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REVIEW

Substance P: a pioneer amongst neuropeptides

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Abstract. Hökfelt T, Pernow B, Wahren J (Karolinska Institutet, Stockholm, Sweden). Substance P: a pioneer amongst neuropeptides. *J Intern Med* 2001; **249:** 27–40.

A brief overview of recent developments in the substance P field is provided, in addition to a historical introduction. It is emphasized that there are multiple tachykinins and tachykinin receptors and that there are examples of coexistence of several tachykinin peptides and of several tachykinin receptors in single cells, and there is evidence for tachykininergic cotransmission. The distribution and functional significance of tachykinins in the gastrointestinal tract and in sensory neurones, and interactions with other peptides and transmitters, are reviewed. The recent production of knock-out mice for either substance P or the NK1 receptor is discussed, as well as the exciting concept of substance P receptor internalization. Finally, the development of specific substance P antagonists is summarized, and possible clinical implications discussed, and, in particular, a recent study which reports that a substance P antagonist shows clinical efficacy in depression.

Keywords: antagonists, depression, gut, internalization, neuropeptides, receptors, sensory neurones.

Introduction

The present article is mainly a summary of presentations at a symposium on substance P (SP) at Nobel Forum, Karolinska Institutet in Stockholm, November 1999. In fact, it is mainly based on material supplied by the speakers. Our intention is to present the status of ongoing SP research, and also to provide a perspective on this peptide. We hope that the article reflects at least some of the present excitement in the field. Interestingly, many of the breakthroughs in the neuropeptide field in general have been associated with SP, for example the first cloned neuropeptide receptor and, recently, the first reported clinical efficacy of a neuropeptide antagonist in a major central nervous system (CNS) disease. Also, in the very first study on SP published in 1931, it was reported that this factor was present both in intestine and brain [1], clearly showing that focus was on the nervous system and, in a way, 'foreseeing' the later so much discussed brain–gut concept. The present summary is, by necessity, an incomplete account of the progress in the field, but how should we be able to provide, in one article, a comprehensive presentation of a molecule that was discovered almost 70 years ago?

Early history

In 1929, a 24-year-old pharmacologist from Karolinska Institutet went to Dr Henry Dale's lab at the National Institute for Medical Research in London

for postdoctoral training. His name was Ulf von Euler. At that time, Dale, later Sir Henry, had published papers indicating that acetylcholine might play a role in neurotransmission; for his discoveries in this field he was awarded the Nobel Prize in Physiology or Medicine together with Otto Loewi in 1936. In 1929 this was something completely new, and only few physiologists and hardly any neurophysiologists believed in the hypothesis of chemical neurotransmission. But von Euler did. Sir Henry had at that time also discovered histamine; another reason for von Euler to choose Dale's laboratory for further training.

The topic suggested by Dale to the young Swede was the distribution of acetylcholine in the gastrointestinal tract, to be tested on a piece of rabbit intestine in an organ bath. Von Euler obtained, as expected, a good stimulatory effect of the extracts, but to his astonishment not all activity was abolished when atropine was added to the bath. His tutor did not seem completely convinced, because he asked his senior assistant John Gaddum to help out, and together they started the work which resulted in the first paper on SP, published in the *Journal of Physiology* in 1931. There they described that both brain and intestine contain an atropine-resistant factor, which stimulates smooth muscle and lowers blood pressure [1]. They found that the activity was retained when the extract was evaporated to a dry powder, which they called substance P.

After his return to Stockholm, von Euler became professor of physiology at Karolinska Institutet. He published a few more papers on the new compound, but soon left this area for, as we know, major discoveries of other factors, such as prostaglandin and noradrenaline. When Bengt Pernow came to von Euler's lab as a junior assistant in 1949, von Euler suggested that he should continue the work on SP. This work eventually resulted in a doctoral thesis [2], which included both purification of SP and studies on its distribution and biological actions. Pernow found an uneven distribution of SP in the brain, with much higher levels in grey than in white matter, and by far the highest concentrations were in some areas of the mesencephalon (substantia nigra) and diencephalon, particularly in the hypothalamus. In the spinal cord, the concentrations were higher in the dorsal than in the ventral halves, and SP was also present in peripheral nerves, particularly in autonomic nerves, spinal ganglia and the sympathetic trunk. In the gastrointestinal tract, the muscle layer with its nerve plexuses showed higher concentrations of SP than the other layers. In children with Hirschprung's disease, which is characterized by a total lack of ganglionic cells in the distal, but not proximal, part of the rectosigmoid, the distal part lacked SP almost completely, whilst the proximal part contained normal amounts [3], thus providing further evidence for a neuronal localization of SP. In Austria, in parallel, Fred Lembeck [4], in his elegant and pioneering studies, presented strong evidence that SP is a sensory neurotransmitter.

Identification of substance P: a breakthrough

It was Susan Leeman and her colleagues in Boston who, in the early 1970s, identified SP as an undecapeptide [5] and were the first to synthesize the compound [6] and to set up a radioimmunoassay [7]. Now the effects of SP could be tested in physiological models [8, 9], antibodies could be used to monitor SP with radioimmunoassay [10] and in immunohistochemical studies [11, 12, 13, 14, 15, 16], and release of SP could be demonstrated [17, 18], thus fulfilling several of the criteria for SP being a neurotransmitter [see 19, 20, 21, 22].

The tachykinin family

It is now recognized that SP is not the only member of the so-called tachykinin family [23, 24] (Fig. 1).

