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The Tachykinin Peptide Family

CINZIA SEVERINI, GIOVANNA IMPROTA, GIULIANA FALCONIERI-ERSPAMER, SEVERO SALVADORI, AND VITTORIO ERSPAMER†

Consiglio Nazionale delle Ricerche-Institute of Neurobiology and Molecular Medicine, Rome, Italy (C.S.); Department of Human Physiology and Pharmacology "Vittorio Erspamer", University "La Sapienza", Rome, Italy (G.I., G.F.-E., V.E.); and Department of Pharmaceutical Sciences, Università degli Studi, Ferrara, Italy (S.S.)

Address correspondence to: Cinzia Severini, Consiglio Nazionale delle Ricerche-Institute of Neurobiology and Molecular Medicine, Viale Marx 15/43, I-00137, Rome, Italy. E-mail: c.severini@in.rm.cnr.it

†This is the last unfinished review written by professor Vittorio Erspamer before he died suddenly in October 1999. His collaborators are proud to present this review on his behalf and to honor his memory as an enthusiastic and intuitive researcher who enriched the knowledge of new and unimagined agents and actions all over the world.

Abstract——**The tachykinin peptide family certainly represents one of the largest peptide families described in the animal organism. So far, more than 40 tachykinins have been isolated from invertebrate (insects, worms, and molluscs), protochordate, and vertebrate (skin, gastrointestinal tract, peripheral and central nervous system) tissues. Substance P (SP), first identified by bioassay as early as 1931 but sequenced only in 1971, several years after the elucidation of the structure of eledoisin from molluscan tissues and of physalaemin from amphibian skin, may be considered as a prototype of the tachykinins. Hitherto, as many as 19 tachykinins have been isolated from amphibian integument, and eight additional peptides have been isolated from amphibian gut and brain. Counterparts of skin tachykinins in mammalian tissues are SP, neurokinin A, and neurokinin B. Three main receptor subtypes for the tachykinins have been identified (NK1, NK2, and NK3), but their number is probably destined to increase. It is obvious that the peripheral and central effects of the tachykinins may substantially vary depending on the activation of different receptor subtypes. Matters are further complicated by the frequent capacity of the single tachykinins to bind, although with different affinity, to more receptors. It has been recognized that tachykinins have a variety of effects in physiological and pathological conditions, and there is evidence suggesting intrinsic neuroprotective and neurodegenerative properties of these neuropeptides. This review provides an update on the current body of knowledge regarding tachykinin occurrence and distribution in the animal kingdom, from the lowest invertebrates to man, and the physiological and pharmacological actions of tachykinins outlining the pregnant importance of this large peptide family.**

I. Introduction

Seventy years ago, von Euler and Gaddum described an unidentified substance present in alcoholic extracts of equine brain and intestine that in the rabbit displayed a potent stimulant action on the jejunum and a hypotensive action that was distinct from all compounds then known to stimulate the gut and that was referred to as "P" on the tracings and the protocols.

Using semipurified preparations, numerous biological studies of its activity were carried out, but many efforts have been made to isolate the active substance. After some unsuccessful attempts on horse intestine, substance P (SP) was isolated in a pure form from bovine hypothalamus, and 40 years later, its structure was established by Chang and Leeman (1970). SP was then isolated in a pure form and sequenced also from horse intestine (Studer et al., 1973). SP was one of the most extensively studied active substances during the half-century since its discovery, and for many years, it was believed to be the only mammalian tachykinin considered to be a neuropeptide. This belief was firmly put to rest only in 1983 with the discovery of neurokinin A (NKA) and neurokinin B (NKB) (Kangawa et al., 1983; Kimura et al., 1983) that differ from SP in their pharmacological activity, both peripheral and central, and in their preference for different tachykinin receptor subtypes.

The story of the identification of SP was very similar to that leading to the discovery of nonmammalian tachykinins. In 1947, while investigating the occurrence of biogenic amines, especially serotonin in the posterior salivary glands of a Mediterranean octopod, *Eledone moschata*, an unidentified substance was found that again lowered blood pressure in rabbits and dogs, stimulated isolated preparations of intestinal smooth muscle, and caused profuse salivation in dogs and rats (Erspamer, 1949). The structure of this substance, first called moschatin and then eledoisin, was established in 1962 (Anastasi and Erspamer, 1962; Erspamer and Falconieri Erspamer, 1962). In the same year, it was found that extracts of the skin of the South American leptodactylid frog *Physalaemus biligonigerus* (formerly *fuscumaculatus*) also displayed eledoisin-like activity. Also the elucidation of the structure of physalaemin (Anastasi et al., 1964; Erspamer et al., 1964) recalled that of eledoisin and similarly was followed in rapid succession by the identification of a number of other related peptides in the skin, in the brain and gut of amphibians, and in brain and gut of submammalian species (from birds to agnata).

[2] Abbreviations: SP, substance P; NKA, neurokinin A; NKB, neurokinin B; RIA, radioimmunoassay; SP-LI, substance P-like immunoreactivity; HPLC, high-performance liquid chromatography; 5-HT, 5-hydroxytryptamine; TK, tachykinin; TAN, tonically autoactive, giant neuron.

These peptides, all called tachykinins, represent the largest known peptide family, including members occurring in different animal species from low invertebrates to mammals. The possible occurrence of authentic tachykinins in invertebrates was confirmed by the isolation of two tachykinins from the salivary glands of a mosquito (Champagne and Ribeiro, 1994) and by the occurrence in nervous structures of the insect *Locusta migratoria* of four related peptides (the locustatachykinins) having structure homology with the vertebrate tachykinins (Schoofs et al., 1990a,b).

The identification of the locustatachykinins was soon followed by the isolation of similar peptides in other insects and in crabs, echinoid worms, and molluscs. It will be shown that locustatachykinin-like peptides, the number of which is destined to grow, have full citizenship right in the tachykinin peptide family, which with more than 40 members represents one of the largest, if not the largest, family in the peptide world.

The purpose of this review is, first, to keep the reader up to date on the different occurrences, species distributions, and localizations of the numerous members of the tachykinin peptide family. Second, because the identification and study of nonmammalian tachykinins has contributed conspicuously to the explosive progress of knowledge in the field of mammalian active tachykinins, we put in evidence the extensive pharmacological studies on the nonmammalian tachykinins (TKs) (eledoisin, physalaemin, and kassinin) whose availability preceded by years that of the corresponding mammalian peptides.

II. Occurrence and Species Distribution of Tachykinin-Like Peptides

At present among the numerous families of neuropeptides, which are evolutionarily the oldest neurotransmitters, perhaps even older than acetylcholine and catecholamines, four tachykinin-like peptides seem to occupy a very important position.

A. Invertebrate Tachykinin-Like Peptides

It is possible separate the tachykinin-like peptides in invertebrates into three groups: a) tachykinins identified by RIA and/or immunohistochemistry, occasionally accompanied by HPLC separation, but not isolated or sequenced; b) tachykinin-related peptides of the locustatachykinin type, isolated and sequenced, which have a C-terminal Arg-NH_2 residue instead of the usual Met-NH_2 residue present in all of the classical vertebrate tachykinins; and c) authentic tachykinins having structure and biological activity identical with those of the vertebrate tachykinins.

a. Substance P-like immunoreactivity (SP-LI) was localized in the primitive nervous system of *Hydra* (Taban and Cathieni, 1979; Grimmelikhuijzen et al., 1981; Pierobon et al., 1989), in the cerebral ganglion of the locust (Benedeczky et al., 1982), in the central nervous system of the cockroach *Periplaneta americana* (Verhaert and De Loof, 1985), in the retina and eyestalk neurones of the lobster *Palinurus interruptus* (Mancillas et al., 1981), in the eye stalk of the fiddler crab *Uca pugilator* (Fingerman et al., 1985), in the somatogastric system of the crab *Cancer borealis* and the lobster *P. interruptus* and *Homarus americanus* (Goldberg et al., 1988), in tissues of the earthworm *Lumbricus terrestris* (Aros et al., 1980; Kaloustian and Edmands, 1986), in the adult nervous system of the fly *Sarcophaga bullata* (Sivasubramanian, 1990), in the cricket *Teleogryllus communis* (Lembeck et al., 1985), in the brain and central ganglia of the bowfly *Calliphora vomitoria* and of *Drosophila* (Lundquist et al., 1994), in the brain, corpora cardiaca, and corpora allata of the insect *Leucophaea madeirae* (El-Salhy et al., 1983), in the central nervous system of the mollusc *Limulus polyphemus* (Mancillas and Selverstone, 1985), and in the nervous system of several parasitic trematode worms (Bush and Gupta, 1988) including *Schistosoma mansoni* (Gustafsson, 1987), *Diphyllobathrium dendriticum* (Gustafsson et al., 1986), *Fasciola hepatica* (Magee et al., 1989), and *Diclidophora merlangi* (Maule et al., 1989).

In a large number of invertebrate phyla from coelenterates to molluscs, in addition to the usual SP-like tachykinin, an NKA-like peptide has also been found. However, it is highly improbable that authentic SP or authentic NKA is present in invertebrates. First, because retention time of the invertebrate tachykinins in elution from reverse-phase HPLC columns never coincided with retention time of the mammalian tachykinins; and second, because authentic SP and/or NKA was never found, even in lower submammalian species (amphibian, fish, and agnata). It has also been shown that radioimmunoassay and immunohistochemical techniques are often insufficient to distinguish between structurally related peptides because of frequent lack of selectivity of the pertinent antisera. Callitachykinin II, for example, was recognized not only by an antiserum to the locustatachykinin (in both peptides the C-terminal residue is Arg-NH_2) but also by an antiserum to the amphibian kassinin having, like all other classical tachykinins, the C-terminal residue Met-NH_2 (Lundquist et al., 1994). Moreover, preincubation of locustatachykinin antibody with SP and preincubation of SP antibody with locustatachykinin blocked subsequent immunolabeling of the somatogastric nervous system in *C. borealis*, indicating that a member of the locustatachykinins is likely to be the source of the previously identified SP in the nervous system (Blitz et al., 1995).

b. Schoofs et al. (1990a,b) first described the occurrence in insects, more precisely in extracts of brain, corpora cardiaca-corpora allata, and suboesophageal ganglion of *Locusta migratoria* of five peptides, the locustatachykinins, which exhibited sequence homologies (up to 45%) with the vertebrate tachykinins, especially with amphibian and fish

tachykinins. The locustatachykinins were completely inactive in all bioassay preparations used for the vertebrate tachykinins but showed a myotropic action in the insect intestine, eliciting a potent contraction of the cockroach hindgut (Winther et al., 1998).

The prediction of Schoofs' group in their first paper (Scoofs et al., 1990b) that "the peptides discovered in this study may be just the first in a whole series of substances from arthropod species to be identified as tachykinin family peptides" was correct even beyond any expectation. Up to the present, as many as 20 locustatachykinin-like peptides were isolated not only from various other arthropods, but also from an echinoid worm and from molluscs (Nassel, 1999). Table 1, reporting the present, probably provisional situation, shows that invertebrate tachykinin-like peptides are linear peptides with 8 to 15 amino acid residues and that, with the exception of the Leucophaea tachykinin-related peptide LemTRP10, they have at their C terminus an amidated Arg residue instead of the amidated Met residue, which is peculiar without exception to all classical tachykinins, including the invertebrate tachykinins (eledoisin and sialokinin I and II). Lem TRP1 is present also in two elongated forms with 17 (LemTRP2) and 19 (LemTRP3) amino acid residues, respectively (Winther et al., 1999).

In the light of appearance on the screen of the locustatachykinins and of the fact that locustatachykinin antisera may cross-react with SP, it is probable that in several and perhaps in most cases, the SP-LI described in a variety of invertebrates must be ascribed to locustatachykinin-like peptides. Thus, the locustatachykinin-like peptides of invertebrates must be considered authentic tachykinins, being either the primitive representatives of the tachykinin peptide family from which the vertebrate tachykinins have evolved by simple substitution of the C-terminal residue Arg-NH_2 with Met-NH_2 or an evolutionary adaptation for the invertebrates of a common ancestral tachykinin prototype already possessing the C-terminal Met-NH_2 residue.

c. Authentic tachykinins with the classical C-terminal pentapeptide sequence Phe-(Tyr/Ile)-Gly-Leu-Met-NH_2 occur in non-neuronal, epithelial cells of the posterior salivary glands of the Mediterranean octopods *E. moschata* and *Eledone aldovrandi* (eledoisin, up to 100 nmol/g wet tissue) (Erspamer and Falconieri Erspamer, 1962) and in the salivary glands of the mosquito *Aedes aegypti* (sialokinins I and II) (Champagne and Ribeiro, 1994): eledoisin, pGlu-Pro-Ser-Lys-Asp-Ala-Phe-Ile-Gly-Leu-Met-NH_2; sialokinin I, Asn-Thr-Gly-Asp-Lys-Phe-Tyr-Gly-Leu-Met-NH_2; sialokinin II, Asp-Thr-Gly-Asp-Lys-Phe-Tyr-Gly-Leu-Met-NH_2.

These peptides display the full spectrum of activity of the mammalian tachykinins and bind to the same receptors. It is remarkable that eledoisin occurs only in *Eledone* but not in the strictly related *Octopus vulgaris.* Yet, the salivary glands of both *Eledone* and *Octopus* contain large amounts of biogenic amines: serotonin (up to 2 μmol/g), octopamine, tyramine, and histamine.

TABLE 1
Amino acid sequence of invertebrate tachykinin-related peptides

Source/Peptide	Primary Structure	Reference
Locusta migratoria		Schoofs et al., 1990a,b
LomTK I	Gly-Pro-Ser-Gly-Phe-Tyr-Gly-Val-Arg-NH_2	
LomTK II	Ala-Pro-Leu-Ser-Gly-Phe-Tyr-Gly-Val-Arg-NH_2	
LomTK III	Ala-Pro-Gln-Ala-Gly-Phe-Tyr-Gly-Val-Arg-NH_2	
LomTK IV	Ala-Pro-Ser-Leu-Gly-Phe-Tyr-Gly-Val-Arg-NH_2	
Culex salinarius		Clottens et al., 1993
CusTK II	Ala-Pro-Ser-Gly-Phe-Met-Gly-Met-Arg-NH_2	
CusTK III	Ala-Pro-Tyr-Gly-Phe-Thr-Gly-Met-Arg-NH_2	
Anodonta cygnea		Fujisawa et al., 1993
AncTK	pGlu-Tyr-Gly-Phe-His-Ala-Val-Arg-NH_2	
Urechis unicintus		Ikeda et al., 1993
UruTKI	Leu-Glu-Gln-Ser-Gln-Phe-Val-Gly-Ser-Arg-NH_2	
UruTKII	Ala-Ala-Gly-Met-Gly-Phe-Phe-Gly-Ala-Arg-NH_2	
Calliphora vomitoria		Lundquist et al., 1994
CavTKI	Ala-Pro-Thr-Ala-Phe-Tyr-Gly-Val-Arg-NH_2	
CavTKII	Gly-Leu-Gly-Asn-Asn-Ala-Phe-Val-Gly-Val-Arg-NH_2	
Leucophea maderae		Muren and Nassel, 1996
LemTRP1	Ala-Pro-Ser-Gly-Phe-Leu-Gly-Val-Arg-NH_2	
LemTRP4	Ala-Pro-Ser-Gly-Phe-Met-Gly-Met-Arg-NH_2	
LemTRP5	Ala-Pro-Ala-Met-Gly-Phe-Gln-Gly-Val-Arg-NH_2	
LemTRP6	Ala-Pro-Ala-Ala-Gly-Phe-Phe-Gly-Met-Arg-NH_2	
LemTRP7	Val-Pro-Ala-Ser-Gly-Phe-Phe-Gly-Met-Arg-NH_2	
LemTRP8	Gly-Pro-Ser-Met-Gly-Phe-His-Gly-Met-Arg-NH_2	
LemTRP9	Ala-Pro-Ser-Met-Gly-Phe-Gln-Gly-Met-Arg-NH_2	
Cancer borealis		Christie et al., 1997
CabTRP1a	Ala-Pro-Ser-Gly-Phe-Leu-Gly-Met-Arg-NH_2	
CabTRP1b	Ser-Gly-Phe-Leu-Gly-Met-Arg-NH_2	
Penaeus vannamei		Nieto et al., 1998
PevTRP	Ala-Pro-Ser-Gly-Phe-Leu-Gly-Met-Arg-NH_2	

L. migratoria (arthropod): brain, corpora cardiaca-corpora allata, subesophageal ganglion; *C. salinarus* (artropod): nervous tissue; *A. cygnea* (bivalve mollusc): central nervous system; *Urechis uncinatus* (echinoid worm): nervous tissues; *C. vomitoria* (artropod): nervous tissues; Leucophea maderae (artropod): brain and midgut; *Cancer borealis* (artropod): stomatogastric nervous system; *Peneaeus vennamei* (artropod): central nervous system.

B. Prevertebrate Tachykinin-Like Peptides

1. Amphioxus lanceolatus. Radioimmunoassay combined with HPLC suggests the occurrence of small amount of SP-LI in brain and spinal cord of this prevertebrate species (Lembeck et al., 1985).

2. Tunicata (Protocordata). Using immunohistochemical techniques only, the presence of an antigen related to substance P has been demonstrated in the neuronal ganglion (Fritsch et al., 1979), gill epithelium (Fritsch et al., 1980), and alimentary tract (Fritsch et al., 1982) of the ascidian *Ciona intestinalis.* More recently, the occurrence of tachykinins in *C. intestinalis* tissues was re-examined by O'Neil et al. (1987) using specific antisera for the C terminus (C) of SP and the N terminus (N) of mammalian SP and NKA, completed with immunohistochemistry and reverse-phase HPLC of the tissue extracts. It was found that only C-SP-LI (not N-SP-LI) occurs both in cells of the ganglia and in peripheral neurons, together with but separately from N-NKA-LI. Only C-SP-LI was found in endocrine cells of the pharynx. However, we conclude that already at the prevertebrate stage of chordate evolution, the tachykinin family is represented by at least two distinct members that are provided by separate cell populations, none of which was identical with either mammalian SP or mammalian NKA.

C. Submammalian Vertebrate Tachykinins

The formidable enlargement of the tachykinin peptide family is consequent to systematic studies conducted on the one side on the amphibian skin and on the other side on the brain and intestines of submammalian vertebrates, mainly in their cold-blooded classes: reptiles, amphibians, fish, and agnata. The story of the group of the amphibian skin peptides began in 1964 with isolation and structure elucidation of physalaemin (Erspamer et al., 1964), followed by the systematic screening of peptide contents in the skin of as many as 600 amphibian species from all over the world, which resulted in the discovery and isolation of numerous neuropeptides, belonging to a dozen distinct families, among which is that of the tachykinins (with 21 members).

The fruitful search for tachykinin peptides in brain and gut of submammalian vertebrates started in 1986 with the isolation and structure elucidation of scyliorhinins I and II from dogfish intestine (Conlon et al., 1986a). At the end of 1998, a list of as many as 24 novel tachykinins was available, 12 from brain and 12 from gut. Skin tachykinins and brain/gut tachykinins will be discussed separately.

