0031-6997/83/3502-0085$02.00/0
PHARMACOLOGICAL REVIEWS

Vol. 35, No. 2
Printed in U.S.A.

Substance P

BENGT PERNOW

Department of Clinical Physiology, Karolinska Institute, Stockholm, Sweden

I. Introduction

THE PEPTIDES constitute the newest class of molecules considered to play key roles as transmitters or modulators in the central and peripheral nervous system. The list of these peptides is steadily growing and now includes more than 20 different molecules, some of them being structurally related (261). Of all these peptides, Substance P (SP) has the longest history and is probably the best characterized as far as distribution, release, and biological properties are concerned.

The story of SP began in 1931 (164) and important works on its distribution and pharmacological effects were performed during the following 30 years. These studies, however, were restrained by the lack of chemically pure preparations and specific analytical methods. The isolation and chemical characterization of SP in 1970 (71, 72), followed by its synthesis (624), was, therefore, an important milestone in the scientific saga of SP and opened possibilities to develop highly specific immunohistochemical and radioimmunological techniques for detailed mapping of the distribution and release of SP. These discoveries substantially stimulated the research on SP, which has exploded during the last few years (fig. 1).

This review will describe our present knowledge of the distribution of SP in various organs, its release and pharmacological properties, and will discuss briefly the putative physiological role of the peptide. The main emphasis will be on the progress made after 1970, whereas studies performed earlier will be mentioned only briefly since they have already been reviewed extensively (658, 403, 58). The reader is also referred to other reviews, which describe various aspects on progress in SP research (484, 471, 382), and to symposia entirely or partly devoted to SP (566, 590, 645, 168, 438, 385, 530). Finally, the continuous follow-up of the entire literature on SP by Skrabanek and Powell (582) is invaluable for all scientists interested in this field.

II. Studies before 1970

In 1931, von Euler and Gaddum (164) reported that extracts of equine brain and intestine contained a hypotensive and spasmogenic factor, which could be separated from all biologically active principles known at that time. It differed qualitatively from acetylcholine and its biological effects were not blocked by atropine. Furthermore, the new active substance, which was prepared in a form of dry powder and called preparation P, showed chemical and biological characteristics, which distinctly separated it from histamine and adenine nucleotides. Early findings suggested that the new compound, provisionally named Substance P (187), might be a protein (160).

A. Chemistry

1. Extraction. SP was originally extracted by mincing the tissue in acid ethanol. After stirring for 1 hour the alcohol was evaporated under pressure. Lipids were removed by ether in which SP is insoluble (160, 161, 164). A simpler method used later (162, 513, 5) was to suspend the minced tissue in acidified water and boil for a few minutes. After neutralization the extract could then be immediately tested on various biological preparations.

SP could be precipitated from aqueous solutions by ammonium sulphate (162), while most other biologically active compounds such as histamine, 5-hydroxytryptamine (5-HT), and adenosine nucleotides remained in the supernatant.

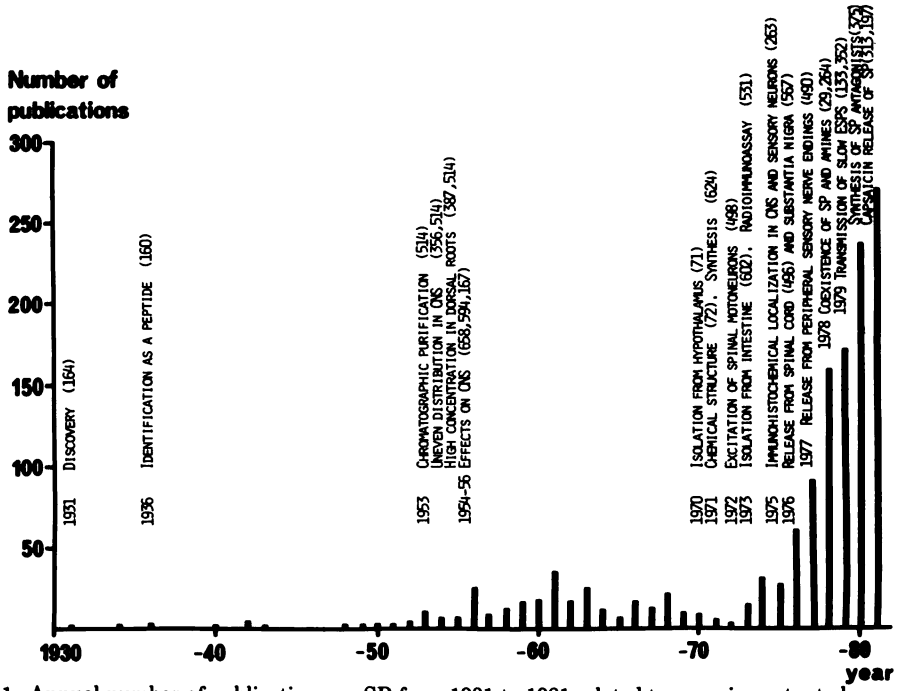

FIG. 1. Annual number of publications on SP from 1931 to 1981 related to some important observations.

2. Purification. The first attempt to purify SP was made in 1953 when Pernow (514) obtained a high degree of purity by absorption of SP from alcoholic solutions on aluminium oxide columns and elution with water. A further passage on a cellulose column and elution with butanol/acetic acid/water yielded a highly active preparation that contained 13 amino acids (514). Further use of advanced purification procedures such as gel filtration, ion exchange chromatography, and high voltage electrophoresis yielded SP preparations close to purity (176, 34, 662).

3. Chemical Properties. Partly purified SP was easily soluble in water and alcohol but insoluble in ether and chloroform. It tolerated boiling at pH 1 to 7 but was rapidly destroyed in alkaline environment (161, 162, 164). During paper electrophoresis it traveled to the cathode at pH < 10 and its isoelectric point was near 9 (516).

The biological activity of SP was destroyed by trypsin and pepsin (160) but resistant to carboxypeptidases (176). The SP destroying activity of chymotrypsin was about 200 times greater than that of trypsin (515). The weak effect of the trypsin preparations used possibly was due to the presence of traces of chymotrypsin. Extracts of several organs such as the kidney, spleen, intestine, liver, and lung destroyed SP (216, 515, 136).

Since no physicochemical or biological differences could be observed between SP prepared from brain and intestine, they were considered identical (148).

B. Occurrence and Tissue Distribution

1. Bioassay. Detailed studies on the occurrence of SP in various organs were performed in the 1930s and 1950s. These studies were based entirely on bioassay of crude tissue extracts. In the first studies (160, 164) the hypotensive effect of SP in the rabbit and spasmogenic effect on isolated rabbit jejunum were used as test of activity. Later the isolated guinea-pig ileum was found to be more suitable (127, 513) and thereafter used for routine assay. The most sensitive assay preparation was, however, the isolated goldfish intestine with the microtechnique suggested by Gaddum and Szerb (188), which allowed the detection of microunits of SP. (One unit of SP corresponded to 2 to 4 times the threshold dose on the isolated rabbit jejunum in a 30-ml Tyrode's solution (162) or 7 to 10 times the threshold dose on the guinea-pig ileum in a 3-ml organ bath (514).

2. Nervous System. SP can be extracted from the brain of all vertebrate species from fish to mammals, including man (514, 166, 659, 593, 368). The concentration of SP seemed to be lower in species with highly differentiated brains, since SP in whole brain was found to increase in the following order—man, cat, rabbit, guinea-pig, rat, duck, pigeon, and chicken (213). A detailed mapping of the occurrence in various parts of the mammalian brain including man was performed in the 1950s (356, 514, 5, 660, 504). Generally, the SP concentration was much higher in the grey than in the white matter. The cerebellum contained negligible amounts. Relatively low concentrations also were found in the cortex, except for the anterior cingulate gyrus and the somatomotor, somatosensory, and olfactory areas, where high or moderate concentrations were identified.

In the subcortical areas considerably higher SP concentrations were found than in the cortex. However, the distribution was very uneven. The highest values were found in the substantia nigra, but also in the hypothalamus, globus pallidus, caudate nucleus, putamen, and

central grey matter high concentrations were present. The medulla oblongata generally contained moderate amounts, but with certain regions such as the area cinerea and area postrema again showing high concentrations.

In the spinal cord, the dorsal half was found to contain more SP than the ventral. As in the brain, more SP was present in the grey matter than in the white, except for the dorsal columns, where much more SP was found than otherwise in the white matter. The dorsal roots were 5 to 10 times richer in SP than the ventral roots.

SP was also found in peripheral nerves, although much less than in most parts of the central nervous system (514). The highest concentrations were observed in the spinal ganglia and cervical sympathetic trunk, whereas the thoracic trunk and the vagus contained smaller amounts. Small but significant amounts were also found in the stellate and nodose ganglion as well as in the sciatic, splanchnic, phrenic, and splenic nerves.

After section of the sciatic nerve, a significant decrease of SP occurred in the distal stump, while the SP concentration rose in the proximal part to about 600% of the control (276). From this observation Pamela Holton concluded: "It seems that substance P behaves more like the substances associated with transmission from nerve endings than those which are not" (277).

The subcellular distribution of SP was studied both in the central nervous system and in peripheral nerves. In broken cell preparation of brain tissue, two thirds of the total SP activity could be found in the mitochondrial fraction (396).

SP was present together with acetylcholine, histamine, and 5-HT in the mitochondrial subfraction, which contains the synaptic vesicles of the nerve endings (186, 297). In peripheral nerves, the SP activity was found in the microsomal fraction, where it evidently was stored in a conjugated inactive form, since the activity could be elicited only after sulphuric acid treatment (165).

3. Gastrointestinal Tract. SP was found in the intestine of mammals (164), fish (166), and frogs (403). Detailed studies on the distribution of SP in the gastrointestinal tract (127, 513) showed that SP occurred in all parts with low concentrations in the oesophagus and stomach, high in the duodenum and jejunum, and progressively decreasing amounts in the small intestine towards the caecum. In the colon and rectum the concentration was moderate. Large species differences occurred with the highest concentrations in the monkey (approximately 50 units/g in the duodenum).

All tissue layers of the intestine were found to contain SP, particularly in the muscularis mucosae with the submucous nerve plexus, whereas the concentration was equally distributed in the external muscle layers. Lower concentrations were found in the mucosa (127, 513). In the gut, SP also seemed to be connected to nervous structures, since in Hirschsprung's disease low concentrations of SP were found in the distal aganglionic segment of the rectosigmoid colon while the proximal, neuroanatomically normal part showed high values (143).

C. Pharmacological Actions

Von Euler and Gaddum (164) reported that SP stimulates the intestinal smooth muscle and lowers blood pressure. During the following 30 years, studies on the pharmacology of SP were mainly concentrated on the gastrointestinal tract and circulation, while the effect of SP on the nervous structures was studied less intensively.

1. Intestine. SP was found to effectively stimulate smooth muscle in all parts of the gastrointestinal tract as well as in the isolated uterus, ureter, and urinary bladder, whereas the gall bladder was almost resistant to SP. These effects were shown in mammals, birds, and amphibia (160, 514, 403). The spasmogenic effect of SP was not blocked by atropine, hexamethonium, nicotine, cocaine (514), antihistamines (127), or 5-HT antagonists (185), which suggested that SP acts directly on the smooth muscle fibers (514). With the Trendelenburg technique, a peristaltic reflex could be elicited at a lower threshold when SP was present (205). Hexamethonium inhibited the effect of SP on peristalsis, indicating that SP, in addition to its direct effect on smooth muscle, probably also stimulates nerve fibers of the peristaltic reflex arc (18).

Intravenous or local application of SP in the rabbit stimulated the intestinal motility in vivo (205). In man, intravenous infusion of 1000 units during a 20-minute period increased both segmental and peristaltic motility as illustrated by cineradiology (408). A transient motility was also initiated in patients with intestinal paralysis (408).

2. Circulation. The hypotensive effect of SP was found to be due to a peripheral vasodilatation and no cardiotropic effects were observed (160). The fall in blood pressure following intravenous or close-arterial injections was dose-dependent and not blocked by atropine, antihistamine, or ganglionic blocking agents (514, 414).

Also, close-arterial injection in man of a few units of SP in the forearm elicited a rapid and transient vasodilatation (414). Intravenous infusions in man led to a bright red flush in the face, a fall in blood pressure, and tachycardia (135). SP was about 100 times more potent as a vasodilator than bradykinin (414). Cardiac output and stroke volume were increased, when SP was infused intravenously in man (135).

3. Nervous System. The observations of high concentrations of SP in certain areas of the central nervous system early initiated pharmacological studies on SP in the brain. Particular attention was focused on the observations that crude SP preparations elicited sedative effects in mice (658) and wild hares (594), measured as a reduction in spontaneous and induced motor activity. SP was also found to antagonize morphine and methylamphetamine (658), prolong hexobarbitone sleeping time (658), and protect against convulsions induced by

strychnine and picrotoxin (658) or tetanus toxin (591). Later, Stern and Hucović (592) repeated all these experiments with partly purified SP preparations. Their preparations failed to antagonize convulsions caused by strychnine or to prolong the hexobarbitone sleeping time but were still able to antagonize morphine analgesia when SP was given in small doses (1000 units/kg).

Intracerebroventricular injection of partly purified SP in rabbits and cats elicited various neurotropic effects such as inhibition of spontaneity, licking, and stupor. The most constant finding was, however, a long-lasting stimulation of respiration (167). Local application of crude SP on the cortex of the cat (94) or injection into the carotid artery (94, 376) increased cortical activity and excitability. In contrast, Caspers and Stern (67) found an EEG-pattern resembling that of sleep when applying SP locally to the cortex. Although these results were conflicting, they indicated that SP exerts significant central neurotropic effects, which stimulated future studies with pure SP.

D. Possible Physiological Role

The widespread distribution of SP and its pronounced pharmacological effects early initiated the discussion on its putative physiological role. Although several proposed functions for SP were pure speculations, some are still relevant. Most authors were anxious to emphasize that the SP preparations used were often far from purity and that some of the effects observed, therefore, might be due to factors other than SP.

The uneven distribution of SP in the central nervous system seemed to indicate clear-cut functional roles for the substance. Of particular importance was the observation of much higher SP concentrations in the dorsal than in the ventral roots of the spinal cord (387, 514), which was the basis for the hypothesis that SP may serve as a transmitter of the primary sensory neuron (387). The observation of large amounts of SP in the intestinal wall and its powerful spasmogenic effects led to early speculation that SP may play a role in the control of intestinal motility (160).

III. Isolation of SP and Its Identification as an Undecapeptide

The first complete purification of SP was done by Chang and Leeman (71) as a "result of a serendipitous finding rather than an intentional effort" (386). While involved in an attempt to isolate a corticotropin-releasing factor from bovine hypothalamus, Leeman and Hammerschlag (384) found a peptide material showing sialogogic activity. (It is interesting to recall that von Euler and Gaddum detected SP while investigating the distribution of acetylcholine in various tissues.) The effect of the "sialogen" was not blocked by atropine or adrenergic blocking agents. It was stable to heat and acid but was destroyed by pepsin. The following year Lembeck and Starke (402) showed that SP stimulated salivary secretion and suggested that the "sialogen" might be identical to SP. Leeman and associates now turned from the corticotropin-releasing factor to the isolation of the newly discovered hypothalamic peptide. The purification steps involved gel filtration, ion exchange chromatography, and high voltage paper electrophoresis. The homogenous peptide thus obtained consisted of 11 amino acids, Lys_1, Arg_1, Pro_2, Gln_2, Gly_1, Met_1, Leu_1, and Phe_2, and the molecular weight was 1348 (71). The pure material had biological properties identical to those reported for partly purified SP preparations. In addition, the physical and chemical properties were very similar.

The amino acid composition of SP was determined by chymotryptic cleavage, Edman degradation, and carboxypeptidase treatment and found to be: H-Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2 (72). The first synthesis of SP was done by Tregear et al. (624) by using the solid-phase procedure. The synthetic material was identical in physical, chemical, and biological characteristics with the naturally occurring material. The synthesis of SP has since been performed by several groups (647, 170, 602, 655) and synthetic SP is now available commercially.

In 1973 Studer et al. (602) isolated SP from horse intestine. The tissue extraction was done according to von Euler (162) and the initial steps in the purification were the same as used by Pernow (514). In the final isolation Studer et al. (602) essentially used the procedure of Chang and Leeman (71). The amino acid composition and sequence of the SP from intestine was identical to that of the sialogogic peptide from bovine hypothalamus (71). This result definitely showed identity between the sialogogic compound and SP of von Euler and Gaddum as well as between SP extracted from intestine and brain.

IV. Aspects on Methodology

The results presented in this review are based on a variety of chemical, immunological, pharmacological, and physiological techniques; most of them are conventional, although others are specially designed for the particular experimental situation. Radioimmunological and immunohistochemical studies have provided quantitative data on the distribution of SP and other peptides, identification of their cellular and subcellular localization, and information on the release of SP in various situations. Since these data are fundamental for our present view of the functional role of SP, some aspects of the methodology will be briefly given.

A. Radioimmunoassay

Due to the small size of the SP peptide, conjugation to a large protein is necessary to elicit an immune response. For this purpose, synthetic SP is coupled, for example, to bovine serum albumin or proteins. The conjugated SP is separated from the nonconjugated by dialysis. At immunization the conjugate is mixed with complete

Freund's adjuvant and injected repeatedly in nanomole quantities into rabbits or guinea-pigs. After about 2 months the antibody titer may be high enough to use the antiserum for assay (531, 478, 655). Also, a standardized and permanent source of monoclonal SP antibodies has been obtained in a culture cell system from a hybrid clone, isolated after fusion of mouse myeloma cells with hyperimmune rat spleen cells (100).

For labeling with radioactive iodine, most groups have used an SP derivative synthesized with a Tyr residue substituted for the Phe[8]. In general, 60% to 80% of the isotope can be attached to the [Tyr[8]]SP. Besides ^{125}I[Tyr[8]]SP, ^{125}I-N$^{\alpha}$-Tyr-SP has also been used as tracer (654).

The specificity of the SP antisera and monoclonal SP antibodies has been analyzed by testing the ability of various SP fragments, SP analogues, and peptides with a structure similar to SP to interfere with the binding of ^{125}I[Tyr[8]]SP or ^{125}I-N$^{\alpha}$-Tyr-SP to the SP antisera raised. The SP fragments SP_{2-11}, SP_{3-11}, and SP_{4-11} have been found to displace the tracer almost identically to SP_{1-11}, while C-terminal SP fragments shorter than SP_{6-11} dramatically reduce crossreactivity (64, 655, 42, 612). This indicates that the binding of the antisera to SP fragments is well correlated to the biological activity of these fragments.

Recently an antiserum has been produced that is specifically directed to the N-terminal part of the SP molecule (379). This antiserum produces only 0.1% and 0.01% crossreactivity with SP_{2-11} and SP_{3-11}, respectively. The same content of SP immunoreactivity in the brain was measured with N- and C-terminal-directed antisera (379).

For the structurally related peptides eledoisin and physalaemin a slight crossreactivity has been reported, amounting to 0.03% to 5% (531, 532, 479, 100). Other peptides, including bradykinin, somatostatin, glucagon, bombesin, met-enkephalin, vasoactive intestinal polypeptide, and gastrin exhibit no or negligible crossreactivity (479, 100, 42). A typical assay (531, 479, 100) is carried out with a sample volume of 0.25 ml and an incubation volume of 2.5 ml. The incubation medium consists of 0.02 M barbital buffer or 0.05 M barbitone/acetate buffer (pH 8.6) to which is added 0.8% bovine serum albumin or 0.1% gelatin in order to reduce adsorption of SP to surfaces of glass or plastic. The rabbit antisera are diluted to give bound/free peptide values near 1. The most sensitive antisera are diluted up to 1:500,000. In order to inhibit peptidases, Trasylol (500 to 1000 KIE, Kallikrein Inaktiverung Einheiten) or other peptidase inhibitor is added to the tubes. Tissue extracts of synthetic SP are preincubated for 4 hours at 4°C with the incubation medium containing the antiserum or antibody. ^{125}I[Tyr[8]] SP (1000 to 1500 cpm) is then added and the incubation continued for another 18 hours at 4°C. Antibody-bound label is separated from unbound by charcoal absorption. Both free and antibody-bound radioactivity is then recorded in a gamma counter. The minimum detectable amount of SP using this radioimmunoassay (RIA) is a few fmol and the interassay coefficient of variation for duplicate samples is less than 10% (42).

B. Immunohistochemistry

The indirect immunofluorescence technique, generally used to identify peptide-containing structures, was introduced by Coons and collaborators (85). A modified protocol often used in studies on SP and other peptides includes fixation of the tissue in formalin, sectioning in a cryostat, and incubation with specific antisera (262). After rinsing, the sections are incubated with fluorescein isothiocyanate-conjugated sheep antirabbit antibodies and examined in a fluorescence microscope. Subsequently markers other than the fluorescent compounds have been introduced, such as horseradish peroxidase, which can be used for studies both at the light and electron microscopic level. The peroxidase-antiperoxidase technique (595) needs no conjugation, since all steps depend on antigen-antibody reactions.

To establish the occurrence of more than one substance (peptides and amines) in a single neuron, either adjacent sections incubated with different antisera are examined or an elution-restaining technique is used (465, 623). With the latter technique the distribution pattern obtained with one antiserum is first studied, thereafter the antibody is washed out with, for example, acid potassium permanganate (623) and the same section is then reincubated with a new antiserum, photographed, and compared with the previous one.

Recently a combination of radioautographic detection of internally labeled monoclonal antibodies with the peroxidase-antiperoxidase technique has been used for simultaneous localization of SP and 5-HT or enkephalin (105). This elegant method seems to solve many problems involved in the simultaneous study of two different antigenic sites in a single preparation and clarifies the coexistence of neuropeptides and classical neurotransmitters in single neuronal cell bodies or nerve terminals.

In the immunohistochemical analyses both rabbit antisera and monoclonal antibodies have been used. Since crossreactivity exists between SP and structurally related peptides such as eledoisin and physalaemin, although to a small extent, most authors have described the material as SP-like immunoreactivity. It also has to be emphasized that negative immunohistochemical results should be interpreted with caution, since the existing intraneuronal levels of the various compounds may be too low to be detected with the techniques used.

V. Central Nervous System

A. Distribution

Extensive radioimmunological and immunohistochemical studies have established the presence of SP in most parts of the central nervous system of all mammals,

including man. A characteristic feature is the uneven distribution with very large accumulation in some areas, while other areas seem completely devoid of SP (for ref. see 103, 411). Detailed RIA studies have confirmed earlier findings that the highest concentrations are present in the mesencephalon, hypothalamus, and preoptic area, whereas insignificant amounts are found in the cerebellum (table 1). (46, 326, 189, 149, 86). The immunohistochemical mapping has so far identified SP in cell bodies in more than 30 areas in the brain including the spinal cord and many parts of the brain stem, as well as in fiber-like structures representing axons and nerve terminals. Experimental studies combining lesion techniques with immunohistochemistry have established several specific SP pathways (fig. 2).

In the following, a brief account on the distribution of SP neurons will be given with main emphasis on the rat central nervous system as described by Hökfelt et al. (263, 260, 476), Cuello and Kanazawa (103), Ljungdahl et al. (411, 412), and Tohyama et al. (296, 556).

In the *telencephalon*, the neocortical areas are mainly devoid of SP immunoreactive cells, while the septal complex as well as the nucleus caudatus and the nucleus interstitialis striae terminalis contain many SP fluorescent cells. The highest density of SP immunoreactivity is found in the central and medial amygdaloid nuclei and in the nucleus caudatus putamen.

Networks of SP positive fibers are also present in the globus pallidus, the substantia innominata, and in the lateral parts of the septum (512). Also, the caudal part of capsula interna shows strong SP fluorescence. Single positive fibers are seen in most cortical areas, particularly in the laminae I and II and the basal layer of the medial frontal cortex. Studies with hemisections extending from the cortex to the base of the brain indicate that the SP immunoreactivity of the frontal cortex derives from a long axonal SP projection originating in a region posterior to the interpeduncular nucleus in the midbrain (507). A well defined distribution of SP fibers is observed in the hippocampal formation (326, 411, 466, 634). This area is of particular interest from the autopharmacological point of view, since both 5-HT and noradrenaline (600), and histamine (15) have been found to occur in high concentrations in the hippocampus. Lesioning of the septal area in the rat significantly reduces the SP concentration in the hippocampus, which suggests that the SP projection to this area arises in or passes through the septum (635). Destruction of the nucleus laterodorsalis tegmenti results in a marked reduction of septal SP fibers in the forebrain, indicating the existence of a long ascending SP neuron system from the nucleus laterodorsalis tegmenti to the lateral septal area (557). SP cell bodies in the amygdaloid complex project to the red nucleus of the stria terminalis and lateral hypothalamus (558).

In the *diencephalon* the preoptic area, the suprachiasmatic area, the ventromedial nucleus, the perifornical area, and pre- and supramamillary areas contain several SP cells. A high density of SP immunoreactive fibers has been found in some areas of the hypothalamus, for example the preoptic nucleus, and SP fibers and terminals are abundant in the median eminence in close connection with the portal vessels supplying the anterior pituitary gland (268). SP axons are also scattered in the pituitary neural lobe (599). Subcellular distribution studies reveal that in the hypothalamus, as in many other areas of the central nervous system, SP is localized in synaptosome particles (129). In the pituitary gland SP is predominantly localized to those cells that also exhibit thyrotropin-stimulating hormone (TSH) (120) or prolactin and gonodotropin immunoreactivity (459). The habenula complex, which provides a major link between the forebrain and more caudal structures, contains SP cell bodies that project to the lateral margin of the interpeduncular nucleus. Lesioning of the medial habenular nuclei results in a drastic decrease in the SP immunoreactivity in the interpeduncular nuclei (287, 461, 150, 260, 462, 99, 636). Also, injections of kainic acid into the habenular complex in the rat, which destroy the habenular cells, deplete SP in the interpeduncular nucleus and dorsal raphe as well as in the habenula (636). These results indicate that most SP in the interpeduncular nuclei is present in axons and nerve terminals originating from the habenula, thus strongly supporting the existence of a habenulointerpeduncular SP pathway. The SP levels in the ventral tegmental area and interpeduncular nucleus in the rat are significantly decreased by electrical stress, whereas SP in the cortex and substantia nigra is not affected (409).

The SP positive cell groups found at the hypothalamic level continue in the central grey of the *mesencephalon*, where the interpeduncular nucleus also contains numerous SP cell bodies. The highest density of SP positive nerve terminals in the rat (46, 326, 411), cat (203), and human (149, 86) central nervous systems has been found in substantia nigra, particularly in the zona reticulata, in the ventral tegmental area, and in the nucleus interpedunculus. The SP fibers in the substantia nigra are both myelinated and unmyelinated and form axon terminals containing intensely labeled granular vesicles (122, 584). These terminals are in continuity with a SP plexus in the ventral tegmental area. Strong immunofluorescence is also seen at the fasciculus retroflexus, i.e., the pathway from the habenula to the interpedunculus nucleus.

Several lesion studies have been performed in order to establish inputs of SP-containing neurons to the substantia nigra (47, 190, 288, 325, 462, 507, 586, 636). Cutting the medial forebrain bundle and medial crus cerebri removes about 90% of SP in the reticular part of the ipsilateral substantia nigra, which probably indicates that the bulk of SP in the substantia nigra is localized in fibers of the striato-nigral tract. This assumption is further supported by the finding of a significant decrease

TABLE 1
Regional distribution of SP in the central nervous system

	Rat			Human		
	Ref. 326 (n = 4)	Ref. 46 (n = 20)	Ref. 128 (n = 6)	Ref. 189 (n = 10–20)	Ref. 149 (n = 3–13)	Ref. 86 (n = 5–10)
Telencephalon						
Somatosensory cortex	19 ± 2*					
Visual cortex	23 ± 4					
Olfactory bulb	62 ± 11	20 ± 5				41 ± 8
Olfactory tubercle	300 ± 14					
Hippocampus	37 ± 4	340 ± 45			104 ± 29	
Amygdala	382 ± 86			340 ± 50	25 ± 12	26 ± 3
Nucleus caudatus	247 ± 28			370 ± 80	138 ± 14	113 ± 12
Putamen				330 ± 60	112 ± 29	81 ± 11
Globus pallidus	332 ± 45			1800 ± 330	877 ± 253	518 ± 151
Septum	405 ± 44	116 ± 14			89 ± 23	
Lateral septal nucleus		360 ± 20				
Internal capsule	137 ± 21					
Retina	66 ± 14					
Optic nerve	65 ± 14					0.6 ± 0.2
Diencephalon						
Hypothalamus (medial)	626 ± 64	208 ± 37				122 ± 22
Hypothalamus (lateral)						135 ± 20
Habenula	377 ± 101					
Mammillary body	207 ± 8					83 ± 14
Pituitary stalk and median eminence						134 ± 21
Posterior pituitary	489 ± 24					
Anterior pituitary	71 ± 2	20 ± 5				
Thalamic nucleus	215 ± 58					35 ± 8
Globus pallidus	332 ± 45			1800 ± 330	877 ± 253	518 ± 151
Lateral geniculate body	82 ± 12	90 ± 13				37 ± 10
Mesencephalon						
Substantia nigra	1725 ± 106		2560 ± 195			
Zona compacta		298 ± 44		4720 ± 480	1264 ± 239	
Zona reticularis		1138 ± 136		4290 ± 490	1535 ± 170	922 ± 156
Zona lateralis		298 ± 39				
Periaqueductal central grey			614 ± 37			130 ± 21
Interpeduncular nucleus	599 ± 63	590 ± 129	994 ± 149		83 ± 25	
Red nucleus			58 ± 12			76 ± 19
Interferior colliculi			94 ± 18			234 ± 19
Pons						
Locus coeruleus			332 ± 50			199 ± 32
Nuclei parabrachiales			546 ± 124			
Motor nucleus V			145 ± 29			
Dorsal raphe nucleus			222 ± 25			
Cerebellum						
Cortex	10 ± 4	2 ± 1	12 ± 1	20 ± 6		1 ± 0.1
Nuclei			29 ± 5			1 ± 0.1
Medulla oblongata						
Nucleus cuneatus			320 ± 32			
Nucleus tractus spinalis V			383 ± 125			
Nucleus tractus solitarii			436 ± 93			
Motor nucleus XII			217 ± 26			
Area postrema			167 ± 36			114 ± 47
Nucleus reticularis gigantocellularis			95 ± 11			
Inferior olive			127 ± 15			8 ± 2
Nucleus raphe magnus			229 ± 23			71 ± 15
Medulla spinalis						
Dorsal horn	1070 ± 160					
Dorsal column	129 ± 29					
Ventral horn	134 ± 33					

* All values have been converted to pmol/g of wet tissue (mean ± SEM).

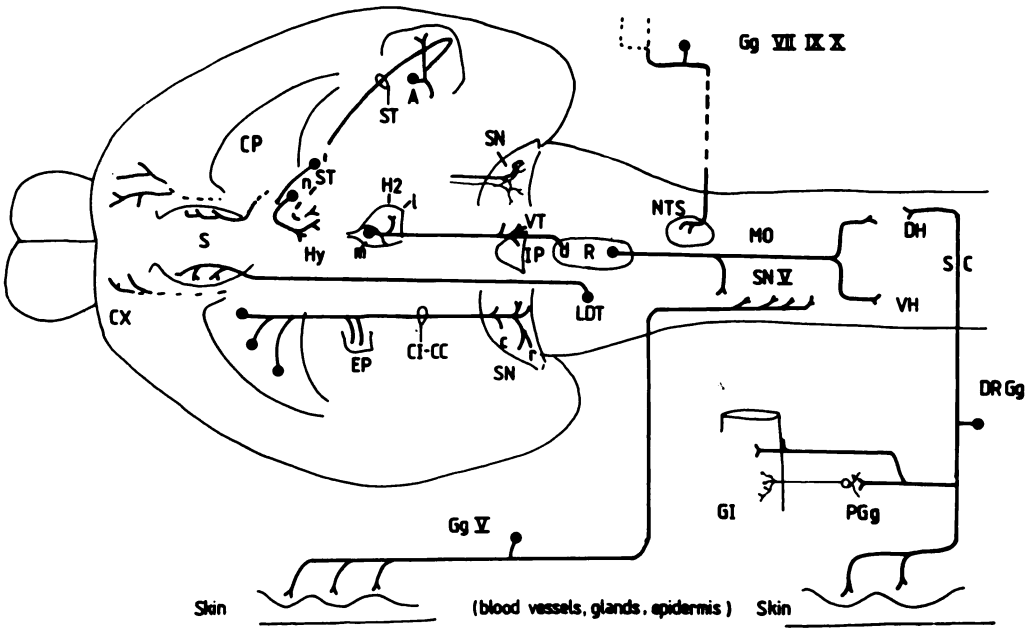

FIG. 2. Schematic representation of SP projecting neurons. Horizontal view of the rat brain. Neurons from the caudate putamen (CP) project heavily to the substantia nigra (SN), pars reticulata (r) and also the pars compacta (c); the fibers run in the capsula interna and crus cerebri (CI, CC). Along their way fibers terminate in the nucleus entopeduncularis (EP). Repetitive synapses of striatonigral SP containing fibers onto presumptive dopaminergic neurons are also indicated. Neurons from medial habenula (m) project to the lateral habenula (l), to the ventral tegmental area (VT) surrounding the nucleus interpeduncularis (IP), and to dorsal raphe (d). H, habenula. Raphe nuclei (R) containing SP (also 5HT and other peptides) project to the spinal trigeminal nucleus (SNV) and to the dorsal horn (DH) and ventral horn (VH) of the spinal cord (SC). In the amygdala (A) neurons from medial amygdala project to central amygdala and probably beyond this nucleus via the stria terminalis (ST). In the stria terminalis SP-containing neurons ramify within the nucleus and also project to the medial preoptic area of the hypothalamus (Hy). Some ascending fibers originating presumably from brain-stem project to the frontal cortex (CX) while the lateral septum (S) receives SP-containing fibers from ventral aspects. The nucleus of the tractus solitarius (NTS) receives fibers presumably from cell bodies located in the sensory ganglia of the VII, IX, and X cranial nerves (Gg VII, IX and X). The nucleus spinalis of the trigeminal nerve, subnucleus caudalis (SNV) is heavily innervated by SP-containing fibers originating in the trigeminal ganglia (GgV) while peripheral branches of these nerves and presumably also those from the dorsal root ganglia (DRg) terminate freely among blood vessels, glands and epidermis of the skin of the trigeminal and spinal cord sensory territories. Some peripheral fibers of the SP-containing sensory neurons terminate synaptically on sympathetic neurons of the prevertebral ganglia (PCg), while free endings terminate in the submucosa and adventitia of the gastrointestinal tract. The central fibers of these neurons terminate in the most superficial layers of the dorsal horn (DH) of the spinal cord (SC). MO, medulla oblongata. [From Cuello et al., 1982 (106) with addition of a long-ascending neuron system from the nucleus laterodorsalis tegmenti of Castaldi to the lateral septal area, and the indication that the amygdalofugal SP pathway in the ST innervates Hy. This reconstruction is based on experimental evidence discussed in the text.]

in the SP concentration in substantia nigra following destruction of the globus pallidus. The striato-nigral projection descends in the internal capsule (507). The existence of a striato-nitral SP pathway is conclusively indicated by the recent observation that ^{35}S-methionine, infused into the corpus striatum in the rat in vivo, is incorporated into SP in the striatum and that the labeled peptide subsequently is transported to the substantia nigra (585).

In the *lower brain stem* SP positive cells are found in various nuclei with a particularly large amount in the medullary raphe nuclei, which project intra-arterially to the spinal cord (36, 270). Also, strong SP fluorescence networks are present in the locus coeruleus, the parabrachial nuclei, the caudal spinal trigeminal nucleus, the solitary tract nucleus, and in the dorsal motor nucleus of the vagal nerve. In the substantia gelatinosa of the spinal trigeminal nucleus several postsynaptic targets for SP-containing terminals have been suggested, including both large lamina I neurons and lamina II interneurons (534). SP neurons project from the ventral medulla to the intermediolateral cell column of the thoracic and upper lumbar segment of the spinal cord, the site of origin of the sympathetic outflow (243).

The immunohistochemical distribution of SP in the *spinal cord* has been extensively studied in the rat, cat, and monkey by using both immunohistochemical techniques (476, 262, 260, 411, 14, 616, 292, 123) and RIA (46, 462, 286). SP-positive nerve cell bodies of varying sizes are present only in the dorsal horn, while SP-immunofluorescent fibers are found in all parts of the grey of spinal cord. The highest density of SP fibers is found in the dorsal horns, particularly in laminae I and II. A strong SP fluorescence is also found in the dorsomedial part of the lateral fasciculus and in the tract of Lissauer. In the area around the central canal and in the ventral horns, distinct but less dense SP networks are present, often in close vicinity of motoneurons and some-

times radiating into the white matter. By electron microscopy SP-positive staining is mostly found in unmyelinated and thinly myelinated small diameter fibers. SP-positive nerve terminals from axodendritic synapses with dorsal horn cells (14, 123).

Quantitative RIA reports up to 10 times higher concentrations of SP in the dorsal parts of the dorsal horns (300 pmol/g of wet weight) than in the ventral horns and up to 100 times higher than in the white matter (46, 462, 286).

The origin of SP in the spinal cord is clearly elucidated by ligation and transection studies. By using bioassay, Takahashi and Otsuka (610) showed that after unilateral ligation and/or section of the dorsal root in the cat, the level of SP in the dorsal horn is markedly lowered, particularly in its dorsal part. In the ligated or sectioned dorsal root, SP is highly accumulated on the ganglion side, whereas its level is lowered in the central part. The substantial decrease of SP in the dorsal horns following unilateral lumbosacral deafferentation by dorsal rhizotomy was confirmed by immunohistochemistry (260, 262, 14, 616) and by RIA (314). SP staining in the deafferented dorsal horn declines to a minimum over the first 10 days and then partially recovers by 1 month (616). These data suggest that SP is biosynthesized in the spinal ganglia and transported in the dorsal root towards intraspinal axon terminals. Tessler et al. (616) have suggested that the return of SP staining depends on the presence of interneurons, which contain SP immunoreactivity (265, 411, 14). Also, a unilateral section of the sciatic nerve in the rat results in a considerable depletion of SP in the dorsal but not in the ventral horn. The onset of this depletion occurs within 7 days and is maintained for 1 to 2 months (314, 16, 640). Dorsal rhizotomy also reduces to some extent the SP content of the ventral horn (314) indicating that some of the SP-containing terminals in this region may originate from primary afferent neurons. Most terminals in the ventral horn, however, seem to have a supraspinal origin, since after total transection of the spinal cord at the upper thoracic level almost all SP positive fibers disappear in the ventral horns below the lesion (260, 411, 273, 327). These fibers evidently emanate from SP cell bodies in the ventral medulla oblongata, since lesion of this area including the nucleus interfasciculus hypoglossi reduces the SP content of the ventral horns of the spinal cord (243). However, even after a combination of dorsal rhizotomy and transection of the spinal cord some SP fluorescence is still present in the spinal cord, evidently originating from intraspinal neurons (574).

In summary, these findings clearly indicate that SP occurs in three distinct populations of neurons in the spinal cord. In the dorsal horns and the spinal trigeminal nucleus SP is largly present in nerve terminals representing central branches of the primary sensory neurons. Nerve terminals in the ventral horns containing SP mainly arise from supraspinal descending neurons located in the lower brain stem and some higher centers. Finally, SP is also present in interneurons and/or propriospinal neurons, their cell bodies being located mainly in the dorsal horn.

The occurrence of SP in the *human central nervous system* has been studied in detail by immunohistochemistry (267, 84) and by RIA (323, 189, 149, 86). The accumulated data show that the distribution in the human brain has a similar pattern to that found in a variety of animal species (table 1). In general, however, the values reported for humans seem to be lower than those in most animals. To some extent these differences may be related to the fact that the tissue obtained from animals can be handled more rapidly than the human material, although SP appears to have a remarkable postmortem stability (189).

In patients with Alzheimer's disease and senile dementia of the Alzheimer type, characterized by structural changes throughout the neocortex, several cortical areas show significantly lower SP immunoreactivity than that seen in control subjects (95).

In the human spinal cord SP immunoreactivity is present, especially in the dorsal horn, as an intense band of immunofluorescent nerve terminals in lamina I and II (104, 366). At the level of entrance of the dorsal roots, the SP fibers are characterized as fine unmyelinated axons. SP is also localized in varicosities and terminals in the intermediomedial nucleus, the reticular nucleus of lamina V, and the ventral horn (104, 366). SP immunoreactive fibers and terminals are, furthermore, found in all major cell columns of the ventral horn in close apposition to motoneurons, forming synaptic junctions predominantly with dendrites (117).

RIA of the lumbar cerebrospinal fluid in man has revealed the occurrence there of SP. The concentrations found in normal adults were 3 to 11 fmol/ml (482) and 50 to 120 fmol/ml (612). The large difference between these values is probably due to methodological factors. Decreased SP concentration has been found in disorders involving the spinal cord and nerve roots (482).

B. Ontogeny

A detailed mapping of the early ontogeny of the SP neuron system has recently been performed (296, 447, 556, 573). SP-positive nerve cells and fibers appear in the rat forebrain, brain stem, and spinal cord as early as gestational days 14 to 19 and show a histochemical maximum on the first postnatal days, tending thereafter to decrease in number as the rats grow (296, 573); this indicates that the development of the SP neurons takes place long before the establishment of normal synaptic transmission (296, 556, 573). A dramatic increase in SP content occurs during the first 3 postnatal weeks, when the SP levels approach and often exceed that of the adult rat (447).

The development of SP in the embryonic spinal cord and dorsal root ganglia is stimulated by nerve growth

factors (572, 343), whereas administration of antinerve growth factor antibodies produces a marked but reversible reduction of the SP content of dorsal root ganglia (444). When sensory neurons from trigeminal, nodose, and dorsal root ganglia containing SP are transplanted to the anterior eye chamber of the rat, a regeneration of new SP-containing plexus in the iris occurs (580). Also, a denervated iris grafted into the anterior eye chamber becomes reinnervated by SP fibers from the host and induces a 5 to 10-fold increase in SP content of the host iris (580). This regeneration is probably also induced by a nerve growth factor mechanism.

C. Release, Pharmacological Effects, and Physiological Significance

The uneven distribution of SP in the central nervous system speaks in favour of specific functions in certain areas, where the substance is particularly accumulated. Numerous studies have, therefore, been devoted to the possible role of SP in the brain and spinal cord. Some of these studies will be reviewed briefly with particular attention to the striato-nigral system and spinal cord.

1. Cortex. Only a few studies have so far been devoted to the effect of synthetic SP on the cortex. SP as well as other peptides such as bradykinin, bombesin, and physalaemin have powerful excitatory actions on cortical Betz neurons. SP is the most potent of these peptides (522). Many of these neurons are also excited by acetylcholine, but SP is more effective as an excitant of Betz cells than acetylcholine. A relationship seems to exist between the sensitivity of cortical structures to acetylcholine and peptides, and it has been suggested that the peptides have a presynaptic, acetylcholine-releasing action (522). Iontophoretic application of SP to the medial amygdaloid nucleus and putamen elicits a strong and prolonged acceleration of the spontaneous neuronal discharge (371). This response is similar to that obtained in other areas such as the spinal cord (250), cuneate nucleus (361), and cortex (522) and is in keeping with the hypothesis that SP may play a role in the modulation of neuronal activity.

2. Hypothalamus—Neurohypophysis. A calcium-dependent, potassium-induced release of SP has been demonstrated in slices from the hypothalamus (299, 362) and from superfused hypothalamic synaptosomes (567). If the plasma levels of SP as well as enkephalins are increased, an acceleration of the orthodromic neural activity occurs in the hypothalamo-neurohypophyseal tract (12), which might indicate an enhanced rate of secretion of pituitary gland hormones. Local application of SP to the guinea-pig hypothalamus in vitro markedly increases the spontaneous firing of hypothalamic neurons (487).

The functional role of SP in the hypothalamus is not clear. However, it has been found that SP stimulates release of growth hormone when given both intraventricularly (632) and intravenously (542), but has no effect on growth hormone release from pituitary gland cells in vitro (542). Also prolactin is released by intraventricular injections of SP (631, 137), while secretion of gonadotropin is inhibited (341). These findings, in addition to the high accumulation of SP immunoreactive fibers close to the blood vessel supply to the hypophysis (268), might suggest that SP is involved in the control of hormonal secretion from the anterior pituitary. This hypothesis is further strengthened by the recent finding that the SP content of the rat's median eminence varies with the oestrus cycle, being highest in dioestrus (9).

3. Striato-nigral Area. Several groups have demonstrated an in vitro and in vivo release of SP in the substantia nigra. Thus, Schenker et al. (567) reported that potassium evokes a calcium-dependent SP release from synaptosomal fractions prepared from the ventral mesencephalon of the rat. Similar results were obtained by Jessell (310) and Torrens et al. (621) on slices of rat substantia nigra. The basal efflux of SP was estimated to be approximately 8 fmol × mg of tissue^{-1} × min^{-1}, e.g., about 0.5% of the tissue store. Increase of the potassium concentration in the superfusing medium to 47 mmol evokes a 5-fold increase in the rate of SP efflux (310). Gamma-aminobutyric acid (GABA), L-glutamic acid, and nicotine inhibit the potassium-induced release of SP, whereas veratridine enhances the reaction. Evidence for an in vivo release of SP was given by Michelot et al. (451) who used push-pull cannulae inplanted in the substantia nigra and the caudate nucleus. This release seem to be dependent on the degree of nerve impulse flow, since it is enhanced during depolarization of cells in the ipsilateral caudate nucleus.

Several observations have indicated an excitatory action of SP in the substantia nigra. Thus, electrophoretic administration of SP into single neurons in the substantia nigra induces a prolonged increase in the neuronal activity (114, 639). Bilateral injections of SP into the substantia nigra in the rat increases basal locomotor activity (338), whereas unilateral microinjection of SP discretely into the reticular part of the substantia nigra elicits dose-dependent circling movements, characterized by slow and undirectional turning on the spot towards the uninjected side (302, 492, 546). On the other hand, SP applied to the zona compacta, zona lateralis, or the medial lemniscus evokes ipsiversive turning (302). Intranigral application of SP, furthermore, causes a substantial increase in stereotyped rearing and sniffing in rats (339). Finally, injection of SP into the substantia nigra of the rat produces a drastic retrograde amnesia for a passive avoidance task (293). It is apparent from these pharmacological studies that the effects induced by local application of SP depend to a large extent on the precise localization of its injection within the substantia nigra and that both excitatory and inhibitory effects can be elicited.