Mammalian tachykinins

Substance P

Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-MetNH_2

Neurokinin A

Hys-Lys-Thr-Asp-Ser-Phe-Val-Gly-Leu-MetNH_2

Neurokinin B

Asp-Met-His-Asp-Phe-Phe-Val-Gly-Leu-MetNH_2

Fig. 1 Structure of the three mammalian tachykinins. (Kindly contributed by Dr C. Maggi.)

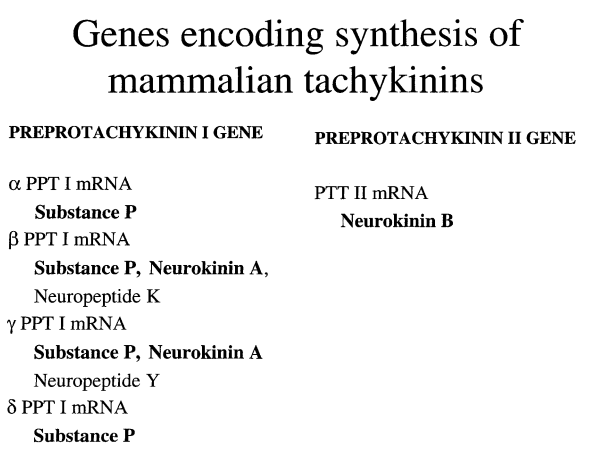

Fig. 2 Genes encoding synthesis of mammalian tachykinins. (Kindly contributed by Dr C. Maggi.)

Two genes exist, the preprotachykinin I (PPTI) gene and the PPTII gene (Fig. 2). The PPTI gene can express four different forms of mRNA through alternative splicing, two of which (the β and γ forms) encode synthesis of both SP and neurokinin A (NKA), whilst the two other, the α and δ forms, encode SP only. The β and γ forms of PPTI mRNA also encode synthesis of neuropeptide K (NPK) and neuropeptide γ (NPγ), which are elongated forms of NKA, although their function has not been fully clarified. It is the PPTII gene that gives rise to neurokinin B (NKB).

Soon after the sequencing of SP, it became evident that nonmammalian species also have peptides with a similar structure to SP. Thus, Erspamer and associates in Italy discovered several peptides with a strong structural homology with SP: physalaemin, eledoisin and kassinin [25].

Substance P receptors

Based on comparative analysis of the pharmacological properties of various tachykinins, it was possible to provide evidence for the existence of three distinct receptors for these messenger molecules [26, 27, 28, 29, 30]. Very soon after, Nakanishi and collaborators [31] were the first to clone an SP-related receptor, the substance K receptor, which was, in fact, the first neuropeptide receptor ever cloned, and thus represented a major breakthrough. Up until then, the presence of neuropeptide receptors was based on binding studies and pharmacological analysis, but now, for the first time, one could envisage the receptor physically. As always, once the gate had been opened, SP and other tachykinin receptors were cloned within a few years [32], as well as many other neuropeptide receptors [33]. Three mammalian tachykinin receptors have now been cloned: the NK1 receptor (Fig. 3) – where SP is the preferred ligand, but where also NKA and NKB can act as ligand (SP > NKA > NKB) – and the NK2 and NK3 receptors. NKA is the preferred ligand for the NK2 receptor (NKA > NKB > SP) and NKB is the preferred ligand for the NK3 receptor (NKB > NKA > SP). Finally, a variant form of the NK3 receptor has been cloned, referred to as NK3B or NK4 receptor.

Substance P antagonists

A crucial step in the analysis of the functional role of messenger molecules is the development of selective and sensitive antagonists. This allows monitoring of the functional role of the endogenous ligand. The first efforts in this direction were made by Karl Folkers, of Austin, TX, who synthesized a number of SP analogues, mainly based on substitution with D-amino acids [34]. These compounds were initially evaluated, principally in Sweden, and found to be useful tools in various experimental paradigms [see 35]. In 1991, Snider *et al.* [36] reported the first nonpeptide NK1 antagonist (CP96 345), and, in a 1997 review, Betancur *et al.* [33] listed more than 20 nonpeptide NK1 antagonists. Since then, many more compounds have been developed, virtually exclusively within the framework of the pharmaceutical industry.

NK1 antagonists and their binding to the receptor

With regard to molecular analysis, the SP NK1 receptor became the first to be characterized in detail, especially by Thue Schwartz's group in Copenhagen, and is now a model for how neurotransmitters, hormones, and receptor-blocking drugs interact. The cloning of the structurally closely related, but pharmacologically distinct, NK2 and NK3 receptors made it possible to design very informative NK1-chimeric receptor constructs by genetic engineering, encompassing segments of

