with Capsaicin

Capsaicin is a standard ingredient in a variety of over-the-counter drugs (e.g., Stimurub, Heat, Capsoderma) used worldwide to relieve muscle ache. In the case of sore muscles after rigorous exercise, beneficial capsaicin effects may be attributed to an increase in microcirculation in the treated area. A likely mediator of this action is CGRP (cf. Holzer, 1988). When muscle ache is due to bruises, such capsaicin ointments probably act as counterirritants. The mechanism of counterirritation is poorly understood. Previously, it was believed to occur at the level of the spinal cord and it was also referred to as "hyperstimulation analgesia" (Melzack, 1975) or "nocigenic inhibition" (Ness and Gebhart, 1991). Recent findings, however, imply a predominantly peripheral mechanism mediated by somatostatin released from vanilloid-sensitive nerve endings (Szolcsányi et al., 1998c). It is not unlikely that "desensitization" by creams containing low concentrations of capsaicin, such as Axsain (0.075% capsaicin; GenDerm Canada Inc., Montreal, Canada; Euroderma Ltd.) and Zostrix (0.025% capsaicin; GenDerm Canada Inc.), reflects counterirritation instead.

B. Desensitization to Capsaicin

Vanilloid-sensitive nerves participate in various reflex responses such as the micturition reflex (cf. Maggi and Meli, 1988). The existence of vanilloid-sensitive nerves in the human urinary bladder is well established and these nerves were shown to play a pivotal role in the reflex control of micturition in humans (Maggi et al., 1989). The micturition reflex is under the inhibitory control of descending supraspinal pathways (cf. Blaivas, 1982). When these inhibitory pathways are disrupted (for example by trauma or multiple sclerosis), the urinary bladder becomes autonomous and the urge to void occurs at low bladder volumes (cf. Blaivas, 1982; De Groat, 1997). This condition is called detrusor hyperreflexia of spinal origin. Capsaicin injected via a catheter into the urinary bladder is beneficial in this condition by decreasing the sensitivity of C-fibers subserving the micturition reflex (cf. De Groat, 1997; Cruz, 1998). Capsaicin is also beneficial in bladder hypersensitivity, a condition caused by an abnormal perception of bladder filling. Detrusor hyperreflexia is a motor form, whereas bladder hypersensitivity is a sensory form of urge incontinence (Stephenson and Mundy, 1994; Heritz and Blaivas, 1996) which, next to stress incontinence only, is the second most common type of incontinence in women (Diakno, 1996; Hampel et al., 1997). A complication of capsaicin instillation into the urinary bladder is the initial hyperreflexic contractions that may cause urethral leakage (cf. Cruz, 1998). Also, most patients report suprapubic pain (cf. Fowler et al., 1994; Cruz, 1998). Both pain and hyperreflexic contractions may be minimized in selected patients by electromotive lidocaine administration (Dasgupta et al., 1998a). However, many patients report intense pain by capsaicin even after lidocaine anesthesia (Das et al., 1996; Cruz et al., 1997b).

A traditional indication for capsaicinoids is toothache (Turnbull, 1850). As discussed above, both eugenol and guaiacol appear to exert their analgesic activity via VRs (Ohkubo and Kitamura, 1997; Ohkubo and Shibata, 1997). Topical capsaicin seems to have a therapeutic value in atypical odontalgia, an unusual chronic orofacial pain condition with no other known effective pharmacological therapy (Vickers et al., 1998), and also in the so-called "burning mouth" syndrome, another disease of unknown etiology (Huang et al., 1996).

Topical capsaicin can ameliorate the symptoms (rhinorrhea, nasal obstruction, pruritus, etc.) of vasomotor rhinitis (LaCroix et al., 1991; Marabini et al., 1991; Stjärne et al., 1991; Filiaci et al., 1994; Wolf et al., 1995; Blom et al., 1997), a common disorder that, as Philip and Togias (1995) succintly put it, is "difficult to define, difficult to treat, and difficult to understand". A recent report from the Karolinska Institute (Stjärne et al., 1998) also describes a lasting amelioration of nasal congestion in patients with birch pollen allergic rhinitis following a single intranasal application of a 30 μM

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capsaicin solution. As an added benefit, capsaicin treatment (once a week for 5 weeks) seems to reduce the size of nasal polyps as well (Filiaci et al., 1996). Interestingly, intranasal capsaicin is also effective in cluster headache (Sicuteri et al., 1989; Fusco et al., 1991; Marks et al., 1993).

