

NEUROPEPTIDES AND INFLAMMATION: The Role of Substance P

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ABSTRACT

The neuropeptide substance P modulates the activities of a number of different leukocytes that characterize both acute and delayed inflammatory responses. Substance P may play a role in the pathogenesis of such diverse diseases as arthritis, asthma, and inflammatory bowel disease.

INTRODUCTION

Substance P (SP) belongs to a family of bioactive peptides, the tachykinins, defined by their common pharmacological properties and conserved carboxyl-terminal sequences, -Phe-X-Gly-Leu-Met-NH₂, where X is a branched aliphatic or aromatic amino acid (Table 1) (1). Moreover, the principal biological activities of SP reside in its carboxyl sequence. There are three mammalian tachykinins, substance P (SP), substance K (SK), and neuromedin K (NMK). SP and SK are derived from a single gene by alternative RNA splicing (preprotachykinin A mRNA, PPT-A), and NMK is also generated from another unique gene (preprotachykinin B mRNA, PPT-B). The level of PPT-A mRNA is higher in the central nervous system than that of PPT-B mRNA, especially in the trigeminal ganglion, caudate nucleus, and olfactory bulb, while PPT-B mRNA is expressed mainly in the hypothalamus and in peripheral tissues such as the duodenum and small intestine (2).

SP, identified by von Euler and Gaddum in 1931, was one of the first

Table 1 Mammalian tachykinins

Peptides	Receptors	Amino acid sequences
Substance P (SP)	SP-P (NK-1)	Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH ₂
Substance K (SK) ^a	SP-K (SP-E, NK-2)	His-Lys-Thr-Asp-Ser-Phe-Val-Gly-Leu-Met-NH ₂
Neuromedin K (NMK) ^b	SP-N (SP-E, NK-3)	Asp-Met-His-Asp-Phe-Phe-Val-Gly-Leu-Met-NH ₂

^a Neurokinin A, neuromedin L, neurokinin alpha.

^b Neurokinin B, neurokinin beta.

neuropeptides described and its effects on diverse tissues have been extensively studied (3, 4). SP is synthesized in dorsal root ganglia (5) and is axoplasmically transported toward the terminal regions of the peripheral sensory nerve branches by distinct mechanisms (6), where it is stored in nerve endings (7). SP released from nerve endings by various stimuli can elicit diverse biological activities, including smooth muscle contraction, vasodilatation, and secretion from distinct glands, all of which contribute to local neurogenic inflammation (4). Furthermore, several studies demonstrate that SP directly stimulates lymphocytes and other leukocytes that participate in the inflammatory response (8-11). The roles of SK and NMK in inflammation are less well described. However, their colocalization with SP in sensory nerve endings suggests they may interact in amplifying localized immune and inflammatory responses in distinct microenvironments such as the skin, upper airway, gut-associated lymphoid tissue, and joints.

This review summarizes the role of substance P in inflammation, the biochemical characteristics of its receptors, and the possible contribution of SP to specific disease states.

THE ROLE OF SUBSTANCE P IN INFLAMMATION

In Vitro Studies

SP and SK may regulate tissue repair processes by enhancing the proliferation of smooth muscle cells, fibroblasts, and endothelial cells (12, 13). The proliferative effect of SP on smooth muscle cells was diminished by preincubation with SP antagonists or simultaneous stimulation with suboptimal concentrations of platelet-derived growth factor, which suggests the possibility of competitive interactions between regulatory signals from neuropeptides and polypeptide growth factors (12). The mitogenic

effects of SP on smooth muscle cells were demonstrated to be cell-surface receptor mediated, with SP binding eliciting a rise in cytosolic Ca^{2+} , suggestive of phosphatidylinositol pathway activation (13). One structural basis for the effects of neuropeptides on cellular proliferation may be the recognized homologies between the amino acid sequences of polypeptide growth factors for fibroblasts, which exert potent mitogenic and angiogenic activities, and those of members of the tachykinin family (14). Moreover, in smooth muscle cells the degree to which DNA synthesis could be induced by SK correlated with the constitutive expression of the proto-oncogene *c-myc*; this suggests that the stimulatory effect of tachykinins may be optimal during cellular differentiation (15). The possibility of opposite effects of SP on cellular proliferation in some circumstances is raised by the discovery that SP acts on human capillary endothelial cells to decrease the mRNA levels of the oncogene *c-sis*, which codes for the B-chain of platelet-derived growth factor (16).