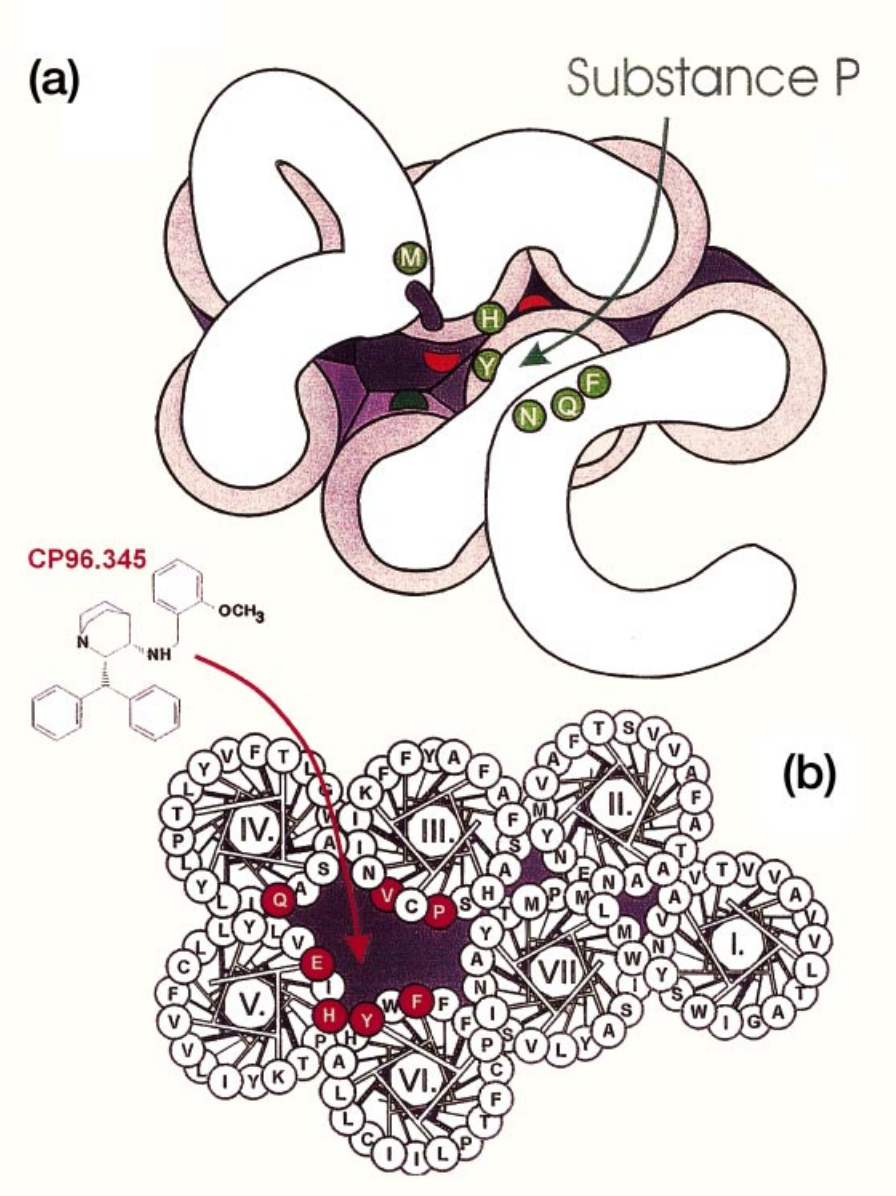

Fig. 3 Differential binding sites for substance P and the nonpeptide antagonist CP-96,345 in the NK1 receptor. The 7-TM receptor (seven transmembrane helical segments) is viewed from outside the cell. In (a), the extracellular loops and the N-terminal extension are indicated in very low resolution and with green circles showing the residues, which by mutagenesis and by cross-linking have been shown to be interaction sites for substance P. The two residues, between which an activating metal-ion sites has been built by genetic engineering and through which Zn(II) can substitute for substance P in activating signal transduction [98], are indicated in orange. Note that this site is located just below the binding site for the larger neuropeptide. In (b), the seven transmembrane segments are shown as helical wheels, and the interaction points for the prototype nonpeptide antagonist CP-96,345 are shown by red circles. Note that the nonpeptide antagonist is binding in a pocket relatively deep between TM-III, -IV, -V, and -VI. There is very little overlap in the binding sites for CP-96,345 and substance P, although they are competitive antagonists. This is because these receptors function as allosteric molecules, where agonists and antagonists stabilize different conformations. (Kindly contributed by Dr T. Schwartz.)

the other tachykinin receptors [37]. This allowed detailed mutational substitutions of multiple residues [38]. With the development of the first nonpeptide NK1 antagonist, CP96 345 (see above), the binding site for such competitive nonpeptide antagonists and SP could be analysed in parallel. It turned out, suprisingly, that the peptide is binding at the extracellular ends of the transmembrane helices and especially in the extracellular loops of the receptor, whereas the small hydrophobic, nonpeptide antagonists are binding more deeply in between the transmembrane segments (Fig. 3) [37, 38]. This principle of interaction for endogenous ligand vs. nonpeptide antagonist later turned out to be a general phenomenon for many peptide hormone and neuropeptide receptors.

Tachykinergic cotransmission

The fact that there are several tachykinin peptides and receptors raises the question, to what extent such peptides and/or the receptors are colocalized and to what extent tachykinin peptides can be coreleased and activate multiple tachykinin recep-

tors. This question has recently been discussed by Maggi [39] and can be summarized as follows. SP and NKA are known to be colocalized and coreleased both from sensory nerves (see below) and enteric neurones, and there is strong evidence for multiple tachykinin receptors on certain target cells. Moreover, NKA is also a good ligand for NK1 receptors. This 'duplication' of the tachykininergic message, together with the expression, at the postjunctional level, of one or more of the three tachykinin receptors, provide a complex situation in terms of tachykininergic cotransmission. Using novel and potent receptor antagonists, it has been possible to precisely analyse the role played by different tachykinin receptors at different neuromuscular junctions in the periphery. Examples have been found in which the message carried by different tachykinin receptors simply summates in producing the final response (e.g. rat urinary bladder), especially if degradation of SP is reduced by the administration of peptidase inhibitors (e.g. guinea-pig bronchus). In other instances (e.g. guinea-pig duodenum), examples of co-operation have been found in which the summation of the message produced by NK1 and NK2 receptors (depolarization of smooth muscle cells) attains the threshold for triggering the final event (firing of action potentials and contraction). Moreover, examples of true specialization have been found (guinea-pig colon). Here the activation of NK1 and NK2 receptors may deliver messages to the same target cells which are distinct both in temporal terms and in terms of effector mechanisms activated to produce the final response. From this analysis, it appears that tachykininergic cotransmission in the peripheral nervous system is quite heterogeneous and that this heterogeneity largely originates from postjunctional factors.