Notalgia paresthetica is a condition characterized by intense pruritus usually accompanied by macular pigmentation over the scapular area (Weber and Poullos, 1988). It is thought to be due to entrapment of the posterior rami of the T2 to T6 spinal nerves. Itching improves in most notalgia paresthetica patients undergoing topical capsaicin therapy (Wallengren, 1991; Leibsohn, 1992; Wallengren and Klinker, 1995). Capsaicin also reduces the severity of pruritus in psoriasis (Bernstein, 1988; Ellis et al., 1993) and in hemodialysis patients with uremia (Breneman et al., 1992; Tarng et al., 1996).

Topical capsaicin has been also tried as an adjuvant analgesic in a variety of neuropathic pain conditions (Table 9), such as postherpetic neuralgia (Bernstein et al., 1987; Bucci et al., 1988; Hawk and Millikan, 1988; Watson et al., 1988), painful diabetic neuropathy (Ross and Varipapa, 1989; Chad et al., 1990; Basha and Whitehouse, 1991; Capsaicin Study Group, 1991; Schefflar et al., 1991; Low et al., 1995), and postmastectomy pain syndrome (Watson et al., 1989; Watson and Evans, 1992; Dini et al., 1993), as well as in osteo- and rheumatoid arthritis (Deal et al., 1991; McCarthy and McCarthy, 1992; Matucci-Cerinic et al., 1995). A critical overview of these clinical trials (at least 50 of them) is out of the scope of this review. Briefly, the therapeutic value of capsaicin in neuropathic pain conditions is difficult to judge because of three reasons. First, controlled capsaicin trials versus placebo are impossible to blind due to the characteristic burning sensation induced by capsaicin. Second, a high placebo response rate was reported in the controlled trials, which may account for some salutary capsaicin effects in the uncontrolled studies. In extreme cases, placebo gives an even better response rate than capsaicin. For example, four of seven lichen simplex chronicus patients reported an improvement of the pruritus following capsaicin treatment (Kantor and Resnick, 1996). However, three of the four patients who reported beneficial effects actually preferred placebo over capsaicin. And third, many patients decide to quit treatment because they find the irritancy of available capsaicin preparations intolerable. Reported withdrawal rates are 30% or higher (cf. Carter, 1991; Rumsfield and West, 1991; Watson, 1994; Rains and Bryson, 1995). C. P. N. Watson (1994), who has conducted the most postmastectomy pain studies and thus has first-hand experience in using capsaicin as an analgesic, concludes: "topical capsaicin is generally not satisfactory as a sole therapy for chronic painful conditions, although it may serve as an adjuvant to other approaches".

TABLE 9
Current clinical indications for use of topical capsaicin

Low-concentration capsaicin creams (0.025% or 0.075%) to relieve pain
Postherpetic neuralgia
Diabetic neuropathy
Postmastectomy pain syndrome
Stump pain
Reflex sympathetic dystrophy
Trigeminal neuralgia
Oral neuropathic pain
Osteoarthritis
Rheumatoid arthritis
Fibromyalgia
Guillain-Barré syndrome
Meralgia paraesthetica
Burning mouth syndrome
High-concentration capsaicin creams (10%) to ameliorate pain
Intractable pain due to bilateral peripheral neuropathy
Capsaicin creams to relieve itch
Psoriasis
Hemodyalisis
Aquagenic pruritus
Vulvar vestibulitis
Notalgia paraesthetica
Brachioradial pruritus
Lichen simplex chronicus
Intranasal capsaicin drops (10 mM)
Cluster headache
Vasomotor rhinitis
Perennial allergic rhinitis
Intravesical capsaicin solution (10 mM)
Bladder hypersensitivity
Spinal detrusor hyperreflexia

See section XII for references.

Those who favor the use of capsaicin creams in clinical practice frequently cite the finding that topical capsaicin effectively desensitizes the rat skin to neurogenic inflammatory agents (McMahon et al., 1991). Human skin is, however, less permeable (by a factor of 4–8) than rat skin to capsaicin (Kasting et al., 1997). In rat skin, capsaicin analogs are extensively metabolized during passage, the primary route of degradation being the hydrolysis of the amide bond (Kasting et al., 1997). This is probably also the case in human skin. Due to a combination of low potency and poor bioavailability, capsaicin applied topically on the skin is not likely to effectively desensitize nerve endings in human skin. This conclusion is supported by the lack of effect of topical capsaicin on the SP immunoreactivity in human skin biopsy samples (Munn et al., 1997). [This is in dramatic contrast to the marked degeneration of SP-containing cutaneous nerve fibers following intradermal injections of high capsaicin doses (Simone et al., 1998).]