The complexity of the actions of SP in the substantia nigra is probably due to its interaction with the classical transmitters. The structural basis for this suggestion is

given by the findings that the SP fibers are in close proximity to dopamine cells and dendrites in the zona compacta of the substantia nigra (412) and that SP-containing boutons are in direct contact with these structures (584). Several observations also clearly indicate a functional interaction between SP and classical transmitters. Thus, injection of SP in the rat lateral ventricle stimulates the synthesis of dopamine, noradrenaline, and 5-HT in various parts of the brain, including the striatum, and accelerates the disappearance of these amines (432). Also, an increase in dopamine metabolites and ^{3}H-dopamine release from the ipsilateral striatum is observed after infusion of SP into the substantia nigra (78, 79, 638). Similarly, SP stimulates uptake and release of dopamine when added to superfused slices of rat substantia nigra (540) or to nigral synaptosomes (576). Intranigral application of SP also enhances the in vivo release of dopamine from nerve terminals in the caudate nucleus (78, 451). In addition, immunoneutralization by local infusion of anti-SP rabbit serum into the substantia nigra results in an inhibition of the release of dopamine in the cat caudate (79). It is also most likely that the behavioral effects, which are induced by local application of SP, are mainly due to a stimulation of the nigro-striatal dopamine system. Thus, SP infusion directly into the ventral tegmental area, which contains the cell bodies of the dopaminergic A10 neurons, elicits a behavioral excitation (597, 300, 340) and intraventricular SP reverses the decrease in locomotor activity induced by the dopamine receptor antagonist, haloperidol (321, 230).

On the other hand, recent findings indicate that SP has no or only a slight effect on dopaminergic neurons in the zona compacta (83, 529), whereas nondopaminergic cells of the zona reticulata are much more sensitive to SP (529), which seems inconsistent with the earlier mentioned behavioral and neurochemical observations. It is possible, however, that the sensitivity or SP receptor density of the cell bodies of neurons in the zona compacta is less than that of their distal dendrites and of reticulata neurons (529), or that SP activates the dopaminergic nigro-striatal pathway indirectly via reticular formation cells (83).

Evidence has also been given for a feedback influence exerted by the dopamine pathways on the SP- as well as on GABA-containing striato-nigral neurons (540). Haloperidol reduces SP-like immunoreactivity in the rat striatum (519, 231) and decreases, in a specific, dose-dependent, and reversible way, SP immunoreactivity in the substantia nigra (231). Similarly, destruction of the nigro-striatal dopaminergic pathway results in a decrease in nigral SP content, which returns to the control level after several weeks (231).

The accumulated data thus suggest an important relationship between the dopaminergic nigro-striatal and SP striato-nigral systems and a role for SP as a dopamine modulator in the sense that SP afferents enhance the firing rate of dopamine neurons. GABA, which is also present in high concentrations in this brain area, has been attributed a transmitter role in inhibitory striato-nigral neurons (489). In fact, the GABA and SP pathways follow a similar course in this area, although a clear anatomical dissociation has been demonstrated between GABA- and SP-containing neurons (47, 190, 311). Both GABA and SP seem to interact synaptically with dopaminergic elements (541, 584). GABA inhibits the potassium-induced SP release from rat nigral slices, while the GABA antagonist, picrotoxin, increases the SP release (309). These findings indicate that GABA may modulate the release of SP from striato-nigral afferent fibers via GABA receptors located on SP-containing presynaptic nerve terminals (309). It is attractive to suggest that an interaction between the dopamin-, GABA-, and SP-ergic systems in the striatal-nigral area contributes to the control of neuronal tone in these extrapyramidal pathways and thereby the locomotor functions.

The functional significance of SP in the substantia nigra is also elucidated by observations of abnormally low SP levels in this area of brains seen post mortem from patients with Huntington's chorea, a disease in which there is a marked atrophy of the basal ganglia (323, 324, 189, 149). Substantial reductions in SP levels are observed also in the globus pallidus (149), caudate nucleus, and putamen (48), i.e., areas known to be most severely atrophied in the choreic brain. There are, therefore, reasons to believe that the drastic reduction of SP-ergic neurons in the basal ganglia in Huntington's disease contributes to the symptomatology of this disease. However, the reduction of SP in the brain stem is not reflected by changes in SP concentration of the lumbar cerebrospinal fluid in patients with Huntington's chorea, Parkinson's disease, progressive supranuclear palsy, or various forms of dyskinesias (93, 482).

Neurons in the substantia nigra respond to noxious radiant heat to both excitation and reduction in firing rate (13). These nociceptive neurons are predominantly located to the zona compacta, an area containing high concentrations of enkephalin (256) as well as of SP (129, 326). It is therefore attractive to speculate that SP in the substantia nigra, besides being involved in the extrapyramidal motor function, is also implicated with nociceptive mechanisms, as seems to be the case in the primary sensory neuron and the trigeminal system (see below).

4. Brain Stem. Application of SP in the lateral ventricle (0.5 to 5 nmol) of the rat (237) or newborn rabbit (651), or into the dorsal surface of medulla (1.5 nmol) in the rabbit (651) elicits a considerable enhancement of respiratory rate and amplitude. The effect of the local application is potentiated by naloxone (651). It is suggested that the stimulatory effect of SP on ventilation is exerted on structures located in the lower brain stem.

5. Spinal Cord. RELEASE. In 1976 Otsuka and Konishi (496) showed that SP is released from the perfused spinal cord of newborn rats either upon electrical stimulation of the dorsal root or by increasing the potassium concen-

tration in the perfusate. The release, which is calcium-dependent, coincides with a simultaneous release of GABA and glycine (1). By combining high pressure liquid chromatography and RIA Akagi et al. (1) were able to identify the radioactive SP released from the isolated rat spinal cord as the undecapeptide SP. SP is also released from the dorsal horn in vitro (312, 197) as well as from dissociated sensory neurons grown in culture. This release is inhibited by enkephalin (463).

A release of SP from the superfused cat spinal cord in vivo has also been demonstrated in response to stimulation of A_2 delta and C fibers of the sciatic nerve (649). This result provides evidence that SP is released in vivo when nociceptive sensory afferents are activated. Morphine, at concentrations known to cause analgesia, completely blocks the release of SP after sciatic nerve stimulation, whereas intraperitoneal administration of naloxone fully restores the evoked release of SP in the presence of morphine (649). Capsaicin, a homovanillylamide derivate, when administered directly into the spinal cord, causes a calcium-dependent release of SP in vitro but not of amino acids such as GABA, glutamic acid, or glycine (197, 617, 1, 649, 50). A similar capsaicin-evoked release of SP in the spinal cord has been demonstrated in vivo (649). The immunoreactive SP released by capsaicin coincides with the authentic undecapeptide SP on high pressure liquid chromatography (1). Daily treatment of rats with subcutaneous capsaicin for 5 days gives an almost complete depletion of SP but not of glutamic acid decarboxylase, the GABA synthesizing enzyme, from the terminals in the substantia gelatinosa and dorsal roots of the spinal cord and medulla oblongata (313, 193). The SP content of the ventral horns, however, remains unchanged (193), indicating that the depletion of SP from the spinal cord induced by capsaicin is limited to terminals in the dorsal horn of the spinal cord.

Capsaicin per se elicits a depolarization of spinal motoneurons which is similar to that of SP (617), and it is likely that this effect of capsaicin is mediated by a release of SP from nerve terminals.

A spontaneous release of SP has been demonstrated also from the spinal trigeminal nucleus, representing 0.2% to 0.4% of the tissue store per minute. Exposure to high potassium concentrations elicits a marked increase in this release, which is calcium-dependent (312). Also capsaicin elicits a SP release from the spinal trigeminal nucleus as well as from the nucleus tractus solitarius (242). On the other hand, the SP content of higher centers of the central nervous system such as the midbrain, hypothalamus, striatum, and cortex is unaffected by capsaicin (193, 242). These findings indicate that capsaicin stimulates SP efflux from regions receiving SP containing sensory afferents but is ineffective in nonsensory SP-containing terminal regions.

PHARMACOLOGICAL ACTIONS. The first evidence that SP exerts a depolarizing action on spinal motor neurons was given by Otsuka et al. (498, 499). They showed that extracts of bovine dorsal root excite the isolated cord of bullfrog and in addition produce a fall in rat blood pressure and stimulation of the guinea-pig ileum. The active principle was soon identified as the undecapeptide SP on the basis of various chemical, immunological, and pharmacological tests (500, 609). The electrophysiological studies were extended by using an isolated spinal cord preparation of the newborn rat (494). Application of SP to the surrounding bath fluid induced a repetitive firing of the spinal motor neurons as revealed by recordings both intracellularly and from the ventral root. After SP had been washed out, recovery took place with a time course of a few minutes. Evidence was given that the effect is due to a direct excitatory action of SP on the spinal motoneurons (349, 350, 502) since the depolarizing effect of SP is observed even when the synaptic transmission is blocked by lowering calcium or by tetrodotoxin (503). The depolarizing potency of SP is up to 10,000 times higher than that of *l*-glutamate (495). The actions of SP on spinal neurons have, since the original observation by Otsuka et al. (498), been studied by many groups (361, 522, 247, 248, 250, 555, 537, 470, 131, 564, 661, 115, 116) preferentially in the rat and cat. In the typical experiment, microiontophoretic application of SP to cuneate or spinal neurons elicits an excitation, which is characterized by a slow and prolonged time course, starting after a delay of several seconds. Also, in the frog spinal cord the effect of SP is slow both in onset and recovery. The maximal depolarization obtained is quite small, the motoneurons rarely reaching the threshold for spike generation (470).

After unilateral dorsal root section a supersensitivity is developed in the deafferented side of the rat spinal cord to iontophoretic application of eledoisin (646), a peptide with a similar amino acid sequence as the C-terminal SP hexapeptide, to which the main biological activity is bound. The development of such supersensitity to SP and analogues is probably attributed to an increase in the number of receptors rather than to an increase in receptor affinity (467).

In cultured mouse spinal neurons, two distinct actions of SP have been reported, one being a rapid desensitizing excitation and the other a depression of excitatory responses to glutamate (633). These findings suggest that SP may serve different functions in interneuronal communication. It may mediate excitatory synaptic events but may also have effects other than that of a conventional neurotransmitter. Varying results have been obtained concerning the effect of SP on membrane resistance. Krnjević (359) observed in spinal motoneurons of the cat that the depolarization induced by SP was accompanied by a decrease in membrane conductance, i.e., an increase in membrane resistance and a reversal potential more negative than the resting. Similar results were obtained in cultured spinal neurons (290, 481) and in neurons of the guinea-pig hypothalamus in vitro (486). Intracellular recordings from lamina V in the cat spinal

cord did, however, not indicate any change in membrane conductance in association to SP-induced depolarization (661). In the frog a reduction in membrane resistance occurs after application of SP (470), which confirms previous findings (497).

The excitatory action of SP described above does not involve more than about half of the cell units in the dorsal horns of the spinal cord or cuneate nucleus (361, 250). This finding, together with the cellular localization of SP particularly in laminae I and II (263), suggests that SP is involved in the central transmission of specific sensory modalities. This problem was first studied by Henry (247), who correlated the effects of SP on spinal dorsal horn neurons to the response of these neurons to natural peripheral stimulation. Henry found that SP selectivity excites those units of the dorsal horn neurons, which respond to noxious radiant heat applied to the skin. SP fails, on the other hand, to excite those cell units which are stimulated by other peripheral sensory modalities such as touch, pressure, or hair movement. These findings were later confirmed and extended (537, 564, 526). In amounts not directly influencing neuronal firing rate, SP specificallly prolongs the response to noxious stimulation and potentiates the late response of dorsal horn neurons to sural C afferent stimulation (537, 564). Units activated by light pressure applied to the rat tail are either depressed or not affected by SP (537). These results seem to indicate that SP rather specifically facilitates nociceptive pathways by increasing their level of excitability or by depolarization.

VI. Peripheral Nervous System

A. Distribution

In the periphery, SP is mainly localized to two systems, the primary sensory neurons and neurons intrinsic to the gastrointestinal tract. The latter localization will be discussed in section IX. Also, in sympathetic ganglia, SP is connected to sensory fibers as is discussed separately in section VIII.

1. Ganglia and Nerves. Numerous SP-containing cell bodies are present in spinal ganglia at all levels (262, 263) as well as in the jugular (260, 334), nodose (422, 196, 334), and trigeminal ganglia (263, 260, 96, 97). These cells are almost exclusively of the small type (B type) and seem to constitute about 20% of all cells in these ganglia. SP-fluorescent axons without or with only a very thin myelin sheath, i.e., C or possibly A delta fibers, are observed between the cells.

SP is present in the vagus (422, 196, 421, 37, 244), sciatic (422, 37, 391), and splanchnic nerves (422). The SP fibers, however, only account for about 10% of all axons in the cat vagus and most of them seem to be unmyelinated (196). Ligation of the vagal nerve results in a considerable accumulation of SP fluorescence proximal to the ligature and a decrease in the distal part (196, 421, 37). The initial accumulation of SP after ligation is, however, followed by a slow progressive decrease of SP in the entire proximal section (314).

SP is also present in the phrenic nerve of rat in quantities similar to that found in the vagus and sciatic nerves (436). Transection of the phrenic nerve close to the diaphragm results in a considerable accumulation of SP proximal to the lesion, indicating a rapid axonal transport of SP from the spinal cord to the diaphragm (436).

2. Organs and Tissues. SP-positive fibers are present in most peripheral tissues. In the human skin (110) the hind paw of the cat (262), and the inferior lip and nostrils of the rat (260, 97) SP immunofluorescent fibers, single or in bundles, are consistently found in the dermis and epithelium, more exceptionally penetrating into the epithelium as apparent free nerve endings. Often the SP fibers are observed in relation to blood vessels, sweat glands, and hair follicles. In the tongue, SP positive fibers are seen in the connective tissue under the epithelium (260), but particularly as nerve terminals in the taste buds of the cat and rat tongue (423, 480). Since the papillae of the tongue, where the taste buds are located, are innervated by the glossopharyngeal nerve, it is interesting to note that SP-immunoreactivity is present in ganglion petrosum and the entire nucleus tractus solitarii (244, 411). In the salivary gland these fibers are observed close to secretory elements. In the nasal mucosa of the cat, peripheral branches of SP neurons have been identified with nerve endings in the respiratory epithelium and around blood vessels (8). SP fluorescent fibers are, furthermore, present in large amounts in the tooth pulp (491), again mainly localized around blood vessels but also as apparently free nerve endings (39, 40).

Trigeminal rhizotomy or electrolytic lesioning of the Gasserian ganglion causes a substantial ipsilateral loss of SP immunofluorescent fibers not only in the substantia gelatinosa of the spinal trigeminal nucleus, but also in the ipsilateral lip (97). More strikingly, sectioning of the peripheral part of the trigeminal nerve produces a total disappearance of all SP-immunoreative fibers of the skin (97). Similarly, crushing or transection of the inferior alveolar nerve results in a disappearance of SP immunoreactivity in the tooth pulp of the denervated side (491), but in an accumulation of SP in axons proximal to the injury (39, 40). Sympathectomy does not interfere with the appearance or frequency of SP fibers in the pulp (491), indicating the sensory character of these fibers in peripheral tissues.

In the tracheobronchial tissue numerous SP fibers are present in both the muscular and submucosal connective tissues. Occasionally SP-fluorescent cells of an endocrine-like type are also found (475).

In the kidney of the guinea-pig a sparse plexus of SP positive fibers is observed mainly in the cortex, mostly following blood vessels, and in the renal pelvis close to

the smooth muscle. Numerous SP fibers are present in the ureter of the cat and guinea-pig, particularly in the proximal part. All layers of the urinary bladder contain SP with dense networks especially in the trigonum area (4, 269).

Treatment of newborn rats with capsaicin, which causes a selective and permanent degeneration mainly of unmyelinated sensory fibers, decreases SP content in various skin areas, in the oral and nasal mucosa, and in the trachea, lungs, and ureter (279).

B. Biosynthesis and Transport

The biosynthesis of SP has recently been studied in dorsal root ganglia (233). When the ganglia are incubated in vitro with ^{35}S-Methionine and ^{3}H-Proline, both amino acids are incorporated into SP after a lag phase of about 1 hour. The biosynthesis is inhibited by cycloheximide, suggesting that SP in dorsal root ganglia is synthesized by a ribosomal process. SP synthesis is also reduced almost completely in ganglia from rats treated neonatally with capsaicin (195).

Several studies have elucidated how SP, after its synthesis on ribosomes in the perikaryon, is delivered to terminal regions where it exerts its biological effects. The early observation by Holton (276) that SP accumulates in the central stump when a peripheral nerve is cut, has later been confirmed in biochemical and immunohistochemical studies (262, 206, 422, 196, 37, 207, 39, 199). The rate of accumulation of SP following ligation at various levels of the vagal and sciatic nerves indicates that about 90% of SP biosynthesized in the sensory ganglion cells is subjected to an axoplasmic transport towards the terminal regions of their peripheral branches. The average velocity of transport of SP in the peripheral direction could be calculated to be about 1 mm per hour in the vagal and sciatic nerves. However, much of SP in these nerves is probably stationary, while a small part is undergoing a rapid transport. The velocity of this moving fraction was calculated to be about 5 mm per hour or 120 mm per day, some even faster. The SP undergoing rapid transport along the axons of peripheral nerves is bound to intra-axonal particles, whereas stationary or slowly moving SP is largely soluble in the axoplasma (37). The axoplasmic transport of SP in the sciatic nerve is completely blocked by capsaicin (199).

The axonal transport of SP has also been studied in rat dorsal ganglion with short segments of dorsal root and peripheral branch attached (232). SP immunoreactivity of the ganglion increases linearly with time and is transported down both branches, the accumulation in the peripheral branch being four times larger than in the dorsal root. The turnover time for SP in the ganglion was calculated to be 3.6 hours. Demecoline, which inhibits axonal transport of neurotransmitter vesicles, causes accumulation of SP in the ganglion due to an inhibition of the transport to the periphery (232). A similar accumulation of SP in the cell bodies of dorsal root ganglion neurons is found if axonal transport is inhibited by colchicine (262). Anisomycin, which blocks ribosomal protein synthesis, inhibits synthesis of SP (232).

In summary, these results clearly indicate that SP in the sensory neurons is synthesized in the cell bodies of dorsal root ganglia and distributed to nerve terminals both in the spinal cord and peripheral tissues by a fast axonal transport.

C. Release

A series of data indicates that SP is released from peripheral, probably sensory, nerve endings in connection with antidromic nerve stimulation. The first observation was made by Olgart et al. (490), who showed that electrical stimulation of the inferior alveolar nerve significantly elevates the concentration of SP immunoreactivity in the superfusate of the cat dental pulp. As a consequence, a 60% depletion of the SP occurs in the pulp (40, 204). A release of SP has also been observed in the eye (see section VII B).

VII. The Visual System

A. Distribution

SP-positive nerve cells and fibers are present in all parts of the peripheral visual system. About one third of the nerve cell population, particularly small-sized cell bodies, in the Gasserian ganglion shows SP immunofluorescence (260, 119, 614). The ophthalmic nerve contains numerous SP-positive nerve fibers. Furthermore, dense networks of SP fibers are found in most layers of the eye. SP positive cell bodies and fibers are frequently seen in the retina of several species such as the pigeon (329), monkey, rat, chicken, and bullfrog (158). In the iris, they occur in the sphincter muscle and in the stroma with fibers running close to the dilator muscle (260, 454, 614, 620). In the ciliary processes, numerous SP fibers are present with a predominantly subepithelial localization, while only a few fibers are observed in the ciliary body (620). Blood vessels in the anterior uvea are often surrounded by SP fibers (620).

After denervation of the trigeminal nerve or electrocoagulation of the ophthalmic branch of this nerve, a complete disappearance occurs of all SP immunoreactive axons in the iris (60, 454, 615, 626). The SP level in the cornea of the adult mouse is reduced 40% by a similar procedure (337). No change in the tissue content of SP is, however, observed after removal of the superior cervical ganglion, which results in a disappearance of all noradrenergic axons (337, 454). Neonatal capsaicin treatment results in an 80% reduction of the SP level in the cornea (337). These results indicate that the eye is supplied by sensory nerves containing SP immunoreactivity. In the retina, in contrast, sensory denervation does not reduce SP immunoreactivity, which indicates that in this

tissue SP is not connected to sensory nerves, but probably to amacrine cells (615), which are known to contain SP (329).

B. Release

Electric or mechanical stimulation of the trigeminal nerve distal to the Gasserian ganglion significantly increases the amount of SP immunoreactivity in the aqueous humor of the eye (28). A similar release of SP is induced by a topical application of nitrogen mustard (62). Furthermore, depolarizing concentrations of potassium induce a calcium-dependent release of SP, as well as of somatostatin, in vitro from the retina of the bullfrog (159).

C. Pharmacological Effects

Injection of SP into the anterior chamber produces miosis in the intact rabbit eye (28). Also, in the isolated iris sphincter muscle of the rabbit a dose-dependent miosis is obtained, the threshold being 2×10^{-10} mol, which is far below that of other known miotic compounds (583, 620). Similar effects are obtained in the isolated bovine pupillary sphincter (82). This constrictor response to SP is not influenced by blockade of muscarinic, nicotinic, or alpha- and beta-adrenergic receptors. Neither does baclofen (see section XIX) affect the SP action in the iris (583). Pretreatment with prostaglandin biosynthesis inhibitors is also without effect (82, 583). Tetrodotoxin, which blocks miosis caused by trigeminal nerve stimulation, does not appreciably influence the effect of SP (437). Furthermore, SP retains its miotic effect even after trigeminal denervation (59). These results indicate that miosis caused by SP is a direct effect on the pupillary sphincter muscle. Intracameral injections of SP in picomole doses, which causes a strong and persistent miosis, does not affect the intraocular pressure or the aqueous humor protein concentration (598). Higher doses of SP, however, elicit both a pressure increase and protein leakage from the blood vessels (28). The increase in intraocular pressure induced by SP can, however, be prevented by iridectomy, which seems to indicate that this effect of SP is caused by a papillary block from the intense miosis (598). The finding that SP induces miosis in doses that do not interfere with local blood flow or pressure support the idea that miosis and circulatory changes induced by painful stimuli are elicited by separate pathways (442).

Infusion of SP into the common carotid artery results in an immediate ipsilateral constriction of the pupil and a transient increase in intraocular pressure simultaneously with a decrease in the contralateral intraocular pressure and arterial blood pressure (598).

Several observations indicate that SP plays an important role as a possible primary mediator of the ocular response to chemical irritants. Thus, topical application of nitrogen mustard to the rabbit eye causes hyperemia, miosis, and ocular hypertension (62). Since SP is simultaneously released, it is suggested that these reactions to nitrogen mustard and similar irritants are due to a local release of SP, presumably from sensory neurons in the anterior uvea (62). Retrobulbar administration of capsaicin, which also releases SP, acutely produces similar symptoms. After a few days of capsaicin treatment, when the SP storage was depleted, nitrogen mustard no longer produced any changes in intraocular pressure (62).

Local application of SP increases spontaneous spike activity and enhances light-evoked excitation of retinal ganglion cells of amphibia (121) and carp (211). SP does not influence the excitation produced by acetylcholine and there are reasons to believe that SP and acetylcholine act on different receptors in the retina (212), as also seems to be the case in the locus coeruleus (221). Since the effect of SP in the retina is not influenced by ganglionic blocking agents, the SP receptors are probably located on the ganglion cell membrane, particularly on cells with an on-component in their light response (212).

VIII. Sympathetic Ganglia

A. Distribution

Immunohistochemical (259, 108, 109, 441, 568) and radioimmunological analyses (352, 200) have shown that prevertebral mammalian sympathetic ganglia contain SP. The number of SP positive nerve fibers varies considerably, however, between different ganglia as well as between species. The inferior mesenteric and coeliac superior mesenteric ganglia show the highest density of SP fluorescence followed by the thoracic and cervical ganglia. Bundles of SP fibers are traversing the ganglion but varicose networks of SP nerve endings are also seen in close contact to the ganglion cells (147, 109), and SP positive boutons are in synaptic contact with principal ganglion cells (441). Also in the coeliac ganglion of the guinea-pig, SP-fibers form axo-dendritic synapses on the postganglionic neurons (348). When a fluorescent dye, true blue, is injected into the inferior mesenteric ganglion, it is transported retrogradely to cell bodies in the dorsal root ganglia L_2 and L_3, where SP immunoreactivity is also observed (108).

The origin of the SP immunoreactive fibers in sympathetic ganglia has been most extensively studied in the inferior mesenteric ganglion. Ligation or cutting of the lumbar splanchnic and intermesenteric nerves leads to a drastic accumulation of SP proximally to the lesion, while an almost total disappearance of varicose and nonvaricose SP fibers occurs in the inferior mesenteric ganglion (352, 109, 441). Cutting the hypogastric, intermesenteric, and colonic nerves, leaving the lumbar splanchnic nerves intact, results in an accumulation of SP immunoreactivity within the inferior mesenteric ganglion (109).

These results indicate that SP is biosynthesized in the cell bodies of the dorsal root ganglia and subjected to axoplasmic transport through the lumbar splanchnic

nerves to the inferior mesenteric ganglion directly or via the intermesenteric nerve. Most of the SP-positive fibers traverse the ganglion on their way from the visceral organ to their cell bodies in the spinal ganglia. Some of these fibers form synpases on sympathetic neurons in the ganglion. The observations with fluroescent dye convincingly indicate the sensory origin of the SP terminals, which is in agreement with electrophysiological studies (see below).

B. Release

SP is released from the prevertebral ganglia by potassium in a calcium-dependent manner (352). A significant increase in the spontaneous release of SP from the mesenteric ganglia is also obtained when capsaicin is added to the incubation fluid (354, 200). This effect of capsaicin is, however, not seen when the calcium is decreased and magnesium increased in the Tyrode solution used as perfusion medium. There is no release of vasoactive intestinal polypeptide, which is also present in large amounts in the mesenteric ganglia (258, 354, 109).

After pretreatment of guinea-pigs with capsaicin (125 mg/kg over 2 days) an almost complete depletion of SP occurs in the sympathetic ganglia and splanchnic nerve (200), which supports the sensory nature of the SP fibers in these nerve tissues.

C. Pharmacological Effects

When applied to neurons of isolated, perfused inferior mesenteric ganglia of guinea-pigs, SP causes a membrane depolarization, which is often accompanied by intense neuronal discharges (133, 352, 134). The SP-induced depolarization is not depressed by tetrodotoxin, *d*-tubocurarine, atropine, or a low calcium/high magnesium medium (133, 354), suggesting that SP acts directly on the postganglionic cell.

Stimulation of preganglionic nerves elicits in the inferior mesenteric ganglion cells a fast cholinergic excitatory postsynaptic potential followed by a slow-developing depolarization. If only the preganglionic fibers in the ventral root are stimulated, a pure cholinergic excitatory postsynaptic potential is elicited, whereas stimulation of the dorsal root produces only a slow noncholinergic excitatory postsynaptic potential (352, 354). There are now several observations supporting the view that the slow excitatory postsynaptic potential is produced by SP. The conductance changes associated with the SP-induced depolarization are similar to those associated with the slow excitatory postsynaptic potential. The depolarization induced when SP is applied to neurons of the inferior mesenteric ganglia is accompanied by an increase in membrane resistance analogous to that involved in synaptically induced noncholinergic depolarization (134). Both responses are insensitive to anticholinergic agents and their ionic mechanisms appear to be similar (133, 352, 354). Exogenous application of SP renders the neuron incapable of generating the slow excitatory postsynaptic potential upon repetitive stimulation of preganglionic fibers (133, 352, 132). Furthermore, capsaicin, which depletes SP from prevertebral ganglia, markedly depresses the slow excitatory postsynaptic potential evoked by stimulation of the lumbar splanchnic nerve (354). Finally, in the inferior mesenteric ganglion of rats the specific SP receptor antagonists, [D-Pro^2, D-Phe^7, D-Trp^9]SP and [D-Arg^1, D-Pro^2, D-Trp^{7,9}]SP, block the noncholinergic, slow, excitatory postsynaptic potential induced by preganglionic nerve stimulation (316, 351).

Both the noncholinergic excitatory postsynaptic potential and the potassium-evoked release of SP from the inferior mesenteric ganglion are depressed by the metenkephalin analogue, [D-Ala^2]Met-enkephalin amide (354). By contrast, the SP-induced depolarization of the ganglion cell is not blocked by this analogue (354). These observations suggest that there exists an enkephalinergic pathway that exerts a presynaptic inhibition of SP-mediated slow excitatory postsynaptic potentials in the mesenteric ganglia (354). Similarly, enkephalin serves as a transmitter of presynaptic inhibition by modulating the release of acetylcholine in the sympathetic ganglia (353).

In summary, these results give strong support for the presence in the prevertebral ganglia of SP nerve endings in contact with sympathetic neurons (fig. 3). The sensory nature of these intraganglionic SP fibers is well established by retrograde tracing and immunohistochemistry as well as by the results obtained after capsaicin application. Convincing evidence is also given that SP is released in the mesenteric ganglia where it causes a membrane depolarization in sympathetic neurons. It may therefore be suggested that SP is the transmitter mediating the noncholinergic potential in prevertebral sympathetic neurons. The accumulated data suggest two possible pathways of SP-containing fibers in the inferior mesenteric ganglion. Sensory SP afferents from the intestinal wall with cell bodies in the spinal ganglia and central terminals in the spinal cord pass the ganglion where they give off collaterals forming synapses on the noradrenaline-releasing cells. As recently pointed out by Dun and Jiang (132), the noncholinergic transmission may also function as a local reflex, whereby sensory information is transmitted from the intestinal wall in SP-ergic fibers to the sympathetic neurons of the ganglion, initiating efferent inhibitory adrenergic signals back to the gut, thus providing a functional connection between sensory and autonomic neurons. The finding that SP is released from peripheral nerve endings in the sympathetic ganglia, therefore, gives new insights on the functional role of sensory neurons as not being merely transducers of afferent sensory information.

While SP in the inferior sympathetic ganglion evidently has a sensory origin, SP in other sympathetic ganglia might have a different origin. Thus, Kessler et

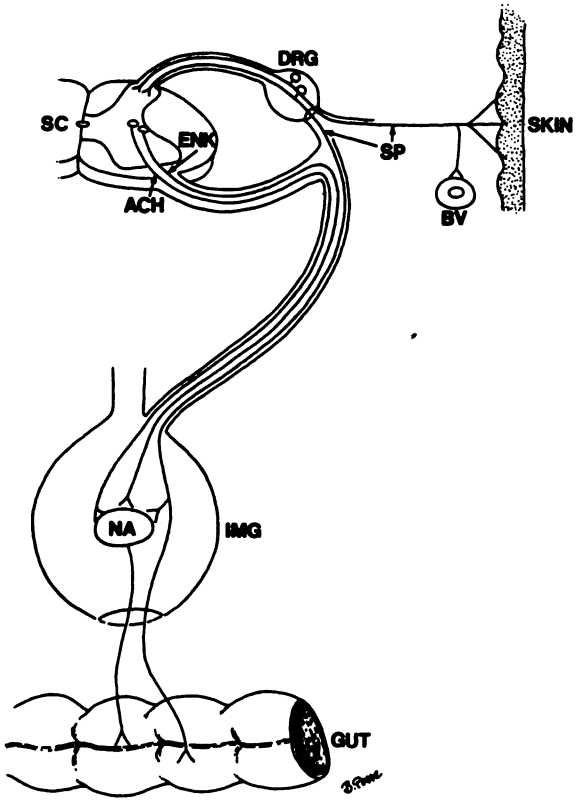

FIG. 3. Schematic representation of somatic and visceral primary afferent neurons and synaptic connections in the inferior mesenteric ganglion (IMG). Sensory SP afferents from both the skin and intestine have their cell bodies in the dorsal root ganglia (DRG) and central terminals in the dorsal horn of the spinal cord (SC). SP-afferents from the intestine pass the IMG where they give off terminals forming synapses on the noradrenaline-releasing cells (NA). ACH, acetylcholine; ENK, enkephalin; BV, blood vessel. [Modified from Konishi et al., 1980 (354), Dalsgaard et al., 1982 (109), and Dun and Jiang, 1982 (132).]

al. (342) showed that denervation of the superior cervical sympathetic ganglion of the neonatal rat, or pharmacological blockade of transmission, causes a remarkable increase in SP immunoreactivity in the ganglion. Conversely, pharmacological stimulation of sympathetic activity reduced SP content of the ganglion. Treatment of rats as neonates with 6-hydroxydopamine, which selectively destroys principal ganglion neurons, profoundly decreased SP in the superior cervical ganglion in the adult rat (344). In cultured ganglia, SP increased more than 50-fold after 48 hours and this rise was dependent on protein and RNA synthesis. Veratridine prevented the increase of SP and tetrodotoxin blocked the veratridine effect, which suggests that sodium influx and membrane depolarization prevent SP elevation (343). As pointed out by Kessler and Black (344), these findings suggest that the level of SP is regulated by the sympathetic impulse activity and may mediate a feedback mechanism tending to stabilize sympathetic activity. In general terms, they suggest that local level of a neuropeptide is dependent on the degree of physiological activity of the neuron.

IX. Gastrointestinal Tract

A. Distribution

The occurrence and distribution of SP in the gastrointestinal wall has been described in detail both qualitatively, with indirect immunofluorescence techniques (477, 509, 604, 269, 569, 175, 266, 88, 89, 38, 374, 434), and quantitatively by RIA (474, 604, 281, 41) (table 2). In general, there is a good agreement between the results obtained from these studies.

The distribution pattern of SP is similar in different parts of the gastrointestinal tract where, as in all other organic systems, SP is almost entirely connected to the nervous structures. Two principal populations of neurons can be distinguished—nerves intrinsic to the gastrointestinal wall and extrinsic nerve fibers. Initially, difficulties arose when trying to determine the origin of peptidergic nerve fibers and nerve cells in the gastrointestinal tract, mainly because peptide levels in cell bodies often are too low to be detected with the indirect immunofluorescence technique. Therefore, tissue culture techniques (569, 315), denervation (175), and transplantation (571, 172, 434) were used to solve this problem. These studies clearly show the presence of SP neurons intrinsic to the intestine. Thus, SP fibers were found mainly in cultures of both the myenteric and submucous plexus from all intestinal segments. Only in the submucous plexus of the caecum was there an absence of SP fibers (569, 315). In segments of ileum, which had been extrinsically denervated, the amount of SP in the external muscle layers, which includes the myenteric plexus, was significantly lower than in the normally innervated ileum (175). The existence of an intrinsic peptidergic neuronal system in the intestine is also suggested by autotransplantation studies. Tissues from the small and large intestine of pre- and postnatal and adult rats, transplanted to the anterior eye chamber, reveal many SP-containing nerve fibers as well as vasoactive intestinal polypeptide- and enkephalin-containing nerve fibers, especially in the circular muscle layer and in the myenteric plexus (571). Similarly, transplants of various tissues from the embryonic avian digestive tract to the chorioallantoic membrane contain numerous SP- and vasoactive intestinal polypeptide-immunofluorescent nerve fibers (172).

Taken together, these observations on different experimental models clearly suggest that the major part of the SP-containing axons and all other peptidergic nerve fibers in the intestinal wall originate from cell bodies within the myenteric and submucosal plexus. It is, however, evident that the gastrointestinal tract is also innervated by extrinsic SP-immunoreactive axons. These axons reach the intestine via the mesenteric nerves and most likely represent sensory nerve fibers passing through prevertebral sympathetic ganglia (259, 422, 88, 441). Furthermore, SP-containing fibers in the vagus terminate in the stomach and the intestine (422, 196, 37, 207). Experimental evidence for the existence of extrinsic

TABLE 2
Distribution of SP in the gastrointestinal tract

	Rat (Ref. 281) Boiled in 1 M acetic acid	Mouse (Ref. 41) Boiled at pH 4	Guinea-pig (Ref. 281) Boiled in 1 M acetic acid	Dog (Ref. 41) Boiled at pH 4	Man (Ref. 43) Boiled in 1 M acetic acid
Oesophagus	1.4 ± 0.2 (6)*				
Proximal		13.7 ± 4.7 (5)	10.4 ± 1.5 (5)	0.28 ± 0.1 (5)	
Distal		6.3 ± 1.7 (5)		0.30 ± 0.1 (5)	
Stomach					
Fundus	24.9 ± 1.6 (6)	14.9 ± 2.8 (5)	39.3 ± 3.7 (8)	0.54 ± 0.1 (5)	
Antrum	9.2 ± 0.6 (6)	6.1 ± 1.8 (5)	81.6 ± 8.2 (5)	0.76 ± 0.2 (5)	
Duodenum	57.6 ± 2.8 (6)				
Proximal		18.1 ± 2.9 (5)	117 ± 8.2 (6)	15.4 ± 3.8 (5)	
Distal		18.9 ± 6.2 (5)		14.6 ± 4.1 (5)	
Jejunum					120 (2)
Proximal	62.4 ± 6.4 (6)	7.2 ± 1.4 (5)	108 ± 8.9 (6)	5.5 ± 1.3 (5)	
Distal			151 ± 8.2 (6)		
Ileum					86 (2)
Proximal		7.6 ± 2.8 (5)	167 ± 17.1 (6)	5.3 ± 1.2 (5)	
Distal	64.5 ± 6.2 (6)	12.4 ± 3.7 (5)	173 ± 14.1 (10)	6.1 ± 1.5 (5)	
Colon					53 (2)
Proximal	27.1 ± 2.8 (6)	9.8 ± 4.4 (5)	122 ± 19.3 (6)	5.4 ± 1.3 (5)	
Distal		12.7 ± 3.4 (5)	107 ± 10.4 (9)	4.2 ± 1.0 (5)	
Rectum		11.5 ± 4.0 (5)	90.5 ± 8.2 (8)	6.3 ± 1.9 (5)	

* Values are given in pmol/g of wet tissue and represent mean ± SEM. Number of observations are given within brackets.

SP innervation of the gut is shown by the finding that crushing the mesenteric nerves 1 month before sacrifice results in a complete disappearance of the SP immunoreactive nerves associated with submucous arteries and also to a small extent SP fibers in the submucous plexus, whereas in all other layers of the intestinal wall extrinsic denervation does not influence the number or distribution of SP fibers (88). Also, bilateral ligation of the splanchnic nerves of spinal ganglionectomy from Th5 to L2 in the cat considerably decreases SP immunoreactivity in Auerbach's plexus in the stomach and duodenum (234).

In the following, a brief summary of the distribution of SP in various parts of the gastrointestinal tract will be given on the basis of results from the papers cited above. The distribution has been studied mainly in the rat and guinea-pig.

In the *oesophagus*, the lower part contains about 10 times more SP than the upper part. SP immunoreactive nerve fibers are particularly numerous in the myenteric and submucous plexus (372). In the *stomach* the highest concentration of SP fibers is seen in the myenteric ganglionic plexus. The longitudinal muscle layer, particularly in the cardia (rat) and antrum (guinea-pig), has a high density as compared to other parts of the gastrointestinal tract. In the submucous layer and in the lamina propria less dense networks are found. In the myenteric plexus, but not in the submucous plexus, single cell bodies show SP immunoreactivity.

In the *small intestine*, the highest density of SP fibers is found in the circular muscle layer and in the myenteric and submucous plexus, while the SP positive fibers occur much less frequently in the longitudinal muscle layer and lamina propria. Also, in the muscularis mucosae and in lamina propria there are numerous SP fluorescent fibers, partly associated with blood vessels. Only a relatively small proportion of neurons, however, contain SP (3% to 5% of total myenteric neurons and 3% to 11% of total submucous neurons) (88, 570). Some SP positive fibers are present at the bases of the mucosal villi and extend in the connective tissue to their tips. SP immunofluorescence has also been observed in epithelial cells, which have been identified as enterochromaffin cells, in the mucosa of both duodenum and colon (477, 509, 240).

SP-positive cell bodies are located in both the myenteric and submucous plexus. Analyses of the projections of the intrinsic and extrinsic SP neurons indicate that cell bodies in the myenteric ganglia supply the circular muscle, the submucosa, and the mucosa, while the submucous ganglia only supply the mucosa. Only SP fibers around the submucosal arteries and some varicose fibers in the submucous ganglia have extrinsic origin (88, 89, 315, 570) (fig. 4).

Also, in the *large intestine*, the highest density of SP fibers if found in the myenteric plexus and in the circular muscle layer. Both in myenteric and submucous plexus they surround the ganglion cells, of which a small number are SP-positive. Numerous SP-positive fibers are also seen in the muscularis mucosae. In the lamina propria, there are few SP fibers.

In the muscle layer of the taenia coli of the caecum frequent SP fibers run in thin nerve bundles or are present as single varicose terminals along the smooth muscle fibers (88, 315, 570, 374)..

Also, in the *human intestine*, there are many SP fibers in the myenteric plexus and a moderate amount in the

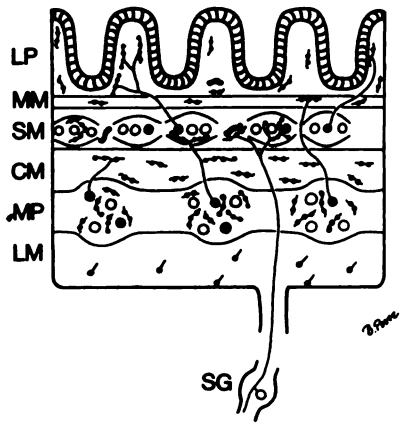

FIG. 4 Schematic representation of the distribution and density of SP cell bodies and nerve terminals in the small intestine. The drawing also indicates the origin of the SP processes. LP, lamina propria; MM, muscularis mucosae; SM, submucosa; CM, circular muscle layer; MP, myenteric plexus; LM, longitudinal muscle layer; SG, sensory ganglion. [Modified from Schultzberg et al., 1980 (570) and Costa et al., 1981 (89).]

submucous plexus (43). In contrast to most animals studied so far, a very dense network of SP immunoreactive nerve fibers is found in the human mucosa, particularly in the lamina propria with single fibers running close to the base of the epithelium. These mucosal fibers, which are most numerous in the ileum, evidently emanate from SP-containing cell bodies in the submucosal plexus, which are more frequently seen in man than in animal species (43).

B. Comparison with Other Peptides

Besides SP, many other peptides have been identified in the gastrointestinal tract. Particular interest has been focused on the distribution of vasoactive intestinal polypeptide, enkephalin, somatostatin, gastrin/cholecystokinin, and neurotensin in the rat (570, 315, 374, 434, 605, 568). It appears from these studies that the general distribution patterns for vasoactive intestinal polypeptide, enkephalin, and SP often exhibit similarities, but also some interesting differences. The vasoactive intestinal polypeptide seems to be the most abundant. In general, the circular muscle layer and the myenteric and submucous ganglion plexus have the highest innervation of peptide immunoreactive fibers. In lamina propria, the vasoactive intestinal polypeptide network is much more dense than for any of the other peptides. SP and enkephalin immunoreactive cell bodies are both present in the myenteric plexus, but enkephalin is rarely seen in the submucous plexus where vasoactive intestinal polypeptide and somatostatin are mostly localized. The SP immunoreactive fibers in the submucous plexus are often seen close to vasoactive intestinal polypeptide immunoreactive cell bodies. Also, in the guinea-pig gut, SP and vasoactive intestinal polypeptide are present in separate subpopulations of peptidergic neurons (535). Gastrin/cholecystokinin immunoreactive neuronal structures are few compared to the peptides mentioned above and some layers, particularly in the upper tract of the guinea-pig, seem to lack gastrin/cholecystokinin. Neurotensin seems to have the most limited distribution of all peptides. It is largely stored in glandular cells (N-cells) in the lamina propria of the small and large intestine (246). In the rat, neurotensin-reactive fibers are localized to the longitudinal and circular muscle layers and to the myenteric plexus (571).

C. Release

When the antrum of the cat stomach is perfused with NaCl or HCl, SP-like immunoreactivity is regularly detected in the perfusate (629). Electrical vagal stimulation causes a substantial increase in this release. Also, SP is released by antral perfusion of acetylcholine as well as by intravenous injections of acetylcholine or adrenaline. Gastrin, somatostatin, and vasoactive intestinal polypeptide are likewise released by these procedures (629). On the other hand, evidence for a release of SP from intrinsic neurons in the intestine is so far only indirect. Thus, high desensitizing concentrations of SP inhibit hyoscine-resistant intestinal contractions evoked by electrical stimulation (174), probably due to a blockade of SP receptors (185). Also, the contractile response of the guinea-pig ileum to cholecystokinin (294) or capsaicin (69) is blocked by desensitizing doses of SP, which suggests that a release of SP may be responsible at least to some extent for this contraction.

D. Pharmacological Effects

SP has a powerful stimulating effect on most smooth muscle layers in the gastrointestinal tract.

In concentrations of 10^{-8} to 10^{-7} M, SP increases basal tension of isolated strips of cat *oesophagus* and greatly enhances the contractile response to electrical nerve stimulation (372). Higher concentrations contract, in a dose-dependent way, the circular muscle and these responses are blocked by the SP antagonist, [D-Pro^2, D-Trp^{7,9}]SP, which has no effect on contractions elicited by electrical stimulation (372). In isolated strips of the circular muscle of the fundus and corpus of the guinea-pig *stomach*, SP evokes a dose-dependent tonic activity in concentrations higher than 10^{-13} M. On the contrary, neither the longitudinal muscle layer of the fundus or corpus nor longitudinal or circular strips from the antrum react to SP (472). The finding that only the circular muscle responds to SP is interesting in view of the observation of a much greater density of SP-positive nerve fibers in the circular than in the longitudinal muscle of the rat stomach (263, 570). Also, in the perfused rat stomach and pylorus, SP has a spasmogenic effect with a threshold dose of about 4 nmol/kg (26, 27).

Close-arterial bolus injections of SP induce an immediate and powerful contraction of the cat stomach and pylorus sphincter in vivo. The duration of this effect is dose-dependent (0.4 to 3 nmol). The contractions cause a total but short cessation of the transpyloric transport

(138). Also, intravenous infusions of SP in dogs with starvation peristalsis result in an increase in the basal muscular tone and depression of the peristalsis. The spike discharge in the electromyogram is markedly stimulated by SP (4 to 400 nmol × kg^{-1} × 100 sec^{-1}) in a dose-dependent manner (452).

The gastric excitatory motor response elicited by antidromic stimulation of the vagal or splanchnic nerves after hexamethonium is markedly reduced by large doses of SP or by pretreatment with [D-Pro^2, D-$Trp^{7,9}$]SP (407, 118). Also, nerve stimulation at high frequencies diminishes the gastric response to subsequent administration of SP, suggesting that the stimulation causes a release of SP that desensitizes SP receptors (118). These findings clearly suggest that SP is involved in the motor responses elicited by extrinsic gastric nerves.