1. Amphibian Skin Tachykinins. Table 2 summarizes the present situation. The great majority of amphibian skin peptides have the classical C-terminal pentapeptide sequence: Phe-X-Gly-Leu-Met-NH_2. However, important exceptions are represented by: 1) some tachykinins from the skin of the Australian frog *Agalychnis callidryas*, namely AC-AR1, -AR2, and -AR3 with the C-terminal pentapeptide sequence Phe-Tyr-Pro-Gly-Met-NH_2 and AC-AR4 with sequence Phe-Tyr-Pro-Val-Met-NH_2; and 2)

TABLE 2
Amino acid sequence of natural amphibian skin tachykinins

Source/Peptide	Primary Structure	Reference
Physalaemus biligonigerus (fuscumaculatus)		Anastasi et al., 1964; Erspamer et al., 1964
Physalaemin (PHYS)	pGlu-Ala-Asp-Pro-Asn-Lys-Phe-Tyr-Gly-Leu-Met-NH_2	
Uperuleia rugosa		Nakajima et al., 1980
[Lys^5, Thr^6]PHYS	pGlu-Ala-Asp-Pro-Lys-Thr-Phe-Tyr-Gly-Leu-Met-NH_2	
Uperuleia marmorata		Anastasi et al., 1975
Uperolein	pGlu-Pro-Asp-Pro-Asn-Ala-Phe-Tyr-Gly-Leu-Met-NH_2	
Uperuleia inundata		Bradford et al., 1996
Uperin	pGlu-Ala-Asp-Pro-Asn-Ala-Phe-Tyr-Gly-Leu-Met-NH_2	
Kassina (Hylambates) maculata		Yasuhara et al., 1981
Hylambatin	Asp-Pro-Pro-Asp-Pro-Asn-Arg-Phe-Tyr-Gly-Leu-Met-NH_2	
[Glu^2, Pro^5]Kassinin	Asp-Glu-Pro-Lys-Pro-Asp-Gln-Phe-Val-Gly-Leu-Met-NH_2	
Kassina senegalensis		Anastasi et al., 1976
Kassinin	Asp-Val-Pro-Lys-Ser-Asp-Gln-Phe-Val-Gly-Leu-Met-NH_2	
Rana margaratae		Lu et al., 1990
Ranamargarin	Asp-Asp-Ala-Ser-Asp-Arg-Ala-Lys-Lys-Phe-Tyr-Gly-Leu-Met-NH_2	
Phyllomedusa bicolor		Anastasi and Falconieri Erspamer, 1970
Phyllomedusin	pGlu-Pro-Asn-Pro-Asn-Arg-Phe-Ile-Gly-Leu-Met-NH_2	
Pseudophryne guntheri		Simmaco et al., 1990
PG-SP1	pGlu-Pro-Asn-Pro-Asp-Glu-Phe-Tyr-Gly-Leu-Met-NH_2	
PG-SP2	pGlu-Pro-Asn-Pro-Asn-Glu-Phe-Tyr-Gly-Leu-Met-NH_2	
PG-KI	pGlu-Pro-His-Pro-Asp-Glu-Phe-Val-Gly-Leu-Met-NH_2	
PG-KII	pGlu-Pro-Asn-Pro-Asp-Glu-Phe-Val-Gly-Leu-Met-NH_2	
PG-KIII	pGlu-Pro-His-Pro-Asn-Glu-Phe-Val-Gly-Leu-Met-NH2	
Agalychnis callidryas		Mignogna et al., 1997
AC-AL	Gly-Pro-Pro-Asp-Pro-Asn-Lys-Phe-Ile-Gly-Leu-Met-NH_2	
AC-AR1	Gly-Pro-Pro-Asp-Pro-Asp-Arg-Phe-Tyr-Pro-Gly-Met-NH_2	
AC-AR2	Gly-Pro-Pro-Asp-Pro-Asp-Lys-Phe-Tyr-Pro-Gly-Met-NH_2	
AC-AR3	pGlu-Pro-Asp-Pro-Asp-Lys-Phe-Tyr-Pro-Glyl-Met-NH_2	
AC-AR4	Gly-Pro-Pro-Asp-Pro-Asn-Lys-Phe-Tyr-Pro-Val-Met-NH_2	

hylambatin from the skin of the South-African frog *Hylambates maculatus* with the C-terminal pentapeptide sequence Phe-Tyr-Gly-Met-Met-NH_2. It is evident that in the C-terminal pentapeptide only the Phe residue at position 5 from the C terminus and Met-NH_2 are immutable.

All of the amphibian skin peptides have a non-neuronal origin, being synthesized in the syncytial cells dressing the wall of the granular glands. These cells are capable of cosynthesizing, costoring, and cosecreting not only peptides belonging to different families, but also amines and alkaloids belonging to various classes and families. The amphibian syncytial cells behave like some mammalian endocrine cells, e.g., the enterochromaffin cells, which may contain both biogenic amines and peptides (substance P, guanylin), and like a number of central and peripheral neurons in which amine messengers coexist with peptide messengers.

2. Brain and Gut Tachykinins. Table 3 summarizes the present situation. All of the tabulated tachykinins, with the exception of ranatachynin D, show the classical C-terminal pentapeptide Phe-X-Gly-Leu-Met-NH_2. Of considerable interest is the fact that in goldfish, cod, and trout NKA-like peptides, the usual acidic Asp residue at position 7 from the C terminus, crucial for receptor NK2/NK3 selectivity, is replaced by the neutral Asn residue.

The list of tachykinins shown in the Table 3 should be completed by authentic NKA occurring in the intestine of the chicken and of the alligator and in the brain of the python, and by authentic NKB found only in the brain of *Rana esculenta.*

Moreover, NKA is present in as many as six submammalian species also by its elongated form, the γ-neuropeptides, as shown in Table 4. From the above sequences, it is evident that none of the submammalian γ-neuropeptides is identical with the corresponding mammalian peptide. Substantial differences in the amino acid composition may be seen not only in the flanking sequence but in all examined fish, even in the NKA-like C-terminal decapeptides.

D. Mammalian Tachykinins

1. Mammalian Tachykinins and Their Biosynthesis. Until now, only three tachykinins have been isolated and sequenced from mammalian tissues: SP, NKA (neuromedin L, neurokinin, and substance k), and NKB (neurokinin and neuromedin k). NKA is present also in two elongated forms, neuropeptide K and neuropeptide-γ (Table 5).

It is hardly conceivable, but of course it is possible, that mammalian tissues contain only three members of the tachykinin family. As a matter of fact, the number of mammalian species in which tachykinin peptides have been isolated is very scanty: horse and guinea pig intestine, porcine spinal cord, and in some additional species (rat and man) preprotachykinins have been detected. RIA or immunohistochemistry, again in a limited number of species and especially in the rat, has detected all other tachykinin locations. This is all. Yet fish present five different tachykinins in the brain and six in the gut, and the four examined amphibian species exhibit as many as nine different tachykinins altogether in the brain and the gut. It is evident that the occurrence even in mammalian tissues of tachykinins other than the three classical ones is likely. Lazarus group (Lazarus and Di Augustine, 1980; Lazarus et al., 1980), using an antiserum specifically recognizing the N-terminal region of physalaemin, was able to detect a physalaemin-LI in a number of tissues of three mammalian species (guinea pig, mouse, and rat) with peaks in guinea pig and mouse gastric fundus, pylorus, and duodenum (up to 18 pmol/g lyophilized tissue). Moreover, physalaemin antiserum caused a clear-cut immunostaining in a population of cells of the Brunner's gland of the guinea pig duodenum, and several other examples of the expression of physalaemin-, eledoisin-, and kassinin-LI may be found in carcinoids (see *Section III.A.4.*).

Mammalian tachykinins are derived from two preprotachykinin genes: the PPT-A gene, which encodes the sequences of SP, NKA, and neuropeptide K and neuropeptide-γ, and the PPT-B gene, which encodes the sequence of NKB (Nawa et al., 1983; Kotani et al., 1986; Bonner et al., 1987; Krause et al., 1987).

The precursor RNA from PPT-A is alternatively processed to yield three different mRNAs (Nawa et al., 1984). The three precursor proteins from which the mRNA codes are designated α-, β-, and γ-PPT; α-PPT, which generates SP; β-PPT, which generates SP, NKA, and neuropeptide K; and γ-PPT, which generates SP, NKA, and neuropeptide-γ. The biological significance of the alternative splicing of PPT-A is unknown. The relative proportion of α-, β-, and γ-PPT mRNAs is markedly species dependent. For example, β-PPT is the predominant form expressed in human basal ganglia (Bannon et al., 1992), whereas α-PPT prevails in the bovine brain (Nawa et al., 1984).

α-PPT mRNA is abundant in the brain, whereas β- and γ-PPT mRNAs are found mainly in peripheral tissues (Nakanishi, 1987). PPT-B mRNA is found in the brain (hypothalamus) and intestine (Kotani et al., 1986). Tachykinins are liberated from their precursors by the action of specific processing proteases. Typical cleavage points are Lys-Arg, Arg-Arg, and Arg-Lys doublets and the cleavage is carried out by six groups of proteolytic enzymes called convertases (Chretien et al., 1989; Steiner et al., 1992; Marcinkiewicz et al., 1993). COOH-terminal amidation after cleavage is generated from the precursor sequence, Gly-Leu-Met-Gly-Lys-Arg, in which Gly acts as the amide donor.

As with all known neurotransmitters, neuronal tachykinins are also released from the nerve ending after a calcium-dependent mechanism in response to application of physiological and nonphysiological stimuli (electrical stimulation, potassium, or capsaicin depolarization) (Maggi et al., 1993). Concerning release, two points are firmly established.

First, like that of biogenic amines, which are considered "rapid transmitters" and which under certain con-

TABLE 3
Amino acid sequence of submammalian vertebrate tachykinins

Source/Peptide		Primary Structure	Reference
Gallus domesticus			
	Gut	Arg-Pro-Arg-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2	Conlon et al., 1988
Alligator mississipiensis			
	Brain	Arg-Pro-Arg-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2	Wang et al., 1992b
Python molurus			
	Gut	Arg-Pro-Arg-Pro-Gln-Gln-Phe-Tyr-Gly-Leu-Met-NH_2	Conlon et al., 1997
Rana catesbeiana			
Ranatachykinis	A) Brain, gut	Lys-Pro-Ser-Pro-Asp-Arg-Phe-Tyr-Gly-Leu-Met-NH_2	Kozawa et al., 1991
	B) Brain	Tyr-Lys-Ser-Asp-Ser-Phe-Tyr-Gly-Leu-Met-NH_2	
	C) Gut	His-Asn-Pro-Ala-Ser-Phe-Ile-Gly-Leu-Met-NH_2	
	D) Gut	Lys-Pro-Asn-Pro-Glu-Arg-Phe-Tyr-Ala-Pro-Met-NH_2	
Rana ridibunda Ranakinin	Brain	Lys-Pro-Asn-Pro-Glu-Arg-Phe-Tyr-Gly-Leu-Met-NH_2	O'Harte et al., 1991
	Gut	His-Lys-Leu-Asp-Ser-Phe-Ile-Gly-Leu-Met-NH_2	Wang et al., 1992a
Bufo marinus Bufokinin	Gut	Lys-Pro-Arg-Pro-Asp-Gln-Phe-Tyr-Gly-Leu-Met-NH_2	Conlon et al., 1998
Amphiuma tridactylum			
	Gut	Asp-Asn-Pro-Ser-Val-Gly-Gln-Phe-Tyr-Gly-Leu-Met-NH_2	Waugh et al., 1995a
	Gut	His-Lys-Asp-Ala-Phe-Ile-Gly-Leu-Met-NH_2	
Oncorhynchus mykiss			
	Brain	Lys-Pro-Arg-Pro-His-Gln-Phe-Phe-Gly-Leu-Met-NH_2	Jensen et al., 1992
	Gut	His-Arg-Ile-Asn-Ser-Phe-Val-Gly-Leu-Met-NH_2	Jensen et al., 1993
Gadus morhua			
	Brain	Lys-Pro-Arg-Pro-Gln-Gln-Phe-Ile-Gly-Leu-Met-NH_2	Jensen et al., 1992
Carassius auratus			
Carassin (13–21)	Brain	His-Arg-Ile-Asn-Ser-Phe-Val-Gly-Leu-Met-NH_2	Conlon et al., 1991; Lin and Peter, 1997
		Lys-Pro-Arg-Pro-His-Gln-Phe-Ile-Gly-Leu-Met-NH_2	
Amia calva			
	Stomach	Ser-Lys-Ser-His-Gln-Phe-Tyr-Gly-Leu-Met-NH_2	Waugh et al., 1995b
Scaphirhyncus platorhyncus			
	Gut	Ser-Lys-Thr-His-Gln-Phe-Tyr-Gly-Leu-Met-NH_2	Wang et al., 1999
Scyliorhinus canicula			
Scyliorhinin I	Gut	Ala-Lys-Phe-Asp-Lys-Phe-Tyr-Gly-Leu-Met-NH_2	Conlon et al., 1986a
Scyliorhinin II	Gut	Ser-Pro-Ser-Asn-Ser-Lys-Cys-Pro-Asp-Gly-Pro-Asp-Cys-Phe-Val-Gly-Leu-Met-NH_2	
	Brain	Lys-Pro-Arg-Pro-Gly-Gln-Phe-Phe-Gly-Leu-Met-NH_2	Waugh et al., 1993
Torpedo marmorata			
Des(Ser^1,Pro^2) Scyliorhinin II	Gut	Ser-Asn-Ser-Lys-Cys-Pro-Asp-Gly-Pro-Asp-Cys-Phe-Val-Gly-Leu-Met-NH_2	Conlon and Thim, 1988
Sphyrna lewinini			
	Gut	Ala-Lys-Phe-Asp-Lys-Phe-Tyr-Gly-Leu-Met-NH_2	Waugh et al., 1995a
Raja rhina			
	Brain	Ala-Lys-His-Asp-Lys-Phe-Tyr-Gly-Leu-Met-NH_2	Waugh et al., 1994
	Brain	His-Lys-Leu-Gly-Ser-Phe-Val-Gly-Leu-Met-NH_2	
Lampetra fluviatilis			
	Brain	Arg-Lys-Pro-His-Pro-Lys-Glu-Phe-Val-Gly-Leu-Met-NH_2	Waugh et al., 1995a
	Brain	His-Phe-Asp-Glu-Phe-Val-Gly-Leu-Met-NH_2	
Petromyzon marinus			
	Brain	Arg-Lys-Pro-His-Pro-Lys-Glu-Phe-Val-Gly-Leu-Met-NH_2	Waugh et al., 1994

TABLE 4
Amino acid sequence of submammalian vertebrate γ-neuropeptides

Source	Primary Structure	Reference
Mammals		
Spinal cord	DAGHGQISHKR His-Lys-Thr-Asp-Ser-Phe-Val-Gly-Leu-Met-NH_2	Kangawa et al., 1983; Kimura et al., 1983
Gold fish		
Brain	SPANAQITRKR His-Lys-Ile-Asn-Ser-Phe-Val-Gly-Leu-Met-NH_2	Conlon et al., 1991
Trout		
Gut	SSANPQITRKR His-Lys-Ile-Asn-Ser-Phe-Val-Gly-Leu-Met-NH_2	Jensen et al., 1993
Phyton		
Gut	DAGYSPLSHKR His-Lys-Thr-Asp-Ser-Phe-Val-Gly-Leu-Met-NH_2	Conlon et al., 1997
Alligator		
Brain	DAGYGQISHKR His-Lys-Thr-Asp-Ser-Phe-Val-Gly-Leu-Met-NH_2	Wang et al., 1992b
Shark		
Gut	ASGPTQAGIVGRKR Gln-Lys-Gly-Glu-Met-Phe-Val-Gly-Leu-Met-NH_2	Waugh et al., 1995a
Bowfin		
Stomach	SGAPQTVPLGRKR His-Lys-Gly-Glu-Met-Phe-Val-Gly-Leu-Met-NH_2	Waugh et al., 1995b

TABLE 5
Amino acid sequence of mammalian tachykinins

Peptide/Source	Primary Structure	Reference
SP		
Bovine hypothalamus	Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2	Chang et al., 1971
NKA		
Porcine spinal cord	His-Lys-Asp-Ser-Val-Phe-Gly-Leu-Met-NH_2	Kimura et al., 1983
NKB		
Porcine spinal cord	Asp-Met-His-Asp-Phe-Phe-Val-Gly-Leu-Met-NH_2	Kangawa et al., 1983
Neuropeptide-γ		
Rabbit intestine	Asp-Ala-Gly-His-Gly-Gln-Ile-Ser-His-Lys-Arg-Lys-Asp-Ser-Val-Phe-Gly-Leu-Met-NH_2	Kage et al., 1988
Neuropeptide K		
Porcine brain	Asp-Ala-Asp-Ser-Ser-Ile-Glu-Lys-Gln-Val-Ala-Leu-Leu-Lys-Ala-Leu-Tyr-Gly-His-Gly-Gln-Ile-Ser-His-Lys-Arg-His-Gly-Gln-Ile-Ser-His-Lys-Arg-Lys-Asp-Ser-Val-Phe-Gly-Leu-Met-NH_2	Tatemoto et al., 1985

ditions may be released massively, release of neuropeptides, considered "slow" transmitters or modulators, is probably discrete and long lasting. Second, at the nerve terminals, especially in brain and in the autonomic nervous system, a release of a single transmitter is improbable, and, at any rate, must represent an exception. The concept of co-release of different peptides, amines, amino acids, and purines is now generally accepted after the immunohistochemical demonstration of the costorage in the granular material of single neurones of more active substances (Hokfelt et al., 1986).

Once released, the tachykinins may be attacked, cleaved, and inactivated by a number of proteolytic enzymes, which, however, act with considerably different intensity on the different tachykinins. The most vulnerable peptide seems to be SP, whereas peptides having at their N-terminal the pGlu residue seem much more resistant to enzyme attack. In the proteolytic degradation of SP, three enzymes seem to display a predominant role: dipeptidyl-amino peptidase, postproline endopeptidase, and cathepsin D (Regoli et al., 1994a).

III. Localization of Tachykinin-Like Peptides

We have previously shown that tachykinins constitute one of the largest families of peptides in the world whose members are present in all animal species, from lower invertebrates to mammals. Tachykinins possess a widespread distribution in the central and peripheral nervous system that is undoubtedly the major source of these peptides. However, tachykinins have also, like numerous other peptides and like all biogenic amines, a limited and species-dependent, but not negligible, distribution in nonneuronal structures represented by the irregular and sparse localizations in which they display known and unknown functions. In the first localization (neuronal cells), the active compounds act as neurotransmitters/neuromodulators, in the second (non neuronal cells) as autocrine, paracrine, or endocrine regulators.

A. Non-Neuronal Localization

1. Amphibian Skin. The most complex and rich localization of non-neuronal tachykinins is certainly the amphibian skin, that may be considered a huge factory and storehouse of a variety of tachykinins. However, an important characteristic of the skin peptides is their extreme irregularity in occurrence and richness: some amphibian species are extremely rich in active peptides, others, even closely related species, are lacking of active peptides. These findings contribute to the incomprehension of the physiological significance of the skin tachykinins and of that of the skin biogenic amines, especially indolealkylamines that are similarly present, sometimes in enormous amounts in the skin. The occurrence in the skin of a such variety of neuropeptides and amines may perhaps be explained by the common embryogenic origin of the integument and the nervous system from the primitive ectoderm.

2. Invertebrate Salivary Glands. The significance of eledoisin, as with that of the large amounts of biogenic amines, occurring in the posterior salivary glands of *E. moschata* but not in the glands of any related *Octopus* species is again completely obscure. On the contrary, the sialokinins occurring in the salivary glands of *A. aegypti* may be interpreted as an evolutionary selection of the peptides as vasodilators, in connection with the habit of feeding in blood of this insect (Champagne and Ribeiro, 1994).

3. Normal Mammalian Tissues. All of the non-neuronal localizations of the tachykinins concern mainly SP, and the occurrence of the peptide was established only by immunohistochemistry, using selective SP-antisera. SP occurring (together with serotonin) in populations of argentaffin/chromaffin and argyrophil/acidophil (with no serotonin) cells of the mammalian and probably also of lower vertebrate (ascidian and fish) intestine may act either as paracrine hormone or as a true hormone after release into the blood stream. It is possible that SP found in mammalian blood originates prevalently from the SP-containing cells of the intestinal mucosa. More than 60 years ago, Vialli and Erspamer (1933) described the so-called "acidophil basigranular cells" of the dog and cat large intestine, and it would be worthwhile checking to see if they are, like the argyrophil cells of the human large intestine, SP-producing cells. The acidophil cells are, in fact, neither argentaffin nor chromaffin, that is they do not contain serotonin. Little is known as to whether and under which conditions SP is released from the endocrine cells of the gastrointestinal mucosa. For example, release of SP from the enterochromaffin cells of the rat caecum mucosa seems to be inhibited by serotonin and calcium-free medium (Simon et al., 1992). Moreover, that SP may be released from the gut endocrine cells into the blood stream is strongly suggested by the evidence that legation of all intestinal blood vessels and evisceration in the cat significantly lowered SP plasma levels (Gamse et al., 1978) and by the fact that portal venous blood contains about 4 times more SP than peripheral blood (Pernow, 1983).