Finally, there are reports that capsaicin may be beneficial in the treatment of the following disease states: Guillain-Barré syndrome (Morgenlander et al., 1990), reflex sympathetic dystrophy (Cheshire and Snyder, 1990), meralgia paresthetica (Puig et al., 1995), stump pain (Rayner et al., 1989), apocrine chromhidrosis (Marks, 1989), burning mouth syndrome (Huang et al., 1996), and vulvar vestibulitis (Friedrich, 1988) (Table 9). As yet, these reports

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should be considered anecdotal due to the very small number of patients (at best, five cases) involved.

A new approach to enhance the clinical effectiveness of capsaicin is to use large doses (a 10% solution instead of the commercially available 0.025% and 0.075% creams) under regional anesthesia (Robbins et al., 1998). Of 10 patients with intractable pain due to bilateral peripheral neuropathy, 9 obtained significant analgesia by this treatment protocol that lasted 1 to 18 weeks. The authors concluded that intermittent use of large-dose capsaicin may decrease chronic analgesic dependence. Another, as yet, clinically untested possibility is epidural vanilloid administration. In the rat, epidural capsaicin caused prolonged, segmental thermal analgesia (Eimerl and Papir-Kricheli, 1987).

We think that the unsatisfactory clinical results with capsaicin are predominantly due to the unfavorable ratio of irritation to desensitization of this drug, which is also apparent in animal studies. Consequently, a drug with improved desensitization to irritation ratio (ideally, causing lasting desensitization with no prior pungency) promises to be of great clinical value. Ongoing clinical trials with RTX indicate (see below) that such vanilloids may in fact be synthesized.

C. Adverse Effects of Topical Capsaicin

The most important adverse effect of capsaicin is the initial burning sensation that it produces (cf. Carter, 1991; Watson, 1994; Cruz, 1998). According to both animal experiments (Craft and Porreca, 1994b; Avelino et al., 1998a) and clinical observations (Fowler et al., 1994; Chandiramani et al., 1996; Dasgupta et al., 1998a), the pain response to capsaicin can be minimized by lidocaine administration without compromising the desired effect, desensitization. However, as we saw above, the real problem with topical capsaicin is not its pungency but rather its poor efficiency to achieve clinically useful desensitization which, of course, cannot be improved with lidocaine.

Capsaicin is usually regarded as a remarkably safe drug and, other than pungency, very few adverse effects have been reported. Initial studies indicated a relatively high frequency (affecting 5–12% of the patients) of respiratory problems (cough and sneezing) (Capsaicin Study Group, 1991; Scheffler et al., 1991; Ellis et al., 1993; Watson et al., 1993), but it may effectively be eliminated by bathing the skin 30 to 40 min after treatment to prevent aerosolization of the unabsorbed, dried capsaicin (Marciniak et al., 1995). It should be noted, however, that one nurse with a history of asthma experienced severe enough congestion, coughing, and shortness of breath when applying capsaicin cream on a patient to require use of her albuterol inhaler (Marciniak et al., 1995). It is recommended therefore that all health care workers, especially those who are asthmatic, wear masks during application of capsaicin creams.

D. Novel, Innovative Clinical Uses

Aspiration pneumonia is among the most common causes of death in the elderly. Both swallowing and cough reflexes are mediated by SP released from vagal sensory nerve endings subserving the pharynx and the upper airways (cf. Lundberg, 1993; Karlsson, 1996). It has been suggested that supplementation of food with capsaicin, which stimulates SP release, may help prevent aspiration pneumonia (Sasaki et al., 1997). An added benefit of this diet is the improved clearance of the esophagus (Gonzalez et al., 1998).

A particularly attractive and, as yet, largely unexplored area for the therapeutic use of vanilloids is weight control. It was concluded in 1986 that those who eat plenty of hot, spicy food have a high metabolic rate and stay lean (Henry and Emery, 1986). In 1990, Cameron-Smith and colleagues indicated the need to "evaluate capsaicin as an antiobesity or slimming agent in humans". More recently, two independent groups have shown that dietary hot pepper increases energy expenditure and diminishes long-term excess energy intake in humans at the same time (Doucet and Tremblay, 1997; Lim et al., 1997). This is entirely in accord with the animal experiments, which have repeatedly shown enhanced oxygen uptake following capsaicin administration (Kawada et al., 1986; Watanabe et al., 1987; Cameron-Smith et al., 1990). The area postrema/nucleus of the solitary tract area is believed to play an important role in satiety (cf. South and Ritter, 1983). The presence of VRs in this area is firmly established (Szallasi et al., 1995a). It is more likely than not that VRs in the area postrema mediate the antiemetic actions of vanilloids, too (Andrews and Bhandari, 1993; Matsuki et al., 1996; Shirosita et al., 1997).