A number of studies have implicated SP in the modulation of cellular activities that characterize both acute hypersensitivity and delayed-type hypersensitivity response. At micromolar concentrations SP evokes the substantial release of histamine from rat serosal or connective-tissue-type mast cells (17). The results of early studies suggested that the effects of SP might be simply analogous to those of other charged basic peptides capable of stimulating mast cells nonspecifically at high concentration (18). However, unlike other basic peptides, the activity of SP exhibited cell specificity and included stimulation of the generation of unstored mediators, such as leukotrienes (19). The mast cell responses elicited by SP were distinguished clearly from those dependent on IgE by the apparent lack of a requirement for extracellular calcium (17). In contrast, basophils failed to release histamine *in vitro* in response to SP at concentrations as high as $10 \mu\text{M}$ (20).

The possible role of substance P in enhancing the cellular responses that define chronic inflammation is suggested by the observation that SP stimulates human monocyte chemotaxis *in vitro* with an EC_{50} of approximately 0.1 pM (21). The chemotactic effect of SP could be blocked by D-amino-acid analogues of SP, but not by the antagonists of the chemotactic peptide *N*-formyl-met-leu-phe (fMLP); this suggests SP specificity (21). SP elicits not only mononuclear leukocyte chemotaxis, but also the generation of thromboxane A_2 , O_2^- and H_2O_2 by *Corynebacterium parvum*-activated macrophages (22). Several other macrophage responses are also evoked by SP, including the down-regulation of membrane-associated 5'-nucleotidase, the stimulation of synthesis and release of lysosomal enzymes, and the release of leukotriene C_4 , prostaglandin E_2 , and thromboxane B_2 (23). These effects of SP on macrophages are receptor medi-

ated, with binding characteristics similar to those described for human peripheral blood T-lymphocytes (23).

The proliferation of both human and murine T-lymphocytes is significantly stimulated by nanomolar concentrations of SP (8, 24, 25). In human T-lymphocytes, SP and the substitute peptide SP(4–11) stimulated increases of as much as 60–70% in [³H]thymidine and [³H]leucine uptake in vitro in the presence or absence of mitogens (8). Similar stimulatory effects on the uptake of [³H]thymidine were observed when SP was incubated with murine lymphocytes from spleen, mesenteric lymph nodes, and Peyer's patches. In addition, SP significantly increased the in vitro production of IgA in lymphocytes from spleen and Peyer's patches by as much as 300% (25). Experiments using a fluorescence-activated cell sorter (FACS) and fluorescein-labeled SP (SP*) demonstrated the specific binding of SP* to approximately 20–30% of the peripheral blood T-lymphocytes (9); thus the mitogenic effects of SP were likely mediated by specific cell-surface receptors present on a distinct lymphocyte subpopulation. Two-color FACS analysis revealed that SP*-reactive T-lymphocytes were distributed between both the helper-inducer (CD4) and the suppressor-cytotoxic (CD8) subtypes with mean frequencies of 20–30% and 10–20%, respectively (9). Both functional and competitive binding studies with substituent SP peptides showed that the carboxyl-terminal sequence was the principal binding determinant of the peptide (9). Furthermore, the majority of B-lymphocytes from distinct immunologic microenvironments such as the spleen and Peyer's patches also showed increased specific SP binding (26).

Recent studies have now indicated that extracts of chronic granulomas isolated from the livers of mice infected with the parasite *Schistosomiasis mansoni* contain immunoreactive SP (27). The authenticity of the SP in these granulomas was determined by radioimmunoassay, by comparisons with standards on HPLC, and by in situ hybridization with oligonucleotide probes derived from the cDNA for the peptide gene (2, 27). Of great interest is the fact that the SP was localized exclusively to the eosinophils in the granuloma, a demonstration that eosinophils from these granulomas express the SP gene and synthesize the molecule (27).

Although the role of SP within these granulomas remains to be explored, these results underscore the potential contribution of SP to diverse chronic inflammatory reactions.

In Vivo Studies

Peptides released from peripheral nerve endings in mammals, including SP and calcitonin-gene-related peptide (CGRP), are among the most potent mediators of smooth muscle contraction and vascular activities. SP contracts strips of guinea pig ileum with a 50% effective concentration (EC₅₀)

of 1–2 nM as well as smooth muscle from other sources (4). In addition, SP also constricts human bronchi *in vitro* (28) and guinea pig pulmonary airways *in vivo* (29). The principal vascular activity of SP is dilatation, which is evident in systemic arteries and arterioles, with striking vasodilatory activity of SP leading to profound hypotension after administration *in vivo* (4). Moreover, in the nose, the local application of SP enhances secretions in humans and rats (28, 29), and in the ferret and human trachea the secretion of epithelial and glycoprotein-rich fluids (30). On the other hand, high concentrations (10–50 μM) of SP are necessary to inhibit surfactant secretion from isolated Type II pneumocytes in rat lungs (31).