At any rate, it is obvious that blood SP must display some function. The most acceptable suggestion is that the peptide acts on the blood vessels either indirectly through release of vasodilator agents from the endothelium (vasodilation) or directly on the vascular smooth muscle, causing generally constriction or even potent stimulation of phasic movements in particular vessels (e.g., rat portal vein). There are sharp species differences in the response of the vessels to tachykinins, depending not only on the vascular beds but also on animal species (see *Section V.I.*).

4. Endocrine Tachykinin-Secreting Tumors. Carcinoids. Carcinoids are tumors of the diffuse endocrine system characterized by a typical growth pattern, silver affinity, and positive immunohistochemical reaction with

specific markers. They can express different biogenic amines (serotonin and histamine), peptides (tachykinins, bradykinin, and enteroglucagon), and prostaglandins (Creutzfeldt, 1996). Among the amines, serotonin is always present in argentaffin/chromaffin carcinoids, originating from the malignant growth of the enterochromaffin cells. They are prevailingly present in the midgut and appendix. Serotonin, however, is lacking in argyrophil nonchromaffin carcinoids, which may be present in the foregut, hindgut, lung (mostly bronchial carcinoids) and various other organs (Creutzfeldt and Stockmann, 1987). Argyrophil carcinoids may originate in the colon and probably in other sites as well, from the population of argyrophil, serotonin-lacking cells described by Sokolski and Lechago (1984). Peptides (bradykinin, enteroglucagon, and especially tachykinins) may occur both in chromaffin (together with serotonin) and in argyrophil nonchromaffin carcinoids (Wilander et al., 1977, 1979).

Because in normal endocrine cells of the gut and other organs only SP has been detected by immunochemistry, as expected, the tachykinin most frequently identified not only in the primary carcinoid tumor but also in its metastases was SP, together with its fragment SP(5–11), both in normal and oxidized form (Gamse et al., 1981; Conlon et al., 1985; Roth et al., 1985; Theodorsson-Norheim et al., 1985; Bishop et al., 1989). However, NKA, together with its fragments NKA(4–10) and NKA(5–10) and its extended form neuropeptide K, may also frequently be present in carcinoids (Roth et al., 1985; Theodorsson-Norheim et al., 1985; Conlon et al., 1986b; Bishop et al., 1989); and, more rarely, an eledoisin-like immunoreactivity (eledoisin-LI) was observed together with a neurokinin B-LI (Theodorsson-Norheim et al., 1985) and an oxidized physalaemin-LI (Conlon et al., 1985).

SP-LI was also found, together with NKA-LI, in bronchial carcinoids (Creutzfeldt and Stockmann, 1987; Bishop et al., 1989), in ovarian carcinoids (Skrabanek et al., 1980; Strodel et al., 1984), and in a medullary carcinoma of the thyroid (Skrabanek et al., 1979).

Tachykinins (NKA-, neuropeptide K-, and eledoisin-like peptides) were produced also by carcinoid tumors in culture (Norheim et al., 1987) and both 5-HT- and SP-LI were found in cytoplasmatic granules isolated from an intestinal argentaffin carcinoid, supporting the view that in this case SP is costored with 5-HT in the granules of the enterochromaffin cells (Alumets et al., 1977).

Pheocromocytomas. Two pheocromocytomas showed SP-LI and SP sulfoxide-LI (Gamse et al., 1981), and another pheocromocytoma showed NKB-LI (Conlon et al., 1985). After subcellular fractionation, SP-LI and catecholamines were enriched in the chromaffin granular fraction, making it unlikely that SP-LI originates from nerve terminals (Gamse et al., 1981).

Lung carcinoma. Only a few data on the content of tachykinins in these carcinoid tumors are available: up to 2 ng/g fresh tissue of SP-like peptides, 1.2 ng/g being represented by authentic SP (Gamse et al., 1981). This content is surprisingly low, compared with the content of serotonin (from 1 μg to 2.5 mg per g fresh tissue) in argentaffin carcinoids (Stacey, 1966). Moreover, Lazarus et al. (1983) have presented evidence that a human small cell carcinoma may contain a tachykinin peptide (1–1.6 ng/g) that has structural and biological activity similar to that of the amphibian physalaemin.

Because carcinoid tumors and their metastases are authentic endocrine glands, releasing into the blood stream biogenic amines and tachykinins, levels of these compounds may increase in plasma and even in urine. TK-LI was found in 75% of the 65 carcinoid patients examined (Norheim et al., 1984). The major component in plasma eluted in ion-exchange chromatography was in a different position from that of the usual tachykinins. Similar results were obtained by Conlon et al. (1986b) in three of four carcinoid patients, with NKA-LI up to 1 nmol/ml plasma and SP-LI up to 345 fmol/ml. Moreover, in urine samples of 79% of the 48 carcinoid patients examined in another study, the TK-LI material was 8 times more elevated than in healthy subjects. The immunoreactive material was heterogeneous, with some components coeluting with oxidized NKA and neuropeptide K (Bergstrom et al., 1995). In the urine of patients with argentaffin carcinoids, the concentration of 5-hydroxyindolacetic acid, the main metabolite of serotonin, was 100 times more elevated than in healthy subjects (Stacey, 1966).

All of these findings on carcinoid tumors demonstrate that tumoral epithelial, non-neuronal cells of the mammalian intestine and other organs are capable of expressing and storing not only SP, as expected, but several other tachykinins as well, certainly NKA and its extended form neuropeptide K, but also NKB and kassinin-, eledoisin-, and physalaemin-like peptides, i.e., peptides occurring in normal tissues only in submammalian species.

Patients with argentaffin carcinoid tumors and their metastases very often exhibit a typical syndrome characterized by flushing, diarrhea, asthma, cyanosis, and right-side valvular disease. Creutzfeldt and Stockmann (1987) have considered tachykinins to be coresponsible only for vasodilation (flushing) and not of the other symptoms. Secretory diarrhea and enhanced motility, important features of the carcinoid syndrome, do not seem to be attributable to SP, but instead to NKA and to eledoisin-like peptides (Makridis et al., 1999).

We can conclude that carcinoid tumors are not pure tachykinin-secreting tumors and do not contribute, unlike other endocrine tumors (gastrinomas and vipomas), to the understanding of the physiological significance and function of the tachykinins.

B. Neuronal Localization

Nervous tissue represents by far the most important localization of the tachykinins in invertebrates and vertebrates. A more or less dense network of tachykininergic fibers, which release their content upon adequate stimulation, permeates all vertebrate tissues close upon

a very rich population of different receptors located on the membrane of neuronal and non-neuronal cells. In certain cerebral areas, the concentration of tachykinins may be on the order of nanomoles.

Data on the distribution and localization of neuronal tachykinins in the CNS and periphery have been obtained by a combination of HPLC with radioimmunoassay and/or by immunohistochemistry (Hokfelt et al., 1975, 1977; Pernow, 1983; Maggio, 1985).

Regional specific antisera directed against the C-terminal region of the tachykinins have been generally used, a condition that is unfortunate for discrimination between the various tachykinins (Maggio, 1988). The low specificity of several antisera and the different tissue extraction methods (Lindefors et al., 1985; Brodin et al., 1986) may explain some differences encountered in the literature on the localization of the three mammalian tachykinins.

Central nervous system. Distribution of the tachykinins in the CNS has been extensively studied only in the rat (Otsuka and Yoshioka, 1993). Data on other species are scanty. As expected, SP is generally cosynthesized, colocalized, and cosecreted with NKA. The values of immunoreactive SP in various areas of the rat brain are: olfactory tubercle, 300 pmol/g of wet tissue; amygdala, 383 pmol/g of wet tissue; nucleus caudatus, 247 pmol/g of wet tissue; globus pallidus, 332 pmol/g of wet tissue; septum, 116 to 405 pmol/g of wet tissue; hypothalamus, 208 to 626 pmol/g of wet tissue; habenula, 377 pmol/g of wet tissue; posterior pituitary, 489 pmol/g of wet tissue; thalamic nucleus, 25 pmol/g of wet tissue; globus pallidus, 332 pmol/g of wet tissue; substantia nigra, 1725 to 2580 pmol/g of wet tissue; periaqueoductal central gray, 590 to 994 pmol/g of wet tissue; locus caeruleus, 332 pmol/g of wet tissue; nuclei parabranchiales, 546 pmol/g of wet tissue; medulla oblungata, 95 to 436 pmol/g of wet tissue; dorsal horn of the spinal cord, 1070 pmol/g of wet tissue; and ventral horn, 134 pmol/g of wet tissue (Kanazawa and Jessell, 1976; Douglas et al., 1982). The concentration of SP may reach values as high as 2 to 3 μg/g of wet tissue.

In rats, both the density and the distribution of SP-containing neurons is changing significantly during the period just before birth, the days after birth, and into the adult period. SP-staminal cells and fibers reached maximum levels between postnatal days 5 and 15. Then density generally decreased (Inagaki et al., 1982; Sakanaka et al., 1982). The distribution of NKA is less known and in the rat brain, seems to be similar to that of SP, with clearly different locations, however, in several regions. As revealed by immunohistochemistry, NKB-containing perikarya were detected in the main and accessory olfactory bulb, some cortical regions, the olfactory tubercule, the n. accumbens, the septum, the neostriatum, several hypothalamic nuclei, the superior colliculus, the substantia nigra, the medullary reticular formation, and the external caudate nucleus (Kanazawa et al., 1984; Merchenthaler et al., 1992). NKB is also located in the spinal cord, predominantly in the dorsal horn, while it is present in negligible amounts in dorsal root ganglia and dorsal roots (Ogawa et al., 1985).

In the cat brain, kassinin-LI (NKA-LI) has a widespread distribution with the highest concentration present in substantia nigra, hypothalamus, and caudate n.; moderate levels in thalamus, brain stem, and spinal cord; and low levels in the cortex and cerebellum. Distribution of NKA-LI paralleled that of SP, although the ratio between the two peptides varied throughout the different areas (Hunter et al., 1985).

In human brain, the areas most rich in immunoreactive SP were: amygdala, 25 to 340 pmol/g of wet tissue; nucleus caudatus, 113 to 370 pmol/g of wet tissue; putamen, 81 to 380 pmol/g of wet tissue; globus pallidus, 518 to 1800 pmol/g of wet tissue; hypothalamus, 125 to 135 pmol/g of wet tissue; substantia nigra, 1264 to 4720 pmol/g of wet tissue; and locus caeruleus, 199 pmol/g of wet tissue (Gale et al., 1978; Emson et al., 1980; Cooper et al., 1981).

Data on the occurrence of SP and SP-like peptides in the frog and fish brain are presented by Inagaki et al. (1981).

Gut. The main sources of the neuronal tachykinins in the gut are: a) the intrinsic enteric neurons of the myenteric plexus, b) the intrinsic enteric neurons of the submucosal plexus, and c) the extrinsic primary afferent fibers. The most quantitatively important source of tachykinins in the gut is the enteric nervous system, which has its cells in the wall of the intestine and supplies all gastrointestinal effector systems. The mammalian gastrointestinal tract contains both SP and NKA and various extended forms of these tachykinins.

Neurons that contain only SP (alone or with other nontachykinin transmitters) are considered to be intrinsic sensory neurons (Holzer and Holzer-Petsche, 1997a, 1997b). In addition to these neurons, extrinsic efferent nerve fibers also display a small, but distinct contribution to the SP/NKA immunoreactivity in the gut. These fibers originate from dorsal root ganglia and reach the periphery via sympathetic or parasympathetic nerves, passing through prevertebral ganglia. The extrinsic efferent nerves project predominantly to the vessels in the intestinal wall but they also supply the lamina propria of the gastrointestinal mucosa. There are considerable species-dependent quantitative differences in the location of the tachykinins in the various gut segments, in the concentration of the peptides and in the density of SP/NKA-containing fibers.

In most species, the highest concentrations of tachykinins in the gut are found in pylorus, gastric fundus, duodenum, and jejunum (Pearse and Polak, 1975; Lazarus et al., 1980; Hunter et al., 1985; Gates et al., 1989). In the guinea pig small intestine, the bulk of SP and NKA, which are stored in the same synaptic vesicles, is associated with the myenteric plexus in longitudinal muscle.

Concerning NKB, the peptide is generally considered to be absent from human, porcine, guinea pig, and rat

intestine, which is consistent with the absence of PPT-B expression in the enteric nervous system of the rat but is in contrast with other results, showing that human and rat intestine contains minute amounts of NKB (Holzer and Holzer-Petsche, 1997a) and even more so with data showing that highly specific antiserum to NK3 receptors detected them in nervous myenteric and submucosal neurons (Grady et al., 1996). SP- but not NKA- and NKB-immunoreactivity is present also in the gall bladder and bile duct and in the pancreas, both around blood vessels and in the acini and the islets (Otsuka and Yoshioka, 1993).

Respiratory tract. RIA and immunohistochemistry have demonstrated the presence of SP and NKA in the respiratory tract of various mammalian fibers. In the trachea and bronchi, SP-immunoreactive fibers have been found in the smooth muscle layer and around local ganglion cells. In the bronchial tree, most of the SP-positive fibers are of vagal origin; but in the lung, the fibers are both of vagal and thoracic spinal origin. (Nilsson et al., 1977; Lundberg et al., 1983; Saria et al., 1985; Manzini et al., 1989).

Blood vessels. Data on the occurrence of SP-containing fibers are rather scanty and old. SP-like immunoreactivity has been observed in fibers of the adventitia and media in various blood vessels, such as feline cerebral arteries (Liu Chen et al., 1986), guinea pig intestinal vasculature (Furness et al., 1982), and rat portal vein (Barja and Mathison, 1982). The majority of SP-containing perivascular fibers are of sensory, capsaicin-sensitive origin.

Urinary system. The distribution of SP and NKA has been extensively studied in renal pelvis and ureter and especially in the urinary bladder of several species (Sharkey et al., 1983; Gibbins et al., 1985; Maggi et al., 1987). Capsaicin treatment results in an almost complete disappearance of the tachykinin-immunoreactive fibers, suggesting that the major sources of tachykinins in the urinary bladder are sensory fibers (Maggi and Meli, 1988; Maggi et al., 1988).

Skin. In human digital skin, SP- and NKA-immunoreactivity is present in free nerve endings in dermal papillae and epidermis (Dalsgaard et al., 1985; Bjorklund et al., 1986). SP-like immunoreactive fibers are also found in the skin of the rat and cat (Hokfelt et al., 1977). Treatment with capsaicin in rats caused a 70% depletion of SP-like immunoreactivity in various skin areas, suggesting that SP is present mainly in primary afferent C-fibers (Holzer, 1991).

Immune system. Tachykinin-containing primary capsaicin-sensitive afferent nerves are present in lymphoid organs, such as thymus, spleen, lymph nodes, and lymphoid aggregates in the lung and nasal mucosa. Their distribution is prevalently perivascular, but some fibers penetrate within the follicles. Both SP and NKA have been detected in rat thymus, spleen, and lymph nodes by radioimmunoassay. In addition to NKA, neuropeptide K and an eledoisin-like peptide also occur in the guinea pig thymus (Geppetti et al., 1987). However, non-neuronal sources of tachykinins are also present in the immune system. Using an anti-NKA antiserum, positive immunostaining was observed in staminal cells throughout the thymic parenchyma of the rat with predominance in the medullary area (Ericsson et al., 1990).

Because endothelial cells express SP-LI (Linnik and Moskowitz, 1989; Ralevic et al., 1990), the vascular endothelium of lymphoid organs may be the source of non-neuronal tachykinins at this level. Moreover, there is evidence that certain immune cells such as eosinophils and macrophages synthesize and release SP (for review, cf. Maggi, 1997).

Blood. Quantitative data on the concentration of immunoreactive SP in blood plasma are very variable with a wide range of values obtained by the different authors, indicating that nonspecific factors are probably interfering with the assay of immunoreactive SP. This is particularly true when unextracted plasma was used. As previously stated, the major part of circulating SP evidently originates from the intestine (Pernow, 1983). Values were as follows: man 70 to 300 and 50 to 620 fmol/ml; dog, 40 to 50 fmol/ml; and calf, 165 and 18 fmol/ml for unextracted and extracted plasma, respectively.

IV. Relationships between Structure/Activity Receptor Selectivity

Tachykinins, yet defined as peptides having the characteristic C-terminal pentapeptide Phe5-Xaa4-Gly-Leu-Met-NH_2, are identified as "aromatic tachykinins" when Xaa is an aromatic amino acid residue (Phe or Tyr) and "aliphatic tachykinins" when Xaa is an aliphatic amino acid residue (Val or Ile). All natural tachykinins are amidated at their C terminus, and this function is crucial for biological activity. Deamidated peptides are virtually inactive (Erspamer, 1994).

Structure/activity relationship studies established that the C-terminal pentapeptide was essential but not sufficient for the biological activity of the tachykinins. In fact, the C-terminal pentapeptide of eledoisin and physalaemin (Bernardi et al., 1964; Regoli et al., 1994b) like that of all other examined tachykinins was virtually inactive. The minimum chain length required for activity was six residues. These studies also recognized the Phe residue at position 5 from the C terminus and the amidation at the C terminus to be crucial for biological activity, both occurring in all vertebrate and invertebrate tachykinins, as well as the presence of the C-terminal Arg-NH_2 in the locustatachykinin-like peptides. The biological activity of the tachykinins depends on their interaction with three G protein-coupled receptors—NK1, NK2, and NK3—which share considerable structural homology, reflecting their common mechanism of action.

Receptors are small proteins of 350 to 500 amino acid residues, belonging to the family of rhodopsin-like mem-

brane structures. The tachykinin receptor displaying higher affinity for SP was termed NK1, the receptor showing higher affinity for NKA was termed NK2, and the receptor showing higher affinity for NKB was termed NK3. It should be emphasized that, up to date, all naturally occurring tachykinins may act as agonists on all three receptor types, although sometimes with considerably different affinities (Regoli et al., 1987, 1994a; Maggi et al., 1993).

Parallel bioassay on a number of isolated and in situ test systems using the natural tachykinins and selective synthetic analogs, radioligand binding studies, and the use of antagonists with increasing potency and selectivity have led to the conclusion that all of the three main tachykinin receptors are heterogeneous entities, with NK1, NK2, and NK3 subtypes (Maggi et al., 1993; Quartara and Maggi, 1997, 1998). The main second messenger system coupled to activate the three known receptor subtypes is the stimulation of phospholipase C, leading to phosphoinositol breakdown and elevation of intracellular calcium (Guard and Watson, 1991). At high tachykinin concentrations, an adenylate cyclase stimulation and cAMP formation may also come into play (Nakajima et al., 1992).

The extracellular loops of these G-protein coupled receptors probably have the specific function of selecting a ligand, whereas the interaction of the ligand with transmembrane domains is responsible for receptor activation. Tachykinin peptides, therefore, presumably contain a sequence that interacts with the extracellular loops of the receptor and a sequence that interacts with transmembrane domains. Recent findings conclusively allowed clarifying the crucial importance for and the influence on receptor selectivity and activity of some key amino acids in the tachykinin sequence (Severini et al., 2000).

A. Residue Occupying Position 7 from the C Terminus

The amino acid in position seven from the C-terminal of tachykinins seems to address the peptide ligand toward the receptor. SP and tachykinins with a neutral or basic residue in this position have a preference for the NK1 receptor. Neutral residues are generally hydrophilic, and proline in position eight from the C-terminal can increase affinity for the NK1 receptor. Tachykinins with an acidic or a couple of acidic residues in position 7 or 6 and 7 from the C-terminal addressed the peptides toward the NK2 and NK3 receptors. Interestingly, the second extracellular loop has four acidic and four basic residues in the rat NK1 receptor, three acidic and two basic residues in the NK2 receptor, and one acidic and five basic residues in the NK3 receptor.

B. Residue Occupying Position 4 from the C Terminus

In all natural tachykinins, position 4 from the C terminus is occupied either by an aromatic amino acid residue (Phe, Tyr) in the aromatic tachykinins or by an aliphatic, branched amino acid residue (Val, Ile) in the aliphatic tachykinins. The presence of an aromatic residue invariably determines selectivity or increases the selectivity of the peptide for the NK1 receptor. This is true not only when a neutral or basic amino acid residue occupies position 7 from the C terminus but also when an acidic residue occupies position 7. The couple of aromatic residues (Phe-Tyr or Phe-Phe) present in the "message domain" of the tachykinins provide specific binding interactions with transmembrane domains of NK1 receptor.