Last, it is noteworthy that capsaicin is bacteriocidal to *Helicobacter pylori* (Jones et al., 1997). In animal experiments, topical capsaicin protects against gastric ulcer formation (cf. Abdel Salam et al., 1994). Thus, ingestion of chili peppers (or supplemetation of the diet with capsaicin) could have a protective effect against gastroduodenal diseases. In the rat, orally administered RTX at a dose as low as 0.6 μg/kg was reported to protect against ethanol- or aspirin-induced gastric mucosal damage (Abdel Salam et al., 1995).

E. RTX, an Improved Vanilloid Drug Undergoing Clinical Trials

RTX was first isolated in 1975 by Hecker and coworkers (Hergenhahn et al., 1975) but it was not until 1989 that it was identified as an ultrapotent vanilloid (De Vries and Blumberg, 1989; Szallasi and Blumberg, 1989a). Nonetheless, euphorbium, the dried latex of *E. resinifera*, has been in medicinal use as an analgesic for two millennia (Fig. 5) (cf. Appendino and Szallasi, 1997). As we saw above, in animal experiments RTX shows a far more favorable ratio of desensitization to irritation

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than does capsaicin (Table 3) (cf. Szallasi and Blumberg, 1990a, 1993b; Blumberg et al., 1993). RTX is also more potent than capsaicin for desensitization (Fig. 21) (cf. Szallasi and Blumberg, 1990a, 1996).

As discussed above, the overactive bladder is one of the few clinical conditions in which capsaicin has fulfilled expectations (cf. Chancellor, 1997; De Ridder et al., 1997). Intravesical capsaicin is as effective as the oral anticholinergic drugs traditionally used to treat bladder hyperreflexia; however, capsaicin bypasses the very unpleasant, atropine-like side-effects of these drugs (cf. Andersson, 1997). Better yet, capsaicin has no effects on the detrusor muscle and therefore it does not increase residual urine (cf. Cruz, 1998). Finally, no premalignant or malignant changes were found in the urinary bladders of patients who had received repeated capsaicin instillations for up to 5 years (Dasgupta et al., 1998b). No wonder that 16 posters featured capsaicin treatment at the last two annual American Urological Association meetings. Intravesical capsaicin, however, has its own side effects (cf. Cruz, 1998), prompting clinicians and pharmacologists to search for better vanilloid drugs. First, as mentioned above, capsaicin induces intense suprapubic pain during intravesical instillation that may be made tolerable by lidocaine in some but not all patients. Second, capsaicin frequently causes a transient worsening of the urinary conditions, before improvement of symptoms due to desensitization of bladder afferents becomes evident (Fowler et al., 1994; Das et al., 1996; Cruz et al., 1997b; De Ridder et al., 1997). And third, in patients with high spinal cord lesions capsaicin might provoke life-threatening autonomic dysreflexia (Geirsson et al., 1995).

Porreca and colleagues (Craft et al., 1993; Craft and Porreca, 1994a,b) found RTX to be approximately 1000 times more potent than capsaicin for desensitizing urinary bladder afferents in the rat. RTX treatment depletes SP from sensory nerves supplying the rat urinary bladder (Fig. 20) (Avelino et al., 1998b) and down-regulates specific RTX binding sites (Fig. 20) (Goso et al., 1993b). These RTX-induced changes are long-lasting in the bladder but completely reversible (Fig. 20). Inspired by these findings, in 1997 two groups working independently initiated the intravesical use of RTX in humans. Turini and colleagues (Lazzeri et al., 1997) compared the action of RTX (30 ml of 10 nM RTX for 30 min) in controls (eight individuals) and detrusor hyperreflexia (six cases) patients. Intravesical RTX in subjects with a normal filling cystometrogram did not produce significant functional changes. RTX, however, did increase the mean bladder capacity in patients with hyperactive bladder from 175 to 280 ml. This improvement persisted up to 4 weeks. Even more important, none of the subjects involved in this study reported any discomfort after RTX treatment. Cruz and coworkers (Cruz et al., 1997a) chose to pursue a more aggressive treatment protocol, 100 ml of 50 to 100 nM RTX solutions for 30 min. Again,