All segments of the *small intestine* are contracted by SP in vitro and in vivo. This effect can be demonstrated in all mammals, including man, as well as in cold-blooded animals. In the isolated guinea-pig ileum, SP increases the tonus in the concentration range 10^{-10} to 10^{-8} M in a dose-dependent way. On this preparation, SP is more potent than acetylcholine, histamine, and barium chloride; the slope of the log dose-response curves is similar (54, 57, 547, 607, 656). When injected close-arterially in the dog ileum in situ, SP causes tonic contractions in doses as low as 0.7 pmol. Higher doses give larger and more persistent contractions, usually reaching maximal amplitude at about 75 to 150 pmol (113). The longitudinal muscle is by far the most sensitive of the external muscle layers to SP. The maximal effect elicited by SP increases from the gastrointestinal sphincter to the ileum and is roughly paralleled by a similar rise in SP concentration in the intestinal segments (280). In the isolated longitudinal muscle of the guinea-pig ileum, application of SP at concentrations of 8 to 10^{-13} M, or higher, to the bath fluid exerts a tonic activation of the muscle (656, 472). Varying opinions have been presented about the effect of SP on the circular muscle layer. Most studies have revealed a striking unresponsiveness of this muscle layer to substances that exert a powerful spasmogenic effect on the longitudinal muscle. This holds for SP, acetylcholine, and 5-HT (for ref. see 472, 285). Thus, SP, in doses that elicit a substantial tonic activation of the isolated longitudinal muscle of the guinea-pig ileum, is without effect on the circular ileum preparation (472, 180). On the other hand, SP (2 nmol) elicits rhythmic contractions on the circular muscle layer of the guinea-pig superimposed on an increased tone elicited by SP, caerulein, bombesin, and 5-HT (285).

In the longitudinal muscle cell of the guinea-pig, SP (10^{-10} to 10^{-8} M) evokes different types of membrane responses comprising bursts of repetitive spikes or slow waves without any change in membrane potential. In higher concentrations, SP induces a membrane depolarization and increase in input resistance (180).

Close-arterial infusion of SP (2.4 pmol/min) into an isolated segment of guinea-pig ileum in situ elicits repetitive peristaltic waves, which expel a considerable volume of intraluminal fluid (283). Similar effects are obtained with 5-HT, caerulein, and bethanechol. In an experimental model, where only isometric contractions of the longitudinal muscle are allowed, SP increases peristaltic efficiency at infusion rates of 2.4 pmol/min, which is more than 100 times lower than those required of 5-HT and bethanechol (284). SP counteracts the paralysis of peristalsis induced by the enkephalin-analogue FK 33-824 (283).

In the isolated *large intestine*, SP has a similar effect as in the small intestine and the dose-response curve in the rat transverse colon does not differ from that of the terminal ileum (280). In the chicken caecum, SP in concentrations of 10^{-9} M and higher causes contractions in a dose-dependent manner (38). The guinea-pig taenia coli is contracted by SP, while electrically induced contractions of the taenia are reduced. In the anaesthetized cat, intravenous SP at a dose of 17 pmol × kg^{-1} × min^{-1} elicits a powerful contraction of the colon in vivo. This effect, however, is seen only in the distal colon, while no effect or a slight inhibitory action is obtained in the proximal part (245).

E. Mode of Action of SP

On the basis of pharmacological studies it was early suggested that the mode of action of SP is twofold, a direct stimulating effect on the smooth muscle and a local neurotropic effect that involves stimulation of afferent fibers in the peristaltic reflex arc (517). The observation that the maximal contraction induced by SP on isolated intestine is not suppressed by atropine, antihistamine, LSD, or ganglionic blocking agents (514) has been repeatedly confirmed (547, 656, 280, 285, 38) and extended by using a variety of compounds such as tetrodotoxin and morphine (57, 547, 38, 180), the muscarinic acetylcholine receptor inhibiting compund, tropamide (285), and guanethidine (38). In the perfused rat stomach, the spasmogenic effect of SP is not modified by atropine, propranolol, methysergide, cimetidine, or verapamil, thus excluding the involvement of acetylcholine, noradrenaline, 5-HT, histamine, or other mediators (27). However, in the circular muscle layer of the guinea-pig ileum, tetrodotoxin reduces or abolishes rhythmic contractions induced by SP, but not the increase in muscular tone (285). It is suggested that the action of SP on the smooth muscle is brought about by facilitation of the myogenically controlled spike activity (278). This assumption is based on the fact that the effect of SP on the isolated rabbit ileum is an increase in the height but not the frequency of the phasic contractions. Furthermore, suppression of the slow wave depolarizations by various procedures also abolishes the effect of SP (278).

Local anaesthetics such as procaine, lidocaine, bupivacaine, and promoxine, which have a depressive action on smooth muscle, cause a dose-related decrease of the

spasmogenic effect of SP as well as of acetylcholine, histamine, and barium chloride (54). The effect of these anaesthetics on the response of the guinea-pig ileum to SP is, however, far less susceptible than to other spasmogenic compounds. The depressant action of local anaesthetics is attributed to their inhibition of calcium influx, and withdrawal of calcium diminishes contractions caused by SP as well as by other spasmogenic factors (54). These findings support the suggestion that SP acts directly on the smooth muscle and indicate that the effect of SP is dependent on the extracellular calcium concentration and involves influx of calcium. The underlying ionic mechanism of the action of SP, furthermore, seems to be a decrease in potassium and chloride conductance (278).

The assumption that SP also acts by neuronal stimulation was based primarily on the results early obtained by Beleslin and Varagić (19), who found that SP enhances the contractions of the guinea-pig ileum induced by acetylcholine. This enhancement is inhibited by hexamethonium, indicating that the effect of SP might partly be due to sensitization of ganglionic cells to acetylcholine. The suggestion that SP activates cholinergic neurons in the gastrointestinal tract has received support in recent time. The first was given by Hedqvist and von Euler (238, 239), who showed that SP enhances contractions of the guinea-pig ileum induced by transmural nerve stimulation. These contractions are inhibited by atropine but not by guanethidine, phentolamine, or propranolol, indicating activation of cholinergic nerves. Since SP much more readily enhances contractions resulting from nerve stimulation than from acetylcholine administration, a prejunctional action of SP on cholinergic transmission was suggested (238, 239). One characteristic of the effect of SP on the longitudinal muscle of the guinea-pig ileum is that its contractile response is not sustained but gradually decreases after achieving its peak response. This phenomenon, known as fading, probably reflects specific desensitization of the SP receptors and, in part, the rate of absorption and metabolism of the peptide (280, 284, 291). Atropine or tropicamide and tetrodotoxin reduce the fading, while physostigmine increases its duration (280, 284, 291). These results seem to further indicate that SP releases acetylcholine and that this release contributes partly to the excitatory effect of SP on the longitudinal muscle of the guinea-pig ileum. In fact, direct evidence for a release of acetylcholine from the myenteric plexus of guinea-pig intestine by SP has recently been given (657). However, not only atropine and tropicamide but also tetrodotoxin and morphine, but not hexamethonium, diminish the contractile effect of SP (284, 113), which indicates that the cholinergically mediated response to SP involves neural conduction.

The stimulatory effect of close-arterial injection of SP on the cat stomach and pylorus in situ is blocked by atropine (138). Also, in the dog ileum in situ, atropine and tetrodotoxin substantially inhibit the tonic contractions caused by low (pmol) doses of SP, but not by higher (nmol) doses (113). These findings further suggest that the motor effects of SP involve activation of intrinsic cholinergic neurons. Besides the cholinergic contraction of the gut, there also exists an atropine-resistant contracture induced by transmural field stimulation. This noncholinergic contraction is significantly reduced by tetrodotoxin, which indicates its neuronal mediation. It is, however, also considerably reduced by addition of [D-Pro^2, D-Trp^{7,9}]SP (373) or by desensitizing the guinea-pig ileum to SP by addition of large amounts of SP itself (210). These observations indicate that SP mediates the atropine-resistant contracture in the gut induced by transmural field stimulation.

Important evidence for the ability of SP to depolarize neurons in the myenteric plexus was given by Katayama and associates (331, 332). Extracellular recordings from single neurons in the myenteric plexus of guinea-pig small intestine in vitro showed that SP applied in picomolar concentrations, either by perfusion or electrophoretically, increases the firing rate of the neurons. Intracellular recordings indicate that SP causes a dose-dependent membrane depolarization associated with an increase in membrane resistance. There is a relation between the SP reversal potential and extracellular potassium concentration, which suggests that SP inactivates the resting potassium conductance of the myenteric neuron. These effects of SP are uneffected by hexamethonium, hyoscine, naloxone, and baclofen (332, 180).

As in the sympathetic ganglia (see above), electrical stimulation of presynaptic nerves in the myenteric ganglia elicits, in addition to cholinergic fast excitatory postsynaptic potentials, slow excitatory postsynaptic potentials, which are not affected by nicotinic or muscarinic blockers. The time course and ionic mechanism of the SP-induced depolarization of the myenteric neurons are similar to that of the slow excitatory postsynaptic potentials (331, 332). Both the SP depolarization and the slow excitatory postsynaptic potentials are reversibly depressed by chymotrypsin, which leaves the fast excitatory postsynaptic potentials and responses to acetylcholine and 5-HT unaffected (460). These results might indicate that SP is involved in the noncholinergic transmission in the myenteric ganglia.

In summary, substantial evidence has now been accumulated that indicates that the SP neurons are an essential part of the enteric neuron system and are involved in the modulation of intestinal motility by several mechanisms: 1) SP-positive nerve cell bodies and fibers are widely distributed in the enteric neuron system, and nerve fibers containing SP are found both around ganglion cells in the ganglionic plexus and in the muscle layers; 2) indirect evidence exists for a release of SP from enteric neurons, which might stimulate the ganglion cells; 3) SP has a powerful stimulating effect on the smooth muscle of the gastrointestinal tract and initiates

peristalsis; 4) SP depolarizes myenteric neurons and mimics synaptically evoked depolarizations within the myenteric plexus; 5) SP releases acetylcholine and activates cholinergic neurons; 6) SP mediates slow noncholinergic depolarizing potentials in the myenteric plexus.

F. Role of SP in the Submucosa and Mucosa

The density of SP fibers in the submucosa and mucosa, especially in connection to blood vessels, secretory glands, and the villi, suggests that SP is involved in the regulation of local blood flow as well as in secretory and absorptive functions. The supply of SP-containing neurons to the submucous blood vessels has an extrinsic origin and is depleted by capsaicin (181). These findings strongly suggest that the extrinsic SP neurons are sensory and it is tempting to postulate that, by analogy with the skin, the SP fibers in the intestinal wall and in other visceral organs are involved in neurogenic vasodilatation. So far, only a few studies have been devoted to the putative role of SP in secretion and absorption. SP has no effect on basal gastric secretion in the dog, but when given during a period of pentagastrin-stimulated secretion, SP (in a dose of 1 nmol $\times$ kg^{-1} $\times$ min^{-1}) significantly reduces acid output. Following withdrawal of SP infusion, gastric secretion quickly returns to the control level (355). In the gut, SP produces a transient increase in transepithelial potential differences and short-current circuits in the serosal surface of guinea-pig ileal mucosa (322), which might indicate that SP stimulates intestinal secretion.

X. Circulation

A. Distribution

In some animals, such as the guinea-pig, practically every vascular bed is supplied with SP-containing sensory fibers. In the skin (263, 110), dental pulp (491), thraceo-bronchial tissue (603, 419), gastrointestinal tract (570), and the brain (260, 139, 141, 111, 181), SP immunoreactive nerve networks are present in the adventitia and at the border between the adventitia and media of the blood vessels, particularly the arterioles, with a distribution pattern typical for vasomotor fibers. The highest density of SP networks is found in the aorta and vena cava close to the heart with decreasing density as more peripheral beds are approached (181).

SP immunoreactive nerve fibers and varicosities have also been identified in the human and guinea-pig heart along blood vessels (538, 642), but also running parallel to myocardial fibers and associated with the conducting system and cardiac ganglion cells (644). In the sinus node of several mammalian species, numerous SP immunoreactive fibers are present in close association to blood vessels (643). Particularly dense networks are also found in the splanchnic vascular bed (181).

SP is widely distributed in pathways known to be involved in the central control of the cardiovascular system. Thus, immunocytochemical evidence has been given for the occurrence of SP fibers in the carotid body (420), carotid sinus nerve (244), sensory ganglion cells of the nodose (422, 196), petrosal ganglia (209), and the vagal nerve (422, 244). SP immunoreactivity is also found in the intermediate zone of the nucleus tractus solitarii (103, 411) and in afferent baro- and chemoreceptor pathways, which transmit information from peripheral receptors to the nucleus tractus solitarii (241). Removal of the nodose ganglion or denervation of the IXth and Xth cranial nerve results in a 50% loss of SP fluorescence in the intermediate and commissural parts of the nucleus tractus solitarii (409, 244), which indicates that the SP content of this nucleus to a large extent emanate from cell bodies in the petrosal and nodose ganglia.

Treatment with capsaicin results in a complete loss of SP immunoreactivity from fibers associated with blood vessels throughout the cardiovascular system with exception of arteries supplying the distal intestine. The SP immunoreactivity is, however, unaffected by 6-hydroxydopamine, which degenerates the noradrenergic nerves to the heart and blood vessels (181). These findings indicate that the SP fibers around blood vessels are of sensory nature.

B. Pharmacological Effects

1. Vascular Effects. SP is one of the most potent vasodilating compounds so far known. Close-arterial injections of SP in doses from 10 nmol cause a dose-related vasodilatation in adipose tissue and skeletal muscle of the dog (518). Similarly, close-arterial infusion of SP increases the blood flow in the hepatic, mesenteric, and iliac arteries of the dog, the threshold infusion rate being less than 1.5 pmol $\times$ kg^{-1} $\times$ min^{-1} (449). In the rat, infusion of SP into the femoral artery increases in a dose-dependent way the outflow of the femoral vein with a threshold of about 0.1 pmol/min. The peak flow is reached within 1 minute, whereafter venous outflow declines despite continuous infusion (397). In the isolated heart of the dog infusion of SP at a rate greater than 30 pmol/min produces an increase of coronary blood flow (415). Also the hypothalamic blood flow is increased significantly by local administration of 40 or 400 pmol, as measured by the ^{133}xenon washout technique (347).

Intravenous infusions of SP in the dog at rates of 0.4 pmol $\times$ kg^{-1} $\times$ min^{-1} or higher increase the blood flow of most vascular beds such as the skin, skeletal muscle, and small intestine (51), while somewhat higher doses are needed to increase the blood flow in the carotid, hepatic, mesenteric, and portal beds (228, 449). Some areas, particularly the renal and cerebral vascular beds, are less sensitive or almost resistant to intravenous SP (228). If the infusions are made in the portal vein, the infusion rate has to be increased to more than 15 pmol $\times$ kg^{-1} $\times$ min^{-1} before any vascular reactions are recorded (228, 449), indicating that the liver has a very high capacity for inactivating SP.

SP also affects isolated strips of arteries. The common

carotid artery seems to be the most sensitive, responding to SP in concentrations of 6×10^{-11} M or higher (91). In the rabbit aorta, SP induces dose-related contractions in a concentration of 2×10^{-6} M or higher (458). These effects are resistant to alpha-adrenoceptor blockade but inhibited by pretreatment with met-enkephalin (458). In isolated arteries of the dog, brought into contraction with noradrenalin, SP exerts a relaxant effect (91). Also, isolated pial arteries of humans (141) or cat (139), which have been contracted by prostaglandin $F_{2\alpha}$, are relaxed by SP in 10^{-8} M concentrations.

Intravenous infusions of SP at rates higher than 0.4 pmol $\times$ kg^{-1} $\times$ min^{-1} cause a transient fall in systolic, diastolic, and mean arterial blood pressure in the dog (51, 520). This effect is, however, not dose-dependent. Thus, when increasing the infusion rate, only a minor additional fall in blood pressure is obtained. Even single doses up to 80 pmol cause only rapid and transient fall in blood pressure. Doses higher than 80 pmol decrease diastolic blood pressure more than the systolic, indicating a fall in systemic vascular resistance and sustained stroke volume. The delay in return, but not the absolute decrease in blood pressure, following single intravenous injections of SP, is dose-related. Even doses as high as 40 nmol, i.e. 10^4 times higher than the threshold dose for a fall in blood pressure, are well sustained by the dog (51). These results indicate a considerable tolerance to SP.

SP is also a potent vasodilating compound in humans. Infusion of 0.7 pmol/min into the brachial artery significantly increases the forearm blood flow (145). This increase is immediate and sustained during the whole infusion period, but the basal flow level is reached again within seconds after the infusion is stopped. The oxygen saturation increases in both cutaneous and muscle blood of the forearm, indicating that SP dilates blood vessels of both the skin and muscle. Intravenous infusions elicit a bright red flushing of the skin, particularly in the head and the neck. The subjects experience a feeling of warmth in the head and temporal pulsations. The tolerance towards SP varies between subjects, but on an average these reactions are obtained at an infusion rate of 70 pmol/min. At these doses a tachycardia and decrease in arterial blood pressure are recorded. Intravenous infusions of more than 80 pmol significantly increase forearm blood flow and venous tone (145), while no effect can be recorded in internal carotid blood flow (561). The capacitance vessels are, however, not affected by arterial infusions of SP (145).

Intradermal injections of SP (10^{-7} to 10^{-5} M) in man induces flare, wheal, and itching, which is similar to the responses to histamine (225). Oral pretreatment with antihistamine (chlorocyclizine) inhibits the effect of SP. After depletion of the local stores of the mast cell bound histamine with compond 48/80, SP is without effect in the skin. When incubated with rat peritoneal mast cells, SP induces histamine release at a concentration of 10^{-5} M (225). This finding is in agreement with observations that SP is a more potent histamine liberator than most other vasoactive peptides (319, 153) and that the vasodilatation induced by SP in the rat hind paw is inhibited to about 50% by antihistamines or compound 48/80 (397). At least some of the vascular effects of SP are, therefore, probably mediated by a release of histamine from the mast cells.

SP has no chronotropic or inotropic effects on the isolated heart of the rabbit, guinea-pig (51), or dog (416). In the intact dog, intravenous SP in doses higher than 2 pmol $\times$ kg^{-1} $\times$ min^{-1} increase in a dose-dependent way cardiac output and stroke volume (51, 520). These effects seem to be entirely due to a decrease in peripheral resistance.

The effects of SP on blood flow and blood pressure are generally not influenced by atropine, alpha- or beta-adrenergic blocking agents, or antihistamines (518, 51, 145, 505). This indicates that the vascular effects of SP generally are not due to cholinergic, adrenergic, or histaminergic receptor stimulation but to a direct effect on the smooth muscle of the vascular wall. There are, however, obvious regional differences. Thus, the vasodilatation induced by SP in the hypothalamus is completely abolished by cholinoreceptor blockade with atropine or mecamylamine. Similarly, blockade of alpha-adrenoceptors by phenoxybenzamine or beta-adrenoceptors by propranolol or destruction of adrenergic hypothalamic nerves with 6-hydroxydopamine completely inhibits SP-induced vasodilatation (347). These data suggest that both cholinergic and adrenergic mechanisms might be involved in the vascular effects of SP in the hypothalamus.

Indomethacin, in a dose sufficient to block the synthesis of prostaglandins, does not influence the SP-induced increase of human forearm blood flow (145) or relaxation of isolated, contracted blood vessels (91). In the anaesthetized dog, on the other hand, indomethacin or meclofenamate reduces the magnitude or shortens the duration of the vasodepressor response to SP (505). These results indicate that in certain conditions or species prostaglandins might participate in the vascular effects of SP. However, the SP-induced vasodilatation and plasma extravasation in the rat hind paw are not affected by indomethacin (397, 388).

2. Effects of Central Administration. Injection of SP into the lateral brain ventricle in the conscious, normotensive rat causes a dose-dependent increase in mean arterial blood pressure and decrease in heart rate (628). These effects are considerably enhanced after sino-aortic baroceptor denervation. In the urethane-anaesthetized rat, injection of SP into the lateral brain ventricle causes an increase in both blood pressure and heart rate (224). The central pressor action of SP is markedly attenuated by intracerebroventricular pretreatment with the gamma-aminobutyric acid derivate baclofen (628). SP injected into the lateral brain ventricle, furthermore,

increases plasma levels of adrenaline and noradrenaline. Peripheral alpha-adrenoceptor blockade by prazosin reverses the central pressor effects of SP to depressor responses (627), which indicates that these effects are mediated by the sympathetic nervous system.

Infusion of the SP receptor antagonist, [D-Pro^2, D-Trp^{7,9}]SP, into the subarachnoid space of the rat causes a fall in blood pressure to levels resembling those after cervical spinal cord transection (413). This antagonist also blocks the increase in blood pressure and heart rate after local application of kainic acid (413). These findings suggest that SP neurons in the vasomotor center of the ventral medulla (209, 244) may play a role in maintaining vasomotor tone (413).

Spontaneously hypertensive rats show a supersensitivity to centrally administered SP with a 2- to 3-fold increase in blood pressure as compared to the normotensive rat. In addition, SP induces tachycardia in the conscious hypertensive rat (628).

The occurrence of SP in the baro- and chemoreceptor afferent fibers (420, 244) and in the nucleus tractus solitarii (103, 411, 241) has initiated pharmacological studies aimed at elucidating the effect and putative role of SP at the first synapse of the baroreceptor reflex. Intracisternal application of SP causes an elevation of the arterial blood pressure (183, 214), which is counteracted by [D-Pro^2, D-Phe^7, D-Trp^9]SP (182). On the other hand, a decrease in spontaneous sympathetic nervous activity, blood pressure, and heart rate is observed after local microinjection of SP in the nucleus tractus solitarii (224).

Local application of capsaicin to the nucleus tractus solitarii produces circulatory effects which are indistinguishable from those of SP. Since capsaicin releases SP, these results have been suggested to indicate a transmitter or neuromodulator role for SP at the first synapse of the baroceptor reflex (224). However, Talman and Reis (611) were unable to observe any effect of SP, microinjected into the nucleus solitarii, on arterial pressure, heart rate, or baroreceptor reflex. They concluded that previously reported cardiovascular effects of local application of SP in this nucleus probably is a consequence of local distortion. Further studies are thus needed to evaluate the functional significance of the presence of SP in the baro- and chemoreceptor afferent fibers.

C. SP and Antidromic Vasodilatation

A series of recent observations have focused interest towards the tentative role of SP as a mediator of antidromic vasodilatation. This phenomenon has been known since 1876 when Stricker showed that stimulation of the peripheral part of a transected dorsal root or the distal end of a cut cutaneous sensory nerve causes arteriolar dilatation (601). Jancsó et al. (304) found that, in addition to vasodilatation, electrical antidromic stimulation of sensory nerves also increases vascular permeability with plasma exudation. The nature of the vasodilator fibers was characterized by Hinsey and Gasser (252), who showed that the increase in blood flows occurs when the C fibers in sensory neurons are stimulated. Later, Celander and Folkow (68) found that the axon reflex taking place in the end branchings of sensory nerves and responsible for the flare component in the triple response of human skin is mediated by pain fibers.

It was already postulated in 1927 by Lewis and Marvin (404) that the antidromic vasodilatation is mediated by a release of neurotransmitter at the sensory nerve endings in the skin. This hypothesis was convincingly supported by Jancsó et al. (304), who showed that the mediator responsible for the extravasation elicited by antidromic stimulation is released from pain sensitive nerve terminals. Since then, many compounds have been suggested in this connection including acetylcholine, adenosine, adrenaline, noradrenaline, adenopine triphosphate, bradykinin, histamine, 5-HT, and prostaglandins. However, since the antidromic vasodilatation and extravasation is not completely blocked by atropine, antihistamines, compound 48/80, adrenergic blocking agents, methysergide, or indomethacin, most of these compounds can be ruled out as the substance released and alone responsible for the mediation of the vascular effects.

It is evident from the above-mentioned data that a compound that should be discussed as a mediator of antidromic vasodilatation must fulfill certain criteria. It has to be present in the C fibers of the sensory neurons, released from peripheral nerve terminals, and possess a high potency as a vasodilator. SP undoubtedly fulfills these criteria. SP is present in unmyelinated primary sensory neurons terminating in the skin (263, 97). A release of SP has been demonstrated from peripheral endings of sensory neurons during antidromic stimulation (28, 490). So far it has, however, not been possible to demonstrate any release of SP in the skin response to antidromic stimulation (52), which is probably due to the very low content of SP in the skin. The vasodilatation induced concomitantly with the release of SP in the dental pulp (490) and the eye (28) can be mimicked by close-arterial administration of SP in concentrations similar to those provided by release from peripheral nerve endings. Similarly, a vasodilatation in the skin is produced by intra-arterial infusion of SP in a dose as low as 0.1 pmol/min (397). The vasodilation in the dental pulp and the oral mucosa, induced both by stimulation of the inferior alveolar nerve and by close-arterial SP infusion, is blocked by [D-Pro^2, D-Phe^7, D-Trp^9]SP and [D-Pro^2, D-Trp^{7,9}]SP (549).

Capsaicin, which causes a substantial reduction of the SP content in those areas of the neuron system where primary afferent fibers are located (313, 197), almost completely abolishes the antidromic vasodilatation (397). Similarly, neonatal capsaicin pretreatment is followed by a complete impairment of neurogenic plasma extravasation (305), which correlates with a decreased SP content of the skin and sensory nerves (197). These

results indicate that the SP-containing pain fibers, which are sensitive to capsaicin, are responsible for the vasodilatation and plasma extravasation induced by antidromic stimulation of peripheral sensory neurons.

The vasodilatation induced by the antidromic stimulation as well as by local administration of SP is partly inhibited by antihistamines and compound 48/80 but not by atropine or indomethacin (397). This finding both confirms earlier observations that antidromic vasodilatation involves a release of histamine from the mast cells (345) and indicates that SP, being released during antidromic stimulation, partly acts by a secondary release of histamine, partly directly on the vascular smooth muscle.

In conclusion, the following data strongly support the assumption that SP is involved in the mediation of antidromic vasodilatation: 1) SP is probably associated with pain sensory fibers, which are actively involved in antidromic vasodilatation and the axon reflex mechanism; 2) SP is released from peripheral endings of sensory neurons during antidromic stimulation; 3) close-arterial administration of SP causes vasodilatation and plasma extravasation thus mimicking the effect of antidromic stimulation; 4) capsaicin depletes SP from chemosensitive primary afferents and blocks antidromic vasodilatation and plasma extravasation; 5) specific SP receptor antagonists inhibit antidromic vasodilatation.

The classical explanation of the axon reflex is that a noxious stimulus generates an afferent impulse, which when reaching a point of ramification, travels antidromically to induce a vasodilatation. According to Lembeck and Gamse (395), the sensory terminal fiber is excited by noxious stimulus over a certain length. The stimulus initiates an orthodromic impulse causing release of SP in the dorsal horn, but also an antidromic impulse causing both vasodilatation and histamine release from the mast cells. Histamine diffuses into the tissue and stimulates both the blood vessels in the neighborhood and new sensory fibers to send orthodromic and antidromic impulses causing a further SP release (395).

However, doubts have recently been cast on the suggestion of an involvement of SP in the antidromic vasodilatation (70). This was based on the finding that acid-acetone extracts of spinal cord possess a much higher cutaneous oedema-inducing activity than can be attributed to their content of SP. This effect was due to an agent in the molecular size range for peptides but not identical to SP. Capsaicin reduces not only SP but also this uncharacterized agent. This still very inconclusive observation, which confirms earlier findings (401), does not rule out SP as a tentative mediator of antidromic vasodilatation.

SP has also recently been discussed in relation to the cutaneous vasodilatation following arterial occlusion ("*reactive hyperemia*"). Thus Lembeck and Donnerer (388) showed that this type of the vasodilatation does not occur in the rat hind paw after sciatic and saphenous denervation and is almost abolished by capsaicin pretreatment. They postulated that the postocclusive cutaneous vasodilatation is caused mainly by a release of SP from peripheral endings of small diameter nerve fibers. This tentative effect of SP probably also involves a release of histamine, since the reactive hyperemia is reduced after histamine depletion with compound 48/80 (388). So far, evidence is only given for a mediation of reactive hyperemia by SP in the skin and it is very likely that this type of hyperemia has different mechanisms in other tissues.

When using capsaicin as a tool in studies on the physiological role of SP it should be kept in mind that not only SP, but other peptides such as vasoactive intestinal polypeptide and cholecystokinin, both present in primary sensory neurons (421, 266), are also depleted in the spinal cord by capsaicin pretreatment (303). This finding indicates that the mode of action of capsaicin is complex. However, of the peptides influenced by capsaicin, only vasoactive intestinal polypeptide causes vasodilatation, but its potency is about 500 times less than that of SP (397). Cholecystokinin is devoid of any vasodilator action (397). This difference in potency strengthens the opinion of SP as an important mediator of antidromic vasodilatation.

Sir Henry Dale suggested in his famous Dixon lecture in 1935 that the same substance may be released from the sensory neuron at the central synapse after orthodromic stimulation and at the periphery after antidromic stimulation. And he added a question: "When we are dealing with two different endings of the same sensory neuron, the one peripheral and concerned with vasodilatation and the other at a central synapse, can we suppose that the discovery and identification of a chemical transmitter of axon-reflex vasodilatation would furnish a hint as to the nature of the transmission process at the central synapse? The possibility has at least some value as a stimulus to further experiment" (107). We now know that SP is released both at the central and distal terminals of the primary sensory neuron. In the spinal cord SP has a pronounced depolarizing effect on certain units, while in peripheral tissues SP is a potent vasodilator. Therefore SP entirely fulfills the criteria outlined by Sir Henry Dale for a compound mediating sensory impulses.

XI. Respiratory Tract

A. Distribution

The occurrence of SP immunoreactivity in the respiratory organs was first described by Nilsson et al. (475), who identified SP nerve fibers in the smooth muscle, connective tissue, and epithelium of the trachea and bronchi of the guinea-pig. A detailed mapping of the distribution of SP immunoreactive nerve fibers in the airways of various species including man has recently been presented (419). SP nerves are found under and within the respiratory epithelium and around blood ves-

sels of the nasal mucosa. Sectioning of the maxillary portion of the trigeminal nerve in cats results in a loss of these nerves. Also, after systemic capsaicin pretreatment of rats and guinea-pigs an almost total loss of SP nerves are seen in the nasal mucosa (428).

Varicose SP nerve fibers are also present close to the respiratory epithelium lining of the epiglottis, larynx, trachea, bronchi, and bronchioli of rat and guinea-pig (419). Several SP fibers are also present in the smooth muscle layers of these tissues and can be followed into the mucosa. Studies of the SP immunoreactivity after chronic vagotomy indicate that the SP innervation of the trachea and stem bronchi originates from the right vagal nerve, while the vagal SP nerves in the lung originate from both sides. Systemic capsaicin pretreatment causes a disappearance of SP nerves in the lower respiratory tract. Following bilateral infranodose capsaicin application on the vagal nerves, the majority of SP nerves disappear in the trachea and stem bronchi, while about 50% of the SP nerves remain in the lungs. This indicates an innervation of the lungs also by nonvagal SP nerves, probably originating from spinal ganglia (419).

B. Pharmacological Effects

In the isolated guinea-pig trachea, SP in 7 to 15 nM concentrations induces an increase in muscular tone. This effect is not influenced by atropine, adrenergic blocking agents, antihistamine, or 5-HT antagonists (475). Also, intravenous injections of SP induce a dose-dependent increase in muscle tone recorded as an elevation in insufflation pressure. A 100% increase in this pressure is obtained with 0.4 nmol of SP or 18 nmol of histamine (475, 424). Intravenous injections of SP (10 nmol/kg) induce extravasation of Evans blue in a variety of tissues, including the epiglottis and larynx. This response to SP is not blocked by mepyramine or cimetidine (424).

C. SP and Airway Hyperreactivity

Recent observations strongly suggest that SP afferents are involved in airway oedema and bronchoconstriction induced by vagal stimulation or local mechanical or chemical irritation.

Antidromic stimulation of the trigeminal nerve induces an atropine- and hexamethonium-resistant vasodilatation in the cat nasal mucosa (428). Also, electrical stimulation of the cervical vagus nerve as well as chemical irritants such as ether and capsaicin causes a subepithelial oedema and rapid increase in insufflation pressure in the rat trachea and bronchial tree (424, 425). These effects are absent in animals pretreated with capsaicin, which causes a loss of vagal sensory SP neurons in the smooth muscle layer of the respiratory tract (419). Furthermore, both vagal stimulation and capsaicin significantly reduce the total amount of SP in the bronchi (419). In addition, the oedema in the lower respiratory tract and the noncholinergic bronchoconstriction induced by antidromic vagus stimulation are abolished by the SP receptor antagonist, [D-Arg^1, D-Pro^2, D-Trp^{7,9}, Leu^{11}]SP (426). These findings clearly suggest that the pulmonary effects elicited by vagal stimulation and capsaicin are due to a local release of SP. They also show that capsaicin is able to induce a desensitization of the airway mucosa to various forms of irritants (425). It is tempting to speculate on the basis of these observations that SP fibers may be involved in the local regulation of airway resistance. They, furthermore, give new aspects on the pathophysiology of airway hyperreactivity.

XII. Secretion

A. Salivary Glands

SP is a potent stimulator of salivation in the dog, rat, guinea-pig, hamster, and hen but not in the cat or rabbit. The first report on the sialogogic effect was given by Vogler et al. (637), who observed that intravenous injections of a highly purified SP preparation induces a profuse salivation. "Copious amounts of watery secretion dropped from nose and mouth for about half a minute" (637). This effect has since been repeatedly confirmed in experiments both in vivo and in vitro (384, 402, 550, 405, 619, 440, 369, 192, 44, 146). Systemic administration of SP in doses higher than 0.07 pmol/kg in the rat starts a secretory response within a few minutes, but the secretion decreases again even during continuous infusion. The effect is dose-dependent and a 50-fold increase in salivary flow has been observed (405). The salivary effect appears to be a direct action of SP on the glands as it is not blocked in vivo by atropine, propranolol, or phenoxybenzamine (384). Parasympathetic denervation of the parotid gland and decentralization of the submaxillary gland in the rat causes a marked sensitization to SP as judged by lower threshold doses and increased secretory responses to submaximal doses (146). Simultaneously, a change in electrolyte composition occurs; the sodium concentration increases while potassium decreases concurrently with the increase in SP-induced salivary flow. A decrease in calcium and protein concentration is observed at the start of SP infusion, soon reaching a low plateau level (440).

The spontaneous release of amylase from parotid gland slices (550, 619, 44) or isolated parotid cells (405) is also stimulated in a dose-dependent way by SP. In both types of preparations the SP stimulation of amylase release is apparent within 1 minute and lasts for at least 40 minutes. The effect is partly calcium-dependent and can be inhibited by 50% upon removal of extracellular calcium (44). The effects are not blocked by adrenergic or cholinergic antagonists (550). The amylase-releasing effect of SP is obtained with all C-terminal fragments of SP even if the undecapeptide is more potent. The C-terminal hexapeptide of SP is the minimum structure which possesses biological activity (44).

SP also causes a rapid and transient release of potas-

sium from rat parotid slices (179). On a molar basis, SP is in this respect much more effective than adrenaline or carbamylcholine. Incubation of the tissue slices with the ATPase inhibitor, oubain, significantly enhances the release of potassium. The response to SP is apparently mediated via a specific receptor since none of the antagonists to adrenergic or cholinergic receptors inhibits the SP-reduced release of potassium (179).

The secretory response of the submandibular gland of the rat to acetylcholine and isoproterenol is markedly inhibited by infusion of SP (440). The mechanism behind this inhibition is not fully understood, but since SP does not modify the binding of specific ligands to glandular autonomic receptors, a postreceptor interaction has been suggested (440). The observations that the pattern of salivary secretion induced by SP is similar to that after autonomic stimulation and that SP is able to influence the effect of parasympathetic and adrenergic agents seem to suggest that SP has a physiological role in the regulation of salivary secretion by modulating the effect of autonomic neurotransmitters (440).

Iontophoretic application of SP evokes changes in the membrane potential as recorded from intracellular microelectrodes inserted into acinar cells of the mouse and rat parotid gland (191, 192). The responses, which are associated with a marked decrease in input resistance, are identical to those evoked by local application of acetylcholine or adrenaline. In the mouse, the effects of both SP and acetylcholine are abolished by atropine. In the rat, however, the parotid acinar cell responses to iontophoretic or superfused SP are resistant to atropine as well as to phentolamine or propranolol, although the latency of the response to SP is greater after autonomic blockade (192). Other peptides, such as caerulein and bombesin, do not evoke any response, indicating a high degree of specificity of the peptide receptors (191).

A conjugate of SP and ^{125}I-labelled Bolton-Hunter reagent binds with a very high affinity to the parotid cell membrane (406). The number of binding sites and specificity obtained with this technique is higher than that reported with ^{125}I- or ^{3}H-SP (466, 229, 578). The demonstration that SP, acetylcholine, and adrenaline have identical actions in the parotid acinar cell membrane might suggest activation of a common effector mechanism, although mediated by separate receptors. This hypothesis is supported by the observation that the amylase-releasing response to SP is not additive to those of acetylcholine or noradrenaline (44). It, therefore, seems likely that in the parotid gland, release of amylase and efflux of potassium ions can be provoked by stimulation of separate alpha- and beta-adrenergic receptors, muscarinic cholinergic receptors, and peptide (SP) receptors, acting via a common ion channel mechanism (536, 191). Although it is not known in detail how activation of these receptors leads to control of the cell responses, there are reasons to believe that calcium is their intracellular second messenger. This assumption is in conformity with the finding by Putney (536) that carbachol, phenylephrine, and SP each are capable of producing a release of potassium and secretion of amylase from rat parotid slices and that the presence of one of these agonists renders the tissue incapable of a subsequent response to either of the other two. It was further shown, that the potassium release response to all compounds is mediated by a receptor-controlled calcium influx, which suggests that the adrenergic, muscarinic, and peptide receptors in the salivary gland regulate the same calcium influx sites (536).

Some observations seem to indicate that SP, besides directly activating specific receptors, also interacts with intrinsic parasympathetic nerve fibers to release acetylcholine. A SP-induced release of endogenous acetylcholine has earlier been reported in both the brain and the gut (512). Similarly, Gallacher and Petersen (191) found that the effects of SP on mouse acinar cells are not blocked by hexamethonium or tetrodotoxin, which suggests that SP is acting at parasympathetic nerve endings to release acetylcholine. This is in conformity with the observation that SP is both structurally and functionally related to the chorda tympani and possibly involved in the parasympathetic innervation of the submaxillary gland of the rat (544).

B. Pancreatic Secretion

SP stimulates exocrine pancreatic secretion both in vivo and in vitro. This was first shown by Starke et al. (587) with crude SP extracts and has later been confirmed and extended with synthetic SP (618, 3, 355). Close-arterial infusion of SP in a dosage of 15 pmol $\times$ kg^{-1} $\times$ min^{-1} increases the output of pancreatic juice by 160% in the dog (618). When given as intravenous infusion in the fasted dog, 1 nmol SP $\times$ kg^{-1} $\times$ hour^{-1} causes a slight but significant increase in pancreatic secretion (355). Pancreatic bicarbonate, amylase, and protein outputs likewise increase, mainly as a result of the rise in total pancreatic outflow (618, 355). SP appears to stimulate amylase secretion from pancreatic acinar cells by activation of calcium outflux and accumulation of cyclic guanosine monophosphate (cGMP) (3, 579), which indicates that the secretagogue effect of SP in the exocrine pancreas is mediated through activation of guanylate cyclase. However, when SP is given during a period when pancreatic secretion is stimulated by feeding, duodenal acidification, secretin, or caerulein, SP significantly inhibits the secretion (355).

SP also affects the endocrine pancreatic secretion. Both in vitro (142) and in vivo (45, 429) studies have shown that SP inhibits the release of insulin induced by glucose and arginine, as well as the basal insulin plasma level. Large doses of SP inhibit the in vitro release of glucagon from the perfused rat pancreas (142), whereas hyperglucagonemia and hyperglycemia are observed in vivo after intravenous administration of SP to the rat (45). Also, close-arterial infusions of SP to the pancreas

increase plasma concentration of glucagon and, in contrast to intravenous administration, also of insulin (328). Although this result might suggest that the glucagon and insulin release is stimulated by endogenous SP, caution should be exercised when interpreting these results since the action of SP and other peptides evidently depend on the nature of the stimulus.

C. Choleresis

Caval infusion of SP in doses higher than 0.4 pmol × kg^{-1} × min^{-1} reduces basal hepatic bile output as well as biliary excretion of electrolytes and amylase in the anaesthetized dog (274, 430). The decrease in choleresis, however, never exceeds 50% even with high doses of SP (15 pmol × kg^{-1} × min^{-1}). The anticholeretic effect occurs within 2 minutes after the start of infusion. After termination of the SP infusion, bile flow gradually increases to levels which are higher than in the basal state. This postinfusion choleretic effect has a duration of about 10 minutes (274). Also the bile output of sodium, potassium, and chloride decreases after SP infusions at rates higher than 2 pmol × kg^{-1} × min^{-1}. Output of bile acids and biliary concentration of electrolytes is, however, unaffected (431).

SP also effectively blocks the choleresis induced by cholecystokinin and vasoactive intestinal polypeptide (430). Cholecystokinin in a dosage of 0.3 Ivy units × kg^{-1} × min^{-1} in the dog increases the bile output by 90%. When an infusion of 15 pmol SP × kg^{-1} × min^{-1} is superimposed, bile flow returns to the control level. Simultaneously, bile content of sodium, potassium, and amylase, also increased by cholecystokinin and vasoactive intestinal polypeptide, is normalized by SP.

Besides somatostatin, SP is the only peptide known to have an anticholeretic effect. The mode of action of this effect is not quite clear. Obviously, different mechanisms are involved in bile formation. A bile acid dependent bile secretion occurs at the canalicular level. Bile is also secreted under the influence of peptide hormones both at the canalicular level under the influence of cholecystokinin and at the ductural level under the influence by secretin. The inhibiting effect of SP on the cholecystokinin-stimulated choleresis seems to indicate that SP affects the bile acid independent canalicular bile fraction (432).

XIII. Kidney Function and Water Homeostasis

SP is one of the most potent natriuretic and diuretic compounds so far described. Infusion of 0.4 pmol × kg^{-1} × min^{-1} or higher into the renal artery of the dog results in a dose-related increase in renal blood flow, urine volume, sodium and potassium excretion, and a decrease in urinary osmolality, while glomerular filtration remains unchanged. At high doses (40 pmol × kg^{-1} × min^{-1}) glomerular filtration rate even decreases (455, 218, 219). In the rat, infusion of 0.05 pmol/min in the aorta above the renal arteries produces an increase in urine flow and urinary sodium excretion (10). Proximal tubular reabsorption is reduced while glomerular filtration rate, renal plasma flow, or intrarenal hydrostatic pressures are not affected (10). The effective dose of SP is 0.04 to 0.08 pmol/ml in the dog (455) and rat (10), which means that on a molar basis SP is about 100 times more potent than bradykinin as regards increases in urine flow and sodium excretion (217). Similarly, the threshold dose of SP is less and its maximal effect greater than those of the prostaglandins E_1 and E_2 (455). In contrast, intravenous infusion of SP in low doses (0.04 to 4 pmol × kg^{-1} × min^{-1}) does not influence renal function while higher doses (40 pmol × kg^{-1} × min^{-1}) reduce glomular filtration rate, diuresis, and excretion of sodium and potassium simultaneously with a decrease in renal blood flow in the dog (218). In the rat, intravenous injections of 20 pmol of SP causes a transient decrease in urine flow and increase in urine conductance (625). This difference in the efficacy of intra-arterial and intravenous SP is likely to result from the rapid removal of SP from the circulation (see ref. 398).

These observations seem to indicate a possible role of SP in the regulation of water metabolism, evidently by both renal and central mechanisms. Thus, infusion of SP in low dose (0.4 pmol × kg^{-1} × min^{-1}) into the artery of anaesthetized dogs causes a dose-related increase in urinary cyclic adenosine monophosphate (cAMP) excretion simultaneously with a decrease in free water clearance (219). The increase in cAMP is not obtained at intravenous administration of SP, which seems to indicate a kidney origin of the increase in urinary cAMP following local administration of SP.

Intravenous infusions of SP significantly stimulate the release of antidiuretic hormone (ADH) in doses that decrease the urine flow but fail to alter blood pressure, plasma osmolality, or urinary excretion of cAMP (219). Intrarenal infusions of SP do not change plasma ADH levels. Taken together these findings suggest that SP may exert its effects on water reabsorption by an adenyl cyclase-cAMP mechanism similar to ADH (219).

XIV. SP in Blood

SP-like activity has been detected by radioimmunoassay in blood plasma from man and various animals. The following plasma levels have been reported: in man 70 to 300 pmol/l (478), 50 to 620 pmol/l (654), and 30 pmol/l (581); in the dog 40 to 150 pmol/l (478); in the rat 425 pmol/l for unextracted and 9 pmol/l for acid acetone extracted plasma (383); in the calf 165 and 18 pmol/l for unextracted and extracted plasma, respectively (383); and in the cat 3 to 165 pmol/l (198). The range is large in most materials which, together with the differences in values obtained by various authors, indicates that probably nonspecific factors are interfering with the assay of immunoreactive SP. This is particularly true when unextracted plasma is used (383). Furthermore, there is evidence indicating that endogenous circulatory SP is in a

molecular form different from the synthetic undecapeptide. The observation that synthetic SP added to plasma is well absorbed on charcoal while very little of endogenous SP is absorbed (63), might speak in favor of this suggestion. SP produced and secreted in the body probably becomes associated with large proteins in blood, which protects it from rapid degradation.

In healthy men, the SP levels in plasma appear to vary during sleep, ranging from undetectable to 430 pmol/l with a mean of 77 pmol/l (581, 532, 533). The first peak appears about 1 hour after sleep onset and subsequent peaks follow at about 1 ½ hour intervals. Plasma SP level is not related to rapid eye movement (REM) or non-REM sleep phases. It coincides with plasma prolactin peaks, but is not correlated to growth hormone, adrenocorticotrophin, and cortisol. Plasma SP level increases in the conscious dog in response to a high-protein meal (301). A considerable increase in plasma SP is obtained also by bombesin. Atropine pretreatment both lowers basal SP level and inhibits the increase induced by meal and bombesin (301). This finding might indicate that the release of SP is modulated by cholinergic mechanisms.

High SP plasma levels have been reported in patients with certain malignancies. In metastatic carcinoid tumours of the ileum, SP concentration of peripheral venous blood is substantially increased (581, 227). The primary tumour in the gut and its metastases also contain large amounts of SP (227). The plasma SP is raised even when the urinary 5-HT metabolite, 5-hydroxyindolacetic acid, is normal (533). The finding of elevated SP levels in the carcinoid syndrome is interesting in view of the fact that SP can elicit all the main symptoms of this syndrome such as flushing, tachycardia, increased intestinal motility, and bronchoconstriction. Also, the medullary carcinoma of the thyroid, a tumour related to the carcinoid, is associated with an increase in plasma SP (533).

The major part of circulating SP evidently originates from the intestine. Thus, ligation of all intestinal blood vessels as well as evisceration in the cat significantly lowers SP plasma level (198). The fact that portal venous blood normally contains about four times more SP than peripheral blood speaks in favour of this assumption. Other sources of circulating SP might be the nervous system. The cerebrospinal fluid contains significant concentrations of SP (612) but to what extent SP from the central nervous system reaches circulation is unclear. A release of SP also occurs from peripheral sensory nerves (490, 28), but the released compound is subjected to a rapid local inactivation (204) and is, therefore, probably not contributing to the circulating SP.