C. Residue Occupying Position 6 from the C Terminus

The presence of a Pro residue in position 6 from the C terminus causes a profound decay of biological activity. The negative contribution of Pro6 could be related to a distortion in the interaction of the C-terminal sequence of the peptide (Phe-Xaa-Gly-Leu-Met-NH2) with all tachykinin receptors. In the *Pseudophryne güntheri* tachykinins, a Glu residue occupies position 6 from the C terminus. The couple of acidic residues Asp7-Glu6 present in PG-SP1 and PG-KII could, therefore, be responsible for the marked shift of receptor selectivity toward the NK3 receptor. This shift is quite evident for PG-KII (an aliphatic tachykinin) and much less evident for PG-SP1 (an aromatic tachykinin) in which the Phe-Tyr sequence induces NK1 receptor selectivity.

D. Amino Acid Substitutions in the C-Terminal Tripeptide

To date, six natural peptides have single or double amino acid substitutions in the C-terminal tripeptide Gly-Leu-Met-NH_2: Pro (AC-AR2, AC-AR4) or Ala (ranatachykinin D) for Gly; and Val (AC-AR4) or Pro (ranatachykinin D) or Gly (AC-AR2) or Met (hylambatin) for Leu. None of these substitutions affected the peptides' receptor selectivity, only their receptor affinity or potency.

E. Pro Residue in the N-Terminal Sequence

The Pro residue is a well represented residue in the natural tachykinins. It is nearly always located in the N-terminal moiety of the peptide sequence and has a clear-cut preference for positions 8 and 10 from the C terminus. In the majority of natural NK1 receptor-preferring tachykinins, a Pro residue is present at position 8, adjacent to the crucial neutral or basic residue occupying position 7. Proline in this position could modify the conformation of the C-terminal sequence of the tachykinin peptides and helps to increase their affinity and selectivity for the NK1 receptor. Cascieri et al. (1992) have suggested that all tachykinins containing Pro at position 8 from the C terminus, for example SP, have greatly reduced affinity for NK2 and NK3 receptors, and they have attributed this behavior to the preferred conformation of the Pro-containing peptides for the NK1 receptor and unfavorable for NK2 and NK3 receptors.

V. Tachykinin-Like Peptides: Pharmacological Actions

The TKs display a number of potent pharmacological actions in the periphery and in the central nervous system. In the present chapter, analysis is limited essentially to the pharmacological actions of the nonmammalian TKs (eledoisin, physalaemin, and even kassinin) available in pure form several years before the structures of SP, NKA, and NKB were elucidated. As a consequence, the pharmacology of the TKs is based largely on the study of amphibian physalaemin, kassinin, and on molluscan eledoisin.

Whereas results obtained with eledoisin and kassinin, multireceptor agonists, do not exactly mimic results obtained with either NKA or NKB, results obtained with physalaemin, a selective NK1 agonist, are perfectly superimposable, with negligible quantitative differences, on those later obtained with SP, the mammalian selective NK1 receptor agonist.

A. Cardiovascular System

1. Systemic Arterial Blood Pressure Tachykinins administered to the anesthetized dog by the parenteral route are the most potent among all known hypotensive agents. On the dog blood pressure, physalaemin was 2 to 2.5 times less potent than SP, 10 to 20 times more potent than kassinin, and 3 to 4 times more potent than eledoisin (Erspamer, 1981). When administered by rapid intravenous injection, physalaemin was 200 to 1000 times more potent than bradykinin, and 600 to 2000 times more potent than histamine (Bertaccini et al., 1965). Physalaemin was very effective in antagonizing the pressor effects of noradrenaline and angiotensin II given at doses 100 and 10 times higher, respectively.

The rabbit was again extremely sensitive to physalaemin and even more so to SP. Eledoisin was approximately 10 times less active than SP; kassinin, 20 times less active; NKA and NKB, 200 and 2000 times less active, respectively (Bianchi Porro et al., 1965; Holzer-Petsche et al., 1985).

Intravenously injected in the sheep, eledoisin showed a hypertensive response of slow onset, probably attributable to some arousal of the animals (Ormas et al., 1975).

In cat and in rat, the effect of physalaemin was considerably less intense, with high variability and tachyphylaxis. Finally, in the decapitated chicken, physalaemin regularly elicited a biphasic response consisting of a brief hypotensive phase followed by a more intense and sustained dose-related pressure increase (Bertaccini et al., 1965). The rise in pressure observed in sheep and chicken was blocked by sympatholytic drugs and by pretreatment with reserpine, thus, indicating a release of catecholamines from the adrenal medulla and/or other stores.

Conversely, the hypotensive effect of the tachykinins was not modified by any of the usual autonomic blocking agents, thus suggesting a direct effect on the vascular smooth muscle.

Physalaemin, ranakinin, SP, and NKB produced a dose-dependent decrease in arterial blood pressure in the toad, *Bufo marinus.* A selective NK1 antagonist had no effect on the blood pressure fall elicited by ranakinin and SP, suggesting the existence of an NK1 receptor subtype different from mammalian NK1 receptor (Courtice et al., 1993).

In the bowfin *Amia calva*, a teleost fish, the bolus injection into the bulbus arteriosus of 0.1 to 10 nmol/kg of the bowfin SP resulted in a significant and dose-dependent rise in vascular resistance and blood pressure and a fall in cardiac output without changes in heart rate. Those effects lasted 5 to 10 min (Waugh et al., 1995b). Similarly, in the teleost fish rainbow trout, both the trout SP and the trout NKA at intraaortic doses of 1 nmol/kg increased systemic and celiac vascular resistance leading to hypertension, bradycardia, and decrease of cardiac output. After in vitro perfusion of the aortic and celiac mesenteric vascular bed, the peptides dose dependently increased the vascular resistance. It may be concluded that in teleost fish, the fish tachykinins are potent vasoconstrictor agents (Kagstrom et al., 1996).

In the conscious, unanaesthetized dogfish *Scyliorhinus canicula,* intravenous injection of either dogfish SP or scyliorhinin I (up to 5 nmol) produced no change in arterial blood pressure, pulse amplitude, and heart rate. Injection of greater amounts of the peptides (10–50 nmol) produced a slight increase in blood pressure (Waugh et al., 1993). However, in the unrestrained spiny dogfish *Squalus acanthia,* the intravenous injection of scyliorhinin I and NKA caused hypotension, due to a general vasodilation, with transient increase in mesenteric blood flow and a prolonged increase in celiac blood flow. The peptides did not increase heart rate (Kagstrom et al., 1996).

In human volunteers, eledoisin given by rapid intravenous injection (threshold 15–20 pmol/kg) decreased blood pressure, caused spinal fluid hypertension, increased the rate of respiration and caused skin vasodilation, particularly in the head. Rise in blood pressure produced by 3 to 5 nmol/kg angiotensin or 40 to 60 nmol/kg noradrenaline was inhibited or reversed by 1 to 2 nmol/kg eledoisin injected 5 s previously (Sicuteri et al., 1963).

In other experiments, the intravenous infusion of 0.6 nmol/kg/min eledoisin or 0.2 nmol/kg/min physalaemin produced only a 20 mm Hg pressure fall that lasted 5 min. Basal levels of pressure returned despite the continued infusion (De Caro et al., 1966).

SP also decreased blood pressure. A significant difference from the basal level was found at an infusion rate of 200 pmol/kg/min or higher (Eklund et al., 1977).

These results were substantially confirmed by Evans et al. (1988), who found that both SP (3 pmol/kg/min)

and NKA (64 pmol/kg/min) did not change systolic blood pressure, whereas diastolic pressure fell significantly only after SP infusion. Moreover both peptides increased heart rate and body temperature, with skin flushing. SP was 6 to 20 times more potent than NKA.

Heart. Electrocardiogram tracings recorded from anesthetized dogs given an intravenous infusion or a subcutaneous injection of physalaemin, at doses approximately 1000 higher than the threshold hypotensive dose, produced only moderate electrocardiographic changes mainly attributable to hypotension (Bertaccini et al., 1965). In a detailed study, the following percentage changes in a number of cardiovascular parameters have been observed in dog after intravenous injection of 4 pmol/kg physalaemin: heart rate, +17.8; mean systemic arterial pressure, −22.3; mean pulmonary arterial pressure, +1.8; mean left atrial pressure, −1; mean right atrial pressure, +0.5; myocardial contractile force, +17.5; cardiac output, +52; total peripheral resistance, −65.2; and pulmonary vascular resistance, −35.4 (Nakano et al., 1968). Similar results were obtained with eledoisin (Nakano, 1964, 1965).

The effect of SP was substantially the same as that observed with physalaemin. At infusion rates ranging from 3 to 450 pmol/kg/min, SP invariably induced a dose-dependent increase of cardiac output mostly due to a larger stroke volume. SP at concentrations up to 50 pmol/ml had no effect either on the isolated guinea pig auricles or the perfused rabbit heart, suggesting that SP has no direct effect on the heart (Burcher et al., 1977).

2. Regional Circulation. Coronary bed. Physalaemin and, to a considerably lesser extent eledoisin and SP (Losay et al., 1977) displayed a very potent vasodilator action on the dog coronary vascular bed not only when given by intracoronary administration, but also when given by intravenous infusion. A transient, 50% increase in coronary flow was obtained by rapid intracoronary injection with 0.1 pmol/kg and a 100% increase with 1 pmol/kg of physalaemin. Eledoisin was 200 times less active and nitroglycerin, 10,000 times less active.

Eledoisin infused intracoronarily at a rate of 6 pmol/kg/min increased sinus coronary outflow by 20%, coronary sinus oxygen tension by 10%, and similarly increased stroke flow and cardiac oxygen consumption, without affecting mean arterial pressure and heart rate (Lochner and Parratt, 1966). Increase in coronary flow and decrease in coronary vascular resistance also was observed after intravenous infusion of eledoisin (Beretta Anguissola et al., 1966).

Skeletal muscle. The vessels of the skeletal musculature of the hindlimbs of dogs were by far the most sensitive to tachykinins of any vascular bed. Doses of eledoisin as low as 10 fmol injected into the peronal artery caused an increase in blood flow, both in the intact and denervated gastrocnemius plantaris muscle. Denervation enhanced the potency of eledoisin (Bergamaschi and Glasser, 1963, 1964). Physalaemin was 50 times more potent than eledoisin and 50,000 times more potent than nitroglycerin (Bergamaschi et al., 1966; Fregnan and Glasser, 1968). In other experiments, close arterial injection of SP caused a dose-related vasodilation in adipose tissue and skeletal muscle of the dog only with doses starting from 10 nmol (Pernow and Rosell, 1975).

SP was also a potent vasodilator in humans. Infusion of 0.7 pmol/kg/min into the brachial artery significantly increased the forearm blood flow, with increases of oxygen consumption in both cutaneous and muscle blood. At the infusion rate of 70 pmol/kg/min, there was a bright red flushing of the skin, particularly in the neck and head, with a subjective feeling of warmth in the same regions, accompanied by tachycardia (Eklund et al., 1977). No effect of SP could be seen on internal carotid blood flow (Samnegard et al., 1978).

Liver. Portal or femoral infusions of SP, eledoisin, and physalaemin (2–20 pmol/kg/min) increased blood flow in the hepatic artery and vein of the dog. Portal infusions were less effective, thus, indicating a highly inactivating capacity of the liver. Hepatic arterial and venous pressures decreased, whereas sinusoid and portal pressure increased during peptide infusion. As a consequence, hepatic arterial and outflow resistances decreased. SP was the most potent peptide, followed by physalaemin (38%) and eledoisin (10%). When given by close arterial infusion, the peptides also consistently increased blood flow in the hepatic artery (Melchiorri et al., 1977). These observations were confirmed by Takaori et al. (1989), who found that intravenous physalaemin (5 pmol/kg) caused dose-dependent increases in mesenteric arterial blood flow (70%) and portal venous blood flow (77%) in the dog.

Lung. Intravenous eledoisin (0.1–1 nmol/kg) did not increase, and sometimes slightly reduced, pulmonary arterial pressure in the guinea pig; it always increased pressure in the rabbit. In the isolated, blood-perfused rabbit lung preparation, eledoisin produced a potent vasoconstriction from threshold doses of 0.01 to 0.1 pmol/kg. Tachyphylaxis was obvious. Bradykinin was 1000 times less effective (Hauge et al., 1966). In the dog NKA was much more potent than SP in decreasing tracheal vascular resistance (Salonen et al., 1988).

Skin. The skin vasculature of the dog was far less sensitive than the vessels of the musculature. In man, injection of 0.2 nmol eledoisin into the brachial artery produced digital vascular responses consisting of an increase in the skin temperature and a consistent increase in total digital volume, despite a decrease in inflow volume. Responses seem to indicate a closure of the arteriovenous anastomoses (De Pasquale and Burch, 1966).

Infusion of SP into the rat femoral artery dose dependently produced vasodilation (threshold 0.1 pmol/kg/min) that was inhibited by mepyramine (Lembeck and Holzer, 1979).

Brain. Eledoisin infused intravenously in the dog at 0.01 nmol/kg/min decreased cerebral blood flow (−22%), with an increase (+20%) in vascular resistance (Beretta Anguissola et al., 1966). In human subjects, the intravenous infusion of eledoisin (1–15 pmol/kg/min) influenced neither the cerebral blood flow and vascular resistance nor the cerebral metabolic rate of oxygen and glucose (Bianchi Porro et al., 1965).

Mechanism of vasodilation and hypotension. All of these findings demonstrate that in some mammalian species, exogenous tachykinins display a potent dilation of regional musculature accompanied by the fall of systemic blood pressure, in other mammalian and nonmammalian species the peptides display inconstant and variable effects: hypotensive/hypertensive or even frank hypertensive responses. Thus, the intervention of endogenous tachykinins in the regulation of blood pressure and regional circulation is certainly possible but irregular and unpredictable. At any rate, it is hardly conceivable that the tachykinins display a significant role in the cardiovascular system similar to that of noradrenaline, serotonin, angiotensin, prostacyclins, etc. This does not exclude that in man the tachykinins may contribute to the control of vascular tone of the cutaneous vessels of some areas. So, it has been suggested that tachykinin (SP and NKA) release is co-involved in the pathogenesis of flushing episodes (not accompanied by edema!) occurring in the carcinoid disease. To our knowledge, tachykinin antagonists have never been used in this disease. The trial could be rewarding from both a pathogenetical and a therapeutical point of view.

The striking hypotensive effects of the tachykinins observed in some animal species, as a consequence of intense vasodilation in several peripheral vascular beds, must be considered a direct effect of the peptides on the blood vessel wall. However, Regoli et al. (1987), D'Orléans-Juste et al. (1985, 1986), and Mastrangelo et al. (1987), in agreement with previous observations (Furchgott, 1983, 1984) on other vasodilators, found that in isolated strips of arteries and veins that were maximally contracted by noradrenaline, the relaxing effect of the tachykinins could be obtained only when the endothelium was intact. This clearly indicated that the site of action of the tachykinins (like that of acetylcholine, bradykinin, neurotensin, and bombesin) was not the smooth muscle cell, as formerly believed, but the endothelium. Thus, the tachykinins may act to promote the release of endogenous factors (prostacyclins, endothelium-delivered relaxing factors, and nitrous oxide) from the endothelium that is able to reduce the tone of the arterial smooth muscle fibers. However, whereas dog carotid artery without endothelium is insensitive to all tachykinins, thus, suggesting an exclusive location of its receptors in the endothelium, this is not true for the other vascular preparations. The rabbit pulmonary artery, for example, may be relaxed or contracted by tachykinins, depending on its basal tone. At high tone levels (premedication with noradrenaline), relaxation was predominant; whereas at low tone levels, contraction occurred. Relaxation may be due to the activation of NK1 receptors (SP, physalaemin) located in the endothelium, and contraction may be caused by activation of NK2 receptors (NKA, kassinin) located on the arterial smooth muscle. In the rabbit pulmonary artery possessing endothelium, the relaxing potency of SP was 3 to 4 times higher than that of NKA or kassinin; in the artery without endothelium, the contracting potency of SP was 30 to 120 times lower than that of NKA or kassinin (D'Orléans-Juste et al., 1986). Similarly, in the rat portal vein (with intact endothelium and not pretreated with noradrenaline), contraction elicited by NKB and kassinin was brought about by NK3 receptors, presumably located on the smooth muscle membrane (Mastrangelo et al., 1987).

In the intact animal, response of the vasculature to tachykinins is complex, depending on the animal species, density in the smooth muscle cells, and the endothelium of the different receptor types, as well as the kind of tachykinins administered or released. Whereas in dogs and rabbits, the NK1-preferring tachykinins (SP, physalaemin) regularly cause a dose-dependent intense hypotension with no sign of tachyphylaxis, the same tachykinins in cats, sheeps, rats, and pigeons may produce moderate hypotension with obvious tachyphylaxis, hypotension/hypertension, or frank hypertension, thus, indicating a complex activation of different receptor types, and perhaps a different availability of relaxing factors in the endothelium (tachyphylaxis).

Capillary permeability. The capillary permeability was enhanced by intradermal injection of eledoisin and physalaemin in the rat, guinea pig, and man. Eledoisin was 2 to 10 times more potent than histamine in all animal species and more potent than bradykinin in man (De Caro, 1963). Intradermal injection of eledoisin at doses exceeding 1 pmol in man caused pain, local edema, and erythema; whereas when injected intraperitoneally, intramuscolarly, or subcutaneously into human subjects at doses up to 17 to 50 nmol, the peptide failed to elicit pain (Kantor et al., 1967). Similarly, intradermal injection of 0.1 to 10 μM SP in man induced flare, wheal, and itching, which was similar to the response induced by histamine and, like histamine, was blocked by antihistaminic drugs (Hagermark et al., 1978).

Introduced in the mouse pleural cavity, SP (ED_{50} = 14 nM) caused a long-lasting recruitment of leukocytes and a small but evident exudation. These effects were partially inhibited by NK1 receptor antagonists and were mediated by nitric oxide (Frode-Saleh et al., 1999). Finally, close-arterial infusion of SP, from doses as low as 0.1–0.5 pmol/min in the rat skin, induced vasodilation and plasma extravasation (Lembeck and Holzer, 1979).

Of great interest is that intradermal injection of SP (30–300 pmol) induced a dose-dependent edema on wild-

type mice, whereas in NK1 receptor knockout mice, the peptide was inactive. The reaction in wild mice was reduced by the histamine antagonist mepyramine, indicating that edema induced by the tachykinin, although totally dependent on NK1 receptor-mediated mechanism, contains a mast cell-dependent component (Cao et al., 1998).

Intravenously injected in the rat, NKA induced a dose-dependent extravasation of Evans blue in stomach, duodenum, jejunum, caecum, and colon but had no effect in ileum. NKB equipotently produced extravasation in the stomach but had no effect in other parts of the gut. SP was ineffective (Lordal et al., 1996).

NKA (100–400 pmol/kg/min) given by intravenous infusion in an in situ perfusion model of the anesthetized rat stimulated duodenal motility and increased duodenal mucosal bicarbonate secretion, fluid output, and mucosal permeability. Effects were dependent on NK2 receptor activation (Hallgren et al., 1997). Plasma extravasation was induced by SP and bradykinin also in mouse gastrointestinal tract and pancreas (Figini et al., 1997). On the contrary, neither capsaicin nor SP (0.1–1 mg/ml) nebulized or given intravenously (0.75 nmol/kg) in rabbits produced significant increases in tracheal or bronchial Evans blue concentration, indicating that SP does not activate the microvascular leakage in the major airways of the rabbit (Matheson et al., 1997).

B. Gastrointestinal Tract

1. Motility. The stimulant action on intestinal motility of crude extracts, together with their hypotensive action, was at the root of the discovery first of SP and then of eledoisin and physalaemin.