Tachykinins in the gut

The distribution and function of SP in the gastrointestinal tract has been studied extensively over the last 25 years, and was recently summarized by Holzer and Holzer-Petsche [40, 41] (Fig. 4). For a more general overview on the localization and distribution of tachykinins and other neuropeptides in the gastrointestinal tract, see Furness and Costa [42]. The present status of the distribution and role of tachykinin in the gastrointestinal tract can be summarized as follows. For references, the reader is referred to the above mentioned reviews [40, 41].

The preprotachykinin-A gene-derived peptides SP and neurokinin A (NKA) are expressed in distinct neural pathways of the mammalian gut (Fig. 4). When released from intrinsic enteric or extrinsic primary afferent neurones, tachykinins have the potential to influence intestinal effector systems by interaction with the three different types of tachykinin receptors. The NK1, NK2 and NK3 receptors are expressed by enteric neurones, intestinal muscle, epithelium, vasculature and immune system in a cell-specific manner and enable SP and NKA to influence motility, electrolyte and fluid secretion, as well as vascular and immune functions in a peptide- and region-specific fashion.

Most prominent amongst the gastrointestinal effects of tachykinins is their action on motility, and they can not only stimulate but also inhibit motility, the net response depending on the type and site of tachykinin receptors that are activated. Nerve-independent facilitation of gastrointestinal motor activity is brought about by NK1 receptors on interstitial cells of Cajal and NK2 receptors on muscle cells. NK3 receptors are largely confined to enteric neurones and mediate predominantly cholinergic contraction of the intestinal musculature within the enteric nervous system. SP and NKA can also depress motor activity through release of inhibitory transmitters such as nitric oxide (an effect exterted via NK3 receptors and in particular NK1 receptors on inhibitory motor pathways).

Chemical and mechanical stimulation of the mucosa or distension of the muscle excites primary sensory neurones within the myenteric plexus, which release tachykinins and activate ascending excitatory and descending inhibitory motor pathways (via NK3 receptors). In addition, intrinsic sensory neurones form self-reinforcing networks in which they communicate via NK3 and, to some extent, NK1 receptors. Further on, tachykinins acting via NK3 receptors contribute to transmission from ascending interneurones to excitatory motor neurones, whereas transmission to inhibitory motor neurones involves NK1 receptors. In many instances, particularly in the ascending excitation of the circular muscle, tachykinins synergize with acetylcholine in the transmission process.

Tachykinins also participate in the neural control of secretory activity in the intestine. Thus, tachyki-