Before one can attribute physiologic or pathophysiologic effects to SP in any system, there must be evidence of local availability at functionally relevant concentrations and of stereospecific receptors in the target tissues of the same species. Much of the work with SP has been carried out with rats, guinea pigs, and rabbits, and only to a much lesser extent with humans. Physiologic systems for producing and delivering SP to sites of its purported actions have been elucidated only partially. For example, the recent demonstration of SP and CGRP in nasal secretions after local antigen challenge also lends credence to a role for neuropeptides in allergic reactions (32).

SUBSTANCE P RECEPTORS

The observed diverse tachykinin activities are believed to be mediated by at least three different classes of receptors (33). SP and the amphibian tachykinin physalaemin are more potent than kassinin in contracting smooth muscle in the guinea pig ileum (SP-P prototype), but far less potent in their action on the vas deferens or bladder (SP-E prototype). A third tachykinin receptor in the mammalian nervous system, SP-N, preferentially binds the agonist succinyl [Asp⁶, Me-Phe⁸]SP(6–11). Recent extensive studies using radiolabeled ligands indicated that SP-P, SP-E, and SP-N correspond to the specific receptors for SP, SK, and NMK, respectively (34). The conformational orientation of several tachykinin C-terminal heptapeptides have been extensively analyzed, with a number of studies demonstrating that the tertiary structures of the different tachykinins exhibit significant variability. However, the partial alpha-helical structure of -Phe-Phe-Gly-Leu- in substance P is common to all the tachykinins. This conformational structure may be an important feature in SP binding to SP-P receptors. This also indicates that SK and NMK, which have their own receptors, can at higher concentrations also stimulate SP receptor activity (35, 36).

The SP receptor has recently been biochemically characterized from the human B-lymphoblast cell line (IM-9) (37–40), rat brain membranes (41), and bovine brainstem membranes (42). Our previous studies using cross-linking techniques indicated that the lymphocyte SP receptor consisted of three distinct proteins of molecular weight 33, 58 and 78 kilodaltons (kd) (37–39). In contrast, the molecular weight of the SP receptor in rat brain is 46 kd when measured by using photoaffinity labeling with [$3'$ - 125 I D-Try⁶, 4'-N₃Phe⁸, Nle¹]-SP (41), and > 1000 and 55–60 kd by gel filtration (42). The reason for the differences of molecular weight among these reports is unclear, but part of the difference may be due to receptor glycosylation: our previous report clearly indicates that the lymphocyte SP receptor is highly *N*-glycosylated (39).

Recently, the SK receptor from bovine intestine was cloned using a novel strategy (43): a cDNA from bovine stomach was constructed in a lambda phage vector designed for the *in vitro* transcription of cDNA inserts. The resulting cDNAs were used to generate a mixture of synthetic mRNAs, which were then evaluated for expression of functional SK receptors following injection into *Xenopus* oocytes. The resulting mixture was taken through a series of fractionations until a single clone expressing a functional SK receptor was obtained. The SK receptor identified reveals a high degree of homology to a family of “seven-transmembrane domain” proteins that includes the muscarinic acetylcholine receptor, the serotonin receptor, and the alpha- and beta-adrenergic receptors (44). Despite this advance, the molecular differences among the three tachykinin receptors have yet to be defined. Of interest, with respect to the proliferative activities of SK, are the structural similarities between the cDNA for the SK receptor and the product of the human oncogene *mas*, first isolated using total genomic DNA and a novel tumorigenicity assay (45, 46).¹ This similarity is intriguing given that both SP and SK are also mitogenic for cultured smooth muscle cells (12, 13, 47) and fibroblasts (12) *in vitro*. The possible relationship between tachykinin receptors and oncogene products will need to be explored by structural modifications of the receptor genes and examination of the resulting functional alterations in receptor activity.