In mammalian and submammalian vertebrates, the tachykinins provoke, with few exceptions, a contractile response by the gut. The excitatory motor effects were evident in all sections of the gut, from esophagus to the rectum, and in all muscular layers, including the longitudinal muscle, the circular muscle, and the muscularis mucosae. However, motor effects may be sharply different depending on the animal species, the gut sections, the different receptor types activated, and the mechanisms involved in the motor response (direct effect on the smooth muscle and indirect effect, through implication of the many neurotransmitters and hormones that are active on the gut motility) (Holzer-Petsche, 1995; Holzer and Holzer-Petsche, 1997a, 1997b; Maggi et al., 1997).

a. In Vitro Experiments. As many as 16 isolated gastrointestinal preparations from eight animal species were assayed with eledoisin and physalaemin (Erspamer and Falconieri Erspamer, 1962; Bertaccini et al., 1965). Response was always stimulation, but intensity and reproducibility of response varied conspicuously dependent on the animal species and the various segments of the intestinal tract. After synthetic SP and, later on, NKA and NKB became available, an enormous amount of work appeared on the action of mammalian tachykinins on isolated preparations of intestinal muscle and the receptors herein involved (Holzer and Holzer-Petsche, 1997a).

b. In Vivo Experiments. Dog. The first phenomenon observed a few minutes after a subcutaneous administration of 25 to 200 nmol/kg eledoisin was vomiting accompanied by profuse salivation. Vomiting was at first alimentary, and then the emesis episodes of increasing severity resulted in the ejection of masses of mucus, sometimes spotted with blood. Shortly after commencement of vomiting, there was a discharge of formed stools, which was soon followed by evacuation of watery stools containing mucus and blood accompanied by violent tenesmus. The tremendous gastrointestinal stimulation and profound depression of the animal lasted unchanged for 1 h and then gradually decreased. During the night, there was complete recovery, and for a few days afterward, the dog showed unusual voracity. In dogs given a subcutaneous dose of 0.15 mg/kg atropine sulfate 30 min before the injection of 20 nmol/kg eledoisin, the alkaloid failed to block stimulation of either salivary glands or gastrointestinal smooth muscle (Erspamer and Glasser, 1963).

Subcutaneous injections of 100 to 250 nmol/kg of physalaemin elicited phenomena similar to those provoked by 25 to 100 nmol/kg eledoisin, but vomiting and diarrhea accompanied by profuse salivation were less severe and recovery was rapid. Doses of 25 to 50 nmol/kg physalaemin caused moderate salivation, moderate evacuation of formed stools, and inconstant vomiting. No consistent gastrointestinal effects, except slight salivation, appeared after doses of 10 nmol/kg physalaemin (Bertaccini et al., 1965).

The general effects produced by rapid intravenous injection of physalaemin in dogs were studied by Bertazzoli and Cheli (personal communication). No obvious effects were elicited by 0.1 nmol/kg of physalaemin; after 0.3 nmol/kg, the only appreciable effect was that the dog had difficulty in sitting (tenesmus?), and this lasted only for a few minutes; 0.6 nmol/kg produced the same effect and, in addition, one or more evacuations of the bowel during the first 5 min; 2.5 nmol/kg caused not only diarrhea but also salivation, lasting 5 to 10 min. Increased salivation and diarrhea were accompanied by vomiting in dogs treated with 12 nmol/kg of physalaemin. All symptoms disappeared within 15 to 20 min. Similar doses of physalaemin (2.5 nmol/kg) given intravenously every day for 10 successive days elicited the same phenomena.

In the anesthetized dog, intravenous infusion of eledoisin at a rate of 40 pmol/kg/min produced neither salivation nor evacuation of the bowel. Infusion rates of 80 to 250 pmol/kg/min, on the other hand, caused salivation and evacuation of liquid stools. Stimulation of both salivary glands and gastrointestinal smooth muscle was atropine-resistant. High infusion rates produced a

more or less evident cutaneous vasodilation (Erspamer and Glasser, 1963).

In conscious dogs, eledoisin and physalaemin displayed a potent stimulating effect on the mechanical and electrical activity of the gut. Low doses of the peptides (2.5–15 ng/kg/min) caused an increase in frequency and duration of the interdigestive myoelectric complexes, and an increase in coordinated mechanical activity. High doses provoked the appearance of a diffuse spike activity, accompanied by intense local motor activity. Pacesetter potentials were not affected (Caprilli et al., 1975). After intravenous injection of 1 nmol/kg physalaemin in anesthetized dogs with a gastric pouch a striking increase in tonus of the gastric pouch was observed, accompanied by stimulation of rhythmic contractions, lasting 10 min. In the ileum a slight initial increase in tonus was followed by intense stimulation of rhythmic activity, lasting 10 to 15 min (Bertaccini, 1982).

In other experiments on the in situ jejuneal loop of the anesthetized dog, physalaemin was twice as potent as CCK in stimulating motility, 15 times as potent as human gastrin I, 50 to 100 times as potent as either bradykinin or carbachol, and more than 300 times as potent as acetylcholine, histamine, and serotonin. Only caerulein overcame physalaemin (by 3 times) in its stimulating effect (Mantovani et al., 1969).

In keeping with these results, the tachykinins administered by close intra-arterial injection increased the motility of gastric antrum and proximal duodenum. Physalaemin and eledoisin (5 pmol/ml) were the most potent peptides, followed by SP (50–60%) and neurokinins A and B (10–12%) (Kuwahara and Yanaihara, 1987). The effect of SP on gastric motility was found to differ depending on the sites and vagal innervation of conscious dogs (Shibata et al., 1994).

Cat. Physalaemin, eledoisin, and SP administered via the splanchnic artery produced both motor and mechanical effects in the stomach of anesthetized cats, characterized by successive phases of initial distension, sustained contraction, and late distension. At low doses, distension was the dominant effect. The sustained contraction and late distension phase were accompanied by phasic contractions. Atropine abolished the sustained contractions but had no effect on phasic contractions and distension phases (Lidberg et al., 1983; Barber et al., 1987).

Guinea pig. Tachykinins influenced peristaltic motor activity in isolated segments of the guinea pig small intestine. NK2 and particularly NK3 agonists facilitated intestinal peristalsis, whereas SP first stimulated and then inhibited peristalsis. The facilitatory effect of SP was prevented by atropine and seems to involve NK2 receptors, whereas the secondary inhibitory effect is due to NK1 stimulation (Holzer-Petsche, 1995).

Rat. The tachykinins displayed a potent spasmogenic action on the rat stomach, as measured from the bulk of duodenal effluent. In terms of threshold doses (0.1–1 nmol/kg intravenous), eledoisin was the most potent peptide, SP was the least potent, and phyllomedusin was by far the most effective with regards to the duration of effect (Bertaccini and Coruzzi, 1977; Bertaccini, 1980). After infusion into the celiac artery at doses of 0.06 to 20 nmol/min, both SP and NKA caused contraction of the stomach, NKA being 10 times more potent than SP (Holzer-Petsche et al., 1987).

The complexity of the effects of the tachykinins on the gastrointestinal propulsion was evident after the intraperitoneal injection of the peptides. It was shown that 3 min after a test meal, both SP ($>$ 0.75 nmol/kg) and NKA ($>$ 8.8 nmol/kg) inhibited both gastric emptying and intestinal transit. The inhibitory effect was reversed to a stimulant effect by pretreatment with atropine. After 10 min, SP dose dependently enhanced intestinal propulsion, an effect that was atropine resistant. From the above experiments, it clearly seems that the gastrointestinal propulsion was dose- and time-dependent, with variable involvement of the autonomic nervous system (Holzer, 1985).

Sheep. Intravenous eledoisin (50–250 pmol/kg) stimulated both in anesthetized and in awake and standing animals the motility of all sections of the stomach producing an increase in tone and in rhythmic movements. The effect was immediate, atropine resistant, and lasted from 5 to 30 min, depending on the dose. The omasum was the most sensitive section. SP was at least 10 times less active than eledoisin (Ormas et al., 1977).

Toad. On the *B. marinus* intestine, bufokinin was the most potent agonist (EC_{50} = 0.35 μM), producing a long-lasting contraction similar to that evoked by physalaemin, SP, and kassinin. Surprisingly, these effects were not inhibited by highly selective NK1 receptor antagonists (Liu et al., 1999b).

Fish. The stimulant effect of the tachykinins was also evident in the fish gut. Both SP and NKA produced contraction of the vascularly preferred cod stomach; SP (pD2 7.05) was almost 6 times more potent than NKA (Jensen, 1997). The same peptides displayed an excitatory effect on the circular muscle of the cod intestine, suggesting that tachykininergic neurons are involved in the ascending excitatory reflex of peristalsis (Karila et al., 1998).

2. Secretions. Salivary secretion. The potent sialagogic effect of substance P (Haefeli and Hurlimann, 1962) and of amphibian tachykinins (Bertaccini and De Caro, 1965; Emmelin and Lenninger, 1967) was recognized several years before the sialagogic principle in a bovine hypothalamic extract was identified as substance P by Chang and Leeman (1970).

Physalaemin displayed a powerful sialagogic effect in dogs and rats. In dogs (with cannulated submaxillary gland), the threshold dose of the peptide was 0.5 nmol/kg when injected into the femoral vein and 0.1 nmol/kg when injected through the ipsilateral carotid artery. The

threshold dose by intravenous infusion was 0.1 to 0.4 nmol/kg/min. Salivary flow never lasted more than 5 to 8 min after the injection. Response was dose dependent and tachyphylaxis was lacking. The effect was appreciable even at a very low systemic blood pressure. High amounts of parasympathetic and sympathetic blocking drugs failed to block salivation induced by physalaemin, thus, indicating a direct point of action of the peptide on the acinar cells. The sialagogic activity of physalaemin exceeded eledoisin by three times, carbachol by seven times, and other known sialagogic agents by more than 100 times (Bertaccini and De Caro, 1965).

These results were confirmed by Emmelin et al. (1969), who found that physalaemin stimulated secretion from the dog parotid and submaxillary glands (threshold doses: 0.5 and 12 nmol/kg, respectively). Considerably lower doses (threshold: 5–10 pmol/kg) elicited a pressure increase in both submaxillary and parotid ducts. The contraction of the myoepithelial cells of the ducts was due to a direct effect of the peptide, because the usual autonomic blocking agents did not abolish it.

In rats, the threshold sialagogic subcutaneous dose of physalaemin was 0.1 to 0.3 nmol/kg. There was a satisfactory dose-response relationship, and the stimulatory effect could be repeated for 2 to 3 h. By intravenous infusion (threshold: 0.2 nmol/kg), salivary stimulation lasted as long as the infusion was continued (Bertaccini and De Caro, 1965). Increase in salivary flow produced both by intravenous injection (0.1–1 nmol/kg) and infusion (1 nmol/kg/min) was associated with an increase in amylase and electrolyte secretion. The flow rate of the submaxillary gland was maintained throughout the period of infusion, whereas the parotid flow rate tended to decrease, finally ceasing entirely. Again, autonomic blocking drugs failed to modify the response to physalaemin (Schneyer and Hall, 1968).

By close intra-arterial injection, the threshold sialagogic dose of physalaemin was much lower (1.5 pmol) for the submaxillary gland than for the parotid gland (62 pmol). An increase in flow of saliva occurred along with a pressure rise in the duct of the submaxillary gland (Thulin, 1976).

Chronic intravenous administration of physalaemin (10 nmol/kg twice daily for 15 days) caused a moderate enlargement of the parotid and submaxillary glands, with a 50% increase in weight. Eledoisin was inactive at doses up to 500 nmol/kg (Bertaccini et al., 1966; Cantalamessa et al., 1975). Eledoisin and physalaemin also caused salivary secretion in the hen at intravenous doses of 0.3 to 0.5 nmol/kg. Physalaemin was 8 to 10 times more potent than eledoisin (Lembeck and Starke, 1968).

As far as mammalian tachykinins are concerned, results obtained mainly with SP confirmed and extended results obtained with physalaemin. In summarizing, it was established that: a) SP potently stimulated salivation in the dog, ferret, rat, and guinea pig; whereas in humans, cat, rabbit, mouse, and hamster, it was virtually inactive (Lembeck and Starke, 1968; Leubeck et al., 1968; Iwabuchi et al., 1992; Tobin and Ekstrom, 1992). b) The principal effect of SP and NKA was to enhance the flow of salivary fluid, which is poor in protein, through a predominant action on the secretory structures of the acini. c) The secretion of fluid was stimulated in all salivary glands of the rat, but the submaxillar glands were most sensitive to SP, whereas the parotid glands and particularly the sublingual glands were less sensitive (Ekstrom et al., 1983). d) SP also produced changes in the output of salivary components, as shown by the increase in the secretion of K^+ and Cl^- ions, in discharge of proteins, glycoproteins, proteolytic enzymes, amylase, kallikrein, and mucus (Holzer and Holzer-Petsche, 1997b). e) The rank order of potency in causing salivary secretion in rats after intravenous injection was: physalaemin = uperolein > eledoisin > SP = kassinin > NKA $\gg$ NKB (Holzer-Petsche et al., 1985).

The greater potency of physalaemin and uperolein in comparison with SP may be explained by the considerably higher resistance to enzyme attack offered in the two first peptides by their N-terminal pGlu residue (Yanaihara et al., 1977). It is generally accepted that the secretory action of the tachykinins in the rat salivary glands is mediated by the NK1 receptor (Holzer and Holzer-Petsche, 1997b).

In slices of rat parotid glands, the tachykinins caused a rapid, concentration-dependent (threshold 10–15 pmol/ml) increase in K^+ efflux and amylase release, independent of any effect on tissue concentrations of cyclic AMP or cyclic GMP levels. Simultaneously, there was a stimulation of phosphatidylinositol turnover (Rudlich and Butcher, 1976; Hanley et al., 1980). Physalaemin and substance P were emulative, eledoisin was one-half as potent, and kassinin was 10 times less potent (Brown and Hanley, 1981).

Gastric acid and biliary secretions. Physalaemin did not influence the gastric acid secretion in fasting dogs at the maximum tolerated intravenous dose (30 nmol/kg). Gastric acid secretion of the rat was stimulated very little, if at all. In the cod, a teleost fish, physalaemin and eledoisin potently stimulated gastric pepsin secretion (ED_{50} = 1 mg/kg/min); SP was 1000 times less active (Holstein and Cederberg, 1986). In the isolated porcine nonantral stomach preparation, 0.1 μM SP induced a 2-fold increase in acid secretion and a 3- to 4-fold increase in pepsinogen secretion. Similar effects were displayed by NKA, whereas capsaicin was inactive (Schmidt et al., 1999). This result may indicate that release of endogenous SP/NKA is insufficient to affect the above parameters. In the dog, the peptide caused changes in bile flow that were associated with contraction of the gall bladder and not with a stimulated secretory activity. In fact, when the hepatic and cystic ducts were cannulated separately, physalaemin increased the

flow in the cystic duct but decreased the flow in the hepatic duct. In the rat, the peptide was completely ineffective (Bertaccini et al., 1967).

In agreement with the above results it was found that SP also attenuated basal and hormone-stimulated (cholecystokinin and vasoactive intestinal peptide) overall bile flow and output of bile acids, Na^+, K^+, Cl^-, and bicarbonate (Starke et al., 1968; Holm et al., 1978; Konturek et al., 1981).

Intestinal secretion. That physalaemin and eledoisin stimulate intestinal secretion is shown by the liquid discharges produced after subcutaneous administration of these peptides. In dogs with a cannulated 25-cm jejunum segment perfused with saline, the intravenous injection of 55 pmol/kg/min SP produced an increase in plasma SP concentration from 6 to 121 fmol/ml, an increase in intestinal secretion of water (from 102 to 275 μl/min) and Na^+ (from 20 to 23.2 μEq/min), and a decrease in Cl^- secretion (from 21.7 to 16.5 μEq/min) (McFadden et al., 1986).

Close intraarterial infusion of SP (causing a plasma concentration of 1–5 μM) to the in vivo feline small intestine regularly evoked a net fluid secretion in vivo, accompanied by release into the blood of vasoactive intestinal polypeptide (Brunsson et al., 1995).

The problem of receptors and mediator systems involved in the in vivo and in vitro secretory effects of the tachykinins is extremely complex. NK1 and NK2 receptors seem to play a predominant role. In the canine intestine, implication of NK2 receptors in addition to NK1 receptors is demonstrated by the greater potency of eledoisin in comparison with physalaemin in eliciting watery discharges.

Pancreatic secretion. Physalaemin displayed a moderate, short-lasting stimulatory action on exocrine pancreatic secretion in the dog after threshold intravenous doses of 0.05 to 0.5 nmol/kg. Eledoisin was 30 times less active. The content of amylase in pancreatic juice was similar to that present in the secretion elicited by cholecystokinin (Bertaccini et al., 1967).

Like eledoisin, kassinin was virtually inactive on the dog pancreas. SP enhanced the basal output of pancreatic juice, amylase, and bicarbonate in dogs (20 pmol/kg/min, by close intraarterial infusion) and rats (Thulin and Holm, 1977; Konturek et al., 1981). However, the effect of the tachykinins was negligible in comparison with that of cholecystokinin.

In dispersed acinar cells prepared from guinea pig pancreas, physalaemin and eledoisin increased outflow of Ca^{2+}, accumulated cyclic GMP, and released amylase without affecting cyclic AMP. The efficacy relative to caerulein was 29% for eledoisin and 17% for physalaemin (May et al., 1978; Jensen and Gardner, 1979). In the isolated rat pancreas, 0.1 to 1 nM SP inhibited cholecystochynin-induced amylase release and secretin-induced flow. Capsaicin displayed the same effects as SP, probably through release of endogenous SP (Kirkwood et al., 1999).

The functional significance of neuronal tachykinins in the gut has been the object of an extensive and thorough investigation (Holzer-Petsche, 1995; Holzer and Holzer-Petsche, 1997a, 1997b). All aspects of the tachykinin function in the gut have been taken in consideration, but it is extremely difficult to draw some firmly established conclusions from the enormous amount of data published. Pharmacological "in vitro" tests have clearly demonstrated that exogenous tachykinins given alone are among the most active substances on the gastrointestinal motility of all examined vertebrate species, generally in the sense of clear-cut stimulation. Things are different in the intact organism. The tachykinins represent only one of the many active agents of peptide and nonpeptide nature (gastrin, cholecystokinin, motilin, enteroglucagon, bombesin, guanylin, neuropeptide tyrosin, opioid peptides, noradrenaline, histamine, serotonin, acetylcholine, prostaglandins, ATP, etc.), and it is virtually impossible at present time to distinguish the part played in the functional control of gut motility by the tachykinins from that played by the array of the above active substances. It is highly probable that the tachykinin co-involvement may be considerably different in the different animal species and in the different gut sections. The use of tachykinin antagonists has given only partial and ambiguous results. In conclusion, whereas, there is no doubt that endogenous SP and NKA may play an important role by interaction with other enteric transmitters in the control of gastrointestinal motor activity, the weight of this role in health and disease remains to be defined.

Exogenous tachykinins seem to cause also secretion of fluid and electrolytes from the intestinal mucosa, and it has been suggested that endogenous tachykinins may play a messenger role in intestinal secretory pathway. The increase in the capillary permeability could contribute to increase in secretions. Moreover, there is evidence that the tachykinins participate in the hypersecretory, vascular, and immunological disturbance, associated with infection and inflammatory bowel disease (Holzer and Holzer-Petsche, 1997a, 1997b). This statement must be again accepted with caution, keeping in mind that the tachykinins are also in the case of secretions and, even more so, of immunological reactions only one of the numerous factors involved in these processes. To remain in the field of gut secretions, in recent years a peptide, guanylin, has been extracted and isolated from various organs, among which is the rat intestine (Currie et al., 1992) in which it seems to be costored with serotonin in some populations of the enterochromaffin cells (Cetin et al., 1994). Guanylin exhibits high sequence and structural homology with *Escherichia coli* heat-stable enterotoxin and, like this toxin, guanylin causes secretory diarrhea in rats, by activation of the guanylate cyclase C receptors. It is evident that guanylin may play an important role in the physiological regulation of elec-

trolyte/water secretion in ion-transporting intestinal epithelium.

Some questions at this point are imperative. Do some enterochromaffin cell populations costore and cosecrete SP and guanylin? Could guanylin be costored with SP also in the nonargentaffin, but argyrophyl/acidophyl cells of the gut? Is it possible that guanylin is present also in carcinoid tumors, contributing to the diarrhea, which is one of the most frequent symptoms in the carcinoid syndrome? Should the answer to these questions be positive, the tachykinin contribution in the regulation of gut secretion could be attributable not to neuronal SP but to SP occurring in the highly neglected epithelial cells of the gut, probably provided with paracrine secretion.

C. Airways System

A detailed description of the effects of the tachykinins on isolated preparations of tracheal and bronchial musculature in the rat, guinea pig, ferret, hamster, and man is presented by Frossard and Advenier (1991), together with a discussion of the receptor types and subtypes involved in the response to the tachykinins. Under physiological conditions, tachykinins contribute, to some extent, in the regulation of the tone of the airways musculature, at least in some animal species.