The physiological significance of SP in blood plasma is not known. The amounts normally present are enough to elicit circulatory effects, but much higher doses than the endogenous plasma levels are needed to stimulate the intestinal motility or salivary and pancreatic secretion. So far no evidence has been given for a role of SP as a circulating hormone and it might be possible that SP in blood is simply a spill-over from the intestine of SP locally released but not destroyed (198).

XV. Storage and Binding

Several early studies were devoted to the storage and binding sites of SP in neuronal tissues. Von Euler (163, 165) showed that about 50% of the total SP in peripheral nerves is present in granular fractions. In brain homogenates a considerable proportion of SP is found in microsomal fractions (297, 552). The synaptosomal localization of SP has later been established by RIA (567) and immunohistochemistry (76, 259, 523, 14). [^{3}H]SP and ^{125}I[Tyr]SP bind with high affinity and reversibility to synaptic membrane fractions from rabbit and rat brain (466, 445, 229). The distribution of such binding within the central nervous system generally correlates with the content of SP immunoreactivity, being high in the hypothalamus, midbrain, and striatum and low in the cortex and cerebellum (466, 229, 563). The binding is specific for SP, shorter fragments of SP, and structurally related peptides such as eledoisin and physalaemin, whereas a variety of other peptides as well as amines and amino acids do not react (229).

The binding sites of SP in synaptic vesicles are extractable with ether and chloroform (445). Furthermore, the binding is reduced by phospholipases but resistant to proteolytic enzymes. These and other observations suggest that the specific SP binding sites in membrane fractions are phospholipids (399, 468). Phosphatidyl serine binds SP (399), which is of interest since this lipid is a constituent of synaptic membranes and vesicles. The number of binding sites for ^{125}I[Tyr^{8}]SP in synaptic vesicles in dorsal roots and spinal cord of the rat is significantly reduced by capsaicin pretreatment (443). A specific and reversible binding of labelled SP has also been demonstrated in the pancreas, where SP binds to acinar cells (578). This binding is much more efficient at low temperatures than at 37°C, probably due to enzymatic degradation of SP in the acinar cells at body temperature. As in the brain, the SP binding sites in the pancreas also bind eledoisin and physalaemin. SP, eledoisin, and physalaemin inhibit the binding of ^{125}I[Tyre^{8}]SP and ^{125}I-physalaemin (308, 578) and the concentration range in which these native peptides inhibit binding of ^{125}I[Tyr^{8}]SP correlates well with the range in which they stimulate biochemical processes such as release of amylase and outflux of calcium (578, 579). There are reasons to suggest at least two functionally distinct types of binding sites for SP in the pancreas—a small number with a high affinity and a larger number of sites with low affinity (579).

Several observations indicate that there probably exist multiple receptors for SP. The specific SP antagonist, [D-Pro^{2}, D-Trp^{7,9}]SP, effectively blocks the spasmogenic and sialogogic actions of SP without affecting the hypo-

tensive effect (30). Different rank orders of potencies have been found among SP and the related tachykinins including their biologically active fragments and synthetic analogues (24, 229, 380, 613). Thus, while the potency of (*p*-hydroxyphenylacetyl)-SP_{7-11} is > SP > [Lys^6, He^8]SP_{6-11} = SP_{6-11} > SP_{7-11} in the guinea-pig ileum, the corresponding rank order of potencies in the rat colon is: [Lys^6, He^8]SP_{6-11} = SP_{6-11} = (*p*-hydroxyphenyl-acetyl)-SP_{7-11} > SP > SP_{7-11} (24). Also, while most C-terminal fragments of SP are equipotent to SP as hypotensive compounds, they are about fives times weaker than SP as sialogogues in vivo (229). Furthermore, the relative potency of the various SP fragments as well as of SP and structurally related tachykinins in competing for [^{3}H]SP binding to rat brain membrane preparations in vitro (229) is different from the corresponding potency found in other test systems (380). Thus, the relative potencies of the tachykinins on the rat vas deferens is in the rank order: kassinin > eledoisin > phyllomedusin > SP = physalaemin; whereas in the receptor binding assay the rank order is: physalaemin > SP > phyllomedusin > eledoisin > kassinin (380).

SP increases adenylate cyclase in the brain (130) and intracellular cGMP level in pancreatic lobules (3). In the parotid gland, on the other hand, SP stimulates secretion without altering cyclic nucleotide levels (550). In parotid slices eledoisin is equipotent with SP in eliciting amylase release, but about 0.02 as potent in producing the same response from pancreatic acinar cells (308).

All these findings seem inconsistent with one SP receptor, but in conformance with the existence of multiple SP receptors. They, furthermore, seem to indicate that the distribution of these receptors varies between different organs, which opens up possibilities to design drugs specific for certain SP-related physiological events.

XVI. Degradation

Several organs have a high capacity to inactivate SP (216, 11, 136, 515, 641, 22). Kidney homogenates seem to be most effective followed by the spleen, liver, and intestine (136, 641). In the kidney, the proximal tubules inactivate SP more than 10 times as fast as the glomeruli, most of the degrading activity being bound to the plasma membranes of the microsomes (641). In the gastrointestinal tract, the inactivating capacity is well correlated to the distribution of SP with the highest proteolytic activity in homogenates of the duodenum and ileum (515).

The capacity of various organs to inactivate SP has further been studied in vivo by analyses of the elimination of SP from the circulation in various vascular beds. By using the rats salivary response as a bioassay, Lembeck et al. (398) showed that the highest degree of elimination of SP occurs in the liver and hind limbs, followed by the kidney. More than 80% of SP infused into the portal vein is degraded by the liver (228, 398, 449). In the kidney in vivo, systemically administered SP is rapidly and completely metabolized, probably by both vascular and tubular elements (61). During intrarenal infusion of ^{125}I[Tyr^8]SP, high levels of radioactive material are found in the urine and renal venous plasma (61). No significant elimination of SP occurs in the lungs (33, 398) or during passage through the cerebral blood vessels (398).

While endogenous SP is stable in plasma, synthetic SP added to human plasma is rapidly destroyed (581, 398, 22, 92). The degradation is temperature-dependent with a much larger loss of activity at 37°C than at 0°C (398). The C-terminal octapeptide (SP_{4-11}) is inactivated at approximately the same rate as SP, at 37°C the half-life in rat plasma being 12 to 15 minutes, while the penta- and hexapeptides are degraded more slowly (92).

The inactivation of SP in various tissues, including blood, is prevented by enzyme inhibitors such as EDTA (56), teprotide (SQ 20881) (378, 533), captopril (SQ 14225) (92), and bacitracin (398). The observation that degradation of SP is inhibited by the nonapeptide SQ 20881 both in plasma (64) and in the brain (378) is of particular interest. This compound has a hypotensive effect, which previously was fully ascribed to a specific inhibition of the angiotensin-converting enzyme, but which also may be due to its capacity to inhibit the degradation of vasodilator peptides such as SP.

Tissue cultures of endothelial cells from human umbilical cord inactivate SP (320), especially if they are suspended or lysed. The degradation is blocked by various enzyme inhibitors such as Trasylol as well as by serine protease and trypsin inhibitors (320). This cleavage of SP by an enzyme bound to the surface of endothelial cells probably contributes to the instantaneous return of the blood flow to its basal level when an infusion of SP is stopped.

Both cytosolic (22, 378) and membrane-bound (330, 22, 31, 381) peptidases capable of degrading SP have been extracted from the brain. No doubt a membrane-bound enzyme is more likely to regulate the SP level at the synapses. In 1975, Benuck and Marks (21) described a partly purified enzyme (neutral endopeptidase) from rat brain, which inactivated SP as shown by bioassay. Two fragments of the undecapeptide were defined, one being Glu^6-Phe^7 or Phe^7-Phe^8 and the other Gly^9-Leu^{10}. The degradation of SP by neutral metalloendopeptidase systems of synaptosomal fractions of the rat brain was later confirmed (22). The basic N-terminal sequence of the SP peptide was found to be important for this inactivation. Similarly, Lee et al. (381) identified and purified a neutral metalloendopeptidase from a membrane fraction of the human brain. This enzyme has a high specificity and affinity for SP, which is cleaved at the bonds Gln^6-Phe^7, Phe^7-Phe^8, and Phe^8-Gly^9. Any of these cleavages leads directly to inactivation of SP. The subcellular distribution of this membrane-bound SP-degrading enzyme parallels that of SP (377). Also, cathepsin D, extracted and purified from calf brain, degrades SP by cleavage at the Phe^6-Phe^7 linkage (20).

On the basis of the present knowledge of the sites of cleavage by SP-degrading enzymes, it has recently been possible to synthesize stable SP analogues (369, 562). Thus, [pGlu^5-MePhe^8-MeGly^9]SP$_{5-11}$ (DiMe-C^7) is resistant to a SP-degrading enzyme purified from brain and to exposure of rat hypothalamic slices (562). This analogue effectively binds to receptor sites in rat brain membranes, but is only 0.1 as potent in contracting the guinea-pig ileum as SP (562). It is equipotent with SP in causing increased locomotor activity and on behaviour in the rat, but the effects are considerably prolonged (588). Similarly, the analogue [pGlu^6-N-MePhe^7, N-MeLeu^{11}] SP$_{6-11}$ is resistant to degradation by proteolytic enzymes but still has an agonistic activity which is comparable to that of SP (369). There are reasons to believe that these enzyme-resistant analogues will be useful tools in future pharmacological SP research.

In contrast to the undecapeptide SP, the C-terminal heptapeptide SP$_{5-11}$ is actively taken up by slices of rat brain and rabbit spinal cord (469) and has about 50 times higher affinity to receptors in synaptic membranes of the rabbit brain than SP$_{1-11}$ (466). These findings might indicate that the undecapeptide is a precursor from which the true neurotransmitter is formed through a N-terminal degradation. From this point of view the observation that dipeptidyl-aminopeptidase IV (DPIV), originally isolated and characterized by Hopsy-Havu and Glenner (289), is able to split the two dipeptides Arg^1-Pro^2 and Lys^3-Pro^4 from the N-terminal end of SP (251, 333), is of particular interest. The kidney has a high DPIV activity, followed by the lung, liver, and adrenal glands (364).

The N-terminal tetrapeptide fraction of the SP molecule has been attributed to a stabilizing role for the molecule. This is based on the observations that the C-terminal hexa- or heptapeptides are more susceptible to inactivation by rat brain homogenate than SP itself (31). Furthermore, the rate of inactivation of the free heptapeptide SP$_{5-11}$ is about 40-fold greater than that of SP$_{5-11}$ stabilized by a blocking group (4-hydroxy-5-indophenyl-propionyl) (31).

XVII. Structure-Activity Relationship

Several studies have been devoted to the relation between biological activity and chain length. These studies are important for the establishment of the amino acid sequence essential for biological effects, which in turn indicates the tentative physiologically active peptide to which the undecapeptide might be a precursor. Furthermore, the potency rank order of SP-fragments has been used as a tool for studies on the SP receptors. SP fragments have also provided a rational basis for the design and synthesis of potent enzyme-resistant SP analogues and specific SP antagonists (table 3).

1. Nervous System. The importance of the C-terminal structure of SP for the motoneuron depolarizing activity was first shown by Konishi and Otsuka (349, 497) on frog spinal cord and on isolated spinal cord of the newborn lamb. Omission of the 1-5 N-terminal amino acids did not cause any significant loss of motoneuron-depolarizing activity. SP$_{6-11}$ and SP$_{7-11}$ were considerably more active than SP$_{1-11}$, while SP$_{8-11}$ and SP$_{9-11}$ were practically inactive. On the other hand, the activity was completely lost when the C-terminal Met was omitted (497).

The analgesic effect, as analyzed with the hot plate test in the mouse, is considerably weaker for the SP fragments than for SP$_{1-11}$. While SP$_{4-11}$ still has some analgesic effect, SP$_{7-11}$ and SP$_{8-11}$ are completely inactive (178).

2. Circulation. On the rat blood pressure, the octapeptide SP$_{4-11}$ has been reported to be twice as potent as SP$_{1-11}$, whereas the sequences SP$_{5-11}$ and SP$_{6-11}$ are either slightly more active or equipotent with SP$_{1-11}$ (622, 92). However, the rat blood pressure is rather insensitive to SP. On the highly sensitive rabbit blood pressure, SP$_{1-11}$ is more active than any of its fragments (Lembeck, personal communication). The replacement of the nat-

TABLE 3

The SP molecule and effect of structural modifications

	N-terminal								C-terminal				References
	1	2	3	4	5	6	7	8	9	10	11		
SP	Arg	Pro	Lys	Pro	Gln	Gln	Phe	Phe	Gly	Leu	Met	NH_2	72
							↑		↑		↑	↑	
						Probably essential residue for receptor binding, but can be replaced by Tyr			Substitution by D-Ala decreases activity considerably		Can be replaced by Eth and Leu	Essential since free acid is inactive	80, 90 157, 375 473
So far most effective SP-antagonist	D-Arg	D-Pro	Lys	Pro	Gln	Gln	D-Trp	Phe	D-Trp	Leu	Leu	NH_2	548
Stable SP-agonists					Glu	Gln	Phe	MePhe	MeGly	Leu	Met	NH_2	562
						Glu	MePhe	Phe	Gly	Leu	MeLeu	NH_2	369

ural residues at the C-terminal, starting from Phe[7] and Phe[8], as well as the removal of the C-terminal amide, practically eliminates the activity (92).

In the hind limb of the dog, the C-terminal octa- and nonapeptides are more potent vasodilators than the undecapeptide, while the C-terminal tripentapeptides possess only weak vasodilator properties (55). Similarly, on isolated strips of the rabbit mesenteric vein no change in constrictive effect is found when the N-terminal residues are substituted, while the substitution of the residues at the C-terminal starting with Phe[7] considerably reduces the venoconstrictive effect (92).

3. Smooth Muscle. All C-terminal SP-fragments larger than the tripeptide possess spasmogenic activity on smooth muscle. Comparative studies on the relative activity of various SP fragments have, however, given conflicting results. Most authors have found the octapeptide SP_{4-11} most active in the isolated guinea-pig ileum (647, 655, 23, 55, 31, 80). On the other hand, Lembeck was unable to find any potentiation by shortening the SP peptide. In the isolated rabbit jejunum, SP_{1-11} was most active with a gradually decreasing potency with shorter C-terminal fragments (Lembeck, personal communication).

The relative potency of different SP fragments on smooth muscle often varies between various preparations. SP_{3-11} is more active than SP_{1-11} in the guinea-pig ileum but weaker in the rat vas deferens. SP_{4-11}, on the other hand, is more potent than SP on the vas deferens but equipotent on the ileum (380). The spasmogenic potency of the C-terminal hexa-, hepta-, and octapeptides of SP has been shown to be similar to or less than that of SP on the guinea-pig and rat ileum but higher on the cow pupillary sphincter and guinea-pig urinary bladder (31).

The one by one replacement of the first six residues from the N-terminal with L-Ala does not change the effect of SP on the isolated ileum, the rabbit mesenteric vein, or rat vas deferens, whereas the activity is considerably reduced when the residues 7–11 are substituted (90). Substitution of Phe[7] with Tyr, or Met[11] with Eth or Leu have virtually no effect on the potency of SP (90). On the other hand, replacement of Phe[7] by Ile or Leu decreases the activity 50- to 500-fold (375, 157), which proves the importance of position 7 for the binding to the receptor. Substitution of Phe[6] with Lys increases the activity about 5 times (73) and acylation of the C-terminal pentapeptide with a 4-hydroxyphenylacetyl group increases the spasmogenic activity in the guinea-pig ileum about 100 times (473).

4. Salivary Secretion. The effect of SP on the salivary gland is also bound to the C-terminal part of the peptide chain. In the rat, the minimum sequence for activity is the C-terminal pentapeptide SP_{7-11}, while the SP_{8-11} and SP_{9-11} are inactive (229, 405). All C-terminal fragments are, however, about five times less active as sialogogues than the undecapeptide (229, 405) and the binding affinity to the parotid cell membrane is well related to their relative potency in stimulating salivation (406). Changing the C-terminal amide to free acid results in an almost complete loss of activity, indicating that the amide is essential for the potency (405).

The release of alpha-amylase from rat salivary glands in vitro is also stimulated by C-terminal SP-fragments, the hexapeptide being the minimum structure for potency. All fragments are, however, less potent than SP_{1-11} (44).

5. Antidiuretic Activity. On intravenous administration, SP_{1-11} is twice as potent as SP_{6-11} in decreasing urine flow and increasing urine conductance, whereas SP_{7-11} and SP_{8-11} are almost ineffective (625).

The studies on the structure-activity relationships thus clearly indicate that the biological activity of SP primarily resides at the C-terminal part of the peptide chain since C-terminal fragments shorter than the pentapeptide SP_{7-11} are generally inactive. They, furthermore, indicate that the C-terminal carboxamide is essential for the activity since the SP free acid is inactive. On the basis of these observations, it has been suggested that the SP receptor interacts only with the six or seven amino acid residues corresponding to the C-terminal part of the undecapeptide molecule, whereas the N-terminal tetrapeptide part may play a role in stabilizing the molecule. However, it has recently been shown that large doses of N-terminal fragments, although inactive on smooth muscle, stimulate salivary secretion in rat, excite cat dorsal horn neurons, and induce stretching behavior in mice (524). On the basis of these observations, Piercey et al. (524) have suggested the nomenclature SP_1 and SP_2 receptors for those insensitive and sensitive for N-terminal fragments, respectively. It has also been shown that the N-terminal tetrapeptide fragment of SP but not the C-terminal fragment is responsible for the histamine-releasing effect of SP (446).

XVIII. Interaction and Coexistence between SP and Classical Neurotransmitters

Interaction between different transmitter systems and also, more recently, coexistence of transmitter or modulator compounds in the same neuron has been attributed an increasing interest in the last few years. SP neurons have been found to interact and/or coexist with neuronal systems containing monoamines, acetylcholine, amino acids, and other neuropeptides.

A. SP and Catecholamines

Almost all catecholamine-containing cell groups in the central nervous system seem to be surrounded by SP-positive terminals (412). The density of these SP networks varies considerably. The largest accumulation of SP fibers is seen around dopamine nerve cells and their dendrites in the substantia nigra and around catecholamine cells in the nucleus commisuralis, nucleus tractus solitarii, and vagus nerve nucleus. The locus coeruleus,

which in the rat is composed almost exclusively of noradrenaline-containing cells, is densely innervated with SP-containing fibers. Occasional SP-immunoreactive fibers are also observed around tyrosine hydroxylase-positive cells in the area postrema and around the dopamine cells in the olfactory bulb (412).

Several areas in the brain receive both SP- and tyrosine hydroxylase-positive terminal networks (412). These include the medial frontal cortex, the amygdaloid complex, the caudate nucleus, various hypothalamic nuclei, the periaqueductal central grey, the solitary tract nucleus, the vagal nerve, and the spinal cord. In some of these areas there is a complete overlap between the two systems, while others show a differential distribution pattern (412).

These histochemical observations seem to indicate that several types of interaction occur between the catecholamines and SP. They also suggest that SP- and catecholamine-containing nerve terminals might together influence neuronal activity in the brain. So far, no evidence for a coexistence between SP and catecholamines has been obtained. Thus, interaction of SP and catecholamines is, in all probability, limited to different types of coupling between two systems.

SP stimulates both synthesis and utilization of catecholamines in the brain. Intracerebroventricular administration of SP induces a significant increase in DOPA formation in several noradrenaline- and dopamine-rich brain regions, such as limbic areas, striatum, hemispheres, and lower brain stem (66). Similarly, SP accelerates the disappearance of dopamine and noradrenaline in these areas (66). The effect of SP on catecholamine synthesis is blocked by naloxone, which might suggest that SP regulates catecholamine metabolism either by influencing the opiate receptors or indirectly by releasing an opioid factor (201).

Also the catecholamine uptake mechanism in the nerve cell might be affected by SP since this peptide inhibits uptake of noradrenaline in hypothalamic synaptosomes both in vitro and in vivo (528). The interaction between SP and the dopaminergic and GABAergic systems in the substantia nigra is discussed in section V.

B. SP and 5-HT

Several regions of the brain contain both 5 HT- and SP-neurons. In some areas, particularly in the medulla oblongata and the spinal cord, strong evidence exists for a coexistence of these compounds in the same neuronal structures. Thus, numerous cell somata in the raphe magnus, pallidus, and obscurus nuclei as well as as in the nucleus interfascicularis hypoglossi contain both 5-HT and SP immunoreactivity (75, 264, 74, 317, 511). The storage of a biologically active amine and a peptide hormone in the same cell is an established phenomenon in endocrine cells. Pearse (508) has termed these cells APUD (amine content and/or precursor uptake and decarboxylation) cells. The first evidence that the APUD concept is valid also for neurons was given by Hökfelt et al. (257), who showed the presence of a somatostatin-like peptide in a population of peripheral sympathetic noradrenergic neurons in the guinea-pig.

The interaction and coexistence between 5-HT and SP are, however, not limited to cell bodies. SP-immunoreactive fibers in the ventral horn of the spinal cord has a distribution similar to that of the 5-HT nerve terminals (263, 411). Treatment with the neurotoxins 5,6-dihydroxytryptamine or 5,7-dihydroxytryptamine cause disappearance of both 5-HT and SP immunoreactivity in the ventral horns, which indicates the coexistence of these two compounds also in nerve endings (264, 29, 74, 577). The coexistence of 5-HT and SP in nerve cells and fibers in the spinal cord has also been established by simultaneous in vivo injections of monoclonal SP antibodies and [^{3}H]5-HT, followed by visualization of the two markers in the same structures by immunohistochemistry and autoradiography (105).

The subcellular storage site(s) of 5-HT and SP in the neurons, where the two compounds coexist, is not completely known, but there is evidence that indicates that they are, to some extent, identical. Generally, 5-HT is present in both small and large granular vesicles (253), whereas SP is stored in subcellular storage particles (163), and these vesicles seem to be of the large type as demonstrated by electron microscopic immunocytochemistry (76, 260, 523). In the ventral horn of the spinal cord 5-HT and SP occur in the same large dense core vesicles (511), which suggest that they may be released simultaneously.

Some cell bodies in the medullary raphe nuclei and adjacent areas also contain, in addition to 5-HT and SP, thyrotropin-releasing hormone (TRH) (317). Other cell bodies in the same area seem to contain one or two of these three putative transmitters. The proportion of 5-HT, SP, and TRH in neuronal cell bodies in the medulla oblongata varies with different levels and nuclei. Overall, there are approximately twice as many 5-HT as SP and TRH cells (317).

In the ventral horn of the spinal cord overlapping networks of 5-HT, SP, and TRH immunoreactive fibers have also been observed. After treatment with 5,6- or 5,7-dihydroxytryptamine an almost complete disappearance of all three types of fibers occurs concomitant with a degeneration of the neuronal elements in which they are present. These findings are consistent with the coexistence of SP, 5-HT, and TRH in descending axons and terminals of bulbospinal neurons (317, 208, 439).

In addition to a structural SP-5-HT interrelation, evidence is also given for a pharmacological interaction between these two compounds in some brain areas. Thus, in superfused slices of rat substantia nigra, SP stimulates the release of 5-HT after addition of labeled tryptophan (539, 540). This effect of SP is blocked by the dopamine antagonist, alphaflupenthixol, which indicates that the SP-evoked release of 5-HT is mediated by dopaminergic

receptors (540). It, furthermore, might indicate that SP-ergic neurons in the substantia nigra may be involved in the local control of 5-HT neurons innervating the basal ganglia.

C. SP and Acetylcholine

Several areas of the brain, such as the habenulo-interpeduncular tract and the hypothalamus, contain both cholinergic and SP neurons with projections from one of these types of neurons to the other (98). Also, the subcellular distribution of these compounds in brain homogenates shows close association of location for the two agents (330, 552). SP- as well as enkephalin-immunoreactivity has also been found in a large proportion of the preganglionic terminals of the avian ciliary ganglion where acetylcholine is the transmitter. A coexistence of acetylcholine with one or both of these neuropeptides seems very likely although it has not yet been demonstrated (152). This association between acetylcholine and SP suggests a functional interaction, which had been supported by observations from several tissues.

1. Central Nervous System. The effect of SP on cholinergic transmission has been most extensively studied in the *Renshaw cells* in the spinal cord (17, 360, 554, 115). In low doses, SP reduces acetylcholine-induced excitation, probably by a postsynaptic mechanism. The inhibitory effect of SP seems to be quite specific for acetylcholine-mediated responses as SP does not inhibit excitation by acidic amino acids such as glutamic acid or homocysteic acid. SP seems to act selectively on the nicotinic receptors in a way similar to exogenous neuromuscular blocking agents. This assumption is supported by the finding that SP does not suppress the response of the Renshaw cells to the muscarinic agonist, acetyl-beta-methylcholine. Furthermore, the inhibitory effect of SP is not blocked by strychnine, a selective antagonist for glycine receptors.

At higher concentrations SP has an excitatory effect probably due to a presynaptic release of acetylcholine from cholinergic terminals. This effect is blocked by the nicotinic blocker, dihydro-beta-erythroidine.

In central neurons, on which the effect of acetylcholine is mediated predominantly by muscarinic receptors, SP has no inhibitory effect (360).

Intracerebroventricular injections of SP or local application in the septum increases the turnover of acetylcholine in the *hippocampus* and *brain stem* (435).

2. Adrenal Medulla Neurons. The acetylcholine- or nicotine-induced release of noradrenaline from isolated cultures of adrenal chromaffin cells is inhibited by SP in 10^{-8} to 10^{-4} M concentrations (410, 545, 35). It is likely that SP inhibits the acetycholine effect on these neurons by interfering with nicotinic receptors by a mechanism similar to that described for the Renshaw cells.

3. Sympathetic Ganglia. Close-arterial injection of SP (2 to 20 nmol) to the superior cervical ganglion partially antagonizes the atropine-resistant contractions of the nictitating membrane in the cat caused by acetylcholine. The increase in blood pressure, induced by local administration of acetylcholine to the ganglion, is also reduced by SP. SP has, however, no effect on the actions of acetyl-beta-methylcholine on the nictitating membrane or blood pressure (81).

4. Intestinal Wall (see section IX). These observations clearly indicate that SP has a modulatory effect on cholinergic transmission in both the central and peripheral nervous system. This modulation seems to be restricted to the cholinergic excitation produced by activation of nicotinic receptors. However, even among these receptors there seems to be a selectivity, since SP does not antagonize the actions of acetylcholine on the nicotinic receptors at the motor end-plate (553).

D. Functional Aspects on Coexistence

The physiological significance of coexistence of an amine and one or several peptides in central and peripheral neurons is at present not clear, but recent observations seem to elucidate how coexisting putative transmitters may exert their action. In the cat, exocrine glands, such as the sweat and salivary glands and glands in the nasal mucosa, are innervated by different populations of neurons, each of them containing an amine and a peptide in coexistence. One population of neurons contains both acetylcholine and vasoactive intestinal polypeptide (417), while a second type contains noradrenaline and an avian pancreatic polypeptide-like peptide, possibly the neuropeptide Y (427). The parasympathetic neurons containing vasoactive intestinal polypeptide innervate blood vessels, myoepithelial cells, and secretory elements (318), while the sympathetic neurons containing neuropeptide preferentially innervate blood vessels (427). Acetylcholine causes both secretion and vasodilatation, whereas vasoactive intestinal polypeptide only has a vasodilatory effect. When the two compounds are administered together, a marked potentiation of both secretion and blood flow occurs (418). Evidence is given that a release of vasoactive intestinal polypeptide is responsible for the atropine resistant vasodilatation in the exocrine glands, but also enhances the response caused by acetylcholine. Neuropeptide Y, which has a direct vasoconstrictory action, i.e., acts in the same direction as its co-transmitter noradrenaline in the blood flow regulation, blocks the neuronal as well as vasoactive intestinal polypeptide-induced, atropine resistant vasodilatation (418). These observations point to at least one physiological function of neuropeptides, namely to potentiate the effect of coexisting classical transmitters.

XIX. SP and the Tachykinins

Tachykinins represent a peptide family with a widespread distribution in invertebrates and vertebrates. The different compounds belonging to this group have been isolated and described by Erspamer and associates (154, 155, 169). They all show pronounced structural similar-

TABLE 4
Chemical structure of tachykinins

Substance P	Arg-Pro-Lys-Pro-Gln-Gln-	Phe	-Phe-	Gly-Leu-Met	$-NH_2$
Physalaemin	pGlu-Ala-Asp-Pro-Asn-Lys-	Phe	-Tyr-	Gly-Leu-Met	$-NH_2$
Uperolein	pGlu-Pro-Asp-Pro-Asn-Ala-	Phe	-Tyr-	Gly-Leu-Met	$-NH_2$
Phyllomedusin	pGlu-Asn-Pro-Asn-Arg-	Phe	-Ile-	Gly-Leu-Met	$-NH_2$
Eledoisin	pGlu-Pro-Ser-Lys-Asp-Ala-	Phe	-Ile-	Gly-Leu-Met	$-NH_2$
Kassinin	Asp-Val-Pro-Lys-Ser-Asp-Gln-	Phe	-Val-	Gly-Leu-Met	$-NH_2$

ities (table 4) in the sense that they all possess the common C-terminal sequence, Phe-X-Gly-Leu-Met-NH_2. Also the spectrum of activity of these compounds on smooth muscle and secretory organs is similar, but the relative potency varies greatly (table 5). SP is more potent as a vasodilator while eledoisin and kassinin are considerably more active than SP on certain types of smooth muscle. Physalaemin is the most potent sialogogue. When given as close-arterial infusions, all these peptides increase blood flow in the hepatic, mesenteric, and iliac vascular beds (449). Generally SP is the most active compound. Like SP, physalaemin is less active when infused into the portal vein than into the femoral vein, while the threshold dose of eledoisin as vasodilator is the same when given into the portal or femoral vein (449). This indicates that the liver has a considerable capacity to inactivate SP and physalaemin but not eledoisin.

Similar to SP, the C-terminal octa- and nonapeptide analogues of eledoisin are more potent than the undecapeptide (156). However, the hexapeptide physalaemin$_{6-11}$ has approximately the same spasmogenic effect on the guinea-pig ileum but only 50% of the hypotensive action of the undecapeptide (25). A structure-activity comparison between SP, eledoisin and physalaemin on the isolated guinea-pig ileum indicates that their analogues containing eight C-terminal amino acids are more active than the corresponding undecapeptides (23). This is particularly the case for physalaemin, where physalemin$_{4-11}$ is about 15 times more active than physalemin$_{1-11}$. SP$_{4-11}$ is about 2.5 times more active, while the eledoisin$_{4-11}$ is only about 20% more active than the corresponding undecapeptides. For the 5–11 C-terminal chain the situation is completely different. While SP$_{5-11}$ is about as active as the undecapeptide, the potency of eledoisin$_{5-11}$ and physalaemin$_{5-11}$ is much lower than for the respective undecapeptides (23).

It is still unclear, whether SP, eledoisin, and physalaemin act on the same or different receptors. Exposure of the guinea-pig ileum to large concentrations of SP (10^8 to 10^9 M) render the gut insensitive to subsequent stimulating doses of SP (185, 174) but also to eledoisin and physalaemin (392). The spasmogenic action of eledoisin is, however, unlike that of SP and physalaemin, being partly atropine-sensitive, and the atropine-resistant component of its action recovers from SP tachyphylaxis at a different rate to SP (380). The fact that SP, physalaemin, and eledoisin exhibit different patterns of recovery from SP desensitization might be explained by a difference in peptide-receptor binding and should not be taken as evidence for the existence of subpopulations of SP receptors (548). Also the guinea-pig pancreas possesses receptors, which interact with SP, eledoisin, and physalaemin but not with other pancreatic secretagogues (308).

On the basis of the different potencies of the tachykinins relative to SP (table 5), the peripheral organs have been divided into two subclasses (298, 380). In one group, termed the SP-P-type, typified by the guinea-pig ileum, the rank order is: physalaemin ≧ SP ≧ eledoisin = kassinin. The second type (SP-E), typified by the rat vas deferens, has the rank order: eledoisin = kassinin > SP = physalaemin. Whether this difference in rank order of potency reflects subdivisions of SP receptors remains to be shown, but some recent observations seem to indicate that this might be the case. Thus, atropine reduces the effects of eledoisin but not of SP and the cross-tachyphylaxis between SP-P and SP-E agonists is not complete in all situations (380). Finally, replacing the C-

TABLE 5
*Relative potency of physalaemin (Phys), kassinin (Kas), eledoisin (Eled), and substance P (SP)**

Test preparation	Phys	Kas	Eled	SP
Dog blood pressure	100	3–8	25–30	150–250
Rabbit blood pressure	100	4–7	20–40	150–270
Rat blood pressure	100	10	50	100
Man blood pressure	100			450
Hepatic arterial blood flow (dog)	100		30	200
Portal venous blood flow (dog)	100		100	400–500
Rat salivary secretion	100	6–7	30–50	20–25
Rat stomach	100	50–100	90–135	50–65
Rat colon	100	150–300	150–300	20–50
Guinea-pig ileum	100	10–40	30–120	20–60
Rabbit large intestine	100	13–40	30–80	30–75
Human stomach	100		170–215	8–12
Human taenia coli	100		300–600	20–35
Rat urinary bladder in situ	100	18–50	50–100	25–50
Hamster urinary bladder	100	3000	3000	50–100
Dog urinary bladder	100	400–4000	1000–5000	30
Man urinary bladder	100	1000–3000	1000–2000	15–30
Guinea-pig trachea	100		100–150	15–25
Spinal motoneurons (frog)	100		135	13

* From Erspamer, 1981 (154).

terminal Met carboxamide in SP with the corresponding methyl ester yields an analogue, which is approximately equipotent with SP in the SP-P system but almost ineffective in the SP-E system (298). SP receptor antagonists have also been used to elucidate the possibility of subpopulations of SP receptors (548). [D-Arg^1, D-Pro^2, D-Trp^{7,9}, Leu^{11}]SP blocks the contractions caused by SP, eledoisin, and physalaemin on various organs. However, the affinity constant (pA_2) for the antagonist versus SP is significantly different on the guinea-pig ileum and rat urinary bladder, indicating the existence of at least two subpopulations of SP receptors. When tested on the hamster urinary bladder, which is very sensitive to eledoisin but rather insensitive to physalaemin and SP (154), it was found that the SP receptor antagonist did not block eledoisin-induced contractions or those elicited by high concentrations of physalaemin or SP. These findings indicate that the hamster urinary bladder lacks SP receptors but has a tachykinin receptor, which has a high affinity for eledoisin and is not blocked by [D-Arg^1, D-Pro^2, D-Trp-^{89}, Leu^{11}]SP (548).

XX. SP Antagonists

The availability of antagonists that specifically block SP receptors would facilitate the analysis of the physiological significance of SP and its role in pathophysiological situations. Such compounds might also possess therapeutic properties or serve as models in the development of a new type of drugs.

Two routes have been used in the search for SP antagonists. One has been a conventional screening of various compounds. So far baclofen is the only nonpeptide substance of interest in this connection. A more successful approach has been to modify the SP structure in order to obtain analogues that competitively block SP receptors.

A. Baclofen

In 1975, Saito et al. (555) reported that the GABA derivative beta-(4-chlorophenyl)-GABA (baclofen, lioresal), which suppresses mono- or polysynaptic reflexes in the central nervous system, effectively antagonizes the depolarizing action of SP on spinal motoneurons. These findings were confirmed by Henry and Ben-Ari (249), who showed that baclofen depresses the rate of discharge from single spinal units in the cat during excitatory responses to SP. Baclofen also reduces the magnitude of the responses of single units in the spinal cord to application of noxious radiant heat to the hindleg (249). This is of interest in view of the finding that units, responding to noxious heat stimuli, are also excited by SP (247, 248). In addition, baclofen depresses the excitatory responses of spinal units to iontophoretic application of glutamate (249).

In the rat cerebral cortex, baclofen, administered by cationic current, also depresses SP evoked excitation, but acetylcholine excitation is affected to the same extent (521). Also, in the locus coeruleus, iontophoretically applied baclofen nonspecifically decreases the excitation elicited by SP, acetylcholine, and glutamate (221). In the isolated spinal cord, on the other hand, perfusion with baclofen abolishes specifically the effect of SP and does not affect to a significant extent the responses to acetylcholine or glycine (503). Furthermore, the excitatory activity of SP on neurons from the guinea-pig hypothalamus, but not that of acetylcholine, is antagonized by baclofen (487).

The increase in blood pressure, induced by intracerebroventricular injections of SP in spontaneously hypertensive rats, is markedly attenuated by pretreatment with baclofen, while the blood pressure increase to central angiotensin is unaffected (628). This finding indicates different pathways for the cardiovascular responses to centrally administered angiotensin and SP.

In the guinea-pig ileum baclofen only partially and nonspecifically blocks the action of SP (173).

In summary, baclofen in the central nervous system effectively blocks the excitatory activity of SP. Since in many areas the excitatory responses to other compounds such as acetylcholine and glutamate are inhibited as well, baclofen may not be considered a specific SP antagonist, but is rather exerting a general depressant effect.

B. SP Analogues

Efforts to constitute SP analogues with apparently specific SP receptor blocking characteristics have recently been successful (375, 171, 549, 30, 65). The principle, to modify a peptide in order to reduce the agonistic activity and to achieve an effective inhibitor, is not new. Substitution of amino acid residues, essential for the biological activity of the luteinizing hormone-releasing hormone, with D-amino acids is known to confer antagonistic activity (87). By replacement of the important Phe^7 in the SP molecule by D-Phe^7, a peptide is obtained with weak agonistic but some, although weak, antagonistic effect (650). However, when several residues especially in the C-terminal part of SP_{1-11} are substituted with D-amino acid residues, specific SP antagonists are obtained with no or negligible agonistic activity. These are [D-Leu^8, D-Leu^9]SP (375), [D-Pro^2, D-Phe^7, D-Trp^9]SP (171), [D-Pro^2, D-Trp^{7,9}]SP (151, 275, 30), and [D-Arg^1, D-Pro^2, D-Trp^{7,9}, Leu^{11}]SP (548). Of these analogues, the two last mentioned are the most potent.

These SP antagonists have been characterized pharmacologically in several organs.

On isolated *smooth muscle* such as the guinea-pig ileum (30) or taenia coli (373) the dose-response curve for SP is shifted to the right in the presence of one of the above-mentioned analogues in the organ bath fluid in a manner suggesting competitive inhibition. Of these, [D-Arg^1, D-Pro^2, D-Trp^{7,9}, Leu^{11}]SP is the most potent antagonist so far discovered, with little or no smooth muscle-contracting effect. Apart from SP, the analogues also inhibit the contractile effects of physalaemin and eledoisin but not

that of histamine, acetylcholine, 5-HT, bradykinin, Lys[8]-vasopressin, or prostaglandin $F_{2\alpha}$ (373). The slow contraction induced by SP, physalaemin, and eledoisin in the iris sphincter muscle is also greatly inhibited by pretreatment with [D-Pro[2], D-Trp[7,9]]SP (373).

It is well known, that even after cholinergic and adrenergic blockade, electrical nerve stimulation still induces a contraction of smooth muscle such as the intestine, urinary bladder, and sphincter pupillae muscle. In the guinea-pig taenia coli and rabbit sphincter pupillae muscle the contractions are greatly reduced by [D-Pro[2], D-Trp[7,9]]SP (373). On the other hand, the SP antagonist does not inhibit the corresponding response to electrical stimulation in the urinary bladder of the guinea-pig (373).

[D-Arg[1], D-Pro[2], D-Trp[7,9], Leu[11]]SP markedly reduces vagally as well as capsaicin-induced, noncholinergic bronchoconstriction in the guinea-pig (426). This suggests that activation of sensory neurons of vagal origin induces a local release of SP, which in turn causes bronchoconstriction (475).

When administered intravenously, the SP receptor antagonists inhibit SP- and physaelamin-stimulated *salivary secretion* of the rat without being secretagogues per se (30). The analogue dose needed to reduce secretion to about 50% of the control is of the order of 0.8 mmol/kg.

The *hemodynamic effects* induced by close-arterial infusions of SP in the dental pulp and oral mucosa of the rat are inhibited by [D-Pro[2], D-Phe[7], D-Trp[9]]SP, while the effect of acetylcholine is not influenced (549). Similarly, the antidromic vasodilatation elicited in these tissues by electric stimulation of the inferior alveolar nerve (549) or the saphenous nerve (390) is also inhibited by this SP analogue.

In the rabbit eye, [D-Pro[2], D-Trp[7,9]]SP inhibits the aqueous flare response and miosis induced by intravitreal administration of SP or by infrared irradiation of the iris (275). This analogue also effects the SP-induced relaxation of cerebral arteries, previously contracted by prostaglandin $F_{2\alpha}$, by shifting the dose-response curve of SP towards higher concentrations (140). [D-Pro[2], D-Trp[7,9]]SP also significantly alters the vasodilatation induced by SP on cat pial arteries in situ (140).

In the *central nervous system* the SP analogues also seem to specifically antagonize SP. Thus, [D-Pro[2], D-Trp[7,9]]SP completely blocks the excitatory effect of SP on neurons in the locus coeruleus even in a dose which produces no effect of its own (151). The specificity of this activity is indicated by the fact that the SP analogue does not inhibit the glutamate- or acetylcholine-induced stimulation of the locus coeruleus neurons (151). The dose-dependent, vasopressor response to intracisternal injection of SP in the rat is counteracted by [D-Pro[2], D-Phe[7], D-Trp[9]]SP (182) and the antagonist in itself causes a fall in blood pressure (413). Furthermore, the pressor response induced by the excitotoxic agent, kainic acid, is attenuated by [D-Trp[2], D-Trp[7,9]]SP (413).

In the isolated rat spinal cord, [D-Arg[1], D-Pro[2], D-Trp[7,9], Leu[11]]SP in concentrations higher than 7 μmol markedly reduces the size and duration of the SP-induced depolarization of motoneurons without having any agonistic action on the spinal neurons (653). The response to L-glutamic acid is only slightly depressed by this SP analogue, and the response to GABA is not affected (653). Also, in neurons of the inferior mesenteric ganglia from the guinea-pigs, the slow membrane depolarization induced by exogenously applied SP or elicited by repetitive presynaptic stimulation is specifically and reversibly inhibited by the SP analogue [D-Pro[2], D-Phe[7], D-Trp[9]]SP (316). On the other hand, Salt et al. (560) were unable to find any antagonizing effects of [D-Pro[2], D-Trp[7,9]]SP on the SP-induced depolarization on isolated rat spinal cord or the caudal trigeminal nucleus in vivo.

Single intrathecal injections of [D-Pro[2], D-Phe[7], D-Trp[9]]SP and [D-Pro[2], D-Trp[7,9]]SP significantly increase latency of the hot-plate test and a blockade of the noxious responses to SP as well as the scratching and biting behaviour of the hind legs normally seen after intrathecal administration of SP (393, 527, 2). These findings presumably indicate that the anti-nociceptive effect of [D-Pro[2],D-Trp[7,9]]SP is due to blockade of SP receptors in the spinal cord and support the hypothesis that SP plays a role in the transmission of nociceptive information.

The mechanism by which these analogues exhibit their antagonistic effects is not known, but is assumed to involve competition with endogenously released SP at its receptor site. It has recently been shown that [D-Pro[2], D-Trp[7,9]]SP also releases histamine from mast cells (606). This finding may explain some of the so-called SP agonistic actions of these analogues and should give rise to the pretreatment with antihistamines before studies of their SP antagonistic effects (426).

The SP antagonists are also important when characterizing SP receptors in various organs. [D-Pro[2], D-Trp[7-9]]SP does not block all effects of SP; it is very effective in antagonizing the secretory or neurotropic actions of SP, but does not influence its hypotensive effects (30). The antagonistic potency of these analogues also varies between different smooth muscle preparations. Thus, the affinity constant for [D-Arg[1], D-Pro[2], D-Trp[7,9], Leu[11]]SP versus SP is different for the guinea-pig ileum and rat urinary bladder preparation, respectively (548). These findings strongly suggest that there are several subpopulations of SP receptors as has also been claimed by others who used different SP agonists (524, 380).

In addition to a SP antagonistic activity, [D-Pro[2], D-Trp[7,9]]SP apparently also has a "neurotoxic" effect, which involves not only SP neurons (272). After injection of 0.7 nmol of this SP analogue into the ventral tegmental area in the rat a marked decrease of SP-like immunoreactivity occurs in the caudate nucleus and in the zona reticulata of the ipsilateral substantia nigra. The dopa-

mine cells in the zona compacta of the same area also exhibit changes. It is not yet known whether other SP analogues give similar morphological changes but no such effects are seen after injections of SP even in larger doses (272). The "neurotoxic" action may be the reason why high doses of [D-Pro^2, D-Trp^{7,9}]SP administered intrathecally cause irreversible motor blockade (2).

XXI. SP and Nociception

A. Evidence that SP Is Involved in Nociception

Numerous morphological, neurophysiological, and pharmacological observations have focused interest on a putative role for SP in the transmission of pain.

1. The apparent selective *localization of SP* in small-size cell bodies of sensory ganglia and fine caliber, probably unmyelinated, axons in the superficial layer of the spinal cord (262, 523) and in the spinal trigeminal nucleus system (260, 97) gives a structural basis for an involvement of SP in nociception. In the spinal trigeminal nucleus, SP immunoreactive fibers have a pattern which is consistent with the neuroanatomical distribution of the pain fibers of the trigeminal nerve (103). The occurrence of unmyelinated or fine caliber SP fibers in the dental pulp (491) is of particular interest from the viewpoint that the pulp afferents are considered to transmit only pain sensation (7). Lesion of the trigeminal ganglion or rhizotomy results in a depletion of SP immunoreactivity in the ipsilateral spinal trigeminal nucleus (97). Similarly, 2 weeks after transection of the inferior alveolar nerve no SP fibers are observed in the dental pulp (491).

2. *Neurophysiological evidence* has been given for an association of SP with pain sensory fibers (248, 6, 537, 564, 649). Microiontophoretic application of SP to single units located in the dorsal horn of the spinal cord selectively excites those cells, which respond to peripheral noxious stimulation, and, in addition, prolongs the response of dorsal horn neurons to such stimulation (248, 564) or to afferent volleys in A delta or C fibers (537). Electrical stimulation of the sciatic nerve at intensities strong enough to recruit A delta and C fiber afferents considerably increases the release of SP from the mammalian spinal cord (649). In the trigeminal nucleus caudalis only neurons which are activated by noxious stimulation are excited by iontophoretic application of SP (6).

However, SP is evidently not involved in all nociceptive units. In those areas in the substantia gelatinosa of the cat, where enkephalin selectively reduces excitation by noxious skin stimuli, SP is without effect (131). Furthermore, in the medullary nucleus reticularis gigantocellularis of the cat, which is an important link in the nociceptive pathway, SP is not able to enhance the response to a nociceptive stimulus (226).