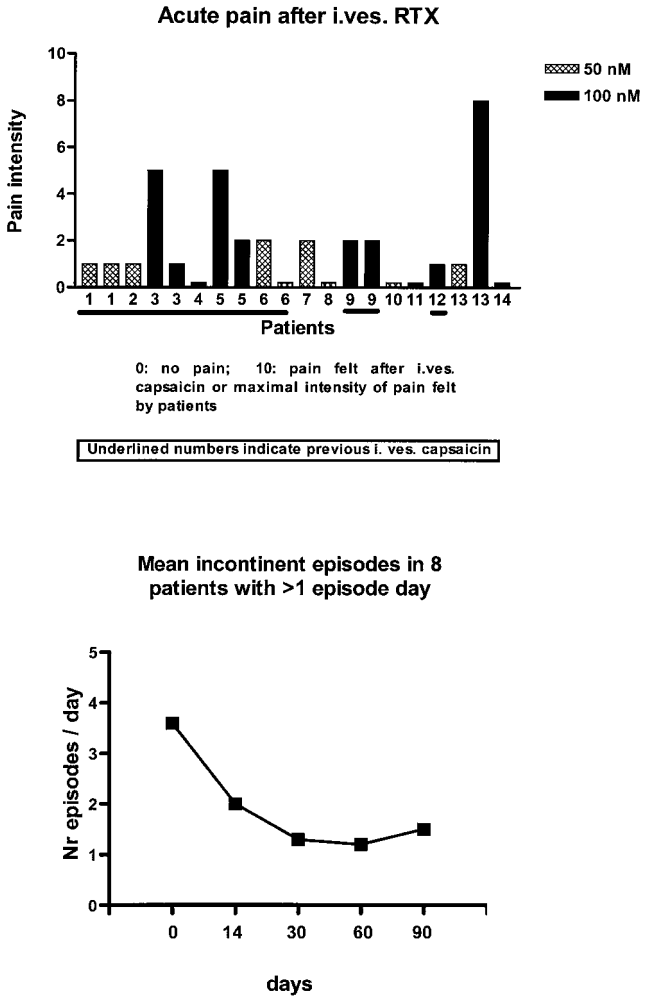

FIG. 23. Upper panel. Acute suprapubic pain experienced by patients following intravesical RTX treatment. Of the 14 patients, 9 received intravesical capsaicin previously. They were asked to rate the pain evoked by RTX on a subjective scale of 0 to 10, where 10 represents the intensity of pain response to intravesical capsaicin. All the patients reported no pain at all or minimal discomfort only in response to 50 nM RTX. Lower panel. Average of incontinent episodes in 8 patients who have received a single intravesical RTX treatment: note the remarkable and long-lasting improvement in the condition of the patients. Figure is a courtesy of Dr. Francisco Cruz, University of Porto, Porto, Portugal.

discomfort evoked by RTX was minimal (Fig. 23, upper panel) and the improvement was even longer lasting (up to 3 months following a single treatment; Fig. 23, lower panel). Based on these reports, a recent editorial in *The Journal of Urology* (Chancellor, 1997) concluded that "Perhaps we should go directly to RTX as the preferred intravesical drug to inhibit the sensory c-fiber. RTX appears to have efficacy similar to capsaicin but with much less acute side effects". Clearly, intravesical RTX results in a lasting improvement in the life of patients with urge urinary incontinence (Fig. 23, lower panel) (Cruz et al., 1998). Patients who previously had to rely on adult diapers became "dry" (continent) again following a single intravesical treatment with RTX (Fig. 20). No wonder that the RTX results met with the enthusiasm of the urology community.

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XIII. Vanilloids: Carcinogens, Anticarcinogens, or Neither?

A. Capsaicin

Given the broad human exposure to capsaicin (both dietary and medicinal), reports that capsaicin may be mutagenic, may promote tumor formation, or may act as a complete carcinogen deserve serious and critical consideration. Before going into details, it is important to emphasize that there is a concensus in the literature that it is not capsaicin itself but its liver metabolites that may be hazardous (cf. Surh and Lee, 1995, 1996). Consequently, topical capsaicin solutions and creams are most likely safe to use. Fowler and colleagues (Dasgupta et al., 1998b) followed 20 patients for 5 years who had received an average of six intravesical treatments with capsaicin. None of the bladder biopsies showed any premalignant (metaplasia or dysplasia) or malignant (in situ, papillary or invasive cancer) changes.