In spontaneously breathing guinea pigs, graded doses of eledoisin (2–5 nmol/kg, intravenous) produced a dose-dependent reduction of the tidal volume associated with temporary tachypnoea. With 5 nmol/kg, the tidal volume approached zero, although the movements of the respiratory muscle had increased. Eledoisin was one-half as active as serotonin.

In mechanically respired guinea pigs, eledoisin reduced the inflation volume in a dose-dependent manner; a 50% reduction was obtained at a dose of 1 nmol/kg. Serotonin was 3 to 4 times less active (Gjuris and Westermann, 1965). These results were confirmed by Nilsson et al. (1977), who observed that intravenous injection of SP produced a dose-dependent elevation of insufflation pressure. SP (0.4 nmol/kg) increased the pressure by 100%, an effect that required doses of histamine 40 times higher. Similarly, Hua et al. (1984), found that kassinin, eledoisin, and NKA potently increased insufflation pressure. Physalaemin was less potent, and NKB still less so. Moreover, in guinea pig airways, physalaemin, eledoisin, and SP provoked an increase in microvascular permeability to protein by activating receptors localized in the endothelial cells, as assessed by Evans blue extravasation (Rogers et al., 1988). It is suggested that both NK1 and NK2 receptors are involved in the response to tachykinins by the guinea pig tracheobronchial tree (Ireland et al., 1991; Maggi et al., 1991).

In rats, the broncho-constrictor action elicited by the intravenous injection of tachykinins was inhibited largely by atropine (suggesting a release of acetylcholine), and by methysergide (suggesting a release of 5-HT from pulmonary mast cells). Eledoisin and kassinin were slightly but significantly more potent than the neurokinins but much more potent than SP (Joos et al., 1986). In anesthetized rabbits, peripheral administration of either SP or NKA (0.2–2 nmol/kg) produced a dose-related increase in rapidly adapting pulmonary stretch receptor activity without any significant changes in total lung resistance. NKA was less potent than SP, NKB was practically inactive. This effect is mediated by activation of both NK1 and NK2 receptors (Matsumoto et al., 1997).

In the anesthetized dog, challenge with aerosolized NKA (0.1–1%) produced a dose-dependent increase in lung resistance, a decrease in dynamic lung compliance, reduced tidal volume, and increased respiratory rate. Experiments with selective tachykinin antagonists suggest that in addition to the NK2 receptor the NK1 receptor may also be involved in the response to NKA by the dog respiratory tract. Cholinergic reflexes may play a small, but significant role in this response (Sherwood et al., 1977).

In humans, infusion of SP had little effect on airway function. A small increase in airway resistance was observed at low dose and converted to bronchodilation at high doses (3.2 pmol/kg/min) (Evans et al., 1988). Inhaled SP also failed to increase airway resistance in both normal and asthmatic subjects. NKA conversely induced a fall in specific airways conductance in patients with mild asthma, suggesting an activation of NK2 receptors (Joos et al., 1986). In man, it is rather doubtful from the available data that the tachykinins play some role in disease, such as asthma, and that tachykinin receptor antagonists may have a future in therapeutics of respiratory disease. One of the major symptoms of the carcinoid syndrome is asthmatic attack. It would be important to know whether these attacks benefit from administration of tachykinin antagonists.

D. Urogenital Tract

Exogenous tachykinins at extraphysiological concentrations produced variable degrees of stimulation of smooth muscle preparations of the urogenital tract, especially the urinary bladder and displayed differences in their agonistic potency not only depending on the different animal species but also on the various segments of the urinary tract. The different kinds of responses seem to be mediated through different types of receptors and seem to be brought about both by a direct effect on the bladder's smooth muscle and by an effect on intramural sensory nervous pathways ("micturition reflex") (Maggi and Meli, 1986; Maggi et al., 1986a, 1986b; Maggi 1991). Activation of rat bladder motility and the micturition reflex may be provoked by intravenous injection and by topical application of tachykinins on the serosal surface of the bladder.

Intravenous injection of the peptides elicited a phasic contraction of the bladder (increase of internal pressure) and an activation of a series of rhythmic contractions. Kassinin was the most potent peptide, followed by NKA (30%), NKB (20%), and SP (1%). Thus, endogenous tachykinins together with other active substances may contribute to the tone and motility, including micturition reflex, of the urinary bladder, the ureters, and the urethra, but the part actually displayed by the tachykinins remains to be established.

E. Immune System

The influence of the tachykinins on the immune system has been carefully reviewed in recent articles (Hartung and Toyka, 1989; McGillis et al., 1990; Eglezos et al., 1991; Maggi, 1997). Although there is increasing evidence that the tachykinins (especially SP and, subordinately NKA) play a role in neuro-immunomodulation, i.e., in the control and regulation of the immune response by the central and peripheral nervous system, the actual relevance and importance of the tachykinins in immunomodulation is uncertain. Tachykinins are merely one of the numerous factors that may directly or indirectly influence the immune system: all or nearly all peptidergic and aminergic neurotransmitters, the derivatives of arachidonic acid, and all of the many active substances synthesized and released by the immune cells.

Data supporting the influence of the tachykinins on the immune system are as follows: a) Occurrence of SP immunoreactive fibers in organs of the immune system, such as lymph nodes, thymus, and bone narrow. b) Presence of SP receptors in thymus and spleen and, above all, expression of these receptors in human circulating lymphocytes and monocytes, rabbit polymorphonucleates and leukocytes, guinea pig macrophages. c) Clear-cut action of SP, in vitro and in vivo, on B- and T-cell proliferation, immunoglobulin secretion, cellular chemotaxis, and lymphocyte migration.

The integrity of the immune system is essential for life. The involvement of tachykinins in the control of the immune system in health is very difficult to be established and, at any rate, it seems to be of limited importance: knockout mice with disrupted preprotachykinin A gene were in good health. However, it is necessary to distinguish between physiology and pathology of the immune system. In pathological conditions, such as inflammatory processes, things are even more complicated, because of the enormous cascade of biochemical events that take place during inflammation and immune reaction. It is certainly possible that certain immune cell types are able to synthesize and release tachykinins (of extra-neuronal source) and that NK1 receptors play a role in mediating extravascular migration of granulocytes into inflamed tissues in response to various stimuli (Maggi, 1997). In human skin, exogenous tachykinins may cause wheal and flare, due to release of histamine, and may evoke plasma leakage through the capillaries in several but not all tissues. SP may also degranulate mast cells, but this is an effect attributable not to the intact SP molecule, but to its N-terminal segment acting on receptors independent from the classical tachykinin receptors. To demonstrate the complexity of participation of SP in inflammation, it has been shown in a recent paper (Wallace et al., 1998) that in a model of acute colitis in the rat and guinea pig, NK1 antagonists, although reducing the infiltration of granulocytes during the first 12 h after induction of colitis, failed after repeated administration during a 3-day period to affect granulocyte recruitment or severity of tissue injury.

F. Central Nervous System

The overall presence of tachykinins with their receptors in the CNS of mammals and in that of all examined submammalian species (see neuronal localization) constitutes the most pregnant and incontrovertible evidence that these peptides play in the CNS a very important role as neurotransmitter/neuromodulatory agents, as demonstrated by neurophysiological evidences directly showing this importance (Otsuka and Yoshioka, 1993).

In the CNS, tachykinins occur in large amounts particularly in areas involved in the central control of several peripheral autonomic functions (blood pressure, respiration, micturition, gastrointestinal motility, etc.), of essential functions (e.g., drinking behavior), of the affective and emotive life (stereotyped behavior, motility, anxiety, aggression, and pain), and of higher cerebral functions (learning and memory).

Blood pressure. Eledoisin (0.1–1 nmol/kg) injected into the cerebral ventricles of anesthetized rats produced a biphasic cardiovascular response that consisted of an initial fall of systemic blood pressure (8–15 mm Hg) followed by a rise (20–22 mm Hg). Abolition of the fall in blood pressure by phentolamine suggests central inhibition of sympathetic tone to vessels that afford peripheral resistance, whereas blockade of the delayed hypertensive phase by propranolol indicates a central activation of cardiac adrenoreceptors (Pearson et al., 1969). Different effects were obtained when 1 μg of eledoisin was intracerebroventricularly administered in conscious rats. The peptide, in fact, produced a long-lasting rise in blood pressure (18 mm Hg) that was accompanied by behavioral excitement. Pretreatment with phentolamine, but not propranolol or morphine, prevented the pressor response, thus, indicating an α-adrenergic-mediated vasoconstriction (Lambert and Lang, 1970). Effects produced by intracerebroventricular injection of SP (10 nmol) were similar: increase in blood pressure, heart rate, and sympathetic efferent activity with visceral vasoconstriction and hindlimb vasodilation. The cardiovascular responses were accompanied by a behavioral defense reaction, including increased locomotion, scratching, skin biting, and

grooming (Unger et al., 1988). Also NKA (10 nmol) elicited increase in blood pressure and heart rate (via sympathetic activity) (Takano et al., 1990).

Respiration. Intracerebroventricular injections of SP in rats (3–30 nmol) induced a dose-dependent stimulation of minute ventilation due to increase in total volume, although respiratory frequency was slightly reduced (Hedner et al., 1984). Similar respiratory effects were produced by application of SP on the dorsal surface of the medulla oblungata in newborn rabbits (Yamamoto and Lagercrantz, 1985).

Gastric acid secretion. The NK3 receptor preferring tachykinins (kassinin, NKB, and PGKII) given to rats by intracerebroventricular injection (0.01–10 nmol/rat) elicited a dose-related inhibition of gastric acid secretion. Kassinin and eledoisin were the most potent peptides, the other peptides showing the following order of relative potency: kassinin > NKB = PGKII ≫ NKA > SP and physalaemin (inactive). Subcutaneous doses up to 20 nmol of eledoisin or kassinin were ineffective (Improta and Broccardo, 1990; Improta et al., 1996).

Gastric emptying. Administration of 0.1 nmol of either eledoisin or kassinin produced a 35 to 40% inhibition, and 10 nmol caused a 100% inhibition of gastric emptying of a liquid meal. The relative potency of other examined tachykinins was as follows: NKA, 50%; physalaemin, NKB, and PGKII 0.3%, thus suggesting a predominant involvement of NK2 receptors (Improta and Broccardo, 1990; Improta et al., 1996). In general, tachykinins are less potent in their inhibition of gastric emptying and gastric secretion than either bombesin or opioid peptides.

Colonic propulsion. Intracerebroventricular injections of PG-KII (0.1–100 ng/rat; threshold 1 ng/rat), a selective NK3 receptor agonist, produced a dose-related inhibition of colonic propulsion in the rat. Senktide had a weaker, but evident action, whereas NKB, the classical mammalian selective NK3 agonist was inactive, up to 10 μg/rat (Broccardo et al., 1999). The interpretation of this surprising result is obscure. It is tempting to suggest the existence of different NK3 receptor subtypes.

Food intake. Intracerebroventricular injections of eledoisin and physalaemin (100–1000 pmol) did not reduce intake of milk or solid food by rats. Some inhibition of milk intake, observed at 100 ng doses, was accompanied by increased grooming and locomotion (Massi et al., 1986).

Thermoregulation. At doses up to 10 nmol, intracerebroventricular administration of tachykinins (kassinin, eledoisin, physalaemin, and SP) had no effect on the temperature of rats kept at room temperature (Broccardo and Improta, 1988).

Sexual behavior. In ovariectomized, estrogen-treated female rats, bilateral injection of SP (50–1000 pmol) into the midbrain gray matter produced a rapid, long-lasting (3-h) increase in lordosis score, similar to that produced by luteinizing hormone-releasing hormone (Dornan et al., 1987). Similarly, injections of SP (10–200 pmol) into the medial-preoptic-anterior-hypothalamic area in rats significantly shortened the interval to initiate copulation and reduced ejaculation latency (Dornan and Malsbury, 1989).

Drinking behavior. The effects of tachykinins on all aspects of drinking behavior were 1000 times less intense by peripheral than by central administration.

Intracerebroventricular pulse administration of eledoisin (threshold, 10 pmol/rat) potently inhibited water intake evoked by intracerebroventricular angiotensin II (100 pmol/rat), water deprivation, and cell dehydration. SP and physalaemin were far less potent; kassinin and NKA caused only a long-lasting inhibition of drinking due to cell dehydration. Brain areas sensitive to the antidipsogenic effect of eledoisin versus angiotensin II-induced drinking are the nucleus preopticus medialis, the nucleus anterior hypothalami, and the subfornical organ (Massi et al., 1988, 1990).

Eledoisin, kassinin, and, to a lesser extent, physalaemin caused release of vasopressin, with ensuing antidiuresis. SP was ineffective. Vasopressin release (particularly evident upon injection of the peptide into the hypothalamic paraventricular nucleus) seems to be mediated by central angiotensin, once NK3 receptors are activated (Polidori et al., 1989; Massi et al., 1991).

Kassinin, eledoisin, and, to a lesser extent, NKA (100 nmol/rat, intracerebroventricularly) displayed a potent and long-lasting inhibitory effect on salt intake. SP, physalaemin, and neurokinin B were far less effective, and thus, did not suggest NK1 receptor involvement, because SP was only poorly effective. The medial region of the amygdala seems to be main site of action of the tachykinins for inhibition of salt intake (Massi and Epstein, 1989; Massi et al., 1990).

The effects of the tachykinins on drinking behavior in other mammalian species (rabbit and sheep) were considerably less intense and less constant. However, in cats, intracerebroventricular injections of eledoisin (100 pmol) caused a remarkable (60%) and long-lasting (over 60 min) inhibition of angiotensin-induced drinking. Eledoisin was at least four times more potent than SP. Kassinin appeared virtually inactive (Barocelli et al., 1988).

In sharp contrast to the rat, tachykinins displayed a potent dipsogenic effect in the pigeon and, less evident, in the duck (De Caro et al., 1978, 1980). Physalaemin stimulated water intake even at intracerebroventricular doses as low as 10 pmol/pigeon. At the highest tested doses (1 nmol), the animal drank more water within a few minutes than would normally be consumed during a period of 16 to 24 h. The dipsogenic potency of physalaemin was 10 times less than that of angiotensin II, similar to that of eledoisin and kassinin, but 10 times greater than that displayed by NKA, and 100 times greater than that possessed by NKB. This order of potency does not seem consistent with the tachykinin re-

ceptor subtypes so far proposed. The dipsogenic effect of the tachykinins cannot be attributed to activation of angiotensin II receptors, because drinking was not reduced by administration of angiotensin II antagonists (De Caro et al., 1978, 1988; Massi et al., 1987).

The selective agonists of NK3 receptors (NKB, senktide, and PG-KII) potently inhibited ethanol intake in genetically alcohol-preferring rats; at intracerebroventricular doses from 10 pmol/rat PG-KII was 3 times more potent than senktide. At doses of 100 pmol/rat, only alcohol intake was inhibited in food-deprived rats, not food intake or prandial drinking, indicating that the effect on alcohol intake was behaviorally selective. PG-KII, a NK3 receptor tachykinin agonist, inhibited angiotensin II-induced drinking only at doses of 300 to 1000 pmol, producing also evident competitive behavior, locomotion, and inhibition of digestive behavior (Ciccocioppo et al., 1997).

Micturition reflex. Intracerebroventricular injection of SP (30 nmol) or capsaicin (25 μg) elicited the micturition reflex in the rat, probably by acting directly on the brain micturitian centers (Dib et al., 1998).

Stereotyped and motor behavior. At intracerebroventricular doses of 0.6 nmol, SP(1–7) inhibited not only nociception but also aggressive and grooming behavior, while stimulating, like SP, investigative motor behavior. The C-terminal peptide fragment [pGlu^6] SP(7–11) exerted opposite effects (Hall and Stewart, 1984). In producing a rigorous reciprocal hindlimb scratching accompanied by extensive grooming behavior, there was an impressive (approximately 1000 times) difference in responsiveness to intracerebroventricular injection of SP as a function of the genetic strain and age of mice, the old animals (4–5 months) being less sensitive than the young (1–2 months) animals (Hall et al., 1985).

Moreover, whereas the intracerebroventricular injection of all tested tachykinins (SP, physalaemin, NKA, eledoisin, and kassinin) produced in mice an enhancement of grooming and scratching behavior and a reduction of sniffing behavior, only SP increased hindlimb rearing behavior. This effect, unique to SP, was shared by the N-terminal metabolic fragment SP(1–7) and, very surprisingly, also by SP(1–6) (Hall et al., 1987).

The intracerebroventricular, but not intravenous, injection in gerbils of the two SP-like, NK1 receptor agonists, [Sar^9,Met$O_2{}^{11}$]SP or DAla-[Pro^9,Leu^{10}]SP, and GR 73632, elicited in gerbils a characteristic repetitive hind paw tapping, which was not associated with an increase in locomotor activity and seemed to be involuntary. Peak response occurred within 5 to 10 min. GR 73632 was 70 times more potent than [Sar^9,Met$O_2{}^{11}$]SP (ED_{50} 0.7 nmol/gerbil), but the response induced by GR 73632 was less intense. Responses were significantly and dose dependently antagonized only by CNS penetrating NK1 receptor antagonists (Bristow and Young, 1994; Rupniak and Williams, 1994).

At intracerebroventricular doses of 100 to 400 pmol, the NK1 agonist GR 73632 significantly increased also in the guinea pig the locomotor activity. The effect was abolished by NK1 receptor antagonists and by haloperidol (Mason et al., 1992).

Results obtained by subcutaneous or intraperitoneal injection of SP (5 nmol) in mice were at variance. In fact, the peptide decreased spontaneous locomotor activity and counteracted amphetamine-induced hyperactivity. Spontaneous exploratory behavior was also lowered. It is possible that brain monoamines are implicated in these effects, with acceleration of dopamine turnover and retardation of serotonin turnover. Smaller doses of SP showed an antinociceptive morphine-like action in the hot plate test. These results are consistent with SP having a tranquilizing action in mice (Starr et al., 1978).

Aggressive behavior. The intracerebroventricular injection of 0.6 nmol/kg SP or of the N-terminal fragment SP(1–7) reduced fighting in mice made aggressive by prolonged isolation. This effect was enhanced by naloxone. In contrast, the shorter C-terminal analog of SP, [pGlu^6]SP(7–11) increased the isolation-induced fighting, an effect that was antagonized by naloxone, demonstrating that the various peptide fragments of the SP molecule can exert opposite effects on a specific behavior and that the different effects of naloxone may be modulated by specific mechanisms (Hall and Stewart, 1984; Hall et al., 1987).

Learning and memory. SP administered subcutaneously influenced dose-dependently passive and active avoidance conditioning in mice. The retention of a single trial passive avoidance task was enhanced by 0.75 pmol/g. Higher or lower doses were less active or ineffective. SP did not alter the rate at which the mice learned an active avoidance task but increased the extinction of learning (Schlesinger et al., 1983). Similarly, in an appetite motivated learning task, mice injected subcutaneously with 0.75 pmol/g SP retained the task better than control animals, suggesting that SP-treated animals better remembered the original task (Schlesinger et al., 1986). These results were confirmed by Hasenohrl et al. (1990), who found that enhancement of inhibitory avoidance learning produced in rats by SP (40 nmol/kg) was reproduced by the N-terminal fragment SP(1–7), but not by the C-terminal fragment [pGlu^6]SP(6–11). Higher or lower doses of SP had no effect, demonstrating that the facilitating effect of the peptide was reflected by an U-shape dose-response function.

Rats given diazepam 20 min before the training on an inhibitory avoidance task showed an impaired retention. The amnesic effect of diazepam was blocked by 50 μg/kg SP and 167 μg/kg SP(1–7) but not by 134 μg/kg SP(6–11). Thus, the amino acid sequence responsible for this effect may be encoded by the N-terminal fragment of SP (Costa and Tomaz, 1998).

Psychological stress: anxiety. The intracerebroventricular infusion of the NK1 agonist GR 73632 (0.1 nmol) in guinea pigs causes not only motor activation but also pronounced and long-lasting audible vocalization, markedly attenuated not only by the antidepressant drugs, but also by the NK1 receptor antagonist L 733.061. Similarly, the CNS-penetrant NK1 receptor antagonists, like the antidepressant and anxiolytic drugs, were able to inhibit vocalization evoked in guinea pig pups by transient maternal separation.