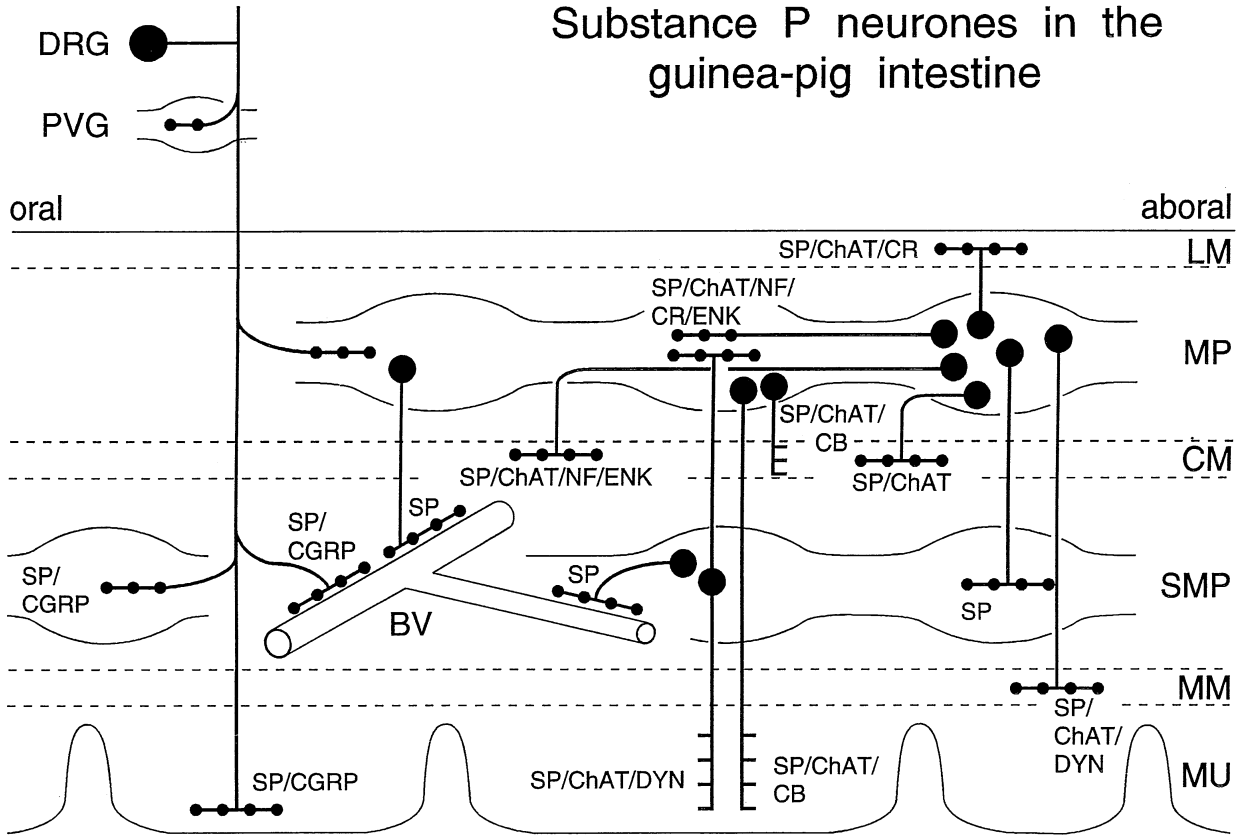

Fig. 4 Schematic summary of the various classes of SP-immunoreactive neurones and their projections within the guinea-pig small intestine, with information on the coexistence with other neuropeptides or neuronal markers. BV, blood vessel; CB, calbindin; CGRP, calcitonin gene-related peptide; ChAT, choline acetyltransferase; CM, circular muscle; CR, calretinin; DRG, dorsal root ganglion; DYN, dynorphin; ENK, enkephalin; LM, longitudinal muscle; MM, muscularis mucosae; MP, myenteric plexus; MU, mucosa; NF, neurofilament protein; PVG, prevertebral ganglion; SMP, submucosal plexus. (From Holzer and Holzer-Petsche [40, > 41], with permission.)

nins can act directly on NK1 or NK2 receptors on enterocytes to stimulate chloride and bicarbonate secretion. In addition, SP and NKA, acting via NK1 and NK3 receptors on enteric neurones, participate in the transmission to secretomotor neurones, which cause ion secretion through release of acetylcholine and/or vasoactive intestinal polypeptide. On the other hand, tachykinins can be released from axon collaterals of intrinsic sensory neurones close to the epithelial effector cells and elicit chloride secretion via an axon reflex type mechanism.

Inflammatory disorders of various aetiologies involve changes in the peptidergic innervation of the gut, and are associated with NK1 receptor upregulation in intestinal blood vessels and lymphoid structures. For example, *Clostridium difficile* toxin A causes extrinsic afferents in the rat and mouse ileum to release SP, which, via NK1 receptors, excites enteric secretomotor neurones and leads to degranulation of mast cells, macrophage and granulocyte activation, hypersecretion, inflammation and necrosis. Thus, in the diseased gut the contribution of tachykininergic neurones seems out of balance and there is a shift away from cholinergic towards tachykininergic regulation. Similarly, in animal experiments, tachykinin receptor antagonists are little active in the normal gut but are able to correct disturbed motility, hypersecretion, tissue homeostasis and pain associated with certain forms of intestinal anaphylaxis, infection and inflammation. Therefore tachykinin receptors are potential therapeutic targets in gastroenterology.

Substance P in sensory neurones

Many lines of evidence suggest that SP plays an

important role in nociception at the spinal level, and some examples are given here. Substance P, synthesized by a subpopulation of small diameter sensory neurones [11], is released in response to peripheral inflammation and noxious stimulation [43–45]. In animals with behavioural hyperalgesia resulting from an experimental polyarthritis, SP can also be released in the dorsal horn by normally ineffective innocuous stimuli [44]. Following inflammation, expression of NK1 mRNA increases within the superficial dorsal horn of the spinal cord [46]. Electrophysiologically, SP applied iontophoretically to the spinal cord has been reported to excite nociceptive but not non-nociceptive neurones [47], and NK1 receptor antagonists have been shown to block 'wind-up' and the increased excitability that follows C-fibre stimulation or a peripheral inflammatory lesion [see, e.g., 48, 49].

Coexistence of substance P, other neuropeptides and glutamate in primary afferents

The concept of cotransmission, as outlined above, can be applied to sensory neurones. Substance P and NKA, colocalized in small dorsal root ganglion (DRG) neurones [50], excite dorsal horn interneurones [9, 51] and synergistically increase spinal excitability, exceeding the additive effect of the two peptides [52, 53]. Thus, colocalized neuropeptides may interact functionally. Using specific antagonists of the NK1 and NK2 receptors, the differential functions of SP and NKA have been demonstrated in the spinal cord. The NK1 antagonist CP-96,345 effectively blocks spinal transmission following activation of both cutaneous [54] and muscle [53] afferents. In contrast, the NK2 antagonist Menarini 10207 blocks spinal hyperexcitability following conditioning stimulation of muscle, but not cutaneous afferents [53, 55]. This differental effect has been confirmed using other specific antagonists [56].

Calcitonin gene-related peptide (CGRP) [57, 58] is another peptide colocalized with SP in many DRG neurones [59]. SP, but not CGRP, injected onto the lumbar spinal cord in rats evokes a brief caudally directed biting/scratching response [60] lasting less than five minutes, but when SP and CGRP are coadministered, a much more prolonged and intense biting/scratching behaviour was observed [59]. A third neuropeptide colocalized in DRGs is galanin [61, 62], which is dramatically upregulated after peripheral nerve injury [63]. Galanin has a complex effect on spinal excitability, but has, especially after nerve injury, an inhibitory function and functions as an endogenous SP and CGRP antagonist [see 64]. Thus, SP containing DRG neurones contain at least two 'intrinsic modulators', one 'enhancer' (CGRP) and one 'attenuater' (galanin).

The principle transmitter in the DRG neurone is, however, glutamate, which is also colocalized with SP [65]. Glutamate appears to have an important role in wind-up [66] and spinal sensitization, and the prolonged facilitation of the flexor reflex following activation of C-afferents can be reduced by a glutamate (NMDA) antagonist [67]. Moreover, when NMDA and NK1 antagonists are coadministered, both wind-up and spinal hyperexcitability are synergistically reduced, suggesting corelease of glutamate and SP and synergistic interaction inducing central sensitization [68, 69].