1. Mutagenesis by Capsaicin. There are several reports both for (Toth et al., 1984; Nagabhushan and Bhide 1985, 1986; Lawson and Gannett, 1989; Azizan and Blevins, 1995) and against (Buchanan et al., 1981; Gannett et al., 1988; Kim et al., 1991; Vinitketkumnuen et al., 1991) capsaicin-induced mutagenesis in *Salmonella typhimurium*. Based on these conflicting findings, three major conclusions may be drawn: 1) capsaicin is not mutagenic unless metabolized by activated liver microsomes (S9 fractions); 2) capsaicin metabolites are weak mutagens (maximal mutagenicity is 3-fold higher than the background value); and 3) to detect mutagenicity, extremely high capsaicin concentrations are needed (up to 7 mM), which are most unlikely to occur in the liver of human beings.

As is the case with bacteria, capsaicin does not cause mutations in mammalian cells (V79 cell line) in culture unless metabolized by S9 hepatic microsomes (Nagabhushan and Bhide, 1985; Lawson and Gannett, 1989). At a concentration of 200 μM capsaicin, only 1.4 mutants per one million survivors can be observed (Lawson and Gannett, 1989). By comparison, an approximately 50-fold lower concentration of benzopyrene induces at least 60 times more mutations (Nagabhushan and Bhide, 1985).

When injected i.p., capsaicin inhibits DNA synthesis in the mouse testis (Nagabhushan and Bhide, 1985). This action, however, was detected using 7.5 mg/kg capsaicin, which is the approximate LD_{50} value of capsaicin given i.p. to the mouse (Glinsukon et al., 1980). Thus, the relevance of this study is highly questionable.

2. Carcinogenesis by Capsaicin: Animal Experiments. Since the initial report by Hoch-Ligeti (1951) that chili pepper consumption may induce liver carcinoma formation in the rat, a number of studies have investigated the possibility that capsaicin may be carcinogenic or at least cocarcinogenic in animal experiments. To mimic natural exposure (and to save cost), most studies employed chili pepper powders instead of pure capsaicin in the diet of animals (Hoch-Ligeti, 1951; Kim et al., 1985; Agrawal et al., 1986). Most of these studies reported a promoter effect by chili pepper on gastrointestinal tumor formation. However, even if one ignores the relevancy of a diet containing 10% (w/w) chili pepper powder (Kawada et al., 1984), the question arises whether the observed carcinogenic effect was in fact due to capsaicin or rather some other constituent in the chili pepper powder?

Inclusion of pure capsaicin into mouse chow was also reported to increase the incidence of gastrointestinal tumors (Toth et al., 1984). Moreover, a capsaicin-containing diet promoted the formation of diethylnitrosamine-induced hepatomas in the rat (Jang and Kim, 1988). Again, the interpretation of these findings is complicated by the extremely high capsaicin doses used. For example, in a frequently cited study (Toth et al., 1984) mice were fed as much as 150 mg/kg capsaicin per diem, which exceeds the usual human capsaicin consumption (0.5–1.0 mg/kg in tropical countries and probably much less than in the United States or Europe) by a factor of 100. Of special importance are the findings that repeated application of capsaicin on shaved mouse skin neither leads to carcinoma formation nor enhances papilloma formation following initiation of the skin by the complete carcinogen 7,12-dimethyl-benzanthracene (Park and Surh, 1997). Quite the contrary, capsaicin seems to inhibit papilloma formation when given before each topical phorbol ester (a typical tumor promoter) application to the initiated skin (Park and Surh, 1997).

In the liver, three major metabolic pathways are believed to generate hazardous, potentially mutagenic and/or carcinogenic capsaicin metabolites (cf. Surh and Lee, 1995; 1996). First, epoxidation of the vanillyl moiety by hepatic cytochrome P-450 (particularly CYP2E1) can produce an arene oxide. Second, demethylation and subsequent oxidation of the vanillyl group can yield (semi)quinone derivatives. And third, oxidation of the phenolic OH group on the vanillyl moiety can form a reactive phenoxy radical. However, liver cells possess very effective self-protective mechanisms such as reduced glutathione as well as conjugation reactions; thus, unless capsaicin is given in high, abusive doses or these self-protective mechanisms are malfunctioning, mutagenic capsaicin metabolites are very unlikely to escape the hepatic detoxification process.

3. May Culinary Hot Pepper Consumption Be a Risk Factor for Stomach Cancer in Humans? The laboratory studies indicating that chili peppers may induce tumor formation in the gastrointestinal tract of rodents (Toth et al., 1984; Kim et al., 1985; Agrawal et al., 1986) have prompted to date three epidemiological studies (Notani and Jayant, 1987; Buiatti et al., 1989; López-Carillo et al., 1994) to compare the incidence of stomach cancer between hot pepper consumers and nonconsumers. Whereas studies carried out in Mexico (López-Carillo et al., 1994) and India (Notani and Jayant, 1987) found a

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significant correlation between chili pepper consumption and risk of having gastric cancer, the Italian study (Buiatti et al., 1989) reached the opposite conclusion: eating hot pepper on a regular basis protected against carcinoma of the stomach. Further studies are needed to confirm or rule out the hazards of hot pepper consumption.