It is concluded that the selective pharmacological blockade of SP receptors is capable of inhibiting behavioral responses to psychological stress in a manner resembling the effect of clinically used psychotherapeutic agents (Kramer et al., 1998). However, no influence on anxiety (upon field assay) was seen in mice with disrupted gene of NK1 receptor (De Felipe et al., 1998).

Abstinence reaction during opioid withdrawal. It has been found that SP may modulate the abstinence reaction to opioid withdrawal. In fact the N-terminal fragment of SP, SP(1–7), may inhibit the intensity of the withdrawal reaction in morphine-dependent rats. Moreover, significant increases in concentration of SP(1–7) were observed in different brain areas during morphine withdrawal, indicating the involvement of the SP system during opioid withdrawal (Zhou et al., 1998).

Action on discrete selected brain areas. Cat subfornical organ. Neither physalaemin nor eledoisin produced activation of neurons in the cat subfornical organ upon direct application onto its surface (Felix, 1967).

Rat cerebral cortex. Iontophoretic application of SP, physalaemin, and eledoisin in the cerebral cortex of rats excited 91% of the spontaneously active neurons tested, including b-cells. Of the tachykinins, 0.8 μM SP was the most potent, immediately followed by physalaemin and, at distance, by eledoisin. No excitation was induced in nonspontaneously active neurons (Phillis and Limacher, 1974).

Rat nigro-striatal system. The bilateral infusion of SP (2.25 nmol) into the substantia nigra produced a strong increase in stereotyped rearing and sniffing, with no concurrent enhancement of locomotion. After successive infusions, rearing response disappeared. In caudate-lesioned rats, behavioral stimulation by SP was blocked, suggesting that the response to SP is mediated through the nigro-striatal dopamine system (Kelley and Iversen, 1979).

In accordance with these results Tan and Tsou (1988) found that intranigral injection of kassinin, eledoisin, and SP (0.5 nmol) produced a marked dose-dependent increase of 3,4-dihydroxyphenylacetic acid and dopamine concentration in the ipsilateral striatum and a number of controlateral circlings. The rank order of activity was kassinin > eledoisin > SP.

SP(1–9) and SP(6–11) at 0.1 to 1 nM concentrations induced an increase in dopamine outflow from rat striatal slices. The effect of SP(6–11) was blocked by an NK1 antagonist, whereas the effect of SP(1–9) was unaffected. The coincubation of the above fragments with intact SP revealed a negative interaction between fragments and substance P (Khan et al., 1998).

In fresh striatal slices, (^{3}H)SP bound specifically to one site from which it was displaced by SP, but not by SP(1–7) or SP(5–11). In contrast, 10 μM SP(1–7) or SP(5–11) induced, like SP, a significant internalization of the NK1 receptor. It is suggested that SP fragments have high affinity with an NK1 receptor conformance, which is different from that labeled by (^{3}H)SP (Michael-Titus et al., 1999).

Hippocampus and entorhinal cortical cortex. Intrahippocampal administration of 10 pmol of SP triggered self-sustained status epilepticus in response to electrical stimulation of the perforant path for periods too brief (7 min) to have any effect in control rats (requiring a 30-min stimulation). The seizures were accompanied by high-amplitude electrographic paroxysmal activity that lasted many hours. Hippocampal damage resembling that known to occur in human epileptic seizures was blocked by SP receptor antagonists (RP 67.580).

In addition, in the status epilepticus, a rapid, dramatic increase of the expression of preprotachykinin A mRNA, of SP in several brain areas, and of the glutamate release from hippocampal slices has been shown. It is concluded that enhanced expression of SP during self-sustained status epilepticus may modulate hippocampal excitability and play a critical role in the maintenance of status epilepticus (Liu et al., 1999a). In hippocampal dentate gyrus granule cells, SP produces a robust enhancement on *N*-methyl aspartate channel function, prolonging the opening of the channels. Thus, SP provokes an enhancement of the excitatory amino acid-mediated excitability (Lieberman and Mody, 1998).

In experiments in vitro on slices of rat entorhinal cortical cortex, it was found that spontaneous epileptiform discharges evoked by the GABA receptor antagonist bicuculline were reduced in frequency and sometimes in duration by SP. Excitatory synaptic potentials mediated by aspartate were not affected by the peptide (Maubach et al., 1998).

Rat ventral tegumental area. SP and related tachykinins administered either intracerebroventricularly (0.1–20 μg) or directly into the ventral tegumental area of the rat mesencephalon (0.5 nmol) caused increased locomotor activity, grooming behavior and wet-dog shakes. Kassinin, eledoisin, and NKA elicited the greatest locomotor activity and wet-dog shakes, whereas SP and physalaemin were not effective in producing full-body grooming (Elliott and Iversen, 1986). Similarly, mice infused with SP (3 nmol) into the ventral tegumental area exhibited a long-lasting increase in spontaneous activity with rearing and sniffing (Kelley et al., 1979).

Rat nucleus tractus solitari. Microinjection of SP (0.7 pmol) or NKA (0.9 pmol) in the nucleus caused prompt, transient hypotension and bradycardia, suggesting that the tachykinins may act as neurotransmitters of the baroreceptors of the nucleus (Nagashima et al., 1989). The same results were obtained with microinjection of either SP or SP(1–7) (60 pmol), and the effects of SP were blocked when cleavage of SP was inhibited by phosphoramidon, which alone failed to block the depressor and bradycardic effect of SP(1–7), suggesting that only the N-terminal fragment was active and not the intact SP molecule (Hall et al., 1989).

Rat nucleus accumbens. SP and [pGlu^6]SP(7–11) attenuated passive avoidance behavior when at picomolar amounts were injected into the n. accumbens. The N-terminal fragment, SP(1–7) had an opposite effect, facilitating passive avoidance behavior (Gaffori et al., 1984). However, intraaccumbens injection of SP at nanomolar doses had no significant behavioral effects in regard to motility and to conditioned place preference (Schildein et al., 1998).

Rat nucleus basalis magnocellularis. SP (1 pmol) injected into the n. basalis magnocellularis region exerted anxiolytic-like effects in the rat as shown by more time spent on the open arms of the plus-maze test and in social interaction. When administered intraperitoneally, SP had a biphasic dose-dependent effect: anxiolytic action at 40 nmol/kg, and anxiogenic action at 400 nmol/kg (Hasenohrl et al., 1998).

Rat spinal cord. On isolated spinal cord of newborn rats, the tachykinins at 10 to 100 nM concentrations caused depolarization of motoneurones. The rank order of potency of the examined tachykinins was physalaemin > NKB = kassinin = SP > NKA (Matsuto et al., 1984).

Bullfrog spinal cord. SP, physalaemin, and eledoisin exerted a strong excitatory action on motoneurones of isolated bullfrog spinal cord. On a molar basis, SP was about 200 times, physalaemin 1500 times, and eledoisin 2000 times more active than L-glutamate in depolarizing the motoneurones. Because the depolarizing action of substance P and related peptides was blocked by Ca^{2+} deficiency by tetrodotoxin, it is likely that the peptides have a direct action on the motoneurones. Moreover, Konishi and Otsuka (1974) suggested SP to be an excitatory transmitter of primary sensory neurons.

Molluscan ganglia. Direct application of physalaemin on isolated esophageal ganglia of the mollusc *Achatina fulica* produced excitation of an identified tonically autoactive, giant neuron (TAN). By bath application of physalaemin (200 μg/ml), the frequency of the TAN′s spike discharge increased more than twice. Micro-drop application (3.5 ng) resulted in the biopotential of TAN showing a slight hyperpolarization followed by a marked depolarization. The excitatory effect of physalaemin clearly was predominant. No other peptides examined, including SP and eledoisin, had any effect (Takeuchi et al., 1976). After trypsin or chymotrypsin treatment, physalaemin lost its effect, and a clear inhibitory effect emerged on the same TAN. Among the peptide fragments obtained by chymotryptic digestion, the tripeptide Lys-Phe-Tyr- appeared to be responsible for the strong inhibitory effect on TAN. The critical concentration of the tripeptide by bath application was 6 to 20 μg/ml, whereas by micro-drop, amounts as low as 0.3–0.5 ng caused a marked inhibition of the TAN biopotential (Takeuchi and Sakai, 1977; Takeuchi et al., 1977a, 1977b).

From the pharmacological data here presented and from other experiments on mutant mice, however, it seems clear that the tachykinins are not essential for life or for most of the above functions and expressions of the CNS activity. Mutant mice had no gross physical abnormalities, were similar in size and weight to wild mice, appeared healthy over a period of at least 6 months, and were fertile with normal litter size and maternal behavior. They also appeared normal on a rotating rod and in open field activity (Cao et al., 1998; De Felipe et al., 1998; Zimmer et al., 1998). Tachykinins are probably a very important link, but only one link, of the extremely complex chain of events underlying the chemical conveyance of information within the CNS. The part played by the tachykinins in the array of transmitters/modulator molecules occurring in the CNS is not yet completely understood. Thus, as pharmacological and physiological data on the role of tachykinins in the CNS are derived essentially from experiments carried out in mice and rats, it is even harder to conceive that they are transferable, sic et simpliciter, to higher mammals and to man.

Moreover, the SP(1–7) fragment seems to be involved not only in analgesia but also in aggressive and grooming behavior (Hall and Stewart, 1984; Hall et al., 1987), aggressive behavior by prolonged isolation (Hall and Stewart, 1984), hindlimb rearing behavior (Hall et al., 1987), learning and memory (Hasenohrl et al., 1998), central elicited hypotension and bradycardia (Hall et al., 1989), diazepam amnesic effect (Costa and Tomaz, 1998), and the abstinence reaction to morphine withdrawal in morphine dependent rats (Zhou et al., 1998).

SP(1–7) may be an endogenous modulator of SP actions in the brain (Herrera-Marschitz et al., 1990). The intranigrally injected fragment acted as a very potent antagonist against responses induced by intranigral injection of SP (dopamine release in the striatum with consequent behavioral effects) and also of physalaemin (Sakurada et al., 1990b). Thus, it seems demonstrated, beyond any doubt, that SP(1–7) may act in the CNS as neurotransmitter/neuromodulator.

All of these findings raise the following questions, with the possibility that the entire problem of the function of tachykinins in the CNS should be revised:

1) To what extent are the central actions of SP attributable to the intact SP molecule and to what extent does

SP require enzymatic cleavage with formation of its metabolite SP(1–7) to become active? SP acts certainly as intact molecule a) in the case that its central effects are reproducible also by other selective NK1 agonists, such for example physalaemin; b) in the case that its action is inhibited by selective NK1 receptor antagonists; and c) in the case that its action is potentiated by enzyme inhibitors, blocking fragmentation of SP. The observation that phosphoramidon (2–2000 pmol) (an endopeptidase inhibitor) and, to a lesser extent, bestatin (an aminopeptidase inhibitor) remarkably increased and prolonged the behavioral responses (scratching, biting, and licking) induced in mice by SP suggests the importance of endopeptidases in terminating the effects of the SP intact molecule (Sakurada et al., 1990a, 1990b). On the contrary, it is probable that only SP(1–7) fragment is active a) when its action is not reproducible by neither SP or other tachykinin agonists; b) when actions of SP(1–7) are not blocked by NK1 antagonists; and c) when the effect of SP is blocked by phosphoramidon, which inhibits formation of SP(1–7). For example, hypotension and bradycardia elicited by injection of SP in the rat nucleus tractus solitarius are blocked by the endopeptidase inhibitors (Hall et al., 1989).

2) Lack of binding of SP(1–7) to any of three classical tachykinin receptors, presupposes the existence of other selective binding sites. Data that, however, need confirmation, have demonstrated the existence of two populations of binding sites in the mouse spinal cord capable of binding reversibly (^{3}H)SP(1–7) (Igwe et al., 1990). Specific agonists for NK1, NK2, and NK3 receptors did not compete at the binding of SP(1–7). These results support the existence of an N-terminal directed SP-receptor. The fact that DAMGO, a μ-opioid agonist, was active in displacing the ligand SP(1–7) is surprising.

The existence of different binding sites for the C-terminal fragment and the N-terminal fragment of SP was indirectly demonstrated also by Khan et al. (1998) who found that the increased dopamine outflow from rat striatal slices produced by SP(6–11) was blocked by a NK1 antagonist, whereas the outflow elicited by SP(1–9) was unaffected.

3) At present, it is not clear which of the amino acid residue(s) in the SP sequence are responsible for the central effects of the heptapeptide. Generally experiments have been carried out with SP(1–7) and SP(1–8), suggesting that the Phe^7 residue plays an important role, but in some experiments, SP(1–6) also proved to be active. SP-like peptides, sharing as many as five to six amino acid residues with the N-terminal heptapetide of SP, are found in reptile brain and intestine (bufokinin) and in fish brain (trout SP and cod SP). It would be of general interest to check whether these N-terminal fragments (1–7) are active in the CNS of the pertinent species and of mammals.

4) Transgenic mice with the disrupted the gene encoding the NK1 receptor (De Felipe et al., 1998) could be an excellent material for investigating if SP (1–7) still displays its central effects and, in this case, to demonstrate unequivocally, the existence of receptors activated only by the N-terminal fragment of SP.

G. Pain

Much evidence has accumulated to suggest that SP is synthesized in the periphery by small-diameter sensory "pain fibers" and then, upon intense peripheral stimulation released into the dorsal horn, as a first step, through activation of NK1 receptors of transmission of pain information into the CNS. As a consequence, there is central hyperexcitability and increased sensitivity to pain. However, SP is also largely present, together with its NK1 receptor, in several brain areas. It is beyond question that brain SP contributes to pain perception and elaboration. But how, and to what extent? To answer this fundamental question it seems opportune to discuss separately the problem of SP and pain in the periphery (until the dorsal horns) and in the brain.

Periphery. The first association of SP with pain was made by Lembeck and Holzer (1979), who suggested that SP, together with other neuropeptides, may be released from the peripheral sensory nerve fibers in the skin, muscle, and joints. This release was thought to be involved in "neurogenic inflammation", a local painful inflammatory response to certain types of injury or infection, such as that caused by the classical irritant capsaicin.

By intraperitoneal administration, SP (0.8–3.2 nmol/mouse) displayed either no analgesic effect (Growcott and Shaw, 1979) or predominantly a clear antinociceptive action. SP antinociception was found at 10 to 20 pmol in the mouse (Stewart et al., 1976), at 0.8 to 3.2 nmol in the mouse (Starr et al., 1978), and at 0.2 to 0.8 nmol/kg in the rat (Mohrland and Gebhart, 1979).

SP injected into the lumbar subarachnoidal space of rats depressed the tail-flick response in a dose-dependent manner (ED_{50} 1.2 nmol/rat). Maximum effect was reached after 20 min and lasted 30 min. The antinociceptice effect of SP was abolished by naloxone (Doi and Jurna, 1981).

At a dose of 7.5 nmol, SP depressed the motor response evoked by supramaximal stimulation of the sural nerve and also reduced the activity of part of the ascending neurons of the spinal cord evoked by stimulation of C-afferent fibers. The depressive effect in ascending nociceptive activity was slow in onset, lasted longer than 60 min, and was abolished by naloxone (Doi and Jurna, 1982). In the superfused spinal cord of rats and cats, iontophoretic application of SP produced a long-lasting excitation of the dorsal horn neurons similar to that elicited by noxious cutaneous stimuli; SP was released from dorsal horns after stimulation of sensory neurons by capsaicin, and this release was completely inhibited by morphine (Yaksh et al., 1980).

Intrathecal injection of SP or NKA (10–100 pmol), which in the mouse caused a dose-dependent reciprocal hindlimb scratching, licking, and biting response directed to the caudal part of the body, also decreased latency in the tail-flick assay but did not alter reaction in the hot plate test. These effects are interpreted as indicative of a nociceptive behavior (Hylden and Wilcox, 1981; Seybold et al., 1982; Gamse and Saria, 1986).

SP, physalaemin, and eledoisin intrathecally injected in rat have been reported to cause hyperalgesia in the tail-flick test. Hyperalgesia produced by the tachykinins was dose-dependent, was maximal 10 to 20 min after injection, and lasted 30 min. The rank order of potency was: physalaemin > SP > eledoisin. Desensitation to the effects of the peptides was observed after three successive injections of the peptide (Moochhala and Sawynok, 1984).

After microdyalisis of SP or NKA into the dorsal horn of anesthetized monkeys, it was observed that neither peptide had significant effects on the background activity or the response to mechanical or thermal stimulation of the skin. However, each peptide produced significant increases in the response to simultaneous or subsequent iontophoretic application of excitatory amino acids (glutamic acid). Thus, it seems that tachykinins facilitate responses of dorsal horn neurons to excitatory amino acids or to cutaneous stimuli (Dougherty et al., 1995).

Similarly, the progressive hypersensitivity of spinal flexor motoneurons induced by repeated peripheral stimulation of inflamed tissues in decerebrated rats was attenuated by the subcutaneous injection of the NK1 antagonist RP 67580, indicating that SP is involved in mediating progressive hypersensitivity during inflammation (Ma and Woolf, 1997).

Recent, decisive demonstration of the important role of SP in nociception has been afforded by experiments with nociceptin and by experiments on mutant mice in which either the preprotachykinin A gene or the gene encoding the NK1 receptor was disrupted. Inoue et al. (1998) demonstrated that the nociceptin/orphanin FQ-induced nociceptive response is brought about in mice by SP release from peripheral nerve endings of nociceptive primary afferent neurons. After intraplantar injection into the hindlimb of mice of nociceptin (EC_{50} = 0.31 fmol), there was a 25 to 70% increase in the flexor-reflex response, which was abolished by pretreatment of mice with an NK1 tachykinin receptor antagonist or with the SP-depleting agent capsaicin, but not by pretreatment with NK2 antagonists. Similarly, nociceptin was completely ineffective in mice with targeted disruption of the NK1 receptor gene.

It has been demonstrated that the knockout mice, which presented a disruption of the gene encoding the NK1 receptor (with consequent blockade of the activity of SP but not of NKA or SP(1–7) (De Felipe et al., 1998), and the mutant mice, which presented a disruption of the preprotachykinin A gene (with consequent lack of expression of SP, SP(1–7), and NKA) (Cao et al., 1998; Zimmer et al., 1998), did not show any changes to acute pain threshold in mechanical, electrical, chemical, or thermal nociceptive tests, but their responses were blunted in tests that involved more intense noxious stimuli. The importance of SP/NKA seems to apply only to a certain "window" of pain intensity, and when the intensity of the pain stimulation was further increased, the response of the knockout mice did not differ from those of wild mice. The fact that behaviorally acute nociceptive threshold (tail-flick and hot plate assays) were not affected by gene disruption would imply lack of any activation of NK1 receptor and of any involvement of SP in the above assays.

In contrast, when sensory nerves are subjected to an intense period of noxious stimulation, normal animals show a "wind up phenomenon", i.e., an amplification and intensity coding of nociceptive reflexes, which indicate a sensitization of the CNS mechanisms by intense stimulation. The "wind up" was completely absent in the NK1 receptor lacking mice. Thus, SP seems to play an unexpected role for full development of stress-induced analgesia and also for the aggressive response to territorial challenge. Mutant mice did not present any change in anxiety tests. However, the fact that aggression but not anxiety (open field assay) was blunted in mutant mice would again indicate that NK1 receptors and SP are not involved in anxiety even if, in another anxiety assay (vocalization in guinea pig pups by transient maternal separation), the NK1 receptor agonists increased vocalization and the NK1 antagonists remarkably attenuated this effect (Kramer et al., 1998).

In agreement with the above data, Zimmer et al. (1998) observed that knockout mice displayed no significant pain responses after formalin injection, but have an increased pain threshold in the hot plate test. In addition, the mutant mice reacted normally in the tail-flick test assay and acetic acid-induced writhing test.

The conclusion is that mutant mice develop hypoalgesia in some assays, but not in others, probably depending on the apparent levels (spinal or supraspinal) in which the involved pain mechanisms are situated. We further suggest that it is possible that enkephalins and SP modulate nociceptive inputs antagonistically and determine whether a nociceptive stimulus is experienced as pain.

Brain. Results obtained by intracerebroventricular injection of SP and other tachykinins, on pain sensation are very complex, conflicting, and open to unexpected speculations.