Tachykinins and synaptic plasticity

Analysis of simple systems has often provided novel and exciting information, and this is the case in recent studies from Sten Grillner's laboratory working on the lamprey, a fish that diverged from the main vertebrate line around 450 million years ago. An SP-like neuropeptide is stored together with serotonin and dopamine in a dense ventromedial nerve plexus in its spinal cord [70, 71]. In this plexus locomotor network, interneurones and motoneurones distribute their dendrites.

The motor pattern underlying locomotion in the lamprey is now known in detail, thanks to extensive studies over many years by Grillner and associates [cf. 72]. Recently involvement of tachykinins has been analysed, and a profound and unusual effect been found. Release of endogenous tachykinins, or administration of an exogenous tachykinin for 10 min, gives rise to a long lasting increase in locomotor activity ($>$ 24 h) [73, 74]. This effect has three phases; an induction phase lasting for 1–2 h, a second phase dependent on protein synthesis from preexisting RNA, lasting for 10–15 h, and a third phase requiring synthesis of new RNA (from DNA).

During the induction phase, activation of TK receptors leads to stimulation of proteinkinase C [73, 75] and an increased NMDA current, presum-

ably due to phosphorylation of NMDA receptors or the incorporation of NMDA receptors/ionophores into the cell membrane. The facilitation of glutamatergic synaptic transmission, especially from excitatory interneurones and reticulospinal cells, constitutes the main factor responsible for the enhanced network activity occurring during the induction phase.

After 1–2 h, the protein synthesis-dependent phase takes over, which is, however, a consequence of events occurring during the induction phase. The NMDA receptor activation leads to Ca^{2+} entrance and increased intracellular levels of cytosolic Ca^{2+}, which is a crucial factor for the induction of the second RNA/protein synthesis-dependent phase. This phase is blocked by protein synthesis inhibitors such as anisomycin acting on the RNA level, whilst the third phase is defined by actinomycin and similar antagonists, which block the synthesis of RNA at the DNA level [76]. These remarkable TK effects, including synaptic plasticity, will thus potentiate the network, giving rise to locomotor activity. Under which behavioural conditions might this occur? Possible conditions include, for example, when the network needs to be recalibrated, when changing body dimensions from a small larva to a full grown lamprey, or during the migration up the river towards the spawning grounds, which occurs at the end of the life cycle.

Studies on knock-out mice

As mentioned above, SP may play a crucial role in the development of inflammatory hypersensitivity and central sensitization. Recent studies on NK1 receptor gene knock-out mice have, however, shown that hyperalgesia develops normally [77], and similar results have been obtained in mice in which the preprotachykinin I gene, which encodes SP, has been disrupted [78, 79]. However, knock-out mice have provided evidence for involvement of SP in a number of other behaviours. Loss of the NK1 receptor results in a reduction of plasma extravasation within peripheral tissues and the loss of the chemoattractant influence of SP on neutrophils [80], and a blunted noxious chemical signalling, including complete loss of the response to capsaicin and a variety of other neurogenic stimuli [81, 82]. This reflects the clinical use of SP antagonists as antiemetics. The second phase of the formalin response is also reduced [77], as can be seen in knock-out mice as well [78, 79]. Further effects include loss of intensity-coding of nociceptive stimuli and wind-up, as well as reduction of stress-induced analgesia, coupled to a more general blunting of the response to danger, including reduced aggression in the resident–intruder test and lowered anxiety to maternal separation [77]. Thus, SP is involved in certain phases of pain behaviour and several other functions.

Receptor internalization

In 1995 Mantyh and colleagues published a remarkable paper in *Science* [83]. Using a highly sensitive and specific antibody to the NK1 receptor, they demonstrated that the receptor protein, normally confined to the cell membrane of a neurone population in the dorsal horn, was dramatically internalized after somatosensory stimulation (Fig. 5). This receptor endocytosis was reversible, so that within 30 min the immunohistochemical image was the same as in normal animals, that is, the receptor protein had returned to the cell membrane. In a series of elegant papers, only a few of which can be discussed here, they have followed up these first findings using NK1 receptor internalization as a marker for SP release. The expression of NK1 receptors in the dorsal horn is modified by peripheral inflammation [84, 85]. Thus, a short noxious mechanical stimulus induces internalization in many more neurones in lamina I after inflammation than in normal rats, and moreover, internalized receptors extend into deeper layers and show an expanded rostrocaudal distribution in rats with inflammation as compared to normal rats. Thus, inflammation causes a ‘reorganization’ of dorsal horn circuits.

A further exciting development in this field is the use of the internalization mechanism to selectively ablate neurones in the dorsal horn that express NK1 receptors [86]. Thus, SP was conjugated to the ribosome-inactivating protein saporin, a cytotoxic compound, and infused into the spinal cord. The conjugate was internalized in lamina I spinal cord neurones expressing the NK1 receptors, and these neurones were killed. In these rats, the response to mild noxious stimuli was unchanged, but there was a marked attenuation of the response to highly noxious stimuli and to mechanical and thermal