3. A considerable amount of *pharmacological evidence* for SP being involved in nociception refers to studies on the effect of capsaicin, a compound that has become a useful tool in the study of pain mechanisms. On acute administration, capsaicin excites peripheral nociceptors, while repeated application desensitizes pain fibers (304). Pretreatment of newborn rats with capsaicin results in a selective and permanent degeneration of small diameter primary sensory neurons, including B-type cells in the spinal ganglia, their terminals in the spinal cord, and thin unmyelinated peripheral fibers (305, 282, 464, 565). Simultaneously, the animals are rendered analgesic to thermal or chemical pain stimuli (305, 282, 53). Long-term administration of capsaicin to the adult rat selectively damages unmyelinated sensory fibers and increases the nociceptive threshold in a reversible manner (306, 236). In the rat, the effect seems to be limited to nociceptive chemical and pressure stimuli without any effect on heat threshold (236), while in the guinea-pig capsaicin treatment results in a profound thermal anti-nociception (49).

In parallel with the effect on unmyelinated fibers and pain mechanisms, capsaicin substantially reduces the content of SP in the primary sensory neuron. Thus, treatment of rats with capsaicin results in a dramatic (80% to 95%) loss of SP immunofluorescence in the substantia gelatinosa and marginal layer of the spinal cord and spinal trigeminal nucleus as well as in peripheral structures such as dorsal roots, saphenous and vagus nerves, and hind paw skin (202, 313, 197, 193, 236, 101, 388, 389). In the rat dosal horn, the extent of depletion of SP following pretreatment with capsaicin is, however, only 40% to 55% (194, 464), which seems to indicate that only about half of the SP present in the dorsal horns has a primary afferent origin. Similar results are obtained in the guinea-pig (49, 53).

A depletion of SP from the central portion of primary afferent neurons is, however, not necessarily required for the inhibition of thermal anti-nociception by capsaicin. Thus, increased escape latency from hot plate in guinea-pigs is already observed within 1 day after capsaicin, while a significant decrease in SP content of dorsal root ganglia is not observed until 4 days after capsaicin (389, 453).

In summary, at least three different actions of capsaicin can be identified. The first effect, presumably occurring within seconds, is a calcium-dependent release of SP from central and peripheral terminals of primary afferents. This is followed, within minutes, by a functional impairment of these fibers. Depletion of SP begins within hours and persists for weeks. Locally applied capsaicin inhibits the axonal flow of SP in the nerve (199).

B. Analgesic Effects of SP

In 1976 Stewart et al. (596) reported that SP given in intracerebroventricular or intraperitoneal injections to mice causes a long-lasting analgesia as indicated by an increase in reaction time to heat stimuli (hot-plate test).

The analgesic effect was prevented by naloxone. Animals treated chronically with morphine showed, however, no analgesic reaction to SP. This rather remarkable observation was later repeatedly confirmed both in mice and rats (433, 589, 608, 630, 125, 357, 450). The analgesic effect of SP is often substantial. Thus, a significant analgesia is recorded with the tail flick test after a microinjection into the periaqueductal grey of only 0.2 nmol per rat, which means that SP is about 25 times more potent than morphine on a molar basis (433). In the hot-plate test 0.7 nmol of SP intraperitoneally showed a similar time course of analgesia as after 150 nmol of morphine in mice (589). In other studies with apparently identical techniques no significant analgesia was, however, observed with SP unless very large doses were used (215, 235, 456). On the contrary, in some studies a decrease in reaction time to hot-plate stimuli has been reported following intracerebroventricular injections of SP in doses of 0.7 nmol per rat (235, 483). Similar results were obtained when SP was administered intrathecally to rats in doses of 15 nmol. This effect was blocked by the SP antagonist [D-Pro^2, D-Trp^{7,9}]SP (393, 2).

These apparently conflicting results are probably a matter of doses, since Frederickson et al. (177) have shown that SP obviously has a dual action on nociception. While small doses of SP (less than 4 pmol given into the lateral ventricle) in mice produces naloxone-dependent analgesia, a hyperalgesia occurs when higher doses (more than 40 pmol) are given even in combination with naloxone. The interpretation of these findings is that small doses of SP probably release endogenous opioid peptides, while higher doses stimulate the neuronal activity in nociceptive pathways. This dose-dependent action of SP in nociception has later been confirmed (485). The varying results presented by different authors may also be due to different observation times. Thus, the immediate response to intraspinal SP is a hyperalgesia (2, 527), whereas a depressed tail flick response to noxious heat and motor response to supramaximal stimulation of the sural nerve in the spinal rat was reported 20 minutes after the injections of large doses (nmol) of SP (126).

SP seems to have no effect on peripheral nociceptors. Thus, Lembeck and Gamse (394) found no stimulatory effect of synthetic SP on paravascular pain receptors, nor did SP modulate the algesic effect of bradykinin or acetylcholine. Furthermore, synthetic SP has practically no excitatory effect on unitary discharge of polymodal nociceptors in the testis of the dog (363).

Intracerebral and intracerebroventricular injections of SP in mice also induce a series of behavioral actions, including locomotor effects and enhanced grooming and scratching (597, 340, 335, 321, 124, 336, 357, 575). The same response is obtained with the C-terminal SP hexapeptide SP_{6-11} but not with the pentapeptide SP_{7-11} (124). The stable SP analogue, [pGlu^5, MePhe^8, Sar^9] SP_{5-11} (DiMe-C7), produces a longer-lasting behavioral stimulation than SP (144). This effect is potentiated by D-amphetamine and haloperidol (144). The closely related peptides eledoisin and physalaemin give the same response as SP but other peptides, such as TRH, neurotensin, and bradykinin, do not (575). Morphine and other analgesic agents administered intraperitoneally effectively prevent in a naloxone-reversible way the SP-induced response (575).

The behavioral effects are, however, considerably more pronounced when SP is given intraspinally than intracerebrally (295, 525, 2). Within a few seconds after intraspinal injections of SP (ED50 = 1.3 pmol) the mice begin to bite their toes and scratch their backs and ears. The sensory nature of the behaviour is apparent, since the mice precisely direct their mouths and paws to the skin surface (525, 527). This noxious stimulus is blocked by intraspinal injection of [D-Pro^2, D-Phe^7, D-Trp^9]SP (393, 527, 2) but not by morphine (526). The SP antagonist also dramatically reduces capsaicin-induced biting and scratching (527). These data provide strong evidence that SP is involved in chemogenic nociceptive mechanisms.

In adjuvant-induced polyarthritis of rats, SP content is particularly raised in those primary afferents that innervate the inflamed and painful areas (391, 395).

The hypothesis that SP is involved in the transmission of impulses related to pain is further supported by the recent observation of a depletion of SP immunoreactivity in the substantia gelatinosa of spinal cord and medulla of patients with familial dysautonomia (510). This syndrome (the Riley-Day syndrome), is characterized by a severe diminution of temperature and pain sensitivity and reduced populations of primary sensory neurons and small fibers in the Lissauer tract (510).

C. Interrelation with Other Neuropeptides

When discussing the putative role of SP in nociception it is important to consider its interrelation and interaction with other peptide systems with which the SP system shows overlapping distribution patterns. Particular interest has been focused on the interaction between *SP and opioid peptides* on the basis of a considerable overlap of nerve terminals containing Met-enkephalin and SP, particularly in areas related to pain and analgesia. Thus, both enkephalin- and SP-positive cell bodies and nerve terminals have been observed in the periaqueductal central grey, the nucleus raphe magnus, the marginal layer and substantia gelatinosa of the spinal trigeminal nucleus of the medulla oblongata, and in all parts of the spinal cord (265, 96, 346, 149, 77, 534). Also, in the human spinal cord, SP- and enkephalin-positive terminals and axon overlap (366).

Transection of the spinal cord at the thoracic level or unilateral dorsal rhizotomy at the lumbar level results in

a marked reduction of SP in the ventral horns but does not seem to influence the distribution or intensity of the enkephalin-positive nerve terminals in the lumbar cord (265). On the other hand, opiate receptors in the spinal cord decrease in number after dorsal rhizotomy (367). These results seem to indicate that at the spinal level the enkephalin systems mainly are either interneuronal or propriospinal, while SP appears to be present both in a supraspinal system innervating mainly the ventral horns and in primary sensory neurons.

Also, in the brain, numerous cell body groups and nerve terminals show both enkephalin- and SP-immunoreactivity. This is particularly true in the hypothalamus, amygdaloid complex, the extrapyramidal system and limbic cortical areas (265, 254, 96, 346). In the primate globus pallidus, dense networks of both enkephalin and SP are present with a somewhat different pattern. Thus, while the enkephalin immunoreactivity is very dense in the external segment, SP immunoreactivity is most prominent in the internal segment (223).

A striking similarity has also been observed between the anatomical distribution of opiate receptor binding sites and presence of SP in the rat brain and spinal cord (312).

The finding that certain neurons in the substantia gelatinosa are innervated by both enkephalin and SP provides the structural basis for a postsynaptic interaction of these peptides in pain modulation. This hypothesis is further elucidated by a series of release studies. The potassium-evoked release of SP from the rat spinal trigeminal nucleus is dose-dependently inhibited by morphine in a naloxone-reversible way (312). In the nucleus raphe magnus, which is implicated in mechanisms of anti-nociception, the content of SP is significantly elevated after administration of morphine (365), which also might indicate an inhibition of SP release. In slices from the rat substantia nigra, on the other hand, morphine is not able to inhibit the potassium-evoked release of SP (312), but enkephalin inhibits release of SP from cultured sensory neurons (463). This type of inhibition is, however, not unique since opiate drugs also inhibit the release of classical transmitters such as noradrenaline (457) and dopamine (415). Nevertheless, these observations speak in favour of the presence of presynaptic opiate receptors on primary afferent nerve terminals containing SP, which inhibit the release of SP and thereby the activation of postsynaptic neurons (265, 312). The existence of such presynaptic receptors on primary afferent terminals is supported by the finding that the density of opiate receptor binding sites in the dorsal horn decreases after dorsal rhizotomy (367). On the other hand the presence of axo-axonal synapses between enkephalin-interneurons and SP primary afferents have not so far been demonstrated with electron microscopy (292).

SP and enkephalin are also present in peripheral nerves such as the vagus (421) and in mammalian sympathetic ganglia (261). The functional interaction between the two peptides in peripheral nervous tissues was elucidated by Konishi et al. (352–354) (see section VII C).

When discussing the functional role of SP in sensory neurons it is important to consider that SP is not the only peptide present in these neurons (fig. 5). Thus, several populations of *peptidergic neurons* belong to the group of small diameter primary afferent fibers. Somatostatin (255, 346), vasoactive intestinal polypeptide (422), gastrin/cholecystokinin (422, 112), and angiotensin II (184) immunoreactivity have been observed in primary afferents in the dorsal horn of the spinal cord and in the spinal ganglia. However, the localization and density of these peptides vary. While SP immunoreactivity is most intense in lamina I and the superficial part of lamina II in the dorsal horn, somatostatin is located somewhat deeper. The gastrin/cholecystokinin fibers have a distribution pattern, which is almost identical to that of SP (303) and recent evidence suggests coexistence of SP and cholecystokinin in neurons of the dorsal root ganglia (112) and in the periaqueductal grey (271).

The amygdaloid complex contains, besides SP, vasoactive intestinal polypeptide, cholecystokinin, neurotensin, enkephalin, and somatostatin (543). Each of these neuropeptides has a characteristic distribution and no coexistence has been identified in cell bodies or nerve fibers (543). There are also neuropharmacological similarities between several of these peptides. Thus, like SP, the vasoactive intestinal polypeptide also depolarizes dorsal horn neurons and increases their excitability (307).

In peripheral branches of the primary sensory neuron, SP is so far the only peptide that has been traced with sufficient certainty. On the other hand, in peripheral nerves, such as the vagus, splanchnic, and sciatic nerve, enkephalin, gastrin/cholecystokinin, somatostatin, and vasoactive intestinal polypeptide immunoreactivity is present besides SP (421). In the vagus nerve complex as well as in the sciatic nerve, SP, gastrin/cholecystokinin, and somatostatin seem to be present in sensory neurons, while enkephalin is apparently contained in axons of motor fibers.

The effect of capsaicin on the SP content and transport in primary sensory neurons has been briefly described above. Recent studies have, however, shown that the effect of capsaicin is not restricted to SP. Thus, the cholecystokinin-like immunoreactivity in the dorsal horn almost completely disappears after capsaicin treatment (303). There is also a reduction in somatostatin- and vasoactive intestinal polypeptide-like immunoreactivity, although to a lesser extent (195, 464, 303). In the dorsal roots of the rat, on the other hand, somatostatin is depleted to 80% to 95%, that is to about the same extent as that of SP (195, 464). Finally, while SP occupies only a minor population of the sensory neurons, the neuro-

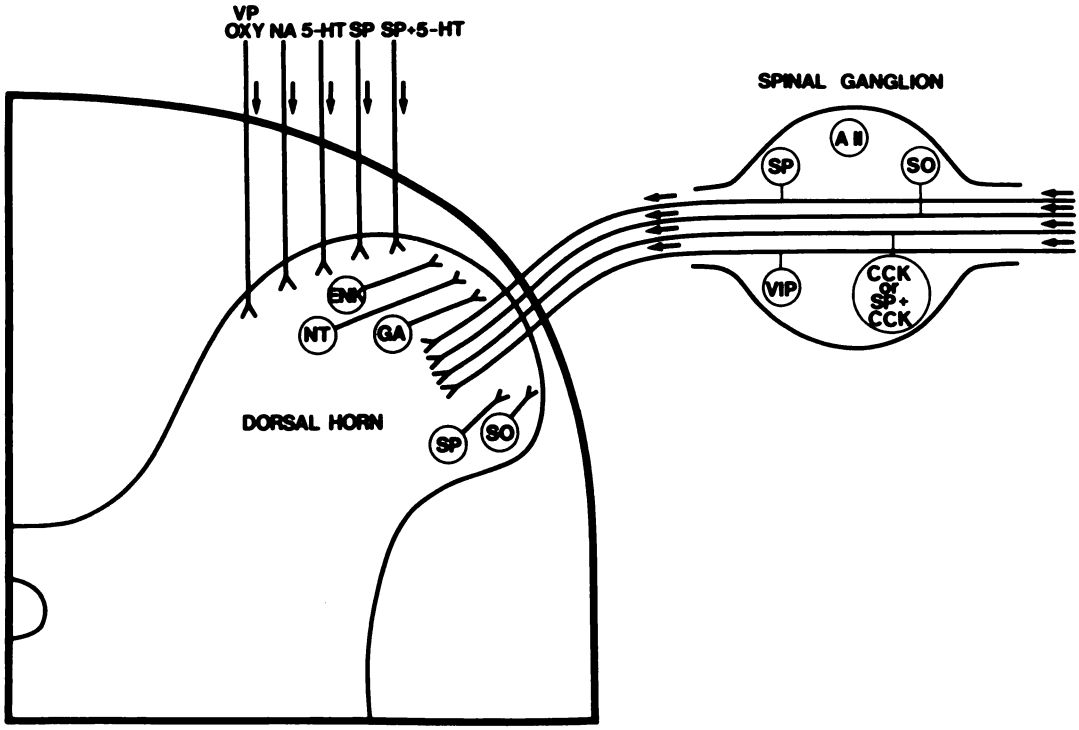

FIG. 5. Schematic illustration of peptidergic afferents to and interneurons in the dorsal horn of the spinal cord. The peptides are present in 1) primary sensory neurons, 2) interneurons, and 3) descending systems. AII, angiotension II; CCK, cholecystokinin; ENK, enkephalin; GA, gastrin; 5-HT, 5-hydroxytryptamine; NA, noradrenaline; NT, neurotensin; OXY, oxytocin; SO, somatostatin; SP, substance P; VIP, vasoactive intestinal polypeptide; VP, vasopressin. [Modified from Hökfelt et al., 1980 (261).]

toxic effect of capsaicin includes up to 95% of the unmyelinated fibers in the rat dorsal roots (370).

It is thus evident that capsaicin, while being a useful tool in the studies on the distribution and function of the unmyelinated fibers in the primary sensory neurons, does not represent a specific neurotoxin for SP-containing sensory fibers. Furthermore, in contrast to systemic and intrathecal administration, intraventricular capsaicin in the rat alters analgesic responsivity but does not appreciably deplete SP in the substantia gelatinosa of the spinal cord and trigeminal nucleus (32). These findings seem to indicate that alteration in several peptide systems may be involved in the analgesic effect of capsaicin.

XXII. Is SP a Neurotransmitter or Modulator?

As early as 1953 (387), a role as a neurotransmitter in various parts of the central and peripheral nervous system was proposed for SP. Special interest has been focused on the primary sensory neurons, particularly nociceptive primary afferents, the sympathetic ganglia, and the SP pathways in the striato-nigral tract, which are probably involved in sensory motor integration. It is, therefore, important to examine to what extent the requirements for a transmitter candidate (448, 493) are met by SP.

A. SP and Transmitter Criteria

1. Location. In the primary sensory neuron, SP is present in unmyelinated nerve fibers of the type known to be involved in transmission of nociceptive information (262, 98, 14, 292). Densely occurring SP nerve terminals in the spinal cord form axo-dendritic synapses with dorsal horn cells (523, 123). In the prevertebral ganglia, SP nerve terminals form similar synapses around the principal cells (441). The extremely large number of SP fibers in the substantia nigra, which synapse upon dendrites in the zona reticulata (584), have their cell bodies in the anterior striatum (47, 288, 325, 586). In all these areas SP is present in synaptosomal fractions and in large granular vesicles in the nerve endings (102, 14, 122, 292, 584).

2. Biosynthesis. SP is biosynthesized in the dorsal root ganglia from where it migrates both centrally to the dorsal horn of the spinal cord and substantia gelatinosa of the medulla and in the peripheral direction of the sensory neurons to the nerve terminals in peripheral tissues (610, 260, 14, 616, 232). Similarly, SP is synthesized in cell bodies of the striatum and transported to nerve terminals in the substantia nigra (585). It is likely that SP, like many other peptides, is formed from a precursor in the cell somata and that appropriate enzyme or enzyme systems produce SP by cleavage from the precursor.

3. Inactivation. Several tissues have a high capacity for inactivating SP. In the nervous system (377) and the gastrointestinal tract (515) this capacity is well correlated to the SP concentration of the tissue. Highly purified enzymatic fractions with a specific SP degradative capacity have been isolated from nervous tissue and their

site of cleavage of the SP molecule has been identified (21, 381).

4. Release. A potassium-evoked release of SP has been well documented in the spinal cord (496, 312, 197, 1), mesenteric ganglia (352), and substantia nigra (567, 310, 621). This release shows a true dependency on the local extracellular calcium concentration (496, 567, 352). Similarly, SP is released from both central and peripheral nerve terminals of sensory neurons during electrical stimulation (496, 490, 28).

5. Mimicry. Local microiontophoretic application of SP causes excitation of those units in the spinal cord that are stimulated by noxious cutaneous stimulation (247). Also, in the substantia nigra SP has an excitatory effect (114, 639). In the inferior mesenteric ganglion and in the myenteric plexus, SP elicits the same type of slow depolarization and increase in membrane resistance as is induced by presynaptic stimulation in the atropinized preparation (133, 352). In peripheral tissues, close-arterial infusion of SP in physiological doses elicits local effects identical to those obtained by antidromic stimulation of sensory nerves (397).

6. Antagonism. SP analogues with specific SP antagonistic but minimal agonistic effects not only block the pharmacological actions of SP in various organs (151, 30, 548), but also influence per se the physiological events. When given intrathecally, these analogues have a significant anti-nociceptive effect (393, 527, 2). Electrically induced, noncholinergic contractions of smooth muscle (373) or antidromic vasodilatation (549, 390) are inhibited by the SP antagonists.

Of these different criteria for a compound being identified as a true transmitter, Orrego (493) proposed identity of action, induced release, and vesicular location as primary, while pharmacological antagonism, transmitter synthesizing system, and differential distribution were considered as secondary criteria. It is apparent that SP fulfills all these requirements to a large extent.

B. Role of SP

1. Sympathetic Ganglia. There is little doubt that the strongest evidence so far accumulated for a transmitter role for SP is found in some mammalian sympathetic ganglia. These experiments have convincingly shown that SP is released from synapses in the prevertebral ganglia and serves as a transmitter generating noncholinergic, slow excitatory, postsynaptic potentials (133, 352).

2. Spinal Cord. On the basis of the above-mentioned facts concerning localization, synthesis, release, and mimicry, SP is frequently mentioned as a candidate for a sensory transmitter. This suggestion is further supported by the observation that stimulation of spinal cord SP receptors produces a sensory experience (525) and that depression of SP release is associated with analgesic mechanisms (312, 463, 648). Furthermore, the recent observation that SP also in spinal neurons produces slow noncholinergic excitatory postsynaptic potentials, which are depressed by [D-Ala^2] Met-enkephalin amide (501), suggests transmitter function of SP in the spinal cord similar to that in the sympathetic ganglion.

On the other hand, several observations are difficult to combine with a true transmitter function but are more in line with a modulating role serving to modify rather than directly transmit the neural message of sensation. The slow and delayed response of local iontophoretic application of SP in the spinal cord (249, 361) does not mimic the fast depolarization expected from primary afferent actions. Furthermore, the threshold nature of depolarization, induced in conjunction with the slow time course (470), seems to indicate that SP acts by modifying the level of excitability of motoneurons rather than transmitting the excitatory impulses. However, in the isolated rat spinal cord in vitro, the delay time for the SP-induced depolarization was not much longer than hundreds of milliseconds (502, 503). Removal of the pia mater by collagenase digestion also reduced the delay in onset of SP action on motoneurons (652). Furthermore, the rat locus coeruleus neurons respond to iontophoretic application of SP with an excitation, which in onset and disappearance is strikingly similar to that produced by classical excitatory transmitters such as acetylcholine (220, 151). Similarly, Vincent and Barker (633) reported rapidly depolarizing responses of cultured spinal neurons to SP. It is probable, therefore, that the earlier described slow onset of the SP action at iontophoretic application in the spinal cord was due to technical problems (222). On the other hand, a relatively slow transmitter process would not be contradictory because the transmission of noxious or temperature messages elicit sustained and slow reactions that need not to be as fast as, for instance, a motor reflex.

The view that the effect of local application of SP is more compatible with a modulator of neuronal excitability than with a transmitter mediating sensory input was further stressed by Krivoy and co-workers (358), who were unable to record any SP-induced spontaneous discharge of motoneurons in the decerebrated low spinal cat. Instead, the postsynaptic response to presynaptic stimuli was altered in the presence of SP. At high doses these alterations were manifested as facilitation of synaptic transmission, whereas at lower doses an inhibition occurred. This concept is also in conformity with the observation that SP has a biphasic influence on response to painful stimuli, with lower doses causing analgesia and higher ones hyperalgesia (177). It also explains why intracerebroventricular administration of SP in the cat causes both behavioral excitation and depression (167).

The recent observation of a coexistence between classical neurotransmitters and peptides in certain neurons is interesting in this connection and might contribute to a better understanding of the functional role of the synaptic peptides. Some mesencephalic dopamine neurons contain cholecystokinin, which facilitates the re-

lease of the amine transmitter (271). In exocrine glands, vasoactive intestinal polypeptide acts by enhancing the physiological response caused by the coexisting acetylcholine (417). These and other observations seem to indicate that neuropeptides often act as modulators to the classical transmitters rather than causing a postsynaptic response by themselves.

Recent observations (640, 559) further question the concept that SP might be a sensory transmitter. As mentioned earlier in this review, it has been shown repeatedly that section of the sciatic nerve results in a substantial depletion of SP in the central terminals of the fine afferents in the cat nerve (314, 16, 640). If SP is an essential transmitter released from C fibers, a decrease of excitatory effectiveness of C fiber stimulation would be expected after depletion of SP in the dorsal horns of the spinal cord. However, Wall et al. (640) were unable to find any change in the central excitatory effect of an afferent C fiber volley 7 to 19 days after the fibers had been cut in the periphery. This observation suggests that the C fiber terminals contain other compounds besides SP, effectively transmitting the excitatory impulses. Also, a 60% depletion of SP in the trigeminal nucleus caudalis by neonatal treatment with capsaicin in the rat did not alter the proportions of neurons responding to noxious or non-noxious stimulation of the face (559). It is, however, quite probable that only small amounts of SP are needed for the SP neurons to function.

Besides SP, several other peptides such as somatostatin, vasoactive intestinal polypeptide, and cholecystokinin have been identified in small sensory neurons and their terminals in the dorsal horn (266). They are all more or less depleted from small sensory neurons after capsaicin treatment (271), which is thus not specifically affecting the SP-containing sensory fibers. Also, application of capsaicin to the rat sciatic nerve leads to a complete block of axoplasmic transport of not only SP, but also somatostatin (199). Therefore, the consideration of SP as the sole transmitter of nociceptive information must wait until a better understanding of the tentative role of the other peptides has been achieved.

3. Substantia Nigra. Ample circumstantial evidence exists for a distinct excitatory striato-nigral SP pathway, where SP is primarily present in the axonal terminals (122, 584, 585). Electron microscopy of SP fibers have shown that each of these fibers have SP immunoreactive boutons with granular vesicles forming synapses with dendrites and perikarya (584). These findings indicate that neurons in the substantia nigra may receive an input from SP-containing afferents. It has also been convincingly shown that SP is released from these terminals during depolarization (567, 311, 621) and after striatal stimulation (451). When applied locally, SP produces behavioural reactions that are influenced by the dopaminergic nigro-striatal pathway (597, 321). These observations suggest the existence of a SP striato-nigral system which, besides the parallel GABA-system, modulates the release and postsynaptic actions of dopamine. However, the recent finding of different types of synaptic contact between SP immunoreactive boutons and neuronal cell bodies in the substantia nigra (584) gives a structural basis for a release of SP at synaptical junctions. Furthermore, the observation that nondopamine cells in the substantia nigra are more sensitive to SP than dopamine neurons (529) indicate functions for SP besides modulating the dopaminergic system.

XXIII. Concluding Remarks

Half a century of research on SP, substantially accelerated during the last 8 years, has brought forward a detailed knowledge of the distribution and properties of SP. With highly sensitive and specific RIA and immunohistochemical techniques as well as novel approaches for raising antisera, a detailed biochemical and morphological characterization of the neuronal systems containing SP has been performed and extended to the ultrastructural level. It has been possible to demonstrate a release of SP in various parts of the central and peripheral nervous system as well as inactivation mechanisms under experimentally well defined situations. However, structurally similar peptides have been identified partly in the same areas as SP, which makes crossreactivity and exact identity of the endogenous peptides still a problem.

In spite of the considerable amount of data on the distribution and actions of SP being accumulated, our knowledge of the function of SP in various organs is still incomplete. The multiplicity and overlap of different systems containing SP and other peptides in various parts of the nervous systems have made it difficult to elucidate the functional role of each of these peptides. The recent finding of a coexistence between SP (and other peptides) and classical neurotransmitters probably requires an expansion of our present concept of chemical transmission. The results so far reached point towards a role of these peptides as modulators rather than conventional transmitters. Thus, observations on an interaction of SP with nicotinic responses seem to indicate that neuropeptides may modulate synaptic transmission by interacting at the receptor level and potentiate the effect of coexisting classical neurotransmitters. SP has been attributed a role as a transmitter or modulator in many parts of the central and peripheral nervous system. The evidence that SP, released from axon collaterals of primary afferents in prevertebral ganglia, serves as a transmitter that generates noncholinergic, slow excitatory postsynaptic potentials seems to be the most convincing so far, and it is possible that similar mechanisms operate also in the spinal cord.

The suggestion that SP is involved in the modulation of intestinal motility is supplied by studies on the distribution of SP nerve cells and fibers in the enteric nervous

system, by indirect demonstration of a release of SP from these neurons,* and by the depolarizing and muscle-stimulating effects of SP in the gastrointestinal tract. However, the precise functional role of SP and its integration with cholinergic and other putative peptidergic mechanisms are still not clear.

It is even more difficult to establish specific roles for SP in the brain. Its uneven distribution and strong excitatory effects in several areas speak in favour of fundamental but still unclear functions. The localization, release, and mimicry of SP has been most intensively studied in the striato-nigral tract and several observations seem to indicate that SP fibers have an excitatory influence on dopaminergic neurons, but the striato-nigral SP fibers also affect other efferent neurons indicating a great complexity in the interaction between different neuronal systems in this area.

The importance of SP in transmission of nociceptive information has been extensively studied, but is still unclear. The recent observation that highly specific SP receptor antagonists are potent anti-nociceptive agents, however, strongly suggests that SP is involved in this mechanism. The discoveries of these antagonists as well as of stable SP analogues constitute important progress and these new compounds have already proven to be effective tools in the study of the physiological significance of SP and possibly also in developing new types of drugs.

Acknowledgments. I am particularly indebted to my collaborators of many years, Dr. Tomas Hökfelt and Dr. Sune Rosell, for constructive discussions and criticism during the preparation of this review. My sincere thanks are also extended to Mrs. Margareta Palmlöw and Mrs. Märta Wretlind for their excellent secretarial help.

The Swedish Medical Research Council and the U.S. Department of Health and Human Services through the National Institutes of Health provided the main support to the studies performed by our research group.

REFERENCES

1. AKAGI, H., OTSUKA, M., AND YANAGISAWA, M.: Identification by high-performance liquid chromatography of immunoreactive-substance P released from isolated rat spinal cord. Neurosci. Lett. **20:** 259–263, 1980.
2. ÅKERMAN, B., ROSELL, S., AND FOLKERS, K.: Intrathecal [D-Pro^2, D-Trp^{7,9}] SP elicits hypoalgesia and motor blockade in the rat and antagonizes noxious responses induced by substance P. Acta Physiol. Scand. **114:** 631–633, 1982.
3. ALBANO, J., BHOOLA, K. D., AND HARVEY, R. F.: The effect of substance P on cyclic AMP and cyclic GMP levels in actively secreting pancreatic lobules. J. Physiol. (Lond.) **275:** 60P–61P, 1978.
4. ALM, P., ALUMETS, J., BRODIN, E., HÅKANSON, R., NILSSON, G., SJÖBERG, N.-O., AND SUNDLER, F.: Peptidergic (substance P) nerves in the genito-urinary tract. Neuroscience **3:** 419–425, 1978.
5. AMIN, A. H., CRAWFORD, T. B. B., AND GADDUM, J. H.: The distribution of substance P and 5-hydroxy-tryptamine in the central nervous system of the dog. J. Physiol. (Lond.) **126:** 596–618, 1954.
6. ANDERSEN, R. K., LUND, J. P., AND PUIL, E.: Enkephalin and substance P effects related to trigeminal pain. Can. J. Physiol. Pharmacol. **56:** 216–222, 1978.
7. ANDERSON, D. J., HANNAM, A. C., AND MATTHEWS, B.: Sensory mechanisms in mammalian teeth and their supporting structures. Physiol. Rev. **50:** 171–195, 1970.
8. ÄNGGÅRD, A., LUNDBERG, J. M., HÖKFELT, T., NILSSON, G., FAHRENKRUG. I., AND SAID, S. J.: Innervation of the cat nasal mucosa with special reference to relations between peptidergic and cholinergic neurons. Acta Physiol. Scand. suppl. 473, p. 50, 1979.
9. ANTONOWICZ, U. JAKUBOWSKA-NAZIEMBLO, B., CANNON, D., AND POWELL, D.: Immunoreactive substance P content in median eminence and pituitary gland during oestrus, dioestrus and after anterior hypothalamic deafferentation. Endokrinologie **79:** 25–34, 1982.
10. ARENDSHORST, W. J., COOK, M. A., AND MILLS, I. H.: Effect of substance P on proximal tubular reabsorption in the rat. Am. J. Physiol. **230:** 1662–1667, 1976.
11. ARVIDSSON, U. B., PERNOW, B., AND SWEDIN, B.: Chromatographic separation of histaminase and substance P-inactivating enzymes in extracts of kidney tissue. Acta Physiol. Scand. **35:** 338–344, 1956.
12. BAERTSCHI, A. J., ZINGG, H. H., AND DREIFUSS, J. J.: Enkephalins, substance P, bradykinin and angiotensin II: Differential sites of action on the hypothalamo-neurohypophyseal system. Brain Res. **220:** 107–119, 1981.
13. BARASI, S.: Responses of substantia nigra neurones to noxious stimulation. Brain Res. **171:** 121–130, 1979.
14. BARBER, R. P., VAUGHN, J. E., SLEMMON, J. R., SALVATERRA, P. M., ROBERTS, E., AND LEEMAN, S. E.: The origin, distribution and synaptic relationships of substance P axons in rat spinal cord. J. Comp. Neurol. **184:** 331–352, 1979.
15. BARBIN, G., GARBERG, M., SCHWARTZ, J.-C., AND STORM-MATHISEN, J.: Histamine synthesizing afferents to the hippocampus region. J. Neurochem. **26:** 258–263, 1976.
16. BARBUT, D., POLAK, J. M., AND WALL, P. D.: Substance P in spinal cord dorsal horn decreases following peripheral nerve injury. Brain Res. **205:** 289–298, 1981.
17. BELCHER, G., AND RYALL, R. W.: Substance P and Renshaw cells: A new concept of inhibitory synaptic interaction. J. Physiol. (Lond.) **272:** 105–119, 1977.
18. BELESLIN, D., AND VARAGIĆ, V.: The effect of substance P on the peristaltic reflex of the isolated guinea-pig ileum. Br. J. Pharmacol. **13:** 321–325, 1958.
19. BELESLIN, D., AND VARAGIĆ, V.: The effect of substance P on the response of the isolated guinea-pig ileum of acetylcholine, nicotine, histamine and 5-hydroxytryptamine. Arch. Int. Pharmacodyn. Thér. **126:** 321–322, 1960.
20. BENUCK, M., GRYNBAUM, A., AND MARKS, N.: Breakdown of somatostatin and substance P by cathepsin D purified from calf brain by affinity chromatography. Brain Res. **143:** 181–185, 1977.
21. BENUCK, M., AND MARKS, N.: Enzymatic inactivation of substance P by a partially purified enzyme from rat brain. Biochem. Biophys. Res. Commun. **65:** 153–160, 1975.
22. BERGER, H., FENCHER, K., ALBRECHT, E., AND NIEDRICH, H.: Substance P: *In vitro* inactivation by rat brain fractions and human plasma. Biochem. Pharmacol. **28:** 3173–3180, 1979.
23. BERGMANN, J., BIENERT, M., NIEDRICH, H., MEHLIS, B., AND OEHME, P.: Uber den Einfluss der Kettenlänge bei C-terminalen Sequenzen der Substanz P—in Vergleich mit analogen Physalaemin-und Eledoisin-Peptiden—auf die Wirksamkeit am Meerschweinchen-Ileum. Experientia (Basel) **30:** 401–403, 1974.
24. BERGMANN, J., OEHME, P., AND BIENERT, M.: Untersuchungen zur biologischen Aktivität und zum Wirkungsmechanismus von Substanz P-analogen an glattmuskulären Organen. Pharmazie **34:** 344–349, 1979.
25. BERNARDI, L., BOSISIO, G., CHILLEMI, F., DE CARO, G., DE CASTIGLIONE, R., ESPAMER, V., GLAESSU, A., AND GOFFREDO, O.: Synthetic peptides related to eledoisin. Experientia (Basel) **22:** 29–31, 1966.
26. BERTACCINI, G., DE CASTIGLIONE, R., AND SCARPIGNATO, C.: Effect of substance P and its natural analogues on gastric emptying of the conscious rat. Br. J. Pharmacol. **72:** 221–223, 1981.
27. BERTACCINI, G., AND CORUZZI, G.: Action of some natural peptides on the stomach of the anaesthetized rat. Naunyn-Schmiedeberg's Arch. Pharmacol. **298:** 163–166, 1977.
28. BILL, A., STJERNSCHANTZ, J., MANDAHL, A., BRODIN, E., AND NILSSON, G.: Substance P: Release on trigeminal nerve stimulation, effects in the eye. Acta Physiol. Scand. **106:** 371–373, 1979.
29. BJÖRKLUND, A. J., EMSON, P. C., GILBERT, R. F. T., AND SKAGERBERG, G.: Further evidence for the possible co-existence of 5-hydroxytryptamine and substance P in medullary raphe neurones of rat brain. Br. J. Pharmacol. **66:** 112P–113P, 1979.
30. BJÖRKROTH, U, ROSELL, S., XU, T-C, AND FOLKERS, K.: Pharmacological characterization of four related substance P antagonists. Acta Physiol. Scand. **116:** 167–173, 1982.
31. BLUMBERG, S., AND TEICHBERG, V. I.: Biological activity and enzymic degradation of substance P analogs: Implications for studies of the substance P receptor. Biochem. Biophys. Res. Commun. **90:** 347–354, 1979.
32. BODNAR, R. J., KIRCHGESSNER, A., NILAVER, G., MULHERN, J., AND ZIMMERMAN, E. A.: Intraventricular capsaicin: Alterations in analgesic responsitivity without depletion of substance P. Neuroscience **7:** 631–638, 1982.
33. BOILEAU, J-C., CAMPEAU, L., AND BIRON, P.: Pulmonary fate of histamine, isoproterenol, physalaemin, and substance P. Can. J. Physiol. Pharmacol.

* At the Substance P symposium in Dublin in April 1983, Holzer, and Gintzler, Jaffe, and Baron were able to show a calcium-dependent release of SP from the myenteric plexus in amounts sufficient to cause a contraction of the longitudinal muscle.

48: 681–684, 1970.
34. BOISSONNAS, R. A., FRANZ, J., AND STÜRMER, E.: On the chemical characterization of substance P. Ann. N.Y. Acad. Sci. 104: 376–377, 1963.
35. BOKSA, P., ST PIERRE, S., AND LIVETT, B. G.: Characterization of substance P and somatostatin receptors on adrenal chromaffin cells using structural analogues. Brain Res. 245: 275–283, 1982.
36. BOWKER, R. M., WESTLUND, K. N., SULLIVAN, M. C., WILBER, J. F., AND COULTER, J. D.: Transmitters of the raphe-spinal complex: Immunocytochemical studies. Peptides 3: 291–298, 1982.
37. BRIMIJOIN, S., LUNDBERG, J. M., BRODIN, E., HÖKFELT, T., AND NILSSON, G.: Axonal transport of substance P in the vagus and sciatic nerves of the guinea pig. Brain Res. 191: 443–457, 1980.
38. BRODIN, E., ALUMETS, J., HÅKANSON, R., LEANDER, S., AND SUNDLER, F.: Immunoreactive substance P in the chicken gut: Distribution, development and possible functional significance. Cell Tissue Res. 216: 455–469, 1981.
39. BRODIN, E., GAZELIUS, B., LUNDBERG, J. M., AND OLGART, L.: Substance P in trigeminal nerve endings: Occurrence and release. Acta Physiol. Scand. 111: 501–503, 1981.
40. BRODIN, E., GAZELIUS, B., OLGART, L., AND NILSSON, G.: Tissue concentration and release of substance P-like immunoreactivity in the dental pulp. Acta Physiol. Scand. 111: 141–149, 1981.
41. BRODIN, E., AND NILSSON, G.: Concentration of substance P-like immunoreactivity (SPLI) in tissues of dog, rat and mouse. Acta Physiol. Scand. 112: 305–312, 1981.
42. BRODIN, E., NILSSON, G., AND FOLKERS, K.: Characterization of two substance P antisera. Acta Physiol. Scand. 114: 53–57, 1982.
43. BRODIN, E., SJÖLUND, K., HÅKANSON, R., AND SUNDLER, F.: Substance P-containing nerve fibers are numerous in human intestinal mucosa. Gastroenterology, in press.
44. BROWN, C. L., AND HANLEY, M. R.: The effects of substance P and related peptides on α-amylase release from rat parotid gland slices. Br. J. Pharmacol. 73: 517–523, 1981.
45. BROWN, M., AND VALE, W.: Effects of neurotensin and substance P on plasma insulin, glucagon and glucose levels. Endocrinology 98: 819–822, 1976.
46. BROWNSTEIN, M. J., MROZ, E. A., KIZER, S., PALKOVITS, M., AND LEEMAN, S. E.: Regional distribution of substance P in the brain of the rat. Brain Res. 116: 299–305, 1976.
47. BROWNSTEIN, M. J., MROZ, E. A., TAPPAZ, M. L., AND LEEMAN, S. E.: On the origin of substance P and glutamic acid decarboxylase (GAD) in the substantia nigra. Brain Res. 135: 315–323, 1977.
48. BUCK, S. H., BURKS, T. F., BROWN, M. R., AND YAMAMURA, H. I.: Reduction in basal ganglia and substantia nigra substance P levels in Huntington's disease. Brain Res. 209: 464–469, 1981.
49. BUCK, S. H., DESHMUKH, P. P., YAMAMURA, H. I., AND BURKS, T. F.: Thermal analgesia and substance P depletion induced by capsaicin in guinea-pigs. Neuroscience 6: 2217–2222, 1981.
50. BUCSICS, A., AND LEMBECK, F.: *In vitro* release of substance P from spinal cord slices by capsaicin congeners. Eur. J. Pharmacol. 71: 71–77, 1981.
51. BURCHER, E., ATTERHÖG, J-H., PERNOW, B., AND ROSELL, S.: Cardiovascular effects of substance P: Effects on the heart and regional blood flow in the dog. *In* Substance P (Nobel Symposium 37), ed. by U.S. von Euler and B. Pernow, pp. 261–268, Raven Press, New York, 1977.
52. BURCHER, E., NILSSON, G., ÅNGGÅRD, A., AND ROSELL, S.: An attempt to demonstrate release of substance P from the tongue, skin and nose of dog and cat. *In* Substance P (Nobel Symposium 37), ed. by U.S. von Euler and B. Pernow, pp. 183–185, Raven Press, New York, 1977.
53. BURKS, T. F., BUCK, S. H., MILLER, M. S., DESHMUKH, P. P., AND YAMAMURA, H. I.: Characterization in guinea pigs of the sensory effects of the putative substance P neurotoxin capsaicin. Proc. West. Pharmacol. Soc. 24: 353–357, 1981.
54. BURY, R. W., AND MASHFORD, M. L.: Interactions between local anesthetics and spasmogens on the guinea-pig ileum. J. Pharmacol. Exp. Ther. 197: 633–640, 1976.
55. BURY, R. W., AND MASHFORD, M. L.: Biological activity of C-terminal partial sequences of substance P. J. Med. Chem. 19: 854–856, 1976.
56. BURY, R. W., AND MASHFORD, M. L.: The stability of synthetic substance P in blood. Eur. J. Pharmacol. 45: 257–260, 1977.
57. BURY, R. W., AND MASHFORD, M. L.: A pharmacological investigation of synthetic substance P on the isolated guinea-pig ileum. Clin. Exp. Pharmacol. Physiol. 4: 453–461, 1977.
58. BURY, R. M., AND MASHFORD, M. L.: Substance P: Its pharmacology and physiological roles. Aust. J. Exp. Biol. Med. Sci. 55: 671–735, 1977.
59. BUTLER, J. M., AND HAMMOND, B. R.: The effects of sensory denervation on the responses of the rabbit eye to prostaglandin E_1, bradykinin and substance P. Br. J. Pharmacol. 69: 495–502, 1980.
60. BUTLER, J. M., POWELL, D., AND UNGER, W. G.: Substance P levels in normal and sensorily denervated rabbit eyes. Exp. Eye Res. 30: 311–313, 1980.
61. CAMPBELL, W. B., AND WARD, P. E.: Renal metabolism of substance P in the dog. Life Sci. 24: 1995–2001, 1979.
62. CAMRAS, C. B., AND BITO, L. Z.: The pathophysiological effects of nitrogen mustard on the rabbit eye. II. The inhibition of the initial hypertensive phase by capsaicin and the apparent role of substance P. Invest. Ophthalmol. Visual Sci. 19: 423–428, 1980.
63. CANNON, D., SKRABANEK, P., AND POWELL, D.: Difference in behaviour between synthetic and endogenous substance P in human plasma. Naunyn-Schmiedeberg's Arch. Pharmacol. 307: 251–255, 1979.
64. CANNON, D., SKRABANEK, P., POWELL, D., AND HARRINGTON, M. G.: Immunological characterization of two substance P antisera with substance P fragments and analogues. Biochem. Soc. Trans. 5: 1736–1738, 1977.
65. CARANIKAS, S., MIZRAHI, J., D'ORLÉANS-JUSTE, P., AND REGOLI, D.: Antagonists of substance P. Eur. J. Pharmacol. 77: 205–206, 1982.
66. CARLSSON, A., MAGNUSSON, T., FISHER, G. H., CHANG, D., AND FOLKERS, K.: Effect of synthetic substance P on central monoaminergic mechanisms. *In* Substance P (Nobel Symposium 37), ed. by U.S. von Euler and B. Pernow, pp. 201–205, Raven Press, New York, 1977.
67. CASPERS, H., AND STERN, P.: Die Wirkung von Substanz P auf das Dendritenpotential und die Gleichspannungskomponente des Neocortex. Pflügers Arch. Gesamte Physiol. Menschen Tiere 273: 94–110, 1961.
68. CELANDER, O., AND FOLKOW, B.: The nature and distribution of afferent fibres provided with the axon reflex arrangement. Acta Physiol. Scand. 29: 359–370, 1953.
69. CHAHL, L. A.: Evidence that the contractile response of the guinea-pig ileum to capsaicin is due to substance P release. Naunyn-Schmiedeberg's Arch. Pharmacol. 319: 212–215, 1982.
70. CHAHL, L. A., AND MANLEY, S. W.: Inflammatory peptide in spinal cord: Evidence that the mediator of antidromic vasodilatation is not substance P. Neurosci. Lett. 18: 99–103, 1980.
71. CHANG, M. M., AND LEEMAN, S. E.: Isolation of a sialogogic peptide from bovine hypothalamic tissue and its characterization as substance P. J. Biol. Chem. 245: 4784–4790, 1970.
72. CHANG, M. M., LEEMAN, S. E., AND NIALL, H. D.: Amino acid sequence of substance P. Nature New Biol. 232: 86–87, 1971.
73. CHANNABASAVAIAH, K., AND SIVANANDAIAH, K.: Peptides related to physalaemin. Indian J. Chem. 12: 1203–1204, 1974.
74. CHAN-PALAY, V.: Combined immunocytochemistry and autoradiography after *in vivo* injections of monclonal antibody to substance P and ^{3}H-serotonin: Coexistence of two putative transmitters in single raphe cells and fiber plexuses. Anat. Embryol. 156: 241–254, 1979.
75. CHAN-PALAY, V., JONSSON, G., AND PALAY, S. L.: Serotonin and substance P coexist in neurons in the rat's central nervous system. Proc. Natl. Acad. Sci. U.S.A. 75: 1582–1586, 1978.
76. CHAN-PALAY, V., AND PALAY, S. L.: Ultrastructural identification of substance P cells and their processes in rat sensory ganglia and their terminals in the spinal cord by immunocytochemistry. Proc. Natl. Acad. Sci. U.S.A. 74: 4050–4054, 1977.
77. CHARNAY, Y., LÉGER, L., AND DUBOIS, P. M.: Distribution de la substance P et de la méthionine-enkephaline dans la partie dorsolatérale du tegmentum pontique chez le chat. Etude par immunofluorescence. C.R. Acad. Sci. 292: 435–440, 1981.
78. CHERAMY, A., MICHELOT, R., LEVIEL, V., NIEOULLON, A., GLOWINSKI, J., AND KERDELHUE, B.: Effect of the immunoneutralization of substance P in the cat substantia nigra on the release of dopamine from dendrites and terminals of dopaminergic neurons. Brain Res. 155: 404–408, 1978.
79. CHERAMY, A., NIEOULLON, A., MICHELOT, R., AND GLOWINSKI, J.: Effects of intranigral application of dopamine and substance P on the in vivo release of newly synthesized [^{3}H]dopamine in the ipsilateral caudate nucleus of the cat. Neurosci. Lett. 4: 105–109, 1977.
80. CHIPKIN, R. E., STEWART, J. M., SWEENEY, V. E., HARRIS, K., AND WILLIAMS, R.: *In vitro* activities of some synthetic substance P analogs. Arch. Int. Pharmacodyn. Ther. 240: 193–202, 1979.
81. CLARK, S. L., AND RYALL, R. W.: Inhibition by substance P of some peripheral actions of acetylcholine in the cat. Br. J. Pharmacol. 76: 595–602, 1982.
82. COHEN, S., DUSMAN, E., BLUMBERG, S., AND TEICHBERG, V. I.: *In vitro* contraction of the pupillary sphincter by substance P and its stable analogs. Invest. Ophthalmol. Visual Sci. 20: 717–721, 1981.
83. COLLINGRIDGE, G. L., AND DAVIES, J.: Actions of substance P and opiates in the rat substantia nigra. Neuropharmacology 21: 715–719, 1982.
84. CONSTANTINIDIS, J., TABAN, CH., GUNTERN, R., AND TISSOT, R.: Etude morphologique de la substance P dans le cerveau humain par immunohisto-fluorescence. Arch. Suisses Neurol. Neurochir. Psychiatr. 127: 323–355, 1980.
85. COONS, A. H.: Fluorescent antibody methods. *In* General Cytochemical Methods, ed. by J.F. Danielli, pp. 399–422, Academic Press, New York, 1958.
86. COOPER, P. E., FERNSTROM, M. H., RORSTAD, O.P., LEEMAN, S. E., AND MARTIN, J. B.: The regional distribution of somatostatin, substance P and neurotensin in human brain. Brain Res. 218: 219–232, 1981.
87. CORBIN, A., AND BEATTIE, C. W.: Inhibition of the pre-ovulatory proestrous gonadotropin surge, ovulation and pregnancy with a peptide analogue of luteinizing hormone releasing hormone. Endocrinol. Res. Commun. 2: 1–23, 1975.
88. COSTA, M., CUELLO, A. C., FURNESS, J. B., AND FRANCO, R.: Distribution of enteric neurons showing immunoreactivity for substance P in the guinea-pig ileum. Neuroscience 5: 323–331, 1980.
89. COSTA, M., FURNESS, J. B., LLEWELLYN-SMITH, I. J., AND CUELLO, A. C.: Projections of substance P-containing neurons within the guinea-pig small intestine. Neuroscience 6: 411–424, 1981.