The intracerebroventricular injection of 2 to 2000 pmol of SP did not affect the hot plate test in mice (Hayes and Tyres, 1979). Similarly, intrathecal SP (10–10000 pmol) in rats did not significantly affect pain threshold in various analgesic tests (paw pressure, tail immersion, and hot plate test). Moreover, Malthe-Sphirenssen et al. (1978) found that neither intracerebroventricular injection nor injection into the periaqueductal gray of high doses of SP (30 nmol) induced analgesia in

rats. Frederickson et al. (1978), in turn, showed that SP produced analgesia in mice when administered in very small doses (1.25–5 pmol/mouse) by intraventricular route. The analgesic effect was blocked by naloxone. At doses greater than 50 pmol, this effect was lost and hyperalgesia appeared when these doses were combined with naloxone, analgesia when combined with baclofen. Thus, SP may have a dual action in brain, releasing endorphin at very low doses and directly exciting neuronal activity in nociceptive pathways at higher doses.

All of these results are, however, in conflict with a considerable amount of observations demonstrating that intracerebroventricular SP or SP injected into discrete brain areas is predominantly an analgesic, pain-blunting substance.

Malick and Goldstein (1978) found that, after injection into the periaqueductal gray, SP (EC_{50} = 0.7 nmol/rat) displayed a long-lasting (30–60 min) analgesic effect in the tail-flick test. SP was approximately 25 times more potent than morphine, and its effect was significantly antagonized by naloxone. Similarly, low doses of SP (10 pmol) applied to the subarachnoid space of the rat potentiated morphine analgesia (0.1 to 0.5 μg) in the rat tail-flick test, either by facilitating release of endogenous opioids or by modulating opioid receptors.

Stewart et al. (1976, 1982) also demonstrated that centrally administered SP displayed a clear-cut analgesic action. A first important observation was that SP antinociception appeared after a lag of approximately 30 min, even after intracerebroventricular injection, suggesting that the peptide may first require, to become active, an enzymic cleavage at the Phe^7-Phe^8 bond, leading to release of the N-terminal fragment SP(1–7) (Hall et al., 1989). This fragment displayed a clear-cut antinociceptive action in the hot plate test, either by intracerebroventricular injection (5 pmol/mouse) or intraperitoneal injection (15–20 pmol/mouse). SP(1–6) and SP(1–4) did not show any significant analgesic effect. Prior treatment with naloxone abolished the effect of SP(1–7). In comparison with intact SP, the SP(1–7) fragment displayed its antinociceptive effect only within a narrow dose range and as expected, had a shorter lag in onset and a shorter duration of action.

Recently, the effects of two amphibian tachykinins, the NK1 receptor agonist PG-SPI and the NK3 receptor agonist PG-KII, and the mammalian tachykinins SP, NKA, and NKB on the reaction time to a painful radiant heat stimulus (tail-flick test in rats) after intracerebroventricular injection were investigated and compared (Improta and Broccardo, 2000). PG-SPI and PG-KII (1, 5 and 10 μg) and SP (10 μg) significantly increased the reaction time, whereas NKA and NKB did not. Like analgesia evoked by exogenous SP, PG-SPI-evoked analgesia was blocked by pretreatment with naloxone. Naloxone left PG-KII antinociception unchanged, but the NK3 receptor selective antagonist markedly reduced it. All of these findings suggest NK1 and NK3 tachykinin receptor system involvement in supraspinal analgesia in rats.

We have discussed in some details this topic on pain because of its great interest in pharmacology, pathology, and therapeutics even if the question "SP equals pain substance?" by Iversen (1998) is still open and has no definite answer, depending on pain intensity (windows!), nature of pain, and methods used to assess response to painful stimuli.

There is evidence that SP plays a role in transmission of pain sensation and its elaboration in the CNS. Evidence is more convincing in the periphery, from sensory nerve endings to the dorsal horns of the spinal cord (SP = pain substance), less so in the CNS, because of a large number of conflicting results and on the still not clear involvement of SP(1–7).

At any rate, in pain control, there is certainly a close interplay between opioid peptides and SP with the concomitant participation of excitatory and inhibitory amino acids, monoamines and other neuropeptides as well. In human beings, the problem of pain is further complicated by its heavy emotional component. The involvement of SP in defense against stress conditions (anxiety and aggression) is highly probable, but again the importance of this involvement remains to be established. It seems that the monoaminergic system plays here a predominant role and that SP or SP(1–7), like other neuropeptides, displays a modulating effect. Again, results obtained in rats and mice are transferable to human beings with caution.

H. Neurogenic Inflammation

Electrical, mechanical, and chemical stimulation of the C-fibers in sensory neurons causes an axon reflex taking place in the branchings of sensory nerves. The consequence is the neurogenic inflammation: pain, vasodilation (flare), and plasma extravasation.

Antidromic vasodilation is mediated by a neurotransmitter at the sensory nerve endings in the skin. Similarly, plasma extravasation elicited by antidromic stimulation also seemed to be provoked by a mediator released from pain sensitive nerve terminals (Jancso et al., 1967).

Among the many transmitters suggested in this connection were acetylcholine, noradrenaline, ATP, bradykinin, histamine, 5-HT, and prostaglandins. At the present time, SP fulfills the criteria for being accepted as the main mediator for all components of antidromic stimulation (Lembeck and Holzer, 1979; Pernow, 1985).

i. SP is present in the C-fibers of the sensory neurons and is released from these fibers during antidromic stimulation.
ii. Close arterial administration of SP causes vasodilation and plasma extravasation, thus, mimicking the effect of antidromic stimulation.

iii. Capsaicin, which depletes SP in sensory neurons, almost completely blocks vasodilation and neurogenic plasma extravasation.

The above criteria were completed and remarkably strengthened by more recent data:

iv. The nociceptin/orphanin-induced nociceptive response is brought about in mice by SP release from peripheral endings of nociceptive primary afferent neurons (Inoue et al., 1998), supporting the view that also pain in neurogenic inflammation is due to release of SP.

v. In mutant mice with disrupted preprotachykinin A gene, neurogenic inflammation produced by topical application of capsaicin was almost absent, whereas in non-neurogenic paw edema produced by complete Freund's adjuvant neurogenic inflammation was the same in wild-type and mutant mice (Cao et al., 1998). However, there is some doubt about the fact that SP is the unique direct or indirect (through release of histamine from the mast cells) agent responsible for the vasodilation and plasma extravasation seen in neurogenic inflammation. Two points deserve attention. The first is that SP is costored and coreleased from sensory nerve endings with calcitonin gene-related peptide, which displays a potent edema producing activity; the second is that the histamine-releasing activity of SP, which remarkably contributes to plasma extravasation and edema, has been attributed not to the intact SP molecule but to its N-terminal fragment (1–7). Moreover there are data, which need confirmation, showing that antidromic stimulation may not always release SP but other active agents.

In summing up, there is little doubt that neurogenic inflammation represents the most striking and credible example of a decisive, if not unique, involvement of SP in a physiopathological process.

I. Miscellaneous Pharmacological Actions

1. Lachrymal Secretion. Physalaemin given intravenously and at threshold doses of 0.03 to 0.3 nmol/kg was a potent stimulant of lachrymal secretion in the dog. At a dose of 1 nmol/kg, which caused an intense drop of blood pressure, the increase in secretion was 400%.

Like those of the dog, the lachrymal glands of the gerbil seem to be very sensitive to SP. In fact, in this animal, the non-natural NK1 agonist, [Sar^9,$MetO_2^{11}$]SP, induced by intravenous injection of doses over 0.007 nmol/gerbil an immediate dose-dependent chromodachryorrhoea, which was blocked by the NK1 antagonist, CP 99,994. Doses of the agonist 180 times higher were required to elicit the same effect by intracerebroventricular injection, demonstrating that the point of attack of the peptide was on peripheral NK1 receptors. The same results were obtained with intravenous injection (0.3 nmol/gerbil) of another synthetic SP-like peptide, GR 73632 (D-Ala[Pro^9,$MeLeu^{10}$]SP). There was no involvement of the cholinergic system. NKA and NKB receptor agonists were ineffective (Bristow and Young, 1994).

The rat was much less sensitive (intravenous threshold 2.5–5 nmol/kg) and the maximum increase in lachrymal secretion never exceeded 100% (De Caro and Cordella, 1965; Bertaccini et al., 1966).

Lachrymal glands of the rabbit seem to be rather insensitive to tachykinins. In fact, eledoisin, given by close intra-arterial injection, at doses up to 1 nmol, failed to modify fluid and protein secretion (Dartt et al., 1988). In humans, given by eye drops, 10 to 20 nmol of physalaemin provoked a 95% increase in lachrymal secretion, with intense conjunctival hyperaemia and sometimes-moderate chemosis (De Caro and Cordella, 1965). The same amount of eledoisin was ineffective in normal human subjects but showed striking stimulatory effects in patients suffering from hypofunctional lachrymal glands. Lachrymal secretion increased up to 200% and the effect lasted for several hours during which it decreased slowly (Impicciatore et al., 1973).

2. Histamine Release. After perfusion of the rat isolated hindlimb with 10 nmol/min of SP, kassinin, eledoisin, and NKA, it was shown that only SP produced a significant increase in the histamine concentration in the perfusion liquid, from 263 to 750 ng histamine/min. The three other tachykinins were inactive (Erjavec et al., 1981; Holzer-Petsche et al., 1985). Similarly, only SP released histamine from peritoneal mast cells, whereas eledoisin and kassinin were ineffective (Erjavec et al., 1981; Pietrowski et al., 1984). In their ability to release histamine from the rat peritoneal mast cells neurotensin, kallidin and SP were the most potent agonists. Surprisingly an undecapeptide SP antagonist behaved as superagonist.

Only compounds with positive charges at their N-terminals caused a noncytotoxic release of histamine from rat mast cells. It is evident that SP acts by a nonspecific mechanism not related to activation of mast cell NK1 receptor and, hence, not related to the tachykinin nature of SP (Devillier et al., 1985, 1989).

A histamine release by 0.1 to 10 μM SP, with consequent flare, wheal, and itching, has been also demonstrated in human skin, where the peptide was 100 times more potent as histamine liberator than in the rat peritoneal cells. The skin reactions were blocked by antihistaminic drugs (Hagermark et al., 1978). In more detail, in human skin (volar surface of the forearm), 6.25 to 25 pmol of SP produced a dose-dependent flare and wheal response. Only peptides having one or more basic residues at their N-terminal region were effective in producing flare: eledoisin and SP(1–7) were 17 and 7 times less active than SP. Wheal production, on the contrary, was not dependent on basic residues: physalaemin was the most potent agent, SP was one-half as potent, and ele-

doisin was 20 times less potent. Pretreatment with a histamine antagonist reduced both flare and wheal responses; pretreatment with capsaicin reduced the flare but not the wheal response, indicating that in response to the tachykinins, both mast cells and SP-containing primary neurons are involved (Foreman et al., 1983). It has been suggested that the histamine-releasing property of SP derives essentially from cooperation between the basic N-terminal tetrapeptide and the C-terminal heptapeptide with the two successive Phe-residues at position 7 and 8 (Mazurek et al., 1981).

To support the validity of this hypothesis, it would be interesting to check the histamine-releasing activity of carassin(12–21) with three basic residues in the N-terminal tetrapeptide, but with the sequence Phe-Val, instead of Phe-Phe and of bufokinin, with 2 basic and 1 acid residue in the N-terminal pentapeptide and the sequence Phe-Tyr, instead of Phe-Phe.

VI. Tachykinins in Human Diseases and Therapeutics

Research on this topic is rather scant and at early stage of advancement. Pharmaceutical companies are highly interested in diseases possibly attributable, at least in part, to excess or deficiency in tachykinin production and/or release. However, because no tachykinin agonists are hitherto known to possess an appreciable capacity to cross the blood-brain barrier, the focus of interest lies, at present time, on the tachykinin antagonists, which are generally of nonpeptide nature and are often brain-penetrating molecules.

A. Tachykinin Receptor Agonists

In patients suffering from arteriosclerosis obliterans of the legs, the effects of eledoisin (15–35 nmol) injected into the femoral artery have been studied (Szam et al., 1966). The angiorheogram and the pulse volume increased considerably in the majority of patients. Fleeting side effects and slight decrease in blood pressure were also observed. Unfortunately this promising clinical trial was not extended: inconveniences of intraarterial infusion discouraged continuation of experiments. Administration, by eye drops, of eledoisin or physalaemin (10–50 nmol, 1–4 times daily) increased lachrymal secretion and ameliorated the Sjögren syndrome and other forms of keratoconjunctivitis sicca due to deficit of lachrymal secretion (De Caro et al., 1969; Jaeger et al., 1985; Jaeger, 1988). Because of the relative rarity of the disease and the existence of other therapeutical approaches, these "orphan" drugs did not further arouse the interest of the pharmaceutical companies. However, in an organ culture of rabbit cornea, it was observed that SP alone (not NKA, NKB, eledoisin, kassinin, or physalaemin) at any concentration (50 ng/ml-50 μg/ml) did not affect epithelial migration but enhanced the stimulant effect (Nakamura et al., 1997). In the search for the SP fragment responsible for the above effects, it has been found that both SP and its C-terminal tetrapeptide, SP(8–11), acted synergistically with insulin-like growth factor 1 on wound healing of rabbit cornea (Nakamura et al., 1999). There was both stimulations of epithelial migration in vitro and of attachment of corneal epithelial cells to a fibronectin matrix. Moreover, the combination of insulin-like growth factor 1, SP(8–11), and integrins by topical application facilitated wound closure in vivo.

As far as it concerns the neurodegenerative and other CNS disorders, it has been suggested that tachykinins may have both neuroprotective and neurodegenerative properties (Raffa, 1998). Among the degenerative diseases of the CNS, in which a deficit of tachykinins is clearly evident, is Huntington's disease or Chorea. In this autosomal hereditary disorder, a marked decrease in immunoreactive SP fiber density in the regions showing the greatest histopathological destruction, particularly in the substantia nigra, has been reported. Of course, whereas SP has nothing to do with the etiology of Huntington's disease, it could be responsible, at least in part, for the symptoms of this disease: choreiform movements, personality disturbances, and cognitive decrease to dementia.

The intervention of tachykinins in other CNS disorders is more controversial: amylotrophic lateral sclerosis, Parkinson's disease (decrease of SP in substantia nigra), schizophrenia (no significant change in SP content in brain areas), and depression (predominant data showing elevated levels of SP).

The problem of how tachykinins participate in the cerebral aging is also a matter of investigation and debate. Here, only a few studies dealing with the relationship between β-amyloid protein and the tachykinins are reviewed. β-Amyloid is a 39- to 43-amino acid polypeptide that is the primary constituent of senile plaques and cerebrovascular deposits in Alzheimer's disease and Down syndrome. Although the protein has been characterized biochemically, neither its primary biological significance nor its role in the pathogenesis of Alzheimer's disease is completely known. It has been shown that, in cultures of rat embryonic hippocampal cells, the β-amyloid protein is neurotrophic in undifferentiated cells and at low concentrations, but it is neurotoxic in mature neurons and at higher concentrations (Yankner et al., 1990). Neurotrophic and neurotoxic effects of the protein were mediated by its fragment 25 to 35, which shows important homologies to the sequences of SP and other tachykinins. However, the problem on the possible involvement of tachykinins (namely SP) in the pathogenesis of Alzheimer's disease cannot be considered solved. In fact, Zhao et al. (1993) found that amyloid β-protein (1–40) was toxic to NB41A3 neuroblastoma cells in serum-free culture, as judged by decreasing cell number and release of lactic dehydrogenase, and that this toxicity was inhibited by the concurrent treatment of the cells with 1 μM physalaemin. In turn, Kimura and Schubert

(1993) observed that amyloid β-protein (1–40) weakly activated, for itself, the tachykinin receptors, but that in the presence of glutamate, the amyloid β-protein produced an activation of both the tachykinin receptors (especially the NK1 receptors) and the phosphatidylinositol turnover. There is the possibility that an overproduction of amyloid β-protein disturbs normal neuronal transmission by activating the SP receptor in synergy with glutamate or by acting as a SP antagonist by itself. The resulting compromised synapses could lead to the dementia of the Alzheimer's disease.

B. Tachykinin Receptor Antagonists

Based on the knowledge of distribution of tachykinin receptors and pharmacological effects of the tachykinins, it may be hypothesized that receptor antagonists may have several therapeutic applications. With regard to NK1 receptor antagonists, their therapeutical use has been hypothesized in the treatment of pain and emesis and, in the periphery, in the treatment of several inflammatory diseases including arthritis, inflammatory and motor diseases, and cystitis (Quartara and Maggi, 1998).

At present time, the only documented clinical trial with tachykinin antagonists, more precisely with a SP antagonist, is that carried out in the treatment of moderate to severe major depression by a large team of researchers, starting from the observation that, like clinically used antidepressant and anxiolytic drugs, also SP antagonists suppressed isolation-induced vocalization in the guinea pig (Kramer et al., 1998).

In a placebo-controlled trial, it has been found that in patients suffering from depression, the SP antagonist MK-869 displayed an antidepressive effect greater than that displayed by the first choice drug in depression, paroxetin, and side effects, always in comparison with paroxetin, were less intense. The mechanism of action of MK-869 is, at present, not completely understood. This TK receptor antagonist does not interact with monoamine systems (inhibition of re-uptake of serotonin and/or noradrenaline) like the known antidepressant drugs do; thus, it cannot be excluded that MK-869 does not act only through NK1 receptor blockade. Moreover, like the known antidepressive drugs, MK-869 acts only after 2 to 3 weeks, suggesting the possibility that all antidepressant drugs act via an as yet unclear "common pathway" mechanism (Wahlestedt, 1998).

The enormous theoretical and therapeutical interest that SP antagonists may represent well tolerated antidepressant drugs is obvious. However, the successful therapeutical use of tachykinin antagonists in humans requires some precise accomplishments: a) knowledge of the tachykinin receptor types and subtypes occurring in the different human organs and tissues and evidence that the antagonists on trial compete exactly with the wanted binding site. This is because of the heterogeneity of all tachykinin receptor types; b) lack of important toxicity (side effects) even by long-term administration. Antagonists are generally synthetic, nonpeptide, and non-natural molecules; c) lack of appreciable agonistic activity; and d) brain-permeability for antagonists destined to act in the CNS.

VII. General Conclusions

We have previously shown that tachykinins constitute one of the largest families of peptides in all of the world whose members are present in all animal species from lower invertebrates to mammals. There is no nervous system, from the most primitive in coelenterates to the most developed and complex human CNS, that is lacking a tachykininergic system.

What is the functional significance of this spectacular display of tachykinin-secreting fibers and their receptors? It is beyond doubt that neuronal tachykinins play an important role in neurotransmission/neuromodulation both in the CNS and in periphery. This is demonstrated by the overall occurrence of tachykinins in the brain and other nervous structures from the lowest invertebrates to mammals. Although important, the tachykinin peptide family represents only one of the numerous peptide and nonpeptide families involved in neurotransmission and neuromodulation. Members of these families are expressed in a variety of tissues, and very frequently a tachykinin is costored and cosecreted by the nerve endings with other peptides or biogenic amines. Moreover, the tachykinins, like all other neuropeptides, may enter in competition, positive or negative, with a number of active extraneuronal compounds originating in blood (bradykinin and angiotensin) or in compact or diffuse endocrine organs.

Tachykinins, with their variable primary structure seem to be adapted to display, in the better way, their function in the different invertebrate and vertebrate phyla. In all examined species, and especially in mammals (the phylum more thoroughly studied), tachykinins elicit a spectrum of biological activity (both in the CNS and in the periphery), which may vary conspicuously in the different species and even in the various strains of single species, again strongly supporting the concept of a general, important functional significance of these peptides.

Transgenic mice with disrupted preprotachykinin A gene or with disrupted NK1 receptor gene are in good health conditions and fertile. This demonstrates that the tachykinins are not essential for life and health, at least in mice but probably in the other mammalian species as well, and points to the well known great adaptability of living organisms and the plasticity of homeostatic mechanisms. At present, we do not know any pathological syndrome attributable entirely or predominantly to excess or defect of tachykinin production and release. No function of the various organs and systems in health and disease seems to depend entirely on the tachykininergic

system, and tachykinins seem to be only one arm of the complex mechanism that regulates body functions.

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