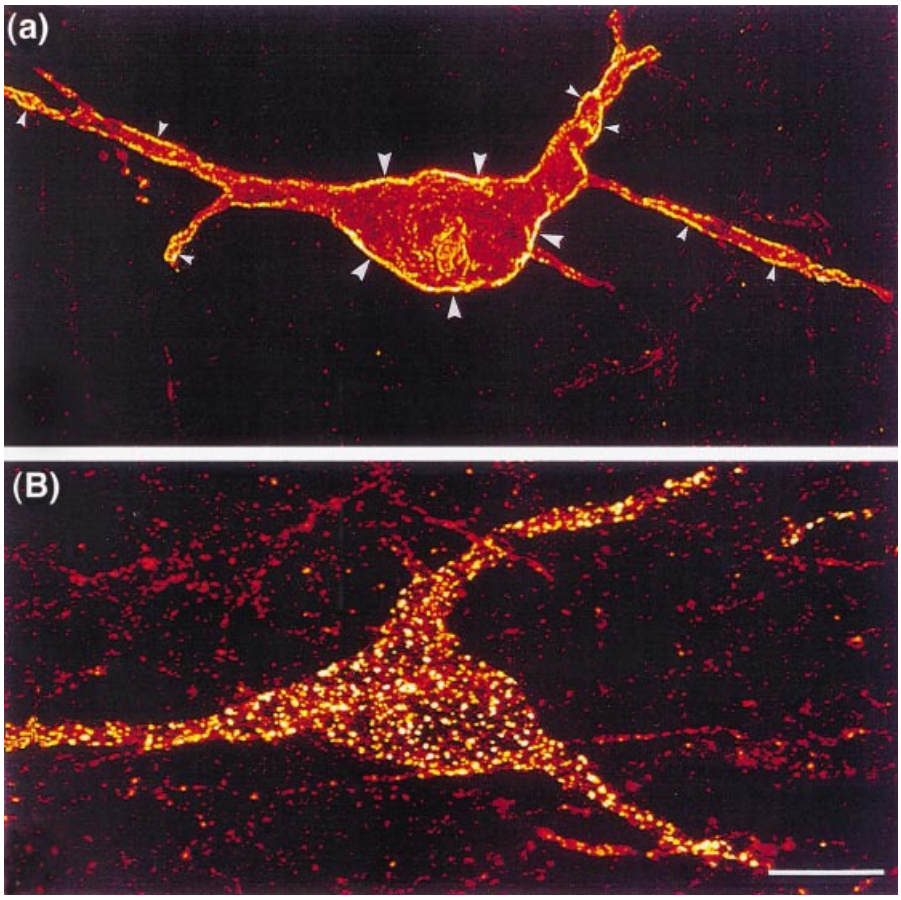

Fig. 5 Fluorescence confocal micrographs of lamina I dorsal horn neurones in the rat lumbar spinal cord, showing NK1-receptor-like immunoreactivity on the unstimulated contralateral side (a) and ipsilaterally to the injection of 100 μg of capsaicin into the hind paw (5 min) (b). On the contralateral side the receptor protein (yellow) can be seen in the membrane of both cell soma (large arrowheads) as well as on the dendrites (small arrowheads). After stimulation, there is a completely different appearance, in that the receptor protein now has been internalized and occupies the entire cytoplasm. (Bar: 20 μm. From Mantyh *et al.* [83], with permission.)

hyperalgesia. This strongly suggests that the NK1 receptor expressing lamina I spinal cord neurones plays an important role in the transmission of highly noxious stimuli and in maintenance of hyperalgesia. In a follow-up study [87], a reduction of thermal hyperalgesia and mechanical allodynia was seen in rats with ablated NK1 receptor neurones after induction of neuropathic and inflammatory pain states. The reason for the clear cut effect seen with lamina I neurone ablation is due to the fact that these neurones, which convey pain information to higher centres, express receptors for numerous transmitters, for example glutamate, several peptides and other transmitters, and thus are able to react to the many messengers released from primary afferents. With the saporin technique, one 'knocks-out' the entire neurone, and does not just pharmacologically block a single selective receptor. In theory this approach could be transferred to treatment of persistent pain in humans. However, much further experimental work is needed in order to be able to use this approach to treat severe clinical pain conditions. In particular, it is necessary to define the long-term consequences of removal of a certain neurone population from the dorsal horn.

Substance P and depression

It has, over past decades, been proposed that drugs acting via a neuropeptide mechanism, in particular neuropeptide antagonists, could have beneficial effects in the treatment of certain diseases. The early hope that a SP antagonist would be efficient in treatment of inflammatory pain has not materialized. It therefore came as a surprise when Kramer, Rupniak and colleagues working at Merck Pharmaceutical Company reported that an NK1 receptor antagonist has antidepressant activity [88]. In fact, the NK1 antagonist MK-869 was as efficient as the reference drug paroxetine, a serotonin reuptake inhibitor (SSRI) of the Prozac type (Fig. 6). Interestingly, the NK1 antagonist was well tolerated, with

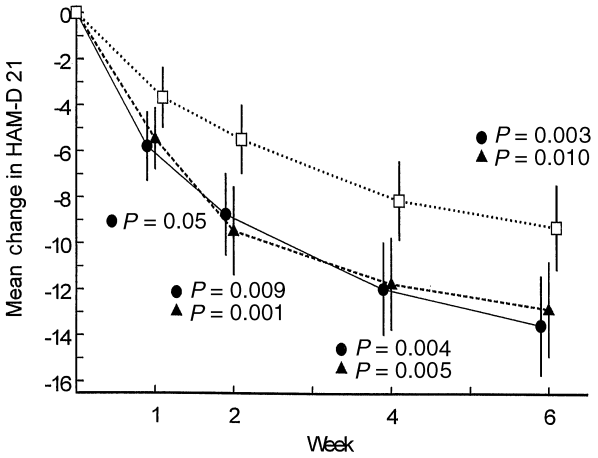

Fig. 6 Comparison between the NK1 antagonist MK-869 (; $N = 66$) and the selective serotonin reuptake inhibitor paroxetine (▲ $N = 68$) vs. placebo (□; $N = 64$) with regard to mean change from baseline (last observation carried forward) and 95% C.I. for the Hamilton depression scale (HAM-D21). (From Kramer *et al.* [88], with permission.)