90. COUTURE, R., FOURNIER, A., MAGNAN, J., ST-PIERRE, S., AND REGOLI, D.: Structure-activity studies on substance P. Can. J. Physiol. Pharmacol. **57:** 1427–1436, 1979.
91. COUTURE, R., GAUDREAU, P., ST-PIERRE, S., AND REGOLI, D.: The dog common carotid artery: A sensitive bioassay for studying vasodilator effects of substance P and of kinins. Can. J. Physiol. Pharmacol. **58:** 1234–1244, 1980.
92. COUTURE, R., AND REGOLI, D.: Inactivation of substance P and its C-terminal fragments in rat plasma and its inhibition by Captopril. Can. J. Physiol. Pharmacol. **59:** 621–625, 1981.
93. CRAMER, H., KOHLER, J., OEPEN, G., SCHOMBURG, G., AND SCHRÖTER, E.: Huntingtons chorea—measurements of somatostatin, substance P and cyclic nucleotides in the cerebrospinal fluid. J. Neurol. **225:** 183–187, 1981.
94. CREPAX, P., AND INFANTELLINI, F.: Analisi elletrofisiologica degli effetti della sostanza P sulla corteccia cerebrale isolata to gatto. Arch. Sci. Biol. **40:** 297–310, 1956.
95. CRYSTAL, H. A., AND DAVIES, P.: Cortical substance P-like immunoreactivity in cases of Alzheimer's disease and senile dementia of the Alzheimer type. J. Neurochem. **38:** 1781–1784, 1982.
96. CUELLO, A. C.: Enkephalin and substance P containing neurones in the trigeminal and extrapyramidal systems. *In* Advances in Biochemical Psychopharmacology, vol. 18, ed. by E. Costa and M. Trabucchi, pp. 111–123, Raven Press, New York, 1978.
97. CUELLO, A. C., DEL FIACCO, M., AND PAXINOS, G.: The central and peripheral ends of the substance P-containing sensory neurones in the rat trigeminal system. Brain Res. **152:** 499–509, 1978.
98. CUELLO, A. C., EMSON, P. C., DEL FIACCO, M., GALE, J., IVERSEN, L. L., JESSELL, T. M., KANAZAWA, I., PAXINOS, G., AND QUIK, M.: Distribution and release of substance P in the central nervous system. *In* Centrally Acting Peptides, ed. by J. Hughes, pp. 135–155, The Macmillan Press, New York, 1978.
99. CUELLO, A. C., EMSON, P. C., PAXINOS, G., AND JESSELL, T.: Substance P containing and cholinergic projections from the habenula. Brain Res. **149:** 413–429, 1978.
100. CUELLO, A. C., GALFRE, G., AND MILSTEIN, C.: Detection of substance P in the central nervous system by a monoclonal antibody. Proc. Natl. Acad. Sci. U.S.A. **76:** 3532–3536, 1979.
101. CUELLO, A. C., GAMSE, R., HOLZER, P., AND LEMBECK, F.: Substance P immunoreactive neurons following neonatal administration of capsaicin. Naunyn-Schmiedeberg's Arch. Pharmacol. **315:** 185–194, 1981.
102. CUELLO, A. C., JESSELL, T. M., KANAZAWA, I., AND IVERSEN, L. L.: Substance P: Localization in synaptic vesicles in rat central nervous system. J. Neurochem. **29:** 747–751, 1977.
103. CUELLO, A. C., AND KANAZAWA, I.: The distribution of substance P immunoreactive fibers in the rat central nervous system. J. Comp. Neurol. **178:** 129–156, 1978.
104. CUELLO, A. C., POLAK, J. M., AND PEARSE, A. G. E.: Substance P: A naturally occurring transmitter in human spinal cord. Lancet **2:** 1054–1056, 1976.
105. CUELLO, A. C., PRIESTLEY, J. V., AND MILSTEIN, C.: Immunocytochemistry with internally labeled monoclonal antibodies. Proc. Natl. Acad. Sci. U.S.A. **79:** 665–669, 1982.
106. CUELLO, A. C., PRIESTLEY, J. V., AND MATTHEWS, M. R.: Localization of substance P in neuronal pathways. *In* Substance P in the Nervous System, ed. by R. Porter, and M. O'Connor (Ciba Foundation Symposium 91), p. 55–83, Pitman, London, 1982.
107. DALE, H. M.: Pharmacology and nerve endings. Proc. R. Soc. Med. **28:** 319–332, 1935.
108. DALSGAARD, C-J., HÖKFELT, T., ELFVIN, L-G., SKIRBOLL, L., AND EMSON, P.: Substance P-containing primary sensory neurons projecting to the inferior mesenteric ganglion: Evidence from combined retrograde tracing and immunohistochemistry. Neuroscience **7:** 647–654, 1982.
109. DALSGAARD, C-J., HÖKFELT, T., SCHULTZBERG, M., LUNDBERG, J. M., TERENIUS, L., DOCKRAY, G. J., CUELLO, A. C., AND GOLDSTEIN, M.: Origin of peptide-containing fibers in the inferior mesenteric ganglion of the guinea pig: Immunohistochemical studies with antisera to substance P, enkephalin, vasoactive intestinal polypeptide, cholecystokinin and bombesin. Neuroscience **9:** 191–211, 1983.
110. DALSGAARD, C-J., JONSSON, C-E., HÖKFELT, T., AND CUELLO, A. C.: Localization of substance P-immunoreactive nerve fibers in the human digital skin. Experientia, in press.
111. DALSGAARD, C-J., RISLING, M., AND CUELLO, A. C.: Immunohistochemical localization of substance P in the lumbosacral spinal pia mater and ventral roots of the cat. Brain Res. **246:** 168–171, 1982.
112. DALSGAARD, C-J., VINCENT, S. R., HÖKFELT, T., LUNDBERG, J. M., DAHLSTRÖM, A., SCHULTZBERG, M., DOCKRAY, G. J., AND CUELLO, A. C.: Coexistence of cholecystokinin- and substance P-like peptides in neurons of the dorsal root ganglia of the rat. Neurosci. Lett. **33:** 159–163, 1982.
113. DANIEL, E. E., GONDA, T., DOMOTO, T., AND OKI, M.: The effects of substance P and met^5-enkephalin in dog ileum. Can. J. Physiol. Pharmacol. **60:** 830–840, 1982.
114. DAVIES, J. E., AND DRAY, A.: Substance P in the substantia nigra. Brain Res. **107:** 623–627, 1976.
115. DAVIES, J. E., AND DRAY, A.: Comparative effects of substance P and morphine on spinal neurones possessing stereospecific opiate receptors. *In* Iontophoretic transmitter mechanisms in the mammalian central nervous system, ed. by R.W. Ryall and J.S. Kelly, pp. 311–313, Elsevier/North Holland Biomedical Press, Amsterdam 1978.
116. DAVIES, J. E., AND DRAY, A.: Depression and facilitation of synaptic responses in cat dorsal horn by substance P administered into substantia gelatinosa. Life Sci. **27:** 2037–2042, 1980.
117. DE LANEROLLE, N. C., AND LAMOTTE, C. C.: The morphological relationships between substance P immunoreactive processes and ventral horn neurons in the human and monkey spinal cord. J. Comp. Neurol. **207:** 305–313, 1982.
118. DELBRO, D., FÄNDRIKS, L., ROSELL, S., AND FOLKERS, K: Inhibition of antidromically induced stimulation of gastric motility by substance P receptor blockade. Acta Physiol. Scand., in press.
119. DEL FIACCO, M., AND CUELLO, A. C.: Substance P and enkephalin-containing neurons in the rat trigeminal system. Neuroscience **5:** 803–815, 1980.
120. DEPALATIS, L. R., FIORINDO, R. P., AND HO, R. H.: Substance P immunoreactivity in the anterior pituitary gland of the guinea pig. Endocrinology **110:** 282–284, 1982.
121. DICK, E., MILLER, R. F., AND BEHBEHANI, M. M.: Opioids and substance P influence ganglion cells in amphibian retina. Invest. Ophthalmol. Visual Sci. **19:** (ARVO suppl.) 132, 1980.
122. DIFIGLIA, M., ARONIN, N., AND LEEMAN, S. E.: Immunoreactive substance P in the substantia nigra of the monkey: Light and electron microscopic localization. Brain Res. **233:** 381–388, 1981.
123. DIFIGLIA, M., ARONIN, N., AND LEEMAN, S. E.: Light microscopic and ultrastructural localization of immunoreactive substance P in the dorsal horn of monkey spinal cord. Neuroscience **7:** 1127–1139, 1982.
124. DOBRY, P. J. K., PIERCEY, M. F., AND SCHROEDER, L. A.: Pharmacological characterization of scratching behaviour induced by intracranial injection of substance P and somatostatin. Neuropharmacology **20:** 267–272, 1981.
125. DOI, T., AND JURNA, I.: Intrathecal substance P depresses the tail-flick response—antagonism by Naloxone. Naunyn-Schmiedeberg's Arch. Pharmacol. **317:** 135–139, 1981.
126. DOI, T., AND JURNA, I.: Intrathecal substance P depresses spinal motor and sensory responses to stimulation of nociceptive afferents—Antagonism by Naloxone. Naunyn-Schmiedeberg's Arch. Pharmacol. **319:** 154–160, 1982.
127. DOUGLAS, W. W., FELDBERG, W., PATON, W. D. M., AND SCHACHTER, M.: Distribution of histamine and substance P in the wall of dogs digestive tract. J. Physiol. (Lond.) **115:** 163–176, 1951.
128. DOUGLAS, F. L., PALKOVITS, M., AND BROWNSTEIN, M. J.: Regional distribution of substance P-like immunoreactivity in the lower brainstem of the rat. Brain Res. **245:** 376–378, 1982.
129. DUFFY, M. J., MULHALL, D., AND POWELL, D.: Subcellular distribution of substance P in bovine hypothalamus and substantia nigra. J. Neurochem. **25:** 305–307, 1975.
130. DUFFY, M. J., AND POWELL, D.: Stimulation of brain adenylate cyclase activity by the undecapeptide Substance P and its modulation by the calcium ion. Biochem. Biophys. Acta **385:** 275–280, 1975.
131. DUGGAN, A. W., GRIERSMITH, B. T., HEADLEY, P. M., AND HALL, J. G.: Lack of effect by substance P at sites in the substantia gelatinosa where met-enkephalin reduces the transmission of nociceptive impulses. Neurosci. Lett. **12:** 313–317, 1979.
132. DUN, N. J., AND JIANG, Z. G.: Non-cholinergic excitatory transmission in inferior mesenteric ganglia of the guinea-pig: Possible mediation by substance P. J. Physiol. (Lond.) **325:** 145–159, 1982.
133. DUN, N. J., AND KARCZMAR, A. G.: Actions of substance P on sympathetic neurons. Neuropharmacology **18:** 215–218, 1979.
134. DUN, N. J., AND MINOTA, S.: Effects of substance P on neurons of the inferior mesenteric ganglia of the guinea-pig. J. Physiol. (Lond.) **321:** 259–271, 1981.
135. DUNÉR, H., AND PERNOW, B.: Circulatory studies on substance P in man. Acta Physiol. Scand. **49:** 261–266, 1960.
136. EBER, O., AND LEMBECK, F.: Uber den enzymatischen Abbau der Substanz P. Naunyn-Schmideberg's Arch. exp. Pathol. Pharmakol. **229:** 139–147, 1956.
137. ECKSTEIN, N., WEHRENBERG, W. B., LOUIS, K., CARMEL, P. W., ZIMMERMANN, E. A., FRANTZ, A. G., AND FERIN, M.: Effects of substance P on anterior pituitary secretion in the female rhesus monkey. Neuroendocrinology **31:** 338–342, 1980.
138. EDIN, R., LUNDBEG, J. M., LIDBERG, P., DAHLSTRÖM, A., AND AHLMAN, H.: Atropine sensitive contractile motor effects of substance P on the feline pylorus and stomach *in vivo*. Acta Physiol. Scand. **110:** 207–209, 1980.
139. EDVINSSON, L., MCCULLOCH, J., AND UDDMAN, R.: Substance P: Immunohistochemical localization and effect upon cat pial arteries *in vitro* and *in situ*. J. Physiol. (Lond.) **318:** 251–258, 1981.
140. EDVINSSON, L., MCCULLOCH, J., ROSELL, S., AND UDDMAN, R.: Antagonism by [D-Pro^2, D-Trp^{7,9}]substance P of the cerebrovascular dilatation induced by substance P. Acta Physiol. Scand. **116:** 411–416, 1982.
141. EDVINSSON, L., AND UDDMAN, R.: Immunohistochemical localization and dilatatory effect of substance P on human cerebral vessels. Brain Res. **232:** 466–471, 1982.
142. EFENDIC, S., LUFT, R., AND PERNOW, B.: Effect of substance P on arginine-induced insulin and glucagon release from the isolated perfused rat pancreas. *In* Substance P (Nobel Symposium 37), ed. by U.S. von Euler and B. Pernow, pp. 241–245, Raven Press, New York, 1977.

143. EHRENPREIS, T., AND PERNOW, B.: On the occurrence of substance P in the rectosigmoid in Hirschprung's disease. Acta Physiol. Scand. **27:** 380–388, 1953.
144. EISON, A. S., IVERSEN, S. D., SANDBERG, B. E. B., WATSON, S. P., HANLEY, M. R., AND IVERSEN, L. L.: Substance P analog, DiMe-C7: Evidence for stability in rat brain and prolonged central actions. Science **215:** 188–190, 1982.
145. EKLUND, B., JOGESTRAND, T., AND PERNOW, B.: Effect of substance P on resistance and capacitance vessels in the human forearm. *In* Substance P (Nobel Symposium 37), ed. by U.S. von Euler and B. Pernow, pp. 275–285, Raven Press, New York, 1977.
146. EKSTRÖM, J., AND WAHLESTEDT, C.: Supersensitivity to substance P and physalaemin in rat salivary glands after denervation or decentralization. Acta Physiol. Scand. **115:** 437–446, 1982.
147. ELFVIN, L.-G., AND DALSGAARD, C. J.: Retrograde axonal transport of horseradish peroxidase in afferent fibers of the inferior mesenteric ganglion of the guinea pig. Identification of the cells of origin in dorsal root ganglia. Brain Res. **126:** 149–153, 1977.
148. ELIASSON, R., LIE, L., AND PERNOW, B.: A comparative study of substance P from intestine and brain. Br. J. Pharmacol. **11:** 137–140, 1956.
149. EMSON, P. C., ARREGUI, A., CLEMENT-JONES, V., SANDBERG, B. E. B., AND ROSSOR, M.: Regional distribution of methionine-enkephalin and substance P-like immunoreativity in normal human brain and in Huntington's disease. Brain Res. **199:** 147–160, 1980.
150. EMSON, P. C., CUELLO, A. C., PAXINOS, G., JESSELL, T., AND IVERSEN, L. L.: The origin of substance P and acetylcholine projections to the ventral tegmental area and interpeduncular nucleus in the rat. Acta Physiol. Scand. Suppl. 452, 43–46, 1977.
151. ENGBERG, G., SVENSSON, T. H., ROSELL, S., AND FOLKERS, K.: A synthetic peptide as an antagonist of substance P. Nature (Lond.) **293:** 222–223, 1981.
152. ERICHSEN, J. T., KARTEN, H. J., ELDRED, W. D., AND BRECHA, N.: Localization of substance P-like and enkephalin-like immunoreactivity within preganglionic terminals of the avian ciliary ganglion: Light and electron microscopy. J. Neurosci. **2:** 994–1003, 1982.
153. ERJAVEC, F., LEMBECK, F., FLORJANC-IRMAN, T., SKOFITSCH, G., DONNERER, J., SARIA, A., AND HOLZER, P.: Release of histamine by substance P. Naunyn-Schmiedeberg's Arch. Pharmacol. **317:** 67–70, 1981.
154. ERSPAMER, V.: The tachykinin peptide family. Trends Neurosci. **4:** 267–269, 1981.
155. ERSPAMER, V., ERSPAMER, G. F., AND LINARI, G.: Occurrence of tachykinins (Physalemin- or Substance P-like peptides) in the amphibian skin and their actions on smooth muscle preparations. *In* Substance P (Nobel Symposium 37), ed. by U.S. von Euler and B. Pernow, pp. 67–74, Raven Press, New York, 1977.
156. ERSPAMER, V., AND MELCHIORRI, P.: Active polypeptides of the amphibian skin and their synthetic analogues. Pure Appl. Chem. **35:** 463–494, 1973.
157. ESCHER, E., COUTURE, R., CHAMPAGNE, G., MIZRAHI, J., AND REGOLI, D.: Synthesis and biological activities of photoaffinity labeling analogues of substance P. J. Med. Chem. **25:** 470–475, 1982.
158. ESKAY, R. L., FURNESS, J. F., AND LONG, R. T.: Substance P activity in the bullfrog retina: Localization and identification in several vertebrate species. Science **212:** 1049–1051, 1981.
159. ESKAY, R. L., LONG, R. T., AND IUVONE, P. M.: Evidence that TRH, somatostatin and substance P are present in neurosecretory elements of the vertebrate retina. Brain Res. **196:** 554–559, 1980.
160. EULER, U. S. v.: Untersuchungen über Substanz P, die atropinfeste, darmerregende und gefässerweiternde Substanz aus Darm und Gehirn. Naunyn-Schmiedeberg's Arch. exp. Pathol. Pharmakol. **181:** 181–197, 1936.
161. EULER, U. S. v.: Preparation of substance P. Skand. Arch. Physiol. **73:** 142–144, 1936.
162. EULER, U. S. v.: Herstellung und Eigenschaften von Substanz P. Acta Physiol. Scand. **4:** 373–375, 1942.
163. EULER, U. S. v.: Substance P in subcellular particles in peripheral nerves. Ann. N.Y. Acad. Sci. **104:** 449–461, 1963.
164. EULER, U. S. v., AND GADDUM, J. H.: An unidentified depressor substance in certain tissue extracts. J. Physiol. (Lond.) **72:** 74–87, 1931.
165. EULER, U. S. v., AND LISHAJKO, F.: Presence of substance P in subcellular particles of peripheral nerves. Proc. Sci. Soc. Bosnia Herzegovina **1:** 109–112, 1961.
166. EULER, U. S. v., AND ÖSTLUND, E.: Occurrence of a substance P-like polypeptide in fish intestine and brain. Br. J. Pharmacol. **11:** 323–325, 1956.
167. EULER, U. S. v., AND PERNOW, B.: Neurotropic effects of substance P. Acta Physiol. Scand. **36:** 265–275, 1956.
168. EULER, U. S. v., AND PERNOW, B. (eds.): Substance P, Nobel Symposium 37, Raven Press, New York, 1977.
169. FALCONIERI ERSPAMER, G., ERSPAMER, V., AND PICCINELLI, D.: Parallel bioassay of physalaemin and kassinin, a tachykinin dodecapeptide from the skin of the african frog *Kassina senegalensis*. Naunyn-Schmiedeberg's Arch. Pharmacol. **311:** 61–65, 1980.
170. FOLKERS, K., CHANG, D., YAMAGUCHI, I., WAN, Y-P., RACKUR, G., AND FISHER, G.: Studies on structure and conformation of substance P toward effecting changes in biological activities. *In* Substance P (Nobel Symposium 37), ed. by U.S. von Euler and B. Pernow, pp. 19–26, Raven Press, New York, 1977.
171. FOLKERS, K., HÖRIG, J., ROSELL, S., AND BJÖRKROTH, U.: Chemical design of antagonists of substance P. Acta Physiol. Scand. **111:** 505–506, 1981.
172. FONTAINE-PERUS, J., CHANCONIE, M., POLAK, J. M., AND LE DOUARIN, N. M.: Origin and development of VIP and substance P containing neurons in the embryonic avian gut. Histochemistry **71:** 313–323, 1981.
173. FOTHERBY, K. J., MORRISH, N. J., AND RYALL, R. W.: Is lioresal (Baclofen) an antagonist of substance P? Brain Res. **113:** 210–213, 1976.
174. FRANCO, R., COSTA, M., AND FURNESS, J. B.: Evidence for the release of endogenous substance P from intestinal nerves. Naunyn-Schmiedeberg's Arch. Pharmacol. **306:** 195–201, 1979.
175. FRANCO, R., COSTA, M., AND FURNESS, J. B.: Evidence that axons containing substance P in the guinea-pig ileum are of intrinsic origin. Naunyn-Schmiedeberg's Arch. Pharmacol. **307:** 57–63, 1979.
176. FRANZ, J., BOISSONNAS, R. A., AND STÜRMER, E.: Isolerung von Substanz P aus Pferdedarm und ihre biologische und chemische Abgrenzung gegenüber Bradykinin. Helv. Chim. Acta **44:** 881–883, 1961.
177. FREDERICKSON, R. C. A., BURGIS, V., HARRELL, C. E., AND EDWARDS, J. D.: Dual actions of substance P on nociception: Possible role of endogenous opioids. Science **199:** 1359–1362, 1978.
178. FREDERICKSON, R. C. A., AND GESELLCHEN, P. D.: Analgesic and other activities of substance P and fragments. *In* Neuropeptides and Neural Transmission, IBRO Monograph Series, vol. 7, ed. by C.A. Marsan and W.Z. Traczyk, 111–120, Raven Press, New York, 1980.
179. FRIEDMAN, Z. Y., AND SELLINGER, Z.: A transient release of potassium mediated by the action of substance P on rat parotid slices. J. Physiol. (Lond.) **278:** 461–469, 1978.
180. FUJISAWA, K., AND ITO, Y.: The effects of substance P on smooth muscle cells and on neuro-effector transmission in the guinea-pig ileum. Br. J. Pharmacol. **76:** 279–290, 1982.
181. FURNESS, J., PAPKA, R. E., DELLA, N. G., COSTA, M., AND ESKAY, R. L.: Substance P-like immunoreactivity in nerves associated with the vascular system of guinea-pigs. Neuroscience **7:** 447–459, 1982.
182. FUXE, K., AGNATI, L. F., ROSELL, S., HÄRFSTRAND, A., FOLKERS, K., LUNDBERG, J. M., ANDERSSON, K., AND HÖKFELT, T.: Vasopressor effects of substance P and C-terminal sequences after intracisternal injection to α-chloralose-anaesthetized rats: Blockade by a substance P antagonist. Eur. J. Pharmacol. **77:** 171–176, 1982.
183. FUXE, K., BOLME, P., JONSSON, G., AGNATI, L. F., GOLDSTEIN, M., HÖKFELT, T., SCHWARCZ, R., AND ENGEL, J.: On the cardiovascular role of noradrenaline, adrenaline and peptide containing neuron systems in the brain. *In* Neurons System and Hypertension, ed. by P. Meyer and H. Schmitt, pp. 1–16, Wiley-Flammarion, New York, 1979.
184. FUXE, K., GANTEN, P., HÖKFELT, T., AND BOLME, P.: Immunohistochemical evidence for the existence of angiotensin II-containing nerve terminals in the brain and spinal cord of the rat. Neurosci. Lett. **2:** 229–234, 1976.
185. GADDUM, J. H.: Tryptamine receptors. J. Physiol. (Lond.) **119:** 363–368, 1953.
186. GADDUM, J. H.: The estimation of substance P in tissue extracts. Proc. Sci. Soc. Bosnia Herzegovina **1:** 7–10, 1961.
187. GADDUM, J. H., AND SCHILD, H.: Depressor substances in extracts of intestine. J. Physiol. (Lond.) **83:** 1–14, 1934.
188. GADDUM, J. H., AND SZERB, J. C.: Assay of substance P on goldfish intestine in a microbath. Br. J. Pharmacol. **17:** 451–463, 1961.
189. GALE, J. S., BIRD, E. D., SPOKES, E. G., IVERSEN, L. L., AND JESSELL, T.: Human brain substance P: Distribution in controls and Huntington's chorea. J. Neurochem. **30:** 633–634, 1978.
190. GALE, K., HONG, J-S., AND GUIDOTTI, A.: Presence of substance P and GABA in separate striatonigral neurons. Brain Res. **136:** 371–375, 1977.
191. GALLACHER, D. V., AND PETERSEN, O. H.: Substance P increases membrane conductance in parotid acinar cells. Nature (Lond.) **283:** 393–395, 1980.
192. GALLACHER, D. V., AND PETERSEN, O. H.: Substance P: Indirect and direct effects on parotid acinar cell membrane potential. Pflügers Arch. Eur. J. Physiol. **389:** 127–130, 1981.
193. GAMSE, R., HOLZER, P., AND LEMBECK, F.: Decrease of substance P in primary afferent neurones and impairment of neurogenic plasma extravasation by capsaicin. Br. J. Pharmacol. **68:** 207–213, 1980.
194. GAMSE, R., LACKNER, D., GAMSE, G., AND LEEMAN, S. E.: Effect of capsaicin pretreatment on capsaicin-evoked release of immunoreactive somatostatin and substance P from primary sensory neurons. Naunyn-Schmiedeberg's Arch. Pharmacol. **316:** 38–41, 1981.
195. GAMSE, R., LEEMAN, S. E., HOLZER, P., AND LEMBECK, F.: Differential effects of capsaicin on the content of somatostatin, substance P and neurotensin in the nervous system of the rat. Naunyn-Schmiedeberg's Arch. Pharmacol. **317:** 140–148, 1981.
196. GAMSE, R., LEMBECK, F., AND CUELLO, A. C.: Substance P in the vagus nerve. Immunochemical and immunohistochemical evidence for axoplasmic transport. Naunyn-Schmiedeberg's Arch. Pharmacol. **306:** 37–44, 1979.
197. GAMSE, R., MOLNAR, A., AND LEMBECK, F.: Substance P release from spinal cord slices by capsaicin. Life Sci. **25:** 629–636, 1979.
198. GAMSE, R., MROZ, E., LEEMAN, S. E., AND LEMBECK, F.: The intestine as source of immunoreactive substance P in plasma of the cat. Naunyn-Schmiedeberg's Arch. Pharmacol. **305:** 17–21, 1978.
199. GAMSE, R., PETSCHE, U., LEMBECK, F., AND JANCSÓ, G.: Capsaicin applied to peripheral nerve inhibits axoplasmic transport of substance P and somatostatin. Brain Res. **239:** 447–462, 1982.

200. GAMSE, R., WAX, A., ZIGMOND, R. E., AND LEEMAN, S. E.: Immunoreactive substance P in sympathetic ganglia: Distribution and sensitivity towards capsaicin. Neuroscience 6: 437–441, 1981.
201. GARCIA-SEVILLA, J. A., MAGNUSSON, T., AND CARLSSON, A.: Opposite effects of naloxone on substance P-induced changes in brain DOPA synthesis and in locomotor activity in rats. J. Neural. Transm. 45: 185–193, 1979.
202. GASPAROVIĆ, I., HADZOVIĆ, S., HUKOVIĆ, S., AND STERN, P.: Contribution to the theory that substance P has a transmitter role in sensitive pathways. Med. Exp. 10: 303–306, 1964.
203. GAUCHY, C., BEAUJOUAN, J. C., BESSON, M. J., KERDELHUE, B., GLOWINSKI, J., AND MICHELOT, R.: Topographical distribution of substance P in the cat substantia nigra. Neurosci. Lett. 12: 127–131, 1979.
204. GAZELIUS, B., BRODIN, E., AND OLGART, L.: Depletion of substance P-like immunoreactivity in the cat dental pulp by antidromic nerve stimulation. Acta Physiol. Scand. 111: 319–327, 1981.
205. GERNANDT, B.: Untersuchung über die biologische Wirkung der Substanz P. Acta Physiol. Scand. 3: 270–274, 1942.
206. GILBERT, R. F. T., AND EMSON, P. C.: Axonal transport of substance P in rat vagus nerve. Neurosci. Lett., Suppl. 1, p. 218, 1978.
207. GILBERT, R. F. T., EMSON, P. C, FAHRENKRUG, J., LEE, C. M., PENMAN, E., AND WASS, J.: Axonal transport of neuropeptides in the cervical vagus nerve of the rat. J. Neurochem. 34: 108–113, 1980.
208. GILBERT, R. F. T., EMSON, P. C., HUNT, S. P., BENNETT, G. W., MARSDEN, C. A., SANDBERG, B. E. B., STEINBUSCH, H. W. M., AND VERHOFSTAD, A. A. J.: The effects of monoamine neurotoxins on peptides in the rat spinal cord. Neuroscience 7: 69–87, 1982.
209. GILLIS, R. A., HELKE, C. J., HAMILTON, B. L., NORMAN, W. P., AND JACOBOWITZ, D. M.: Evidence that substance P is a neurotransmitter of baro- and chemoreceptor afferents in nucleus tractus solitarius. Brain Res. 181: 476–481, 1980.
210. GINTZLER, A. R., AND SCALISI, J. A.: Effects of opioids on noncholinergic excitatory responses of the guinea-pig isolated ileum: Inhibition of release of enteric substance P. Br. J. Pharmacol. 75: 199–205, 1982.
211. GLICKMAN, R. D., ADOLPH, A. R., AND DOWLING, E.: Does substance P have a physiological role in the carp retina? Invest. Ophthalmol. Visual Sci. 19: (ARVO suppl) 281, 1980.
212. GLICKMAN, R. D., AND ADOLPH, A. R.: Acetylcholine and substance P: Action via distinct receptors on carp retinal ganglion cells. Invest. Ophthalmol. Visual Sci. 22: 804–808, 1982.
213. GRABNER, K., LEMBECK, F., AND NEUHOLD, K.: Substanz P im Gehirn verschiedener Species. Naunyn-Schmiedeberg's Arch. exp. Pathol. Pharmakol. 236: 331–334, 1959.
214. GRANATA, A. R., AND WOODRUFF, G. N.: A central hypertensive action of substance P in rats. IRCS Med. Sci. 8: 205, 1980.
215. GROWCOTT, J. W., AND SHAW, J. S.: Failure of substance P to produce analgesia in the mouse. Br. J. Pharmacol. 66: 129P, 1979.
216. GULLBRING, B.: Inactivation of substance P by tissue extracts. Acta Physiol. Scand. 6: 252–255, 1943.
217. GULLNER, H-G., AND BARTTER, F. C.: Participation of substance P in the control of renin release. Life Sci. 24: 2449–2454, 1979.
218. GULLNER, H-G., CAMPBELL, W. B., AND PETTINGER, W. A.: Effects of substance P on renin release and renal function in anesthetized dogs. Life Sci. 24: 237–246, 1979.
219. GULLNER, H-G., CAMPBELL, W. B., AND PETTINGER, W. A.: Role of substance P in water homeostasis. Life Sci. 24: 2351–2360, 1979.
220. GUYENET, P. G., AND AGHAJANIAN, G. K.: Excitation of neurons of the nucleus locus coeruleus by substance P and related peptides. Brain Res. 136: 178–184, 1977.
221. GUYENET, P. G., AND AGHAJANIAN, G. K.: ACh, Substance P and met-enkephalin in the locus coeruleus: Pharmacological evidence for independent sites of action. Eur. J. Pharmacol. 53: 319–328, 1979.
222. GUYENET, P. G., MROZ, E. A., AGHAJANIAN, G. K., AND LEEMAN, S. E.: Delayed iontophoretic ejection of substance P from glass micropipettes: Correlation with time-course of neuronal excitation *in vivo*. Neuropharmacology 18: 553–558, 1979.
223. HABER, S., AND ELDE, R.: Correlation between met-enkephalin and substance P immunoreactivity in the primate globus pallidus. Neuroscience 6: 1291–1297, 1981.
224. HAEUSLER, G., AND OSTERWALDER, R.: Evidence suggesting a transmitter or neuromodulatory role for substance P at the first synapse of the baroreceptor reflex. Naunyn-Schmiedeberg's Arch. Pharmacol. 314: 111–121, 1980.
225. HÄGERMARK, Ö., HÖKFELT, T., AND PERNOW, B.: Flare and itch induced by substance P in human skin. J. Invest. Dermatol. 71: 233–235, 1978.
226. HAIGLER, H. J., AND SPRING, D. D.: Substance P, morphine and methionine-enkephalin: Effects on spontaneous and evoked neuronal firing in the nucleus reticularis gigantocellularis of the rat. Eur. J. Pharmacol. 67: 65–74, 1980.
227. HÅKANSON, R., BENGMARK, S., BRODIN, E., INGEMANSSON, S., LARSSON, L. I., NILSSON, G., AND SUNDLER, F.: Substance P-like immunoreactivity in intestinal carcinoid tumors. *In* Substance P (Nobel Symposium 37), ed. by U.S. von Euler and B. Pernow, pp. 55–58, Raven Press, New York, 1977.
228. HALLBERG, D., AND PERNOW, B.: Effect of substance P on various vascular beds in the dog. Acta Physiol. Scand. 93: 277–285, 1975.
229. HANLEY, M. R., SANDBERG, B. E. B., LEE, C. M., IVERSEN, L. L., BRUNDISH, D. E., AND WADE, R.: Specific binding of ^{3}H-substance P to rat brain membranes. Nature (Lond.) 286: 810–812, 1980.
230. HANSSON, G. R., ALPHS, L., PRADHAN, S., AND LOVENBERG, W.: Response of striatonigral substance P systems to a dopamine receptor agonist and antagonist. Neuropharmacology 20: 541–548, 1981.
231. HANSON, G. R., ALPHS, L., WOLF, W., LEVINE, R., AND LOVENBERG, W.: Haloperidol-induced reduction of nigral substance P-like immunoreactivity: A probe for the interactions between dopamine and substance P neuronal systems. J. Pharmacol. Exp. Ther. 218: 568–574, 1981.
232. HARMAR, A., AND KEEN, P.: Synthesis, and central and peripheral axonal transport of substance P in a dorsal root ganglion-nerve preparation *in vitro*. Brain Res. 231: 379–385, 1982.
233. HARMAR, A., SCHOFIELD, J. G., AND KEEN, P.: Substance P biosynthesis in dorsal root ganglia; An immunochemical study of (^{35}S) Methionine and (^{3}H) Proline incorporation *in vitro*. Neuroscience 6: 1917–1922, 1981.
234. HAYASHI, H., OHSUMI, K., UEDA, N., FUJIWARA, M., AND MIZUNO, N.: Effect of spinal ganglionectomy on substance P-like immunoreactivity in the gastroduodenal tract of cats. Brain Res. 232: 227–230, 1982.
235. HAYES, A. G., AND TYERS, M. B.: Effects of intrathecal and intracerebroventricular injections of substance P on nociception in the rat and mouse. Br. J. Pharmacol. 66: 488P, 1979.
236. HAYES, A. G., AND TYERS, M. B.: Effects of capsaicin on nociceptive heat, pressure and chemical thresholds and on substance P levels in the rat. Brain Res. 189: 561–564, 1980.
237. HEDNER, T., HEDNER, J., JONASON, J., AND LUNDBERG, D.: Evidence suggesting a role for substance P in central respiratory regulation in the rat. Acta Physiol. Scand. 112.: 487–489, 1981.
238. HEDQVIST, P., AND EULER, U. S. v.: Influence of substance P on the response of guinea pig ileum to transmural nerve stimulation. Acta Physiol. Scand. 95: 341–343, 1975.
239. HEDQVIST, P., AND EULER, U. S. v.: Effects of substance P on some autonomic neuroeffector junctions. *In* Substance P (Nobel Symposium 37), ed. by U.S. von Euler and B. Pernow, pp. 89–95, Raven Press, New York, 1977.
240. HEITZ, PH., POLAK, J. M., TIMSON, C. M., AND PEARSE, A. G. E.: Enterochromaffin cells as the endocrine source of gastrointestinal substance P. Histochemistry 49: 343–347, 1976.
241. HELKE, C. J.: Neuroanatomical localization of substance P: Implications for central cardiovascular control. Peptides 3: 479–483, 1982.
242. HELKE, C. J., JACOBOWITZ, D. M., AND THOA, N. B.: Capsaicin and potassium evoked substance P release from the nucleus tractus solitarius and spinal trigeminal nucleus *in vitro*. Life Sci. 29: 1779–1785, 1981.
243. HELKE, C. J., NEIL, J. J., MASSARI, V. J., AND LOEWY, A. D.: Substance P neurons project from the ventral medulla to the intermediolateral cell column and ventral horn in the rat. Brain Res. 243: 147–152, 1982.
244. HELKE, C. J., O'DONOHUE, T. L., AND JACOBOWITZ, D. M.: Substance P as a baro- and chemoreceptor afferent neurotransmitter: Immunocytochemical and neurochemical evidence in the rat. Peptides 1: 1–9, 1980.
245. HELLSTRÖM, P. M., AND ROSELL, S.: Effects of neurotensin, substance P and methionine-enkephalin on colonic motility. Acta Physiol. Scand. 113: 147–154, 1981.
246. HELMSTAEDTER, V., TANGNER, C. H., FENSLE, G. E., AND FORSMANN, W. G.: Localisation of neurotensin-immunoreactive cells in the small intestine of man and various mammals. Histochemistry 53: 35–41, 1977.
247. HENRY, J. L.: Effects of substance P on functionally identified units in cat spinal cord. Brain Res. 114: 439–451, 1976.
248. HENRY, J. L.: Substance P and pain: A possible relation in afferent transmission. *In* Substance P, Nobel Symposium 37, ed. by U.S. von Euler and B. Pernow, pp. 231–240, Raven Press, New York, 1977.
249. HENRY, J. L., AND BEN-ARI, Y.: Actions of the *p*-chlorophenyl derivative of GABA, Lioresal, on nociceptive and non-nociceptive units in the spinal cord of the cat. Brain Res. 117: 540–544, 1976.
250. HENRY, J. L., KRNJEVIĆ, K., AND MORRIS, M. E.: Substance P and spinal neurones. Can. J. Physiol. Pharmacol. 53: 423–432, 1975.
251. HEYMANN, E., AND MENTLEIN, R.: Liver dipeptidyl aminopeptidase IV hydrolyzes substance P. FEBS Lett. 91: 360–364, 1978.
252. HINSEY, J. C., AND GASSER, H. S.: The component of the dorsal root mediating vasodilatation and the Sherrington contraction. Am. J. Physiol. 92: 679–689, 1930.
253. HÖKFELT, T.: The effect of reserpine on the intraneuronal vesicles of the rat vas deferens. Experientia (Basel) 22: 56–57, 1966.
254. HÖKFELT, T., ELDE, R., JOHANSSON, O., LJUNGDAHL, Å., SCHULTZBERG, M., FUXE, K., GOLDSTEIN, M., NILSSON, G., PERNOW, B., TERENIUS, L., GANTEN, D., JEFFCOATE, S. L., REHFELD, J., AND SAID, S.: Distribution of peptide-containing neurons. *In* Psychopharmacology: A generation of progress, ed. by M.A. Lipton, A. DiMascio, and K.F. Killam, pp. 39–66, Raven Press, New York, 1978.
255. HÖKFELT, T., ELDE, R., JOHANSSON, O., LUFT, R., NILSSON, G., AND ARIMURA, A.: Immunohistochemical evidence for separate populations of somatostatin-containing and substance P-containing primary afferent neurons. Neuroscience 1: 131–136, 1976.
256. HÖKFELT, T., ELDE, R., JOHANSSON, O., TERENIUS, L., AND STEIN, L.: The distribution of enkephalin-immunoreactive cell bodies in the rat central nervous system. Neurosci. Lett. 5: 25–31, 1977.
257. HÖKFELT, T., ELFVIN, L. G., ELDE, R., SCHULTZBERG, M., GOLDSTEIN,

M., AND LUFT, R.: Occurrence of somatostatin-like immunoreactivity in some peripheral sympathetic noradrenergic neurons. Proc. Natl. Acad. Sci. **74:** 3587–3591, 1977.