4. Capsaicin: A Potential Antitumor Agent? The potential carcinogenic activity of capsaicin has been a focus of attention, however, the reports that capsaicin might be anticarcinogenic (Miller et al., 1993; Teel, 1993; Zhang et al., 1993, 1997) have received less publicity. Various chemical carcinogens must undergo metabolic activation. Important examples include tobacco-specific nitrosamines, benzopyrene, and aflatoxin. These carcinogens and capsaicin are processed, at least in part, via the same metabolic pathways in the liver (cf. Surh and Lee, 1995, 1996). Consequently, capsaicin may be chemoprotective by retarding the metabolic activation of carcinogens. A recent interesting observation is that capsaicin can inhibit the growth of a number of transformed cell lines (Morré et al., 1995). For example, HL-60 human promyelocytic leukemia cells are unable to divide in the presence of 100 μM capsaicin; however, these cells become resistant to the growth inhibitory action of capsaicin after they have been induced to differentiate with dimethyl sulfoxide. It is believed that the transformed cells express an unusual NADH oxidase isoform that is absent in the normal cells (Morré et al., 1995). This putative enzyme is the suggested target for capsaicin.

B. RTX

Although human contact with *Euphorbium*, the dried latex of *E. resinifera* from which RTX is isolated, has occurred over 2000 years (cf. Appendino and Szallasi, 1997), some concern still surrounds the medicinal use of RTX. This concern stems from the structural similarity between RTX and tumor-promoting phorbol esters (cf. Blumberg et al., 1993). Phorbol esters enhance papilloma formation in mouse skin pretreated (initiated) with a subeffective dose of a complete carcinogen (cf. Hecker, 1968). A receptor for phorbol esters was identified as the enzyme PKC (cf. Blumberg, 1988). The structure-activity relations for phorbol ester binding to PKC have been explored in depth. PKC binding requires the presence of a free OH group at the C20 position (Hecker, 1978). However, RTX is esterified with a homovanillic acid substituent at this position (Hergenhahn et al., 1975). Therefore it is hardly surprising that RTX shows only marginal affinity toward PKC. For example, RTX inhibits the specific binding of [^{3}H]phorbol 12,13-dibutyrate to PKC in rat DRG membranes with a K_i value of 10 μM (Szallasi and Blumberg, 1990b). Furthermore, [^{3}H]RTX binds to partially purified PKC with an affinity of 404 nM (Szallasi et al., 1989b). Evans and coworkers (Dimitrijevic et al., 1995) determined the affinity of RTX for seven recombinant PKC isotypes and reported values of 9 μM and 45 μM for isotypes β_1 and β_2, respectively. The other PKC isoforms did not bind RTX. In 1995, the isolation of a RTX-stimulated, Ca^{2+}-inhibited but phosphatidylserine-dependent kinase from human neutrophils and murine macrophages was reported (Sharma et al., 1995): this so-called "Rx-kinase" bound RTX with an affinity of 4 μM. These affinities should be compared with the affinity of RTX for VRs, which is approximately 20 pM in rat (Szallasi et al., 1992, 1993a,b; Ács et al., 1994a) and 500 pM in human spinal cord (Ács et al., 1994b), respectively. At concentrations at which it saturates VRs, RTX is unlikely to activate PKC. In keeping with this, RTX is inactive in the cellular assays used to detect phorbol ester-like activity (Driedger and Blumberg, 1980).

In theory, RTX applied to the skin and bladder or nasal mucosa might be deesterified at C20 position to yield its parent diterpene, resiniferonol 9,13,14-orthophenylacetate (ROPA). ROPA is approximately 10-fold more potent than RTX for PKC binding (Szallasi et al., 1989b). Also, ROPA is a weak tumor promoter (Hergenhahn et al., 1982). Direct experimentation shows, however, that RTX does not induce hyperplasia in mice (Szallasi and Blumberg, 1989b) nor does it promote the formation of tumors in initiated (that is, pretreated with a subthreshold dose of carcinogen) mouse skin (Zur Hausen et al., 1979).