no statistically significant differences in the frequency of adverse events compared to placebo. Unlike paroxetine, MK-869 did not cause sexual dysfunction. This was a multicentre, 6 week double-blind placebo-controlled study carried out on patients with major depressive disorder using the 14- and 21-item Hamilton depressant (HAM-D) scales. In this study, impressive behavioural and histochemical animal experiments were also presented, providing a rationale for SP being involved in mood control [88, 89]. Interestingly, there is a potential common denominator for SSRIs and NK1 antagonists, in that SP is colocalized in almost half of all serotonin neurones in the dorsal raphe nucleus in the human brain (Fig. 7) [90, 91]. These 5-HT neurones are assumed to be the therapeutic targets for SSRIs. To what extent the colocalization of 5-HT and SP in the brain is of significance for the reported effect of MK-869 in depression remains to be analysed.

The same Merck group has reported that in a second, not yet published, study neither MK-868 nor an SSRI differed from placebo. This disappointing finding should, however, be seen against the background that about 50% of similarly designed studies conducted with antidepressants of the Prozac type have failed to show a difference between test drug and placebo (Kramer, personal communication). Moreover, *post hoc* analysis of this study

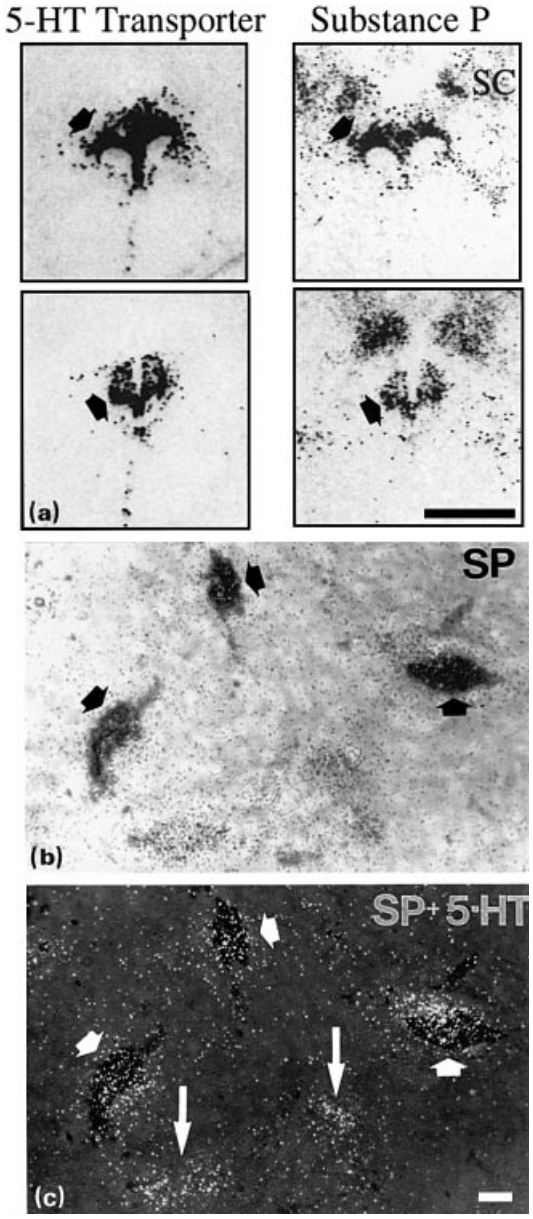

Fig. 7 (a) Film autoradiograph images showing the 5-HT transporter (left column) and substance P (right column) mRNA expression in adjacent sections at two different levels of the human raphe nucleus and adjacent areas. The sections are cut at an ≈ 1000-μm interval, whereby the top section is dorsal to the bottom section. Arrows point to areas with a high degree of colocalization in the dorsal raphe nuclues. SC, superior colliculus. (Bar: 2 mm.) Brightfield (b) and combined bright and darkfield (c) micrographs of a single section of the human dorsal raphe nucleus after double hybridization with probes complementary to 5-HT transporter (5-HT-T) and substance P mRNA. Substance P mRNA probe is labelled with digoxigenin (dark precipitate; best seen in b) and the 5-HT-T mRNA probe is radioactively labelled (white grains; best seen in c). Three cells (short arrows) express both markers, whereas some other cells (long arrows) are only 5-HT-T mRNA-positive. (Bar: 25 μm.)

showed clinically significant antidepressant activity for MK-869 as well as paroxetine in a subset of patients with characteristics predicting a positive response to antidepressant treatment. The Merck group is now conducting several studies in depressed patients with a second, more potent NK1 antagonist. We eagerly await the outcome of these new, ongoing double-blind studies.

NK1 antagonists have also been tested for other therapeutic targets, including different types of pain such as dental pain, osteoarthritis, neuropathic pain and migraine. In all studies, with one exception, no analgesic effect was recorded [92].

Based on animal studies, where NK1 receptor antagonists attenuate both acute and delayed emesis induced by cisplatin in ferrets [93], clinical studies have been carried out with SP antagonists on patients undergoing cancer chemotherapy. Thus, in three clinical trials with three different NK1 antagonists, it has been demonstrated that this treatment is extremely effective in preventing delayed emesis after cisplatin chemotherapy [see 92].

Concluding remarks

The present article describes some aspects of how SP was discovered and the subsequent development of this research field. It is, in a way, prototypical for a large number of other neuropeptides, although most are in different stages of research. An aspect not covered here is a trophic role of SP. In fact, there is strong evidence for such a role not only for SP [94, 95] but also for many other neuropeptides [see 96, 97].

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