258. HÖKFELT, T., ELFVIN, L. G., SCHULTZBERG, M., FUXE, K., SAID, S. I., MUTT, V., AND GOLDSTEIN, M.: Immunohistochemical evidence of vasoactive intestinal polypeptide-containing neurons and nerve fibers in sympathetic ganglia. Neuroscience **2:** 885–889, 1977.
259. HÖKFELT, T., ELFVIN, L. G., SCHULTZBERG, M., GOLDSTEIN, M., AND NILSSON, G.: On the occurrence of substance P-containing fibers in sympathetic ganglia: Immunohistochemical evidence. Brain Res. **132:** 29–41, 1977.
260. HÖKFELT, T., JOHANSSON, O., KELLERTH, J.-O., LJUNGDAHL, A., NILSSON, G., NYGÅRDS, A., AND PERNOW, B.: Immunohistochemical distribution of substance P. *In* Substance P (Nobel Symposium 37), ed. by U.S. von Euler and B. Pernow, pp. 117–145, Raven Press, New York, 1977.
261. HÖKFELT, T., JOHANSSON, O., LJUNGDAHL, Å., LUNDBERG, J. M., AND SCHULTZBERG, M.: Peptidergic neurones. Nature (Lond.) **284:** 515–521, 1980.
262. HÖKFELT, T., KELLERTH, J-O., NILSSON, G., AND PERNOW, B.: Experimental immunohistochemical studies on the localization and distribution of substance P in cat primary sensory neurons. Brain Res. **100:** 235–252, 1975.
263. HÖKFELT, T., KELLERTH, J. O., NILSSON, G., AND PERNOW, B.: Substance P: Localization in the central nervous system and in some primary sensory neurons. Science **190:** 889–890, 1975.
264. HÖKFELT, T., LJUNGDAHL, Å., STEINBUSCH, H., VERHOFSTAD, A., NILSSON, G., BRODIN, E., PERNOW, B., AND GOLDSTEIN, M.: Immunohistochemical evidence of substance P-like immunoreactivity in some 5-hydroxytryptamine- containing neurons in the rat central nervous system. Neuroscience **3:** 517–538, 1978.
265. HÖKFELT, T., LJUNGDAHL, Å., TERENIUS, L., ELDE, R., AND NILSSON, G.: Immunohistochemical analysis of peptide pathways possibly related to pain and analgesia: Enkelphalin and substance P. Proc. Natl. Acad. Sci. **74:** 3081–3085, 1977.
266. HÖKFELT, T., LUNDBERG, J. M., SCHULTZBERG, M., JOHANSSON, O., SKIRBOLL, L., ÄNGGÅRD, A., FREDHOLM, B., HAMBERGER, B., PERNOW, B., REHFELD, J., AND GOLDSTEIN, M.: Cellular localization of peptides in neural structures. Proc. R. Soc. Lond. **210:** 63–77, 1980.
267. HÖKFELT, T., MEYERSON, B., NILSSON, G., PERNOW, B., AND SACHS, C.: Immunohistochemical evidence for substance P-containing nerve endings in the human cortex with an uneven distribution. Brain Res. **104:** 181–186, 1976.
268. HÖKFELT, T., PERNOW, B., NILSSON, G., WETTERBERG, L., GOLDSTEIN, M., AND JEFFCOATE, S. L.: Dense plexus of substance P immunoreactive nerve terminal in eminentia medialis of the primate hypothalamus. Proc. Natl. Acad. Sci. **75:** 1013–1015, 1978.
269. HÖKFELT, T., SCHULTZBERG, M., ELDE, R., NILSSON, G., TERENIUS, L., SAID, S., AND GOLDSTEIN, M.: Peptide neurons in peripheral tissues including the urinary tract: Immunohistochemical studies. Acta Pharmacol. Toxicol. **43:** 79–89, 1978.
270. HÖKFELT, T., SKIRBOLL, L., DALSGAARD, C-J., JOHANSSON, O., LUNDBERG, J. M., NORELL, G., AND JANCSÓ, G.: Peptide neurons in the spinal cord with special reference to descending systems. *In* Brain Stem Control of Spinal Mechanisms, ed. by B. Sjölund and A. Björklund, pp. 89–117, Elsevier/North Holland Biomedical Press, 1982.
271. HÖKFELT, T., VINCENT, S., DALSGAARD, C-J., SKIRBOLL, L., JOHANSSON, O., SCHULTZBERG, M., LUNDBERG, J. M., ROSELL, S., PERNOW, B., AND JANCSÓ, G.: Distribution of substance P in brain and periphery and its possible role as a co-transmitter. *In* Substance P in the nervous system, (Ciba Foundation Symposium 91), ed. by R. Porter, and M. O'Connor pp. 84–106, Pitman, London, 1982.
272. HÖKFELT, T., VINCENT, S., HELLSTEN, L., ROSELL, S., FOLKERS, K., MARKEY, K., GOLDSTEIN, M., AND CUELLO, A. C.: Immunohistochemical evidence for a "neurotoxic" action of [D-Pro^2, D-Trp^{7,9}]substance P, an analogue with substance P antagonistic activity. Acta Physiol. Scand. **113:** 571–573, 1981.
273. HOLETS, V., AND ELDE, R.: The differential distribution and relationship of serotoninergic and peptidergic fibers to sympathoadrenal neurons in the intermediolateral cell column of the rat: A combined retrograde axonal transport and immunofluorescence study. Neuroscience **7:** 1155–1174, 1982.
274. HOLM, I., THULIN, L., AND HELLGREN, M.: Anticholeretic effect of substance P in anesthetized dogs. Acta Physiol. Scand. **102:** 274–280, 1978.
275. HOLMDAHL, G., HÅKANSON, R., LEANDER, S., ROSELL, S., FOLKERS, K., AND SUNDLER, F.: A substance P antagonist, [D-Pro^2, D-Trp^{7,9}]SP, inhibits inflammatory responses in the rabbit eye. Science **214:** 1029–1031, 1981.
276. HOLTON, P.: The substance P and adenosinetriphosphate (ATP) contents of sensory nerve in degenerating nerve. J. Physiol. (Lond.) **143:** 45P., 1958.
277. HOLTON, P.: Substance P concentration in degenerating nerve. *In* Polypeptides which affect smooth muscles and blood vessels, ed. by M. Schachter, pp. 192–194, Pergamon Press, Oxford, 1960.
278. HOLZER, P.: An enquiry into the mechanism by which substance P facilitates the phasic longitudinal contractions of the rabbit ileum. J. Physiol. (Lond.) **325:** 377–392, 1982.
279. HOLZER, P., BUCSICS, A., AND LEMBECK, F.: Distribution of capsaicin-sensitive nerve fibres containing immunoreactive substance P in cutaneous and visceral tissues of the rat. Neurosci. Lett. **31:** 253–257, 1982.
280. HOLZER, P., EMSON, P. C., IVERSEN, L. L., AND SHARMAN, D. F.: Regional differences in the response to substance P of the longitudinal muscle and the concentration of substance P in the digestive tract of the guinea-pig. Neuroscience **6:** 1433–1441, 1981.
281. HOLZER, P., GAMSE, R., AND LEMBECK, F.: Distribution of substance P in the rat gastrointestinal tract—lack of effect of capsaicin pretreatment. Eur. J. Pharmacol. **61.**: 303–307, 1980.
282. HOLZER, P., JURNA, I., GAMSE, R., AND LEMBECK, F.: Nociceptive threshold after neonatal capsaicin treatment. Eur. J. Pharmacol. **58:** 511–514, 1979.
283. HOLZER, P., AND LEMBECK, F.: Effect of neuropeptides on the efficiency of the peristaltic reflex. Naunyn-Schmiedeberg's Arch. Pharmacol. **307:** 257–264, 1979.
284. HOLZER, P., AND LEMBECK, F.: Neurally mediated contraction of ileal longitudinal muscle by substance P. Neurosci. Lett. **17:** 101–105, 1980.
285. HOLZER, P., LEMBECK, F., AND DONNERER, J.: Caerulein, substance P, serotonin, and cholinomimetics induce rhythmic contractions of the intestinal circular muscle. Naunyn-Schmiedeberg's Arch. Pharmacol. **312:** 131–137, 1980.
286. HOMMA, S., SUZUKI, T., MURAYAMA, S., AND OTSUKA, M.: Amino acid and substance P contents in spinal cord of cats with experimental hind-limb rigidity produced by occlusion of spinal cord blood supply. J. Neurochem. **32:** 691–698, 1979.
287. HONG, J. S., COSTA, E., AND YANG, H-Y. T.: Effects of habenular lesions on the substance P content of various brain regions. Brain Res. **118:** 523–525, 1976.
288. HONG, J. S., YANG, H-Y., T., RACAGNI, G., AND COSTA, E.: Projections of substance P containing neurons from neostriatum to substantia nigra. Brain Res. **122:** 541–544, 1977.
289. HOPSY-HAVU, V., AND GLENNER, G.: A new dipeptide naphthylamidase hydrolyzing glycyl-propyl-beta-naphthyl-amide. Histochemie **7:** 197–201, 1966.
290. HÖSLI, L., HÖSLI, E., ZEHNTER, CH., AND LANDOLT, H.: Effects of substance P on neurones and glial cells in cultured rat spinal cord. Neurosci. Lett. **24:** 165–168, 1981.
291. HUIDOBRO-TORO, J. P., CHELALA, C. A., BAHOUTH, S., NODAR, R., AND MUSACCHIO, J.: Fading and tachyphylaxis to the contractile effects of substance P in the guinea-pig. Eur. J. Pharmacol. **81:** 21–34, 1981.
292. HUNT, S. P., KELLY, J. S., EMSON, C., KIMMEL, J. R., MILLER, R. J., AND WU, J-Y.: An immunohistochemical study of neuronal populations containing neuropeptides or γ-aminobutyrate within the superificial layers of the rat dorsal horn. Neuroscience **6:** 1883–1898, 1981.
293. HUSTON, J. P., AND STAUBLI, U.: Retrograde amnesia produced by post-trial injection of substance P into substantia nigra. Brain Res. **159:** 468–472, 1978.
294. HUTCHISON, J. B., AND DOCKRAY, G. J.: Evidence that the action of cholecystokinin octapeptide on the guinea pig ileum longitudinal muscle is mediated in part by substance P release from the myenteric plexus. Eur. J. Pharmacol. **69:** 87–93, 1981.
295. HYLDEN, J. L. K., AND WILCOX, G. L.: Intrathecal substance P elicits a caudally-directed biting and scratching behavior in mice. Brain Res. **217:** 212–215, 1981.
296. INAGAKI, S., SAKANAKA, M., SHIOSAKA, S., SENBA, E., TAKATSUKI, K., TAKAGI, H., KAWAI, Y., MINAGAWA, H., AND TOHYAMA, M.: Ontogeny of substance P-containing neuron system of the rat: Immunohistochemical analysis. I. Forebrain and upper brain stem. Neuroscience **7:** 251–277, 1982.
297. INOUYE, A., AND KATAOKA, K.: Subcellular distribution of the substance P in the nervous tissues. Nature (Lond.) **193:** 585, 1962.
298. IVERSEN, L. L., HANLEY, M. R., SANDBERG, B. E. B., LEE, C.-M., PINNOCK, R. D., AND WATSON, S. P.: Substance P receptors in the nervous system and possible receptor subtypes. *In* Substance P in the nervous system (Ciba Foundation Symposium 91) ed. by R. Porter, and M. O'Connor, pp. 186–205, Pitman, London, 1982.
299. IVERSEN, L. L., JESSELL, T., AND KANAZAWA, I.: Release and metabolism of substance P in rat hypothalamus. Nature (Lond) **264:** 81–83, 1976.
300. IVERSEN, S. D., KELLEY, A., AND STINUS, L.: Dose dependent behavioural stimulation after local infusion of substance P into the ventral tegmental area in the rat. Br. J. Pharmacol. **66:** 113P–114P, 1979.
301. JAFFE, B. M., AKANDE, B., MODLIN, I. M., REILLY, P., AND ALBERT, D.: Cholinergic modulation of substance P release. Dig. Dis. Sci. **27:** 28–32, 1982.
302. JAMES, T. A., AND STARR, M. S.: Effects of substance P injected into the substantia nigra. Br. J. Pharmacol. **65:** 423–429, 1979.
303. JANCSÓ, G., HÖKFELT, T., LUNDBERG, J. M., KIRALY, E., HALÁSZ, N., NILSSON, G., TERENIUS, L., REHFELD, J., STEINBUSCH, H., VERHOFSTAD, A., ELDE, R., SAID, S., AND BROWN, M.: Immunohistochemical studies on the effect of capsaicin on spinal and medullary peptide and monoamine neurons using antisera to substance P, gastrin/CCK, somatostatin, VIP, enkephalin, neurotensin and 5-hydroxytryptamine. J. Neurocytol. **10:** 963–980, 1981.
304. JANCSÓ, N., JANSCÓ-GÁBOR, A., AND SZOLCSÁNYI, J.: Direct evidence for neurogenic inflammation and its prevention by denervation and by pre-

treatment with capsaicin. Br. J. Pharmacol. **31:** 138–151, 1967.
305. JANSCÓ, G., KIRÁLY, E., AND JANCSÓ-GÁBOR, A.: Pharmacologically induced selective degeneration of chemosensitive primary sensory neurones. Nature (Lond.) **270:** 741–743, 1977.
306. JANCSÓ, G., AND KNYIHÁR, E.: Functional linkage between nociception and fluoride-resistant acid phosphatase activity in the Rolando substance. Neurobiology **5:** 42–43, 1975.
307. JEFTINIJA, S., MURASE, K., NEDELJKOV, V., AND RANDIC, M.: Vasoactive intestinal polypeptide excites mammalian dorsal horn neurons both in vivo and in vitro. Brain Res. **243:** 158–164, 1982.
308. JENSEN, R. T., AND GARDNER, J. D.: Interaction of physalaemin, substance P, and eledoisin with specific membrane receptors of pancreatic acinar cells. Proc. Natl. Acad. Sci. **76.:** 5679–5683, 1979.
309. JESSELL, T. M.: Inhibition of substance P release from the isolated rat substantia nigra by GABA. Br. J. Pharmacol. **59:** 486P, 1977.
310. JESSELL, T. M.: Substance P release from the rat substantia nigra. Brain Res. **151:** 469–478, 1978.
311. JESSELL, T. M., EMSON, P. C., PAXINOS, G., AND CUELLO, A. C.: Topographic projections of substance P and GABA pathways in the striato-pallido-nigral system: A biochemical and immunohistochemical study. Brain Res. **152:** 487–498, 1978.
312. JESSELL, T. M., AND IVERSEN, L. L.: Opiate analgesics inhibit substance P release from rat trigeminal nucleus. Nature (Lond.) **268:** 549–551, 1977.
313. JESSELL, T. M., IVERSEN, L.L., AND CUELLO, A. C.: Capsaicin-induced depletion of substance P from primary sensory neurones. Brain Res. **152:** 183–188, 1978.
314. JESSELL, T. M., TSUNOO, A., KANAZAWA, I., AND OTSUKA, M.: Substance P: Depletion in the dorsal horn of rat spinal cord after section of the peripheral processes of primary sensory neurons. Brain Res. **168:** 247–259, 1979.
315. JESSEN, K. R., SAFFREY, M. J., VAN NOORDEN, S., BLOOM, S. R., POLAK, J. M., AND BURNSTOCK, G.: Immunohistochemical studies of the enteric nervous system in tissue culture and *in situ*: Localization of vasoactive intestinal polypeptide (VIP), substance P and enkephalin immunoreactive nerves in the guinea-pig gut. Neuroscience **5:** 1717–1735, 1980.
316. JIANG, Z-G., DUN, N. J., AND KARCZMAR, A. G.: Substance P: A putative sensory transmitter in mammalian autonomic ganglia. Science **217:** 739–741, 1982.
317. JOHANSSON, O., HÖKFELT, T., PERNOW, B., JEFFCOATE, S. L., WHITE, N., STEINBUSCH, H. W. M., VERHOFSTAD, A. A. J., EMSON, P. C., AND SPINDEL, E.: Immunohistochemical support for three putative transmitters in one neuron: Coexistence of 5-hydroxytryptamine, substance P- and thyrotropin releasing hormone-like immunoreactivity in medullary neurons projecting to the spinal cord. Neuroscience **6:** 1857–1881, 1981.
318. JOHANSSON, O., AND LUNDBERG, J. M.: Ultrastructural localization of VIP-like immunoreactivity in large dense-core vesicles of "cholinergic-type" nerve terminals in cat exocrine glands. Neuroscience **6:** 847–862, 1981.
319. JOHNSON, A. R., AND ERDÖS, E. G.: Release of histamine from mast cells by vasoactive peptides. Proc. Soc. Exp. Biol. Med. **142:** 1252–1256, 1973.
320. JOHNSON, A. R., AND ERDÖS, E. G.: Inactivation of substance P by cultured human endothelial cells. *In* Substance P (Nobel Symposium 37), ed. by U.S. von Euler and B. Pernow, pp. 253–260, Raven Press, New York, 1977.
321. JOLICOEUR, F. B., RONDEAU, D. B., DELANGER, F., FOURIEZOS, G., AND BARBEAU, A.: Influence of substance P on the behavioral changes induced by haloperidol in rats. Peptides **1:** 103–107, 1980.
322. KACHUR, J. F., MILLER, R. J., FIELD, M., AND RIVIER, J.: Neurohumoral control of ileal electrolyte transport. II. Neurotensin and substance P. J. Pharmacol. Exp. Ther. **220:** 456–463, 1982.
323. KANAZAWA, I., BIRD, E. D., GALE, J. S., IVERSEN, L. L., JESSELL, T. M., MURAMOTO, O., SPOKES, E. G., AND SUTOO, D.: Substance P: Decrease in substantia nigra and globus pallidus in Huntington's disease. *In* Advances in Neurology vol. 23, ed. by T.N. Chase et al., pp. 495–504, Raven Press, New York, 1979.
324. KANAZAWA, I., BIRD, E., O'CONNELL, R., AND POWELL, D.: Evidence for a decrease in substance P content of substantia nigra in Huntington's chorea. Brain Res. **120:** 387–392, 1977.
325. KANAZAWA, I., EMSON, P. C., AND CUELLO, A. C.: Evidence for the existence of substance P-containing fibres in striato-nigral and pallido-nigral pathways in rat brain. Brain Res. **119:** 447–453, 1977.
326. KANAZAWA, I., AND JESSELL, T.: Post mortem changes and regional distribution of substance P in the rat and mouse nervous system. Brain Res. **117:** 362–367, 1976.
327. KANAZAWA, I., SUTOO, D., OSHIMA, I., AND SAITO, S.: Effect of transection on choline acetyltransferase, thyrotropin releasing hormone and substance P in the cat cervical spinal cord. Neurosci. Lett. **13:** 325–330, 1979.
328. KANETO, A., KANEKO, T., KAJINUMA, H., AND KOSAKA, K.: Effects of substance P and neurotensin infused intrapancreatically on glucagon and insulin secretion. Endocrinology **102:** 393–401, 1978.
329. KARTEN, H. J., AND BRECHA, N.: Localisation of substance P immunoreactivity in amacrine cells of the retina. Nature (Lond.) **283:** 87–88, 1980.
330. KATAOKA, K.: The subcellular distribution of substance P in the nervous tissues. Jpn. J. Physiol. **12:** 81–96, 1962.
331. KATAYAMA, Y., AND NORTH, R. A.: Does substance P mediate slow synaptic excitation within the myenteric plexus? Nature (Lond.) **274:** 387–388, 1978.
332. KATAYAMA, Y., NORTH, R. A., AND WILLIAMS, J. T.: The action of substance P on neurons of the myenteric plexus of the guinea-pig small intestine. Proc. R. Soc. Lond. **206:** 191–208, 1979.
333. KATO, T., NAGATSU, T., FUKASAWA, K., HARADA, M., NAGATSU, I., AND SAKAKIBARA, S.: Successive cleavage of N-terminal Arg^1-Pro^2 and Lys^3-Pro^4 from substance P but no release of Arg^1-Pro^2 from bradykinin, by X-Pro dipeptidyl-aminopeptidase. Biochim. Biophys. Acta **525:** 417–422, 1978.
334. KATZ, D. M., AND KARTEN, H. J.: Substance P in the vagal sensory ganglia: Localization in cell bodies and pericellular arborizations. J. Comp. Neurol. **193:** 549–564, 1980.
335. KATZ, R. J.: Central injection of substance P elicits grooming behavior and motor inhibition in mice. Neurosci. Lett. **12:** 133–136, 1979.
336. KATZ, R. J.: Substance P elicited grooming in the mouse: Behavioral and pharmacological characteristics. Int. J. Neurosci. **10:** 187–189, 1980.
337. KEEN, P., TULLO, A. B., BLYTH, W. A., AND HILL, T. J.: Substance P in the mouse cornea: Effects of chemical and surgical denervation. Neurosci. Lett. **29:** 231–235, 1982.
338. KELLEY, A. E., AND IVERSEN, S. D.: Behavioural response to bilateral injections of substance P into the substantia nigra. Brain Res. **158:** 474–478, 1978.
339. KELLEY, A. E., AND IVERSEN, S. D.: Substance P infusion into substantia nigra of the rat: Behavioural analysis and involvement of striatal dopamine. Eur. J. Pharmacol. **60:** 171–179, 1979.
340. KELLEY, A. E., STINUS, L., AND IVERSEN, S. D.: Behavioural activation induced in the rat by substance P infusion into ventral tegmental area: Implication of dopaminergic A10 neurones. Neurosci. Lett. **11:** 335–339, 1979.
341. KERDELHUÉ, B., KHAR, A., DE NAY, D., LANGLOIS, Y., BERNNARDO, T., LIWSKA, J., AND JUTISZ, M.: Inhibition *in vitro* par la substance P de l'excrétion des gonadotropines induite par le LH-RH à partir de cellules adénohypophysaires de rat en culture. C. R. Acad. Sci. (Paris) **287:** 879–882, 1978.
342. KESSLER, J. A., ADLER, J. E., BOHN, M. C., AND BLACK, I. B.: Substance P in principal sympathetic neurons: Regulation by impulse activity. Science **214:** 335–336, 1981.
343. KESSLER, J. A., AND BLACK, I. B.: Nerve growth factor stimulates development of substance P in the embryonic spinal cord. Brain Res. **208:** 135–145, 1981.
344. KESSLER, J. A., AND BLACK, I. B.: Regulation of substance P in adult rat sympathetic ganglia. Brain Res. **234:** 182–187, 1982.
345. KIERNAN, J. A.: A pharmacological and histological investigation of the involvement of mast cells in cutaneous axon reflex vasodilatation. Q. J. Exp. Physiol. **60:** 123–130, 1975.
346. KISHIDA, T., KITO, S., ITOGA, E., YANAIHARA, N., OGAWA, N., AND WAKABAYASHI, I.: Immunohistochemical studies on the distribution of biologically active oligopeptides in the rat central nervous system. Acta Histochem. Cytochem. **12:** 301–324, 1979.
347. KLUGMAN, K. P., LEMBECK, F., MARKOWITZ, S., MITCHELL, G., AND ROSENDORFF, C.: Substance P increases hypothalamic blood flow via an indirect adrenergic-cholinergic interaction. Br. J. Pharmacol. **71:** 623–629, 1980.
348. KONDO, H., AND YUI, R.: An electron microscopic study on substance P-like immunoreactive nerve fibers in the celiac ganglion of guinea-pigs. Brain Res. **222:** 134–137, 1981.
349. KONISHI, S., AND OTSUKA, M.: The effects of substance P and other peptides on spinal neurons of the frog. Brain Res. **65:** 397–410, 1974.
350. KONISHI, S., AND OTSUKA, M.: Excitatory action of hypothalamic substance P on spinal motoneurons of newborn rats. Nature (Lond.) **252:** 734–735, 1974.
351. KONISHI, S., OTSUKA, M., FOLKERS, K., AND ROSELL, S.: A substance P antagonist blocks non-cholinergic slow excitatory postsynaptic potential in guinea-pig sympathetic ganglia. Acta Physiol. Scand. **117:** 157–160, 1983.
352. KONISHI, S., TSUNOO, A., AND OTSUKA, M.: Substance P and noncholinergic excitatory synaptic transmission in guinea pig sympathetic ganglia. Proc. Jpn. Acad. **55:** 525–530, 1979.
353. KONISHI, S., TSUNOO, A., AND OTSUKA, M.: Enkephalins presynaptically inhibit cholinergic transmission in sympathetic ganglia. Nature (Lond.) **282:** 515–516, 1979.
354. KONISHI, S., TSUNOO, A., YANAIHARA, N., AND OTSUKA, M.: Peptidergic excitatory and inhibitory synapses in mammalian sympathetic ganglia: Roles of substance P and enkephalin. Biomed. Res. **1:** 528–536, 1980.
355. KONTUREK, S. J., JAWOREK, J., TASLER, J., CIESZKOWSKI, M., AND PAWLIK, W.: Effect of substance P and its C-terminal hexapeptide on gastric and pancreatic secretion in the dog. Am. J. Physiol. **241:** G74–G81, 1981.
356. KOPERA, H., AND LAZARINI, W.: Zur Frage der zentralen Übertragung afferenter Impulse. IV. Die Verteilung der Substanz P im Zentralnervensystem. Naunyn-Schmiedeberg's Arch. exp. Pathol. Pharmakol. **219:** 214–222, 1953.
357. KOTANI, Y., OKA, M., YONEHARA, N., KUDO, T., AND INOKI, R.: Algesiogenic and analgesic activities of synthetic substance P. Jpn. J. Pharmacol. **31:** 315–321, 1981.
358. KRIVOY, W. A., COUCH, J. R., STEWART, J. M., AND ZIMMERMANN, E.: Modulation of cat monosynaptic reflexes by substance P. Brain Res. **202:**

365–372, 1980.
359. KRNJEVIĆ, K.: Effects of substance P on central neurons in cats. *In* Substance P (Nobel Symposium 37), ed. by U. S. von Euler and B. Pernow, pp. 217–230, Raven Press, New York, 1977.
360. KRNJEVIĆ, K., AND LEKIC, D.: Substance P selectively blocks excitation of Renshaw cell by acetylcholine. Can. J. Physiol. Pharmacol. **55:** 958–961, 1977.
361. KRNJEVIĆ, K., AND MORRIS, M. E.: An excitatory action of substance P on cuneate neurones. Can. J. Physiol. Pharmacol. **52:** 736–744, 1974.
362. KRONHEIM, S., SHEPPARD, M. C., AND PIMSTONE, B. L.: Substance P release from rat hypothalamus and spinal cord. S. Afr. Med. J. **57:** 827–830, 1980.
363. KUMAZAWA, T., AND MIZUMURA, K.: Effects of synthetic substance P on unit-discharges of testicular nociceptors of dogs. Brain Res. **170:** 553–557, 1979.
364. LABSCH, B., HEINS, J., HILSE, H., BARTH, A., AND OEHME, P.: Die Aktivität der Dipeptidyl-Peptidase IV in verschiedenen Organen und Geweben der Ratte und ihre mögliche Beziehung zum Substanz-P-Metabolismus. Pharmazie **36:** 161–163, 1981.
365. LAKOSKI, J. M., MOHRLAND, J. S., AND GEBHART, G. F.: The effect of morphine on the content of serotonin, 5-hydroxyindole acetic acid and substance P in the nuclei raphe magnus and reticularis gigantocellularis. Life Sci. **27:** 2639–2644, 1980.
366. LAMOTTE, C. C., AND DE LANEROLLE, N. C.: Human spinal neurons: Innervation by both substance P and enkephalin. Neuroscience **6:** 713–723, 1981.
367. LAMOTTE, C. C., PERT, C. B., AND SNYDER, S. H.: Opiate receptor binding in primate spinal cord: Distribution and changes after dorsal root section. Brain Res. **112:** 407–412, 1976.
368. LASZLO, I.: Estimation of substance P in mouse brain. The identification of interfering nucleotides in extract. Br. J. Pharmacol. **20:** 449–461, 1963.
369. LAUFER, R., CHOREV, M., GILON, C., FRIEDMAN, Z. Y., WORMSER, U., AND SELINGER, Z.: Persistent action of N-methylated analogs of substance P on rat parotid slices. FEBS Lett. **123:** 291–294, 1981.
370. LAWSON, S. N., AND NICKELS, S. M.: The use of morphometric techniques to analyse the effect of neonatal capsaicin treatment on dorsal root ganglia and dorsal roots. J. Physiol. (Lond.) **303:** 12, 1980.
371. LE GAL LA SALLE, G., AND BEN-ARI, Y.: Microiontophoretic effects of substance P on neurons of the medial amygdala and putamen of the rat. Brain Res. **135:** 174–179, 1977.
372. LEANDER, S., BRODIN, E., HÅKANSON, R., SUNDLER, F., AND UDDMAN, R.: Neuronal substance P in the oesphagus. Distribution and effects on motor activity. Acta Physiol. Scand. **115:** 427–435, 1982.
373. LEANDER, S., HÅKANSON, R., ROSELL, S., FOLKERS, K., SUNDLER, F., AND TORNQVIST, K.: A specific substance P antagonist blocks smooth muscle contractions induced by non-cholinergic, non-adrenergic nerve stimulation. Nature (Lond.) **294:** 467–469, 1981.
374. LEANDER, S., HÅKANSON, R., AND SUNDLER, F.: Nerves containing substance P, vasoactive intestinal polypeptide, enkephalin or somatostatin in the guinea-pig taenia coli. Cell Tissue Res. **215:** 21–39, 1981.
375. LEBAN, J., RACKUR, G., YAMAGUCHI, I., FOLKERS, K., BJÖRKROTH, U., ROSELL, S., YANAIHARA, N., AND YANAIHARA, C.: Synthesis of substance P analogs and agonistic and antagonistic activities. Acta Chem. Scand. B **33:** 664–668, 1979.
376. LECHNER, H., AND LEMBECK, F.: Einfluss der Substanz P auf die elektrische Aktivität des Gehirns. Naunyn-Schmiedeberg's Arch. exp. Pathol. Pharmakol. **234:** 419–425, 1958.
377. LEE, C. M.: Enzymatic inactivation of substance P in the central nervous system. *In* Substance P in the Nervous System (Ciba Foundation symposium 91), ed. by R. Porter and M. O'Connor, pp. 165–185, Pitman, London, 1982.
378. LEE, C. M., ARREGUI, A., AND IVERSEN, L. L.: Substance P degradation by rat brain peptidases: Inhibition by SQ 20881. Biochem. Pharmacol. **28:** 553–556, 1979.
379. LEE, C. M., EMSON, P. C., AND IVERSEN, L. L.: The development and application of a novel N-terminal directed substance P antiserum. Life Sci. **27:** 535–543, 1980.
380. Lee, C-M., Iversen, L. L., Hanley, M. R., and Sandberg, B. E. B.: The possible existence of multiple receptors for substance P. Naunyn-Schmiedeberg's Arch. Pharmacol. **318:** 281–287, 1982.
381. LEE, C-M., SANDBERG, B. E. B., HANLEY, M. R., AND IVERSEN, L. L.: Purification and characterisation of a membrane-bound substance-P-degrading enzyme from human brain. Eur. J. Biochem. **114:** 315–327, 1981.
382. LEEMAN, S. E., ARONIN, N., AND FERRIS, C.: Substance P and neurotensin. *In* Recent Progress in Hormone Research, vol. 30, ed. by R. O. Greep, pp. 93–128, Academic Press, New York, 1982.
383. LEEMAN, S. E., AND CARRAWAY, R. E.: The discovery of a sialogogic peptide in bovine hypothalamic extracts: Its isolation, characterization as substance P, structure and synthesis. *In* Substance P (Nobel Symposium 37), ed. by U. S. von Euler and B. Pernow, pp. 5–13, Raven Press, New York, 1977.
384. LEEMAN, S. E., AND HAMMERSCHLAG, R.: Stimulation of salivary secretion by a factor from hypothalamic tissue. Endocrinology **81:** 803–810, 1967.
385. LEEMAN, S. E., AND LEMBECK, F. (chairmen): Substance P. *In* Advances in Pharmacology and Therapeutics II, vol. 1: CNS Pharmacology-Neuropeptides, ed. by H. Yoshida, Y. Hagihara, and S. Ebashi, Pergamon Press, Oxford, 1982.
386. LEEMAN, S. E., AND MROZ, E. A.: Substance P. Minireview. Life Sci. **15:** 2033–2044, 1974.
387. LEMBECK, F.: Zur Frage der zentralen Übertragung afferenter Impulse. III. Das Vorkommen und die Bedeutung der Substanz P in den dorsalen Wurzeln des Rückenmarks. Naunyn-Schmiedeberg's Arch. exp. Pathol. Pharmakol. **219:** 197–213, 1953.
388. LEMBECK, F., AND DONNERER, J.: Postocclusive cutaneous vasodilatation mediated by substance P. Naunyn-Schmiedeberg's Arch. Pharmacol. **316:** 165–171, 1981.
389. LEMBECK, F., AND DONNERER, J.: Time course of capsaicin-induced functional impairments in comparison with changes in neuronal substance P content. Naunyn-Schmiedeberg's Arch. Pharmacol. **316:** 240–243, 1981.
390. LEMBECK, F., DONNERER, J., AND BARTHO, L.: Inhibition of neurogenic vasodilatation and plasma extravasation by substance P antagonists, somatostatin and D-Met², Pro⁵ enkephalinamide. Eur. J. Pharmacol. **85:** 171–176, 1982.
391. LEMBECK, F., DONNERER, J., AND COLPAERT, F. C.: Increase of substance P in primary afferent nerves during chronic pain. Neuropeptides **1:** 175–180, 1981.
392. LEMBECK, F., AND FISCHER, G.: Gekreutze Tachyphylaxie von Peptiden. Naunyn-Schmiedeberg's Arch. Pharmacol. **258:** 452–456, 1967.
393. LEMBECK, F., FOLKERS, K., AND DONNERER, J.: Analgesic effect of antagonists of substance P. Biochem. Biophys. Res. Commun. **103:** 1318–1321, 1981.
394. LEMBECK, F., AND GAMSE, R.: Lack of algesic effect of substance P on paravascular pain receptors. Naunyn-Schmiedeberg's Arch. Pharmacol. **229:** 295–303, 1977.
395. LEMBECK, F., AND GAMSE, R.: Substance P in peripheral sensory processes. *In* Substance P in the Nervous System. ed. by R. Porter and M. O'Connor (Ciba Foundation Symposium 91), pp. 35–54, Pitman, London, 1982.
396. LEMBECK, F., AND HOLASEK, A.: Die intracelluläre Lokalisation der Substanz P. Naunyn-Schmiedeberg's Arch. Exp. Pathol. Pharmakol. **238:** 542–545, 1960.
397. LEMBECK, F., AND HOLZER, P.: Substance P as neurogenic mediator of antidromic vasodilatation and neurogenic plasma extravasation. Naunyn-Schmiedeberg's Arch. Pharmacol. **310:** 175–183, 1979.
398. LEMBECK, F., HOLZER, P., SCHWEDITSCH, M., AND GAMSE, R.: Elimination of substance P from the circulation of the rat and its inhibition by bacitracin. Naunyn-Schmiedeberg's Arch. Pharmacol. **305:** 9–16, 1978.
399. LEMBECK, F., MAYER, N., AND SCHINDLER, G.: Substance P in rat brain synaptosomes. Naunyn-Schmiedeberg's Arch. Pharmacol. **301:** 17–22, 1977.
400. LEMBECK, F., MAYER, N., AND SCHINDLER, G.: Substance P: Binding to lipids in the brain. Naunyn-Schmiedeberg's Arch. Pharmacol. **303:** 79–86, 1978.
401. LEMBECK, F., AND STARKE, K.: Substance P content and effect of capillary permeability of extract of various parts of human brain. Nature (Lond.) **199:** 1295–1296, 1963.
402. LEMBECK, F., AND STARKE, K.: Substanz P und Speichelsekretion. Naunyn-Schmiedeberg's Arch. exp. Pathol. Pharmakol. **259:** 375–385, 1968.
403. LEMBECK, F., AND ZETLER, G.: Substance P: A polypeptide of possible physiological significance especially within the nervous system. Int. Rev. Neurobiol. **4:** 159–215, 1962.
404. LEWIS, T., AND MARVIN, H. M.: Observations relating to vasodilatation arising from antidromic impulses, to herpes zoster and trophic effects. Heart **14:** 27–46, 1927.
405. LIANG, T., AND CASCIERI, M. A.: Substance P stimulation of amylase release by isolated parotid cells and inhibition of substance P induction of salivation by vasoactive peptides. Mol. Cell. Endocrinol. **15:** 151–162, 1979.
406. LIANG, T., AND CASCIERI, M. A.: Substance P receptor on parotid cell membranes. J. Neurosci. **1:** 1133–1141, 1981.
407. LIDBERG, P., EDIN, R., LUNDBERG, J. M., DAHLSTRÖM, A., ROSELL, S., FOLKERS, K., AND AHLMAN, H.: The involvement of substance P in the vagal control of the feline pylorus. Acta Physiol. Scand. **114:** 307–309, 1982.
408. LILJEDAHL, S-O., MATTSSON, O., AND PERNOW, B.: The effect of substance P on intestinal motility in man. Scand. J. Clin. Lab. Invest. **10:** 16–25, 1958.
409. LISOPRAWSKI, A., BLANC, G., AND GLOWINSKI, J.: Activation by stress of the habenulo-interpeduncular substance P neurons in the rat. Neurosci. Lett. **25:** 47–51, 1981.
410. LIVETT, B. G., KOZOUSEK, V., MIZOBE, F., AND DEAN, D. M.: Substance P inhibits nicotinic activation of chromaffin cells. Nature (Lond.) **278:** 256–257, 1979.
411. LJUNGDAHL, Å., HÖKFELT, T., AND NILSSON, G.: Distribution of substance P-like immunoreactivity in the central nervous system of the rat. I. Cell bodies and nerve terminals. Neuroscience **3:** 861–943, 1978.
412. LJUNGDAHL, Å., HÖKFELT, T., NILSSON, G., AND GOLDSTEIN, M.: Distribution of substance P-like immunoreactivity in the central nervous system of the rat. II. Light microscopic localization in relation to catecholamine-containing neurons. Neuroscience **3:** 945–976, 1978.
413. LOEWY, A. D., AND SAWYER, W. B.: Substance P antagonist inhibits vasomotor responses elicted from ventral medulla in rat. Brain Res. **245:**

379–383, 1982.
414. LÖFSTRÖM, B., PERNOW, B., AND WAHREN, J.: Vasodilating action of substance P in the human forearm. Acta Physiol. Scand. **63:** 311–324, 1965.
415. LOH, H. H., BRASE, D. A., SAMPATH-KHANNA, S., MAR, J. B., WAY, E. L., AND LI, C. H.: β-Endorphin *in vitro* inhibition of striatal dopamine release. Nature (Lond.) **264:** 567–569, 1976.
416. LOSAY, J., MROZ, E., TREGEAR, G. W., LEEMAN, S. E., AND GAMBLE, W. J.: Reaction of substance P on the coronary blood flow in the isolated dog heart. *In* Substance P (Nobel Symposium 37), ed. by U. S. von Euler and B. Pernow, pp. 287–293, Raven Press, New York, 1977.
417. LUNDBERG, J. M.: Evidence for coexistence of vasoactive intestinal polypeptide (VIP) and acetylcholine in neurons of cat exocrine glands. Morphological, biochemical and functional studies. Acta Physiol. Scand. **112:** suppl. 496, 1–57, 1981.
418. LUNDBERG, J. M., ÅNGGÅRD, A., HÖKFELT, T., AND KIMMEL, J.: Avian pancreatic polypeptide (APP) inhibits atropine resistant vasodilation in cat submandibular salivary gland and nasal mucosa: Possible interaction with VIP. Acta Physiol. Scand. **110:** 199–201, 1980.
419. LUNDBERG, J. M., BRODIN, E., AND SARIA, A.: Origin and distribution of vagal capsaicin-sensitive substance P neurons with special reference to the trachea and lungs. Acta Physiol. Scand. In press, 1983.
420. LUNDBERG, J. M., HÖKFELT, T., FAHRENKRUG, J., NILSSON, G., AND TERENIUS, L.: Peptides in the cat carotid body (glomus caroticum): VIP-, enkephalin-, and substance P-like immunoreactivity. Acta Physiol. Scand. **107:** 279–281, 1979.
421. LUNDBERG, J. M., HÖKFELT, T., KEWENTER, J., PETTERSSON, G., AHLMAN, H., EDIN, R., DAHLSTRÖM, A., NILSSON, G., TERENIUS, L., UVNÄS-WALLENSTEN, K., AND SAID, S.: Substance P-, VIP-, and enkephalin-like immunoreactivity in the human vagus nerve. Gastroenterology **77:** 468–471, 1979.
422. LUNDBERG, J. M., HÖKFELT, T., NILSSON, G., TERENIUS, L., REHFELD, J., ELDE, R., AND SAID, S.: Peptide neurons in the vagus, splanchnic and sciatic nerves. Acta Physiol. Scand. **104:** 499–501, 1978.
423. LUNDBERG, J. M., HÖKFELT, T., ÅNGGÅRD, A., PERNOW, B., AND EMSON, P.: Immunohistochemical evidence for substance P immunoreactive nerve fibres in the taste buds of the cat. Acta Physiol. Scand. **107:** 389–391, 1979.
424. LUNDBERG, J. M., AND SARIA, A.: Bronchial smooth muscle contraction induced by stimulation of capsaicin-sensitive vagal sensory neurons. Acta Physiol. Scand. **116:** 473–476, 1982.
425. LUNDBERG, J. M., AND SARIA, A.: Capsaicin-induced desensitization of the airway mucosa to mechanical, chemical and anaphylactic irritants. Nature (Lond.) **308:** 351–353, 1983.
426. LUNDBERG, J. M., SARIA, A., BRODIN, E., ROSELL, S., AND FOLKERS, K.: A substance P antagonist inhibits vagally induced inflammation and bronchial smooth muscle contraction in the guinea-pig. Proc. Natl. Acad. Sci. **80:** 1120–1124, 1983.
427. LUNDBERG, J. M., AND TATEMOTO, K.: Pancreatic polypeptide family (APP, BPP, NPY and PYY) in relation to α-adrenoreceptor resistant sympathetic vasoconstrictor. Acta Physiol. Scand. **116:** 393–402, 1982.
428. LUNDBLAD, L., ÅNGGÅRD, A., AND LUNDBERG, J. M.: Effects of antidromic trigeminal nerve stimulation in relation to parasympathetic vasodilation in cat nasal mucosa. Acta Physiol. Scand. In press.
429. LUNDQVIST, I., SUNDLER, F., AHREN, B., ALUMETS, J., AND HÅKANSON, R.: Somatostatin, pancreatic polypeptide, substance P, and neurotensin: Cellular distribution and effects on stimulated insulin secretion in the mouse. Endocrinology **104:** 832–838, 1979.
430. MAGNUSSON (HOLM), I., AND THULIN, L.: Effect of substance P on CCK- or VIP-induced choleresis in anesthetized dogs. Acta Physiol. Scand. **106:** 293–297, 1979.
431. MAGNUSSON, I., AND THULIN, L.: Effects of substance P and somatostatin on choleresis in anesthetized dogs. Acta Chir. Scand. **147:** 667–671, 1981.
432. MAGNUSSON, T., CARLSSON, A., FISHER, G. H., CHANG, D., AND FOLKERS, K.: Effect of synthetic substance P on monoaminergic mechanisms in brain. J. Neural Transm. **38:** 89–93, 1976.
433. MALICK, J. B., AND GOLDSTEIN, J. M.: Analgesic activity of substance P following intracerebral administration in rats. Life Sci. **23:** 835–844, 1978.
434. MALMFORS, G., LEANDER, S., BRODIN, E., HÅKANSON, R., HOLMIN, T., AND SUNDLER, F.: Peptide-containing neurons intrinsic to the gut wall. An experimental study in the pig. Cell Tissue Res. **214:** 225–238, 1981.
435. MALTHE-SÖRENSEN, D., CHENEY, S. L., AND COSTA, E.: Modulation of acetylcholine metabolism in the hippocampal cholinergic pathway by interseptally injected substance P. J. Pharmacol. Exp. Ther. **206:** 21–28, 1978.
436. MALTHE-SÖRENSEN, D., AND OKTEDALEN, O.: Substance P-like immunoreactivity in the phrenic nerve and diaphragm: Axonal transport of substance P in the phrenic nerve. Neurosci. Lett. **28:** 269–274, 1982.
437. MANDAHL, A., AND BILL, A.: Ocular responses to antidromic trigeminal stimulation, intracameral prostaglandin E_1 and E_2, capsaicin and substance P. Acta Physiol. Scand. **112:** 331–338, 1981.
438. MARSAN, C. A., AND TRACZYK, W. X. (eds.): Neuropeptides and Neural Transmission, International Brain Research Organization, IBRO Monograph Series, vol. 7, Raven Press, New York, 1980.
439. MARSDEN, C. A., BENNETT, G. W., AND IRONS, J.: Localization and release of 5-hydroxytryptamine, thyrotrophin releasing hormone and substance P in rat ventral spinal cord. Comp. Biochem. Physiol. **72C:** 263–270, 1982.
440. MARTINEZ, J. R., AND MARTINEZ, A. M.: Stimulatory and inhibitory effects of substance P on rat submandibular secretion. J. Dent. Res. **60:** 1031–1038, 1981.
441. MATTHEWS, M. R., AND CUELLO, A. C.: Substance P-immunoreactive peripheral branches of sensory neurons innervate guinea pig sympathetic neurons. Proc. Natl. Acad. Sci. U.S.A. **79:** 1668–1672, 1982.
442. MAUL, E., AND SEARS, M. L.: Objective evaluation of experimental occular irritation. Invest. Ophthalmol. **15:** 308–315, 1976.
443. MAYER, N., GAMSE, R., AND LEMBECK, F.: Effect of capsaicin pretreatment on substance P binding to synaptic vesicles. J. Neurochem. **35:** 1238–1241, 1980.
444. MAYER, N., LEMBECK, F., GOEDERT, M., AND OTTEN, U.: Effects of antibodies against nerve growth factor on the postnatal development of substance P-containing sensory neurons. Neurosci. Lett. **29:** 47–52, 1982.
445. MAYER, N., LEMBECK, F., SARIA, A., AND GAMSE, R.: Substance P: Characteristics of binding to synaptic vesicles of rat brain. Naunyn-Schmiedeberg's Arch. Pharmacol. **306:** 45–51, 1979.
446. MAZUREK, N., PECHT, I., TEICHBERG, V. I., AND BLUMBERG, S.: The role of the N-terminal tetrapeptide in the histamine releasing action of substance P. Neuropharmacology **20:** 1025–1027, 1981.
447. MCGREGOR, G. P., WOODHAMS, P. L., O'SHAUGHNESSY, D. J., GHATEI, M. A., POLAK, J. M., AND BLOOM, S. R.: Developmental changes in bombesin, substance P, somatostatin and vasoactive intestinal polypeptide in the rat brain. Neurosci. Lett. **28:** 21–27, 1982.
448. MCLENNAN, H.: Synaptic Transmission, W. B. Saunders, Philadelphia, 1963.
449. MELCHIORRI, P., TONELLI, F., AND NEGRI, L.: Comparative circulatory effects of substance P, eledoisin and physalemin in the dog. *In* Substance P (Nobel Symposium 37), ed. by U. S. von Euler and B. Pernow, pp. 311–319, Raven Press, New York, 1977.
450. MÉSZÁROS, J., TARCHALSKA, B., GAJEWSKA, S., JANICKI, P., DURIASZ, H., AND SZRENIAWSKI, Z.: Substance P, hexapeptide pGlu^6 (SP_{6-11}), analgesia and serotonin depletion. Pharmacol. Biochem. Behav. **14:** 11–15, 1981.
451. MICHELOT, R., LEVIEL, V., GIORGUIEFF-CHESSELET, M. F., CHÉRAMY, A., AND GLOWINSKI, J.: Effects of the unilateral nigral modulation of substance P transmission on the activity of the two nigro-striatal dopaminergic pathways. Life Sci. **24:** 715–724, 1979.
452. MILENOV, K., OEHME, P., BIENERT, M., AND BERGMANN, J.: Effect of SP on mechanical and myoelectrical activities of stomach and small intestines in conscious dog. Arch. Int. Pharmacodyn. Thér. **233:** 251–260, 1978.
453. MILLER, M. S., BUCK, S. H., SIPES, I. G., AND BURKS, T. F.: Capsaicinoid-induced local and systemic antinociception without substance P depletion. Brain Res. **244:** 193–197, 1982.
454. MILLER, A., COSTA, M., FURNESS, J. B., AND CHUBB, I. W.: Substance P immunoreactive sensory nerves supply the rat iris and cornea. Neurosci. Lett. **23:** 243–249, 1981.
455. MILLS, I. H., MACFARLANE, N. A. A., AND WARD, P. E.: Increase in kallikrein excretion during the natriuresis produced by arterial infusion of substance P. Nature (Lond.) **247:** 108–109, 1974.
456. MOHRLAND, J. S., AND GEBHART, G. F.: Substance P-induced analgesia in the rat. Brain Res. **171:** 556–559, 1979.
457. MONTEL, H., STARKE, V., AND WEBER, F.: Influence of morphine and naloxone on the release of noradrenaline from rat brain cortex slices. Naunyn-Schmiedeberg's Arch. Pharmacol. **283:** 357–369, 1974.
458. MOORE, A. F.: The interaction of enkephalins with substance P on vascular smooth muscle. Res. Commun. Chem. Pathol. Pharmacol. **23:** 233–242, 1979.
459. MOREL, G., CHAYVIALLE, J. A., KERDELHUE, B., AND DUBOIS, P. M.: Ultrastructural evidence for endogenous substance-P-like immunoreactivity in the rat pituitary gland. Neuroendocrinology **35:** 86–92, 1982.
460. MORITA, K., NORTH, R. A., AND KATAYAMA, Y.: Evidence that substance P is a neurotransmitter in the myenteric plexus. Nature (Lond.) **287:** 151–152, 1980.
461. MROZ, E. A., BROWNSTEIN, M. J., AND LEEMAN, S. E.: Evidence for substance P in the habenulo-interpeduncular tract. Brain Res. **113:** 597–599, 1976.
462. MROZ, E. A., BROWNSTEIN, M. J., AND LEEMAN, S. E.: Distribution of immunoassayable substance P in the rat brain: Evidence for the existence of substance P-containing tracts. *In* Substance P (Nobel Symposium 37), ed. by U. S. von Euler and B. Pernow, pp. 147–154, Raven Press, New York, 1977.
463. MUDGE, A. W., LEEMAN, S. E., AND FISCHBACH, G. D.: Enkephalin inhibits release of substance P from sensory neurons in culture and decreases action potential duration. Proc. Natl. Acad. Sci. U.S.A. **76:** 526–530, 1979.
464. NAGY, J. I., HUNT, S. P., IVERSEN, L. L., AND EMSON, P. C.: Biochemical and anatomical observations on the degeneration of peptide-containing primary afferent neurons after neonatal capsaicin. Neuroscience **6:** 1923–1934, 1981.
465. NAKANE, P. K.: Simultaneous localization of multiple tissue antigens using the peroxidase-labelled antibody methods: A study on pituitary glands in the rat. J. Histochem. Cytochem. **16:** 557–560, 1968.
466. NAKATA, Y., KUSAKA, Y., SEGAWA, T., YAJIMA, H., AND KITAGAWA, K.: Substance P: Regional distribution and specific binding to synaptic mem-