XIV. Concluding Remarks, Emerging Principles, and Future Perspectives

Over the past 6 years, vanilloid research has experienced an unprecedented rate of advances. The simple model of a single receptor recognizing a chemically well-defined pharmacophore can no longer be maintained anymore. In 1997, a VR, termed VR1, was cloned. This VR1 is a distinct relative of the TRP family of store-operated calcium channels. Sequences (ESTs) similar to VR1 are also found in nonneuronal tissues. This is entirely in accord with the demonstration of VR-mediated calcium uptake by mast cells and glia. Taken together, these findings imply that capsaicin is much less specific for primary sensory neurons than was thought before and thus "nonspecific" capsaicin actions need to be carefully reevaluated. VR1 is activated not only by vanilloids but also noxious heat and acids, thus it can now be viewed as a molecular integrator of chemical and physical stimuli that elicit pain. The emerging concept is that it is heat that gates VR1, whereas capsaicin and low pH only serve to reduce the heat activation threshold of the receptor. An important implication of this model is that even innocuous (for example room) temperatures are able to stimulate VR1 in the presence of mildly acidic conditions.

In contrast to early reports, capsaicin activates not a single conductance but multiple inward currents that differ in kinetics, affinity for agonists, and sensitivity for antagonists. These both kinetically and pharmacologi-

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cally distinct currents seem to represent VR1 isoforms and/or related receptor subtypes and thus imply heterogeneity of VRs.

VR1 homologs, however, may not necessarily mediate heat-, acid-, and/or vanilloid-sensitivity. The recognition domains for these types of activation are not well understood, and they may not be at all conserved. If there is an extended VR gene family, it may be associated with a very diverse biology. The presence of VRs in several of brain nuclei as well as in nonneuronal tissues lends support to this prediction.

Inflammatory mediators (like bradykinin and prostanoids) have been shown to activate VRs in sensory neurons in an indirect fashion. The intracellular mediator(s) of this actions is(are) unknown. The competitive VR antagonist capsazepine prevents the activation of VRs by inflammatory mediators, which implies the involvement of an endogenous vanilloid. Moreover, capsazepine is also effective in animal models of chronic pain and inflammation. Thus, VRs appear to have a complex regulation, in which exogenous vanilloid drugs, endogenous VR activators, low pH, and heat may facilitate each other's effects.

Another breakthrough discovery in the field was the demonstration that compounds structurally unrelated to typical vanilloids, namely sesquiterpene unsaturated dialdehydes and other bioactive terpenoids, as well as triprenyl phenols, may activate sensory neurons via stimulation of VRs. These novel vanilloids represent new chemical leads for drug development. Also, their very existence implies that the term VRs is somewhat of a misnomer. The identification of endogenous "vanilloids" will give these receptors a rational name.

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Appendix. Mr. and Mrs. Miklós Jancsó (1949) Desensitization of sensory nerve endings (in Hungarian), Kísérletes Orvostudomány (Experimental Medicine) **2(Suppl):**15.

There are compounds that can selectively desensitize sensory nerve endings to noxious chemical stimuli without causing local anesthesia. In the state of desensitization, reflex responses (e.g., corneal reflex) mediated by sensory nerves may be evoked by physical stimuli. However, the same sensory nerves are unresponsive to noxious chemical stimuli.

Capsaicin is the paradigm of such desensitizing agents. Capsaicin effects last for days and protect against various chemicals. Repeated instillation of capsaicin into the eye of rats or guinea pigs results in gradually increasing desensitization which finally becomes complete. Following capsaicin treatment, eyes do not respond to other pungent or tear-inducing agents, either: the sensitivity of eyes to such agents may diminish by a thousand-fold. Repeated capsaicin treatments lead to desensitization of skin and airways as well.

Nicotine is nonpungent in the guinea pig eye pretreated with nicotine, lobeline, coniine, or sparteine. This desensitization by nicotine is, however, short in duration (20 min to 2 h) and specific to the stimulus: it does not protect against capsaicin. Our experiments show that the nicotine family of toxins act on sensory nerves and parasympathetic neurons in a similar fashion. The fact that atropine desensitizes to nicotine highlights the importance of the piperine structure.

Note Added in Proof. Most recently, the cloning of two vanilloid receptor homologs has been reported. One homolog was cloned from rat brain and termed VRL1 for vanilloid receptor-like protein 1 (Caterina et al., 1999). This terminology is, however, confusing as VRL1 does not respond to vanilloids. The other homolog is present in rat kidney where it is believed to sense stretch (Suzuki et al., 1999). This receptor is called stretch-inhibitable nonselective cation channel, or, briefly, SIC. SIC is not sensitive to vanilloids either.

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