branes in rabbit central nervous system. Life Sci. **22:** 259–268, 1978.
467. NAKATA, Y., KUSAKA, Y., AND SEGAWA, T.: Supersensitivity to substance P after dorsal root section. Life Sci. **24:** 1651–1654, 1979.
468. NAKATA, Y., KUSAKA, Y., YAJIMA, H., KITAGAWA, K., AND SEGAWA, T.: Further characterization of the binding of substance P to a fraction from rabbit brain enriched in synaptic membranes. Naunyn-Schmiedeberg's Arch. Pharmacol. **314:** 211–214, 1980.
469. NAKATA, Y., KUSAKA, Y., YAJIMA, H., AND SEGAWA, T.: Active uptake of substance P carboxy-terminal heptapeptide (5-11) into rat brain and rabbit spinal cord slices. J. Neurochem. **37:** 1529–1534, 1981.
470. NICOLL, R. A.: The action of thyrotropin-releasing hormone, substance P and related peptides on frog spinal motoneurons. J. Pharmacol. Exp. Ther. **207:** 817–824, 1978.
471. NICOLL, R. A., SCHENKER, C., AND LEEMAN, S. E.: Substance P as a transmitter candidate. Annu. Rev. Neurosci. **3:** 227–268, 1980.
472. NIEBER, K., MILENOV, K., BERGMANN, J., AND OEHME, P.: Contractile response of longitudinal and circular smooth muscles of guinea pig stomach and ileum to substance P. Acta Biol. Med. **40:** 209–216, 1981.
473. NIEDRICH, H., BIENERT, M., MEHLIS, B., BERGMANN, J., AND OEHME, P.: Studien zum Wirkungsmechanismus glattmuskulär angreifender Peptide. Acta Biol. Med. Germ. **34:** 483–489, 1975.
474. NILSSON, G., AND BRODIN, E.: Tissue distribution of substance P-like immunoreactivity in dog, cat, rat and mouse. *In* Substance P (Nobel Symposium 37), ed. by U. S. von Euler and B. Pernow, pp. 49–54, Raven Press, New York, 1977.
475. NILSSON, G., DAHLBERG, K., BRODIN, E., SUNDLER, F., AND STRANDBERG, K.: Distribution and constrictor effect of substance P in guinea pig tracheobronchial tissue. *In* Substance P (Nobel Symposium 37), ed. by U. S. von Euler and B. Pernow, pp. 75–81, Raven Press, New York, 1977.
476. NILSSON, G., HÖKFELT, T., AND PERNOW, B.: Distribution of substance P-like immunoreactivity in the rat central nervous system as revealed by immunohistochemistry. Med. Biol. **52:** 424–427, 1974.
477. NILSSON, G., LARSSON, L.-I., HÅKANSSON, R., BRODIN, E., PERNOW, B., AND SUNDLER, F.: Localization of substance P-like immunoreactivity in mouse gut. Histochemistry **43:** 97–99, 1975.
478. NILSSON, G., PERNOW, B., FISHER, G. H., AND FOLKERS, K.: Presence of substance P-like immunoreactivity in plasma from man and dog. Acta Physiol. Scand. **94:** 542–544, 1975.
479. NILSSON, G., PERNOW, B., FISHER, G. H., AND FOLKERS, K.: Radioimmunological determination of substance P. *In* Substance P (Nobel Symposium 37), ed. by U. S. von Euler and B. Pernow, pp. 41–48, Raven Press, New York, 1977.
480. NISHIMOTO, T., AKAI, M., INAGAKI, S., SHIOSAKA, S., SHIMIZU, Y., YAMAMOTO, K., SENBA, E., SAKANAKA, M., TAKATSUKI, K., HARA, Y., TAKAGI, H., MATSUZAKI, T., KAWAI, Y., AND TOHYAMA, M.: On the distribution and origins of substance P in the papillae of the rat tongue: An experimental and immunohistochemical study. J. Comp. Neurol. **207:** 85–92, 1982.
481. NOWAK, L. M., AND MACDONALD, R. L.: Substance P decreases a potassium conductance of spinal cord neurons in cell culture. Brain Res. **214:** 416–423, 1981.
482. NUTT, J. G., MROZ, E. A., LEEMAN, S. E., WILLIAMS, A. C., ENGEL, W. K., AND CHASE, T. N.: Substance P in human cerebrospinal fluid: Reductions in peripheral neuropathy and autonomic dysfunction. Neurology **30:** 1280–1285, 1980.
483. OEHME, P., BERGMANN, J., BIENERT, M., HILSE, H., PIESCHE, L., MINH THU, P., AND SCHEER, E.: Biological action of substance P—Its differentiation by affinity and intrinsic efficacy. *In* Substance P (Nobel Symposium 37), ed. by U. S. von Euler and B. Pernow, pp. 327–335, Raven Press, New York, 1977.
484. OEHME, P, BIENERT, M., HECHT, K., AND BERGMAN, J.: Substanz P. Ausgewählte Probleme der Chemie, Biochemie, Pharmacologie, Physiologie and Pathophysiologie. Beitr. Wirkstofforsch. **12:** 1–185, 1981.
485. OEHME, P., HILSE, H., MORGENSTERN, E., AND GÖRES, E.: Substance P: Does it produce analgesia or hyperalgesia? Science **208:** 305–307, 1980.
486. OGATA, N.: Substance P causes direct depolarisation of neurones of guinea pig interpeduncular nucleus *in vitro*. Nature (Lond.) **277:** 480–481, 1979.
487. OGATA, N., AND ABE, H.: Further support for the postsynaptic action of substance P and its blockade with baclofen in neurons of the guinea-pig hypothalamus *in vitro*. Experientia (Basel) **37:** 759–761, 1981.
488. OGATA, N., AND ABE, H.: Substance P decreases membrane conductance in neurons of the guinea pig hypothalamus *in vitro*. Neuropharmacology **21:** 187–189, 1982.
489. OKADA, Y.: Role of GABA in the substantia nigra. *In* GABA in Nervous System Function, ed. by E. Robert, T. N. Chase, and D. B. Tower, pp. 235–243, Raven Press, New York, 1976.
490. OLGART, L., GAZELIUS, B., BRODIN, E., AND NILSSON, G.: Release of substance P-like immunoreactivity from the dental pulp. Acta Physiol. Scand. **101:** 510–512, 1977.
491. OLGART, L., HÖKFELT, T., NILSSON, G., AND PERNOW, B.: Localization of substance P-like immunoreactivity in nerves in the tooth pulp. Pain **4:** 153–159, 1977.
492. OLPE, H-R., AND KOELLA, W.: Rotatory behavior in rats by intranigral application of substance P and an eledoisin fragment. Brain Res. **126:** 576–579, 1977.
493. ORREGO, B.: Criteria for the identification of central neurotransmitters and their application to studies with some nerve tissue preparations *in vitro*. Neuroscience **4:** 1037–1057, 1979.
494. OTSUKA, M., AND KONISHI, S.: Electrophysiology of mammalian spinal cord *in vitro*. Nature (Lond.) **252:** 733–734, 1974.
495. OTSUKA, M., AND KONISHI, S.: Substance P and excitatory transmitter of primary sensory neurons. Cold Spring Harbor Symp. Quant. Biol. **40:** 135–143, 1975.
496. OTSUKA, M., AND KONISHI, S.: Release of substance P-like immunoreactivity from isolated spinal cord of newborn rat. Nature (Lond.) **264:** 83–84, 1976.
497. OTSUKA, M., AND KONISHI, S.: Electrophysiological and neurochemical evidence for substance P as a transmitter of primary sensory neurons. *In* Substance P (Nobel Symposium 37), ed. by U. S. von Euler and B. Pernow, pp. 207–214, Raven Press, New York, 1977.
498. OTSUKA, M., KONISHI, S., AND TAKAHASHI, T.: The presence of a motoneuron-depolarizing peptide in bovine dorsal roots of spinal nerves. Proc. Jpn. Acad. **48:** 342–346, 1972.
499. OTSUKA, M., KONISHI, S., AND TAKAHASHI, T.: A further study of motoneuron-depolarizing peptide extracted from dorsal roots of bovine spinal nerves. Proc. Jpn. Acad. **48:** 747–752, 1972.
500. OTSUKA, M., KONISHI, S., AND TAKAHASHI, T.: Hypothalamic substance P as a candidate for transmitter of primary afferent neurons. Fed. Proc. **34:** 1922–1928, 1975.
501. OTSUKA, M., KONISHI, S., YANAGISAWA, M., TSUNOO, A., AND AKAGI, H.: Role of substance P as a sensory transmitter in spinal cord and sympathetic ganglia. *In* Substance P in the Nervous System (Ciba Foundation symposium 91), ed. by R. Porter, and M. O'Connor, p. 13–34, Pitman, London, 1982.
502. OTSUKA, M., AND YANAGISAWA, M.: The action of substance P on motoneurons of the isolated rat spinal cord. *In* Advances in Pharmacology and Therapeutics, vol. 2, ed. by P. Simon, 181–190, Pergamon press, Oxford and New York, 1979.
503. OTSUKA, M., AND YANAGISAWA, M.: The effects of substance P and baclofen on motoneurones of isolated spinal cord of the newborn rat. J. Exp. Biol. **89:** 201–214, 1980.
504. PAASONEN, M. K., AND VOGT, M.: The effect of drugs on the amounts of substance P and 5-hydroxytryptamine in mammalian brain. J. Physiol. (Lond.) **131:** 617–626, 1956.
505. PALS, D. T., AND MICALIZZI, E. R.: Participation of prostaglandins in the vasodepressor effect of substance P. J. Pharm. Pharmacol. **33:** 110–111, 1981.
506. PAXINOS, G., EMSON, P. C., AND CUELLO, A. C.: Substance P projections to the endopeduncular nucleus, the medial preoptic area and the lateral septum. Neurosci. Lett. **7:** 133–136, 1978.
507. PAXINOS, G., EMSON, P. C., AND CUELLO, A. C.: The substance P projections to the frontal cortex and the substantia nigra. Neurosci. Lett. **7:** 127–131, 1978.
508. PEARSE, A. G. E.: The cytochemistry and ultrastructure of polypeptide hormone-producing cells of the APUD series, and the embryologic, physiologic and pathologic implications of the concept. J. Histochem. Cytochem. **17:** 303–313, 1969.
509. PEARSE, A. G. E., AND POLAK, J. M.: Immunocytochemical localization of substance P in mammalian intestine. Histochemistry **41:** 373–375, 1975.
510. PEARSON, J., BRANDEIS, L., AND CUELLO, A. C.: Depletion of substance P-containing axons in substantia gelatinosa of patients with diminished pain sensitivity. Nature (Lond.) **295:** 61–63, 1982.
511. PELLETIER, G., STEINBUSCH, H. W. M., AND VERHOFSTAD, A. A. J.: Immunoreactive substance P and serotonin present in the same dense-core vesicles. Nature (Lond.) **293:** 71–72, 1981.
512. PEPEU, G.: The release of acetylcholine from the brain: an approach to the study of central cholinergic metabolism. Progr. Neurobiol. **2:** 259–288, 1974.
513. PERNOW, B.: Substance P distribution in the digestive tract. Acta Physiol. Scand. **24:** 97–102, 1951.
514. PERNOW, B.: Studies on substance P. Purification, occurrence and biological actions. Acta Physiol. Scand. **29:** suppl. 105, 1–90, 1953.
515. PERNOW, B.: Inactivation of substance P by proteolytic enzymes. Acta Physiol. Scand. **34:** 295–302, 1955.
516. PERNOW, B.: Substanz P. Z. Vitam-Horm.- Fermentforsch. **7:** 59–71, 1955.
517. PERNOW, B.: Pharmacology of substance P. Ann. N.Y. Acad. Sci. **104:** 393–402, 1963.
518. PERNOW, B., AND ROSELL, S.: Effect of substance P on blood flow in canine adipose tissue and skeletal muscle. Acta Physiol. Scand. **93:** 139–141, 1975.
519. PETTIBONE, D. J., AND WURTMAN, R. J.: D-Amphetamine reduces striatal substance P concentrations by presynaptic release of dopamine. Brain Res. **186:** 409–419, 1980.
520. PHAM-HUU-CHANH, A., PHAM-HUU-CHANH, P., CLAVEL, P., AND LEHMANN-SCHAD, W.: Cardiovascular effects of synthetic substance P. Pharmacology **15:** 341–347, 1977.
521. PHILLIS, J. W.: Is β-(4-chlorophenyl)-GABA a specific antagonist of substance P on cerebral cortical neurons? Experientia (Basel) **32:** 593–594, 1976.
522. PHILLIS, J. W., AND LIMACHER, J. J.: Substance P excitation of cerebral cortical Betz cells. Brain Res. **69:** 158–163, 1974.

523. PICKEL, V. M., REIS, D. J., AND LEEMAN, S. E.: Ultrastructural localization of substance P in neurons of rat spinal cord. Brain Res. **122:** 534–540, 1977.
524. PIERCEY, M. F., DOBRY, P. J. K., EINSPAHR, F. J., SCHROEDER, L. A., AND MASIQUES, N.: Use of substance P fragments to differentiate substance P receptors of different tissues. Regul. Pept. **3:** 337–349, 1982.
525. PIERCEY, M. F., DOBRY, P. J. K., SCHROEDER, L. A., AND EINSPAHR, F. J.: Behavioral evidence that substance P may be spinal cord sensory neurotransmitter. Brain Res. **210:** 407–412, 1981.
526. PIERCEY, M. F., EINSPAHR, F. J., DOBRY, P. J. K., SCHROEDER, L. A., AND HOLLISTER, R. P.: Morphine does not antagonize the substance P mediated excitation of dorsal horn neurons. Brain Res. **186:** 421–434, 1980.
527. PIERCEY, M. F., SCHROEDER, L. A., FOLKERS, K., XU, J.-C., AND HORIG, J.: Sensory and motor functions of spinal cord substance P. Science **214:** 1361–1363, 1981.
528. PIESCHE, L., HILSE, H., BIENERT, M., AND OEHME, P.: Über die Hemmung der ^{3}H-Noradrenalin-Aufnahme in Synaptosomen durch Substanz P. Acta Biol. Med. Germ. **36:** 1351–1354, 1978.
529. PINNOCK, R. D., AND DRAY, A.: Differential sensitivity of presumed dopaminergic and non-dopaminergic neurones in rat substantia nigra to electrophoretically applied substance P. Neurosci. Lett. **29:** 153–158, 1982.
530. PORTER, R., AND O'CONNOR, M. (eds.): Substance P in the Nervous System (Ciba Foundation Symposium 91), Pitman, London, 1982.
531. POWELL, D., LEEMAN, S. E., TREGEAR, G. W., NIALL, H. D., AND POTTS, J. T., JR.: Radioimmunoassay for substance P. Nature New Biol. **241:** 252–254, 1973.
532. POWELL, D., SKRABANEK, P., AND CANNON, D.: Substance P: Radioimmunoassay studies. *In* Substance P (Nobel Symposium 37), ed. by U. S. von Euler and B. Pernow, pp. 35–40, Raven Press, New York, 1977.
533. POWELL, D., SKRABANEK, P., CANNON, D., AND BALFE, A.: Substance P in human plasma. *In* Neuropetides and Neural Transmission, IBRO Monograph Series, vol. 7, ed. by C. A. Marsan and W. X. Traczyk, pp. 105–110, Raven Press, New York, 1980.
534. PRIESTLEY, J. V., SOMOGYI, P., AND CUELLO, A. C.: Immunocytochemical localization of substance P in the spinal trigeminal nucleus of the rat: A light and electron microscopic study. J. Comp. Neurol. **211:** 31–49, 1982.
535. PROBERT, L., DE MEY, J., AND POLAK, J. M.: Distinct subpopulations of enteric *p*-type neurones contain substance P and vasoactive intestinal polypeptide. Nature (Lond.) **294:** 470–471, 1981.
536. PUTNEY, J. W., JR.: Muscarinic, alpha-adrenergic and peptide receptors regulate the same calcium influx sites in the parotid gland. J. Physiol. (Lond.) **268:** 139–149, 1977.
537. RANDIĆ, M., AND MILETIĆ, V.: Effect of substance P in cat dorsal horn neurones activated by noxious stimuli. Brain Res. **128:** 164–169, 1977.
538. REINECKE, M., WEIHE, E., AND FORSSMANN, W. G.: Substance P-immunoreactive nerve fibers in the heart. Neurosci. Lett. **20:** 265–269, 1980.
539. REISINE, T., SOUBRIE, P., ARTAUD, F., AND GLOWINSKI, J.: Application of L-glutamic acid and substance P to the substantia nigra modulates in vivo (^{3}H)serotonin release in the basal ganglia of the cat. Brain Res. **236:** 317–327, 1982.
540. REUBI, J. C., EMSON, P. C., JESSELL, T. M., AND IVERSEN, L. L.: Effects of GABA, dopamine, and substance P on the release of newly synthesized ^{3}H-5-hydroxytryptamine from rat substantia nigra in vitro. Naunyn-Schmiedeberg's Arch. Pharmacol. **304:** 271–275, 1978.
541. RIBAK, C. E., VAUGHN, J. E., SAITO, K., BARBER, R., AND ROBERTS, E.: Immunocytochemical localization of glutamate decarboxylase in rat substantia nigra. Brain Res. **116:** 287–298, 1976.
542. RIVIER, C., BROWN, M., AND VALE, W.: Effect of neurotensin, substance P and morphine sulphate on the secretion of prolaction and growth hormone in the rat. Endocrinology **100:** 751–754, 1977.
543. ROBERTS, G. W., WOODHAMS, P. L., POLAK, J. M., AND CROW, T. J.: Distribution of neuropeptides in the limbic system of the rat: The amygdaloid complex. Neuroscience **7:** 99–131, 1982.
544. ROBINSON, S. E., SCHWARTZ, J. P., AND COSTA, E.: Substance P in the superior cervical ganglion and the submaxillary gland of the rat. Brain Res. **182:** 11–17, 1980.
545. ROLE, L. W., LEEMAN, S. E., AND PERLMAN, R. L.: Satostatin and substance P inhibit catecholamine secretion from isolated cells of guinea-pig adrenal medulla. Neuroscience **6:** 1813–1821, 1981.
546. RONDEAU, D. B., JOLICOEUR, F. B., BELANGER, F., AND BARBEAU, A.: Motor activity induced by substance P in rats. Pharmacol. Biochem. Behav. **9:** 769–775, 1978.
547. ROSELL, S., BJÖRKROTH, U., CHANG, D., YAMAGUCHI, I., WAN, Y-P., RACKUR, G., FISHER, G., AND FOLKERS, K.: Effects of substance P and analogs on isolated guinea pig ileum. *In* Substance P (Nobel Symposium 37), ed. by U. S. von Euler and B. Pernow, pp. 83–88, Raven Press, New York, 1977.
548. ROSELL, S., BJÖRKROTH, U., XU, J-C., AND FOLKERS, K.: The pharmacological profile of a substance P (SP) antagonist. Evidence for the existence of subpopulations of SP receptors. Acta Physiol. Scand. **117:** 445–449, 1983.
549. ROSELL, S., OLGART, L., GAZELIUS, B., PANOPOULOS, P., FOLKERS, K., AND HÖRIG, J.: Inhibition of antidromic and substance P-induced vasodilatation by a substance P antagonist. Acta Physiol. Scand. **111:** 381–382, 1981.
550. RUDICH, L., AND BUTCHER, F. R.: Effect of substance P and eledoisin on K^+ efflux amylase release and cyclic nucleotide levels in slices of rat parotid gland. Biochim. Biophys. Acta **444:** 704–711, 1976.
551. RYALL, R. W.: Sub-cellular distribution of pharmacologically active substances in guinea-pig brain. Nature (Lond.) **196:** 680–681, 1962.
552. RYALL, R. W.: The subcellular distribution of substance P, acetylcholine, serotonin and glutamic acid in brain homogenates. J. Neurochem. **11:** 131–145, 1964.
553. RYALL, R. W.: Modulation of cholinergic transmission by substance P. *In* Substance P in the Nervous System (Ciba Foundation symposium 91), ed. by R. Porter and M. O'Connor, pp. 267–280, Pitman, London, 1982.
554. RYALL, R. W., AND BELCHER, G.: Substance P selectively blocks nicotinic receptors on Renshaw cells: A possible synaptic inhibitory mechanism. Brain Res. **137:** 376–380, 1977.
555. SAITO, K., KONISHI, S., AND OTSUKA, M.: Antagonism between lioresal and substance P in rat spinal cord. Brain Res. **97:** 177–180, 1975.
556. SAKANAKA, M., INAGAKI, S., SHIOSAKA, S., SENBA, E., TAKAGI, H., TAKATSUKI, K., KAWAI, Y., IIDA, H., HARA, Y., AND TOHYAMA, M.: Ontogeny of substance P-containing neuron system of the rat: Immunohistochemical analysis. II. Lower brain stem. Neuroscience **7:** 1097–1126, 1982.
557. SAKANAKA, M., SHIOSAKA, S., TAKATSUKI, K., INAGAKI, S., TAKAGI, H., SENBA, E., KAWAI, Y., HARA, Y., IIDA, H., MINAGAWA, H., MATSUZAKI, T., AND TOHYAMA, M.: Evidence for the existence of a substance P-containing pathway from the nucleus laterodorsalis tegmenti (Castaldi) to the lateral septal area of the rat. Brain Res. **230:** 351–355, 1981.
558. SAKANAKA, M., SHIOSAKA, S., TAKATSUKI, K., INAGAKI, S., TAKAGI, H., SENBA, E., KAWAI, Y., MATSUZAKI, T., AND TOHYAMA, M.: Experimental immunohistochemical studies on the amygdalofugal peptidergic (substance P and somatostatin) fibers in the stria terminalis of the rat. Brain Res. **221:** 231–242, 1981.
559. SALT, T. E., CROZIER, C. S., AND HILL, R. G.: The effects of capsaisin pretreatment on the responses of single neurones to sensory stimuli in the trigeminal nucleus caudalis of the rat: Evidence against a role for substance P as the neurotransmitter serving thermal nociception. Neuroscience **7:** 1141–1148, 1982.
560. SALT, T. E., DE VRIES, G. J., RODRIGUEZ, R. E., CAHUSAC, P. M. B., MORRIS, R., AND HILL, R. G.: Evaluation of [D-Pro^2, D-Trp^{7,9}]substance P as an antagonist of substance P responses in the rat central nervous system. Neurosci. Lett. **30:** 291–295, 1982.
561. SAMNEGÅRD, H., THULIN, L., TYDÉN, G., JOHANSSON, C., MUHRBECK, O., AND BJÖRKLUND, C.: Effect of synthetic substance P on internal carotid artery blood flow in man. Acta Physiol. Scand. **104:** 491–495, 1978.
562. SANDBERG, B. E. B., LEE, C-M., HANLEY, M. R., AND IVERSEN, L. L.: Synthesis and biological properties of enzyme-resistant analogues of substance P. Eur. J. Biochem. **114:** 329–337, 1981.
563. SARIA, A., MAYER, N., LEMBECK, F., AND PABST, M.: Regional distribution and biochemical properties of ^{125}I-Tyr^8-substance P binding sites in synaptic vesicles. Naunyn-Schmiedeberg's Arch. Pharmacol. **311:** 151–157, 1980.
564. SASTRY, B. R.: Substance P effects on spinal nociceptive neurones. Life Sci. **24:** 2169–2178, 1979.
565. SCADDING, J. W.: The permanent anatomical effects of neonatal capsaicin on somatosensory nerves. J. Anat. **131:** 473–484, 1980.
566. SCHACHTER, M. (ed.): Polypeptides Which Affect Smooth Muscles and Blood Vessels, pp. 163–198, Pergamon Press, Oxford, 1960.
567. SCHENKER, C., MROZ, E. A., AND LEEMAN, S. E.: Release of substance P from isolated nerve endings. Nature (Lond.) **264:** 790–792, 1976.
568. SCHULTZBERG, M.: The peripheral nervous system. *In* Chemical Neuroanatomy, ed. by P. Emson. Raven Press, New York, 1983.
569. SCHULTZBERG, M., DREYFUS, C. F., GERSHON, M. D., HÖKFELT, T., ELDE, R. P., NILSSON, G., SAID, S., AND GOLDSTEIN, M.: VIP-, enkephalin-, substance P-, and somatostatin-like immunoreactivity in neurons intrinsic to the intestine: Immunohistochemical evidence from organotypic tissue cultures. Brain Res. **155:** 239–248, 1978.
570. SCHULTZBERG, M., HÖKFELT, T., NILSSON, G., TERENIUS, L., REHFELD, J. F., BROWN, M., ELDE, R., GOLDSTEIN, M., AND SAID, S.: Distribution of peptide- and catecholamine-containing neurons in the gastro-intestinal tract of rat and guinea-pig: Immunohistochemical studies with antisera to substance P, vasoactive intestinal polypeptide, enkephalins, somatostatin, gastrin/cholecystokinin, neurotensin and dopamine β-hydroxylase. Neuroscience **5:** 689–744, 1980.
571. SCHULTZBERG, M., HÖKFELT, T., OLSON, L., ÅLUND, M., NILSSON, G., TERENIUS, L., ELDE, R., GOLDSTEIN, M., AND SAID, S.: Substance P, VIP, enkephalin and somatostatin immunoreactive neurons in intestinal tissue transplanted to the anterior eye chamber. J. Auton. Nerv. Syst. **1:** 291–303, 1980.
572. SCHWARTZ, J. P., AND COSTA, E.: Nerve growth factor-mediated increase of the substance P content of chick embryo dorsal root ganglia. Brain Res. **170:** 198–202, 1979.
573. SENBA, E., SHIOSAKA, S., HARA, Y., INAGAKI, S., SAKANAKA, M., TAKATSUKI, K., KAWAI, Y., AND TOHYAMA, M.: Ontogeny of the peptidergic system in the rat spinal cord: Immunohistochemical analysis. J. Comp. Neurol. **208:** 54–66, 1982.
574. SENBA, E., SHIOSAKA, S., INAGAKI, S., TAKAGI, H., TAKATSUKI, K., SAKANAKA, M., KAWAI, Y., TOHYAMA, M., AND SHIOTANI, Y.: Peptidergic

neuron system in the rat spinal cord. I. Experimental immunohistochemical study. Neurosci. Lett. suppl. **6:** 77, 1981.
575. SHARE, N. N., AND RACKHAM, A.: Intracerebral substance P in mice: Behavioral effects and narcotic agents. Brain Res. **211:** 379–386, 1981.
576. SILBERGELD, E. K., AND WALTERS, J. R.: Synaptosomal uptake and release of dopamine in substantia nigra: Effects of γ-aminobutyric acid and substance P. Neurosci. Lett. **12:** 119–126, 1979.
577. SINGER, E., SPERK, G., PLACHETA, P., AND LEEMAN, S. E.: Reduction of substance P levels in the ventral cervical spinal cord of the rat after intracisternal 5,7-dihydroxytryptamine injection. Brain Res. **174:** 362–365, 1979.
578. SJÖDIN, L., BRODIN, E., NILSSON, G., AND CONLON, T. P.: Interaction of substance P with dispersed pancreatic acinar cells from the guinea pig. Binding of radioiodinated peptide. Acta Physiol. Scand. **109:** 97–105, 1980.
579. SJÖDIN, L., CONLON, T. P., GUSTAVSON, C., AND UDDHOLM, K.: Interaction of substance P with dispersed pancreatic acinar cells from the guinea pig. Stimulation of calcium outflux, accumulation of cyclic GMP and amylase release. Acta Physiol. Scand. **109:** 107–110, 1980.
580. SKAGERBERG, G., EMSON, P. C., AND BJÖRKLUND, A.: Transplantation of irides or sensory ganglia to the anterior eye chamber of the rat: Survival and sprouting of substance P-containing neurones. Neurosci. Lett. **28:** 29–34, 1982.
581. SKRABANEK, P., CANNON, D., KIRRANE, J., LEGGE, D., AND POWELL, D.: Circulating immunoreactive substance P in man. Ir. J. Med. Sci. **145:** 399–406, 1976.
582. SKRABANEK, P., AND POWELL, D. (eds.): Substance P. Churchill Livingstone, Edinburgh, vol. 1, 1977; vol. 2, 1980; and vol. 3, 1983.
583. SOLOWAY, M. R., STJERNSCHANTZ, J., AND SEARS, M.: The miotic effect of substance P on the isolated rabbit iris. Invest. Ophthalmol. Visual Sci. **20:** 47–52, 1981.
584. SOMOGYI, P., PRIESTLEY, J. V., CUELLO, A. C., SMITH, A. D., AND BOLAM, J. P.: Synaptic connections of substance P-immunoreactive nerve terminals in the substantia nigra of the rat. Cell Tissue Res. **223:** 469–486, 1982.
585. SPERK, G., AND SINGER, E. A.: *In vivo* synthesis of substance P in the corpus striatum of the rat and its transport to the substantia nigra. Brain Res. **238:** 127–135, 1982.
586. STAINES, W. A., NAGY, J. I., VINCENT, S. R., AND FIBIGER, H. C.: Neurotransmitters contained in the efferents of the striatum. Brain Res. **194:** 391–402, 1980.
587. STARKE, K., LEMBECK, F., LORENZ, W., AND WEISS, U.: Gallen- und Pankreas-Sekretion unter Substanz P und einem Physalämin-Derivat. Naunyn-Schmiedeberg's Arch. exp. Pathol. Pharmacol. **260:** 269–274, 1968.
588. STARK-EISON, A., EISON, M. S., AND IVERSEN, S. D.: The behavioural effects of a novel substance P analogue following infusion into the ventral tegmental area of substantia nigra of rat brain. Brain Res. **238:** 137–152, 1982.
589. STARR, M. S., JAMES, T. A., AND GAYTTEN, D.: Behavioural depressant and antinociceptive properties of substance P in the mouse: Possible implication of brain monoamines. Eur. J. Pharmacol. **48:** 203–212, 1978.
590. STERN, P. (ed.): Symposium on substance P. Proc. Sci. Soc. Bosnia Herzegonia **1:** 1961.
591. STERN, P., AND HUKOVIĆ, S.: Substanz P und Tetanustoxin. Naturwissenschaften **23:** 538, 1956.
592. STERN, P., AND HUKOVIĆ, S.: Relation between central and peripheral actions of substance P. Med. Exp. **2:** 1–7, 1960.
593. STERN, P., AND KOCIC-MITROVIĆ, D.: Wirkung des Reserpins auf die Menge der Substanz P im Rattengehirn. Naunyn-Schmiedeberg's Arch. exp. Pathol. Pharmakol. **238:** 57–58, 1960.
594. STERN, P., AND MILIN, R.: Tranquillizing effect of substance P. Proc. Sci. Soc. Exptl. Biol. Med. **101:** 298–299, 1959.
595. STERNBERGER, L. A.: Immunocytochemistry, 2nd ed., John Wiley & Sons, New York, 1979.
596. STEWART, J. M., GETTO, C. J., NELDNER, K., REEVE, E. B., KRIVOY, W. A., AND ZIMMERMANN, E.: Substance P and analgesia. Nature (Lond.) **262:** 784–785, 1976.
597. STINUS, L., KELLEY, A. E., AND IVERSEN, S. D.: Increased spontaneous activity following substance P infusion into A10 dopaminergic area. Nature (Lond.) **276:** 616–618, 1978.
598. STJERNSCHANTZ, J., SEARS, M., AND STJERNSCHANTZ, L.: Intraocular effects of substance P in the rabbit. Invest. Ophthalmol. Visual Sci. **20:** 53–60, 1981.
599. STOECKEL, M. E., PORTE, A., KLEIN, M. J., AND CUELLO, A. C.: Immunocytochemical localization of substance P in the neurohypophysis and hypothalamus of the mouse compared with the distribution of other neuropeptides. Cell Tissue Res. **223:** 533–544, 1982.
600. STORM-MATHISEN, J., AND GULDBERG, H. C.: Hydrotryptamine and noradrenaline in the hippocampal region: Effect of transection of efferent pathways on endogenous levels, high affinity uptake and some transmitter related enzymes. J. Neurochem. **22:** 793–803, 1974.
601. STRICKER, S.: Untersuchungen über die Gefässwurzeln des Ischiadicus. Stitz. Ber. Kaiserl. Akad. Wiss. (Wien), **3:** 173–185, 1876.
602. STUDER, R. O., TRZECIAK, H., AND LERGIER, W.: Isolierung und Aminosäuresequenz von Substanz P aus Pferdedarm. Helv. Chim. Acta **56:** 860–866, 1973.
603. SUNDLER, F., ALUMETS, J., BRODIN, E., KAHLBERG, K., AND NILSSON, G.: Perivascular substance P-immunoreactive nerves in tracheobronchial tissue. *In* Substance P (Nobel Symposium 37), ed. by U. S. von Euler and B. Pernow, pp. 271–273, Raven Press, New York, 1977.
604. SUNDLER, F., HÅKANSON, R., LARSSON, L. I., BRODIN, E., AND NILSSON, G.: Substance P in the gut: An immunochemical and immunohistochemical study of its distribution and development. *In* Substance P (Nobel Symposium 37), ed. by U. S. von Euler and B. Pernow, pp. 59–65, Raven Press, New York, 1977.
605. SUNDLER, F., HÅKANSSON, R., AND LEANDER, S.: Peptidergic nervous systems in the gut. Clin. Gastroenterology **9:** 517–543, 1980.
606. SYDBOM, A.: Histamine release from isolated rat mast cells by neurotensin and other peptides. Agents and Actions **12:** 91, 1982.
607. SZELI, J., MOLINA, E., ZAPPIA, L., AND BERTACCINI, C.: Action of some natural polypeptides on the longitudinal muscle of the guinea-pig ileum. Eur. J. Pharmacol. **43:** 285–287, 1977.
608. SZRENIAWSKI, Z., CZLONKOWSKI, A., JANICKI, P., LIBICH, J., AND GOMULKA, S. W.: Substance P: Pain transmission and analgesia. *In* Neuropeptides and Neural Transmission, IBRO Monograph Series, vol. 7, ed. by A. Marsan, and W. Z. Traczyk, pp. 121–129, Raven Press, New York, 1980.
609. TAKAHASHI, T., KONISHI, S., POWELL, D., LEEMAN, S. E., AND OTSUKA, M.: Identification of the motoneuron-depolarizing peptide in bovine dorsal root as hypothalamic substance P. Brain Res. **73:** 59–69, 1974.
610. TAKAHASHI, T., AND OTSUKA, M.: Regional distribution of substance P in the spinal cord and nerve roots of the cat and the effect of dorsal root section. Brain Res. **87:** 1–11, 1975.
611. TALMAN, W. T., AND REIS, D. J.: Baroreflex actions of substance P microinjected into the nucleus tractus solitarii in rat: A consequence of local distortion. Brain Res. **220:** 402–407, 1981.
612. TAMSEN, A., SAKURADA, T., WAHLSTRÖM, A., TERENIUS, L., AND HARTVIG, P.: Postoperative demand for analgesics in relation to individual levels of endorphins and substance P in cerebrospinal fluid. Pain **13:** 171–183, 1982.
613. TEICHBERG, V. I., COHEN, S., AND BLUMBERG, S.: Distinct classes of substance P receptors revealed by a comparison of the activities of substance P and some of its segments. Regul. Pept. **1:** 327–333, 1981.
614. TERVO, K., TERVO, T., ERÄNKÖ, L., ERÄNKÖ, O., AND CUELLO, A. C.: Immunoreactivity for substance P in the Gasserian ganglion, ophtalmic nerve and anterior segment of the rabbit eye. Histochem. J. **13:** 435–443, 1981.
615. TERVO, K., TERVO, T., ERENKÖ, L., ERENKÖ, O., VALTONEN, S., AND CUELLO, A. C.: Effect of sensory and sympathetic denervation on substance P immunoreactivity in nerve fibres of the rabbit eye. Exp. Eye Res. **34:** 577–585, 1982.
616. TESSLER, A., HIMES, B. T., ARTYMYSHYN, R., MURRAY, M., AND GOLDBERGER, M. E.: Spinal neurons mediate return of substance P following deafferentation of cat spinal cord. Brain Res. **230:** 263–281, 1981.
617. THERIAULT, E., OTSUKA, M., AND JESSELL, T.: Capsaicin-evoked release of substance P from primary sensory neurons. Brain Res. **170:** 209–213, 1979.
618. THULIN, L., AND HOLM, I.: Effect of substance P on the flow of hepatic bile and pancreatic juice. *In* Substance P (Nobel Symposium 37), ed. by U. S. von Euler and B. Pernow, pp. 247–251, Raven Press, New York, 1977.
619. TOGARI, A., KATO, T., AND NAGATSU, T.: Effect of substance P C-terminal heptapeptide on amylase release in rat parotid gland. Arch. Oral Biol. **25:** 597–600, 1980.
620. TORNQVIST, K., MANDAHL, A., LEANDER, S., LORÉN, I., HÅKANSON, R., AND SUNDLER, F.: Substance P-immunoreactive nerve fibres in the anterior segment of the rabbit eye. Cell Tissue Res. **222:** 467–477, 1982.
621. TORRENS, Y., BEAUJOUAN, J-C., BESSON, M-J., MICHELOT, R., AND GLOWINSKI, J.: Inhibitory effects of GABA, L-glutamic acid and nicotine on the potassium-evoked release of substance P in substantia nigra slices of the rat. Eur. J. Pharmacol. **71:** 383–392, 1981.
622. TRACZYK, W. Z.: Circulatory effects of substance P, SP_{6-11}, and ($pGlu^6$) SP_{6-11} hexapeptides. *In* Substance P (Nobel Symposium 37), ed. by U. S. von Euler and B. Pernow, pp. 297–309, Raven Press, New York, 1977.
623. TRAMU, G., PILLEZ, A., AND LEONARDELLI, J.: An efficient method of antibody elution for the successive or simultaneous localization of two antigens by immunocytochemistry. J. Histochem. Cytochem. **26:** 322–324, 1978.
624. TREGEAR, G., NIALL, H. D., POTTS, J. T., LEEMAN, S. E., AND CHANG, M. M.: Synthesis of substance P. Nature New Biol. **232:** 87–89, 1971.
625. UKAI, M., NAGASE, T., HIROHASHI, M., AND YANAIHARA, N.: Antidiuretic activities of substance P and its analogs. Experientia (Basel) **37:** 521–523, 1981.
626. UNGER, W. G., BUTLER, J. M., COLE, D. F., BLOOM, S. R., AND MCGREGOR, G. P.: Substance P, vasoactive intestinal polypeptide (VIP) and somatostatin levels in ocular tissue of normal and sensorily denervated rabbit eyes. Exp. Eye Res. **32:** 797–801, 1981.
627. UNGER, T., RASCHER, W., SCHUSTER, C., PAVLOVITCH, R., SCHÖMIG, A., DIETZ, R., AND GANTEN, D.: Central blood pressure effects of substance P and angiotensin II: Role of the sympathetic nervous system and vasopressin. Eur. J. Pharmacol. **71:** 33–42, 1981.
628. UNGER, T., ROCKHOLD, R. W., YUKIMURA, T., RETTIG, R., AND GANTEN, D.: Blood pressure and heart rate responses to centrally administered substance P are increased in spontaneously hypertensive rats. Clin. Sci. **59:** 299s–302s, 1980.

629. UVNÄS-WALLENSTEN, K.: Release of substance P-like immunoreactivity into the antral lumen of cats. Acta Physiol. Scand. **104:** 464–468, 1978.
630. UYENO, E. T., CHANG, D., AND FOLKERS, K.: Substance P found to lower body temperature and aggresion. Biochem. Biophys. Res. Commun. **86:** 837–842, 1979.
631. VIJAYAN, E., AND MCCANN, S. M.: In vivo and in vitro effects of substance P and neurotensin on gonadotropin and prolactin release. Endocrinology **105:** 64–68, 1979.
632. VIJAYAN, E., AND MCCANN, S. M.: Effects of substance P and neurotensin on growth hormone and thyrotropin release *in vivo* and *in vitro*. Life Sci. **26:** 321–327, 1980.
633. VINCENT, J. D., AND BARKER, J. L.: Substance P: Evidence for diverse roles in neuronal function from cultured mouse spinal neurons. Science **205:** 1409–1412, 1979.
634. VINCENT, S. R., KIMURA, H., AND MCGEER, E. G.: Organization of substance P fibers within the hippocampal formation demonstrated with a biotin-avidin immunoperoxidase technique. J. Comp. Neurol. **199:** 113–123, 1981.
635. VINCENT, S. R., AND MCGEER, E. G.: A substance P projection to the hippocampus. Brain Res. **215:** 349–351, 1981.
636. VINCENT, S. R., STAINES, W. A., MCGEER, E. G., AND FIBIGER, H. C.: Transmitters contained in the efferents of the habenula. Brain Res. **195:** 479–484, 1980.
637. VOGLER, K., HAEFELY, W., HÜRLIMANN, A., STUDER, R. O., LERGIER, W., STRÄSSLE, R., AND BERNEIS, K. H.: A new purification procedure and biological properties of substance P. Ann. N.Y. Acad. Sci. **104:** 378–389, 1963.
638. WALDMEIER, P. C., KAM, R., AND STÖCKLIN, K.: Increased dopamine metabolism in rat striatum after infusions of substance P into the substantia nigra. Brain Res. **159:** 223–227, 1978.
639. WALKER, R. J., KEMP, J. A., YAJIMA, H., KITAGAWA, K., AND WOODRUFF, G. N.: The action of substance P on mesencephalic reticular and substantia nigral neurones of the rat. Experientia (Basal) **32:** 214–215, 1976.
640. WALL, P. D., FITZGERALD, M., AND GIBSON, S. J.: The response of rat spinal cord cells to unmyelinated afferents after peripheral nerve section and after changes in substance P levels. Neuroscience **6:** 2205–2215, 1981.
641. WARD, P. E., AND JOHNSON, A. R.: Renal inactivation of substance P in the rat. Biochem. J. **171:** 143–148, 1978.
642. WEIHE, E., AND REINECKE, M.: Peptidergic innervation of the mammalian sinus nodes: Vasoactive intestinal polypeptide, neurotensin, substance P. Neurosci. Lett. **26:** 283–288, 1981.
643. WEIHE, E., REINECKE, M., OPHERK, D., AND FORSSMANN, W. G.: Peptidergic innervation (substance P) in the human heart. J. Mol. Cell Cardiol. **13:** 331–333, 1981.
644. WHARTON, J., POLAK, J. M., MCGREGOR, G. P., BISHOP, A. E., AND BLOOM, S. R.: The distribution of substance P-like immunoreactive nerves in the guinea-pig heart. Neuroscience **6:** 2193–2204, 1981.
645. WHIPPLE, H. E., SILVERZWEIG, S., AND ERDÖS, E. G. (eds.): Structure and function of biologically active peptides: Bradykinin, kallidin and congeners. Ann N.Y. Acad. Sci. **104:** 376–463, 1963.
646. WRIGHT, D. M., AND ROBERTS, M. H. T.: Supersensitivity to a substance P analogue following dorsal root section. Life Sci. **22:** 19–24, 1978.
647. YAJIMA, H., KITAGAWA, K., AND SEGAWA, T.: Studies on peptides. XXXVIII. Structure-activity correlations in substance P. Chem. Pharm. Bull. **21:** 2500–2506, 1973.
648. YAKSH, T. L., FARB, D. H., LEEMAN, S. E., AND JESSELL, T. M.: Intrathecal capsaicin depletes substance P in the rat spinal cord and produces prolonged thermal analgesia. Science **206:** 481–483, 1979.
649. YAKSH, T. L., JESSELL, T. M., GAMSE, R., MUDGE, A. W., AND LEEMAN, S. E.: Intrathecal morphine inhibits substance P release from mammalian spinal cord *in vivo*. Nature (Lond.) **286:** 155–157, 1980.
650. YAMAGUCHI, I., RACKUR, G., LEBAN, J. J., BJÖRKROTH, U., ROSELL, S., AND FOLKERS, K.: Synthesis and biological activity of analogs of substance P, modified for conformational information by D-amino acids. Acta Chem. Scand. B. **33:** 63–68, 1979.
651. YAMAMOTO, Y., LAGERCRANTZ, H., AND EULER, C. V.: Effects of substance P and TRH on ventilation and pattern of breathing in newborn rabbits. Acta Physiol. Scand. **113:** 541–543, 1981.
652. YANAGISAWA, M., KONISHI, S., SUZUE, T., AND OTSUKA, M.: Effect of substance P and capsaicin on isolated spinal cord of newborn rat. *In* Neuropeptides and Neural Transmission, IBRO Monograph Series, vol. 7, ed. by A. Marsan and W. Z. Traczyk, pp. 43–49, Raven Press, New York, 1980.
653. YANAGISAWA, M., OTSUKA, M., KONISHI, S., AKAGI, H., FOLKERS, K., AND ROSELL, S.: A substance P antagonist inhibits a slow reflex response in the spinal cord of the newborn rat. Acta Physiol. Scand. **116:** 109–112, 1982.
654. YANAIHARA, C., SATO, H., HIROHASHI, M., SAKAGAMI, M., YAMAMOTO, K. HASHIMOTO, T., YANAIHARA, N., ABE, K., AND KANEKO, T.: Substance P radioimmunoassay using N-α-tyrosyl-substance P and demonstration of the presence of substance P-like immunoreactivity in human blood and porcine tissue extracts. Endocrinol. Jap. **23:** 457–463, 1976.
655. YANAIHARA, N., YANAIHARA, C., HIROHASHI, M., SATO, H., ILZUKA, Y., HASHIMOTO, T., AND SAKAGAMI, M.: Substance P analogs: Synthesis and biological and immunological properties. *In* Substance P (Nobel Symposium 37), ed. by U. S. von Euler and B. Pernow, pp. 27–33, Raven Press, New York, 1977.
656. YAU, W. M.: Effect of substance P on intestinal muscle. Gastroenterology **74:** 228–231, 1978.
657. YAU, W. M., AND YOUTHER, M. L.: Direct evidence for a release of acetylcholine from the myenteric plexus of guinea pig small intestine by substance P. Eur. J. Pharmacol. **81:** 665–668, 1982.
658. ZETLER, G.: Substanz P, ein Polypeptid aus Darm und Gehirn mit depressiven, hyperalgetischen und Morphinantagonischen Wirkungen auf das Zentralnervensystem. Naunyn-Schmiedeberg's Arch. exp. Pathol. Pharmakol. **228:** 513–538, 1956.
659. ZETLER, G., AND OHNESORGE, G.: Die Substanz P-Konzentration im Gehirn bei verschiedenen Funktionszuständen des Zentralnervensystems. Naunyn-Schmiedeberg's Arch. exp. Pathol. Pharmakol. **231:** 199–210, 1957.
660. ZETLER, G., AND SCHLOSSER, L.: Über die Verteilung von Substanz P und Cholinacetylase im Gehirn. Naunyn-Schmiedeberg's Arch. exp. Pathol. Pharmakol. **224:** 159–175, 1955.
661. ZIEGLGÄNSBERGER, W., AND TULLOCH, I. F.: Effects of substance P on neurones in the dorsal horn of the spinal cord of the cat. Brain Res. **166:** 273–282, 1979.
662. ZUBER, H.: Isolation of substance P from bovine brain. Ann. N.Y. Acad. Sci. **104:** 391–392, 1963.