With regard to the second messenger systems involved in SP receptor activities, it has been demonstrated that SP evokes inositol phospholipid hydrolysis when binding to guinea pig ileum (48), rat hypothalamus (48), salivary glands (49), and lymphocytes (11). Recent studies have also indicated that SP receptors are coupled to guanine nucleotide-dependent regulatory proteins (G-proteins) in rat parotid glands (50) and lymphocytes (S. E. Leeman, D. G. Payan et al, unpublished data). In rat brain slices, SK and NMK also elicit hydrolysis of phosphatidylinositol but not an

¹ See Note Added in Proof on page 352.

enhanced adenylyclase response (51). This finding suggests that, like SP, SK and NMK may regulate inositol phospholipid metabolism as a primary receptor-coupling mechanism. The homology of the predicted SK receptor protein to muscarinic acetylcholine receptors and beta receptors also suggests that SK receptors are coupled to a second-messenger transduction system by a GTP-binding protein (43).

THE ROLE OF SUBSTANCE P IN HUMAN INFLAMMATORY DISORDERS

The evidence supporting the involvement of SP in the pathology of a variety of diseases has been derived from studies of animal models and from humans. Increased concentrations of SP in fluids and tissues of affected systems relative to those without disease, the capacity of SP antagonists to alter the expression of certain immunopathological responses, and changes in the number of SP receptors in diseased tissue constitute the usual proof of involvement. Several studies now suggest that SP plays a role in diseases involving hypersensitivity reactions in the skin and lungs, and in chronic inflammatory disorders such as arthritis and inflammatory bowel disease (Table 2).

Substance P increases the severity of adjuvant-induced arthritis in rats (52). In this animal model, joints that developed more severe arthritis (ankles) were more densely innervated by SP-containing neurons than were joints that developed less severe arthritis (knees) (52). Of interest was that SP infusion into the knee increased the severity of the arthritic response, whereas infusions of an SP antagonist did not (52). When these observations were extended to *in vitro* studies on synoviocytes from patients with rheumatoid arthritis, SP stimulated prostaglandin E₂ and collagenase release from these cells, as well as a proliferative response of the same order of magnitude and over the same concentration range as had

Table 2 Pathogenic involvement of substance P in diseases

Disease	Species	Site	Action
Arthritis	Rat, human	Regional nerves, joint capsule	Synoviocyte, leukocyte stimulation
Bullous pemphigoid	Human	Skin	Mast cell activation
Asthma	Guinea pig, human	Bronchial tissue	Bronchoconstriction, mediator release
Inflammatory bowel disease	Human	Large and small intestines	Altered immune regulation
Carcinoid tumor	Human	Ileum	Altered gut motility and vascularity

been observed for human lymphocytes (8, 53). Moreover, macrophages from these inflamed joints synthesize and secrete interleukin-1 and tumor necrosis factor α when incubated with SP (54).

The release of SP into pulmonary tissues may under certain circumstances be the result of or exacerbate bronchial asthma, which is a complex disorder involving bronchial hyperresponsiveness, type I, III, and IV hypersensitivity responses, inflammatory reactions, gastroesophageal reflux, and possibly the contribution of psychological factors (55). One example of the possible contribution of SP to the pathophysiology of asthma is the axon reflex mechanism (56). Damage to airway epithelium by various mechanisms may expose C-fiber afferent nerve endings such as "cough" receptors and "irritant" receptors. Stimulation of these endings by nonspecific stimuli and inflammatory mediators, such as bradykinin or the leukotrienes, may result in the release of sensory neuropeptides such as SP, SK, and CGRP (57) following an axon reflex with production of cough (58) and bronchoconstriction via cholinergic pathways (59). Thus, further examination of this hypothesis might lead to new strategies for treatment of asthma employing C-fiber ending "stabilizers" (60), specific tachykinin antagonists, and inhibition of reflex bronchoconstriction (61).

In the skin, SP may play a role in certain inflammatory conditions such as bullous pemphigoid and eczema, where immunoreactive SP levels in blisters were significantly increased when compared to blister fluid from patients with allergic skin conditions (62). In addition, following superficial burns in humans, there is an increase in the healing tissues of SP-immunoreactive sensory nerve fibers in connection with blood vessel regeneration (63).

Studies suggest that neuropeptides such as SP and vasoactive intestinal polypeptide (VIP) may play an important role in the pathology of human inflammatory bowel diseases (64, 65). In surgically dissected specimens from patients with Crohn's disease and ulcerative colitis, VIP and SP were quantitated by radioimmunoassay and compared with normal intestinal tissue. Mucosal-submucosal layer concentrations of VIP were significantly decreased in Crohn's disease and ulcerative colitis, while those of SP were significantly increased in left-sided ulcerative colitis (64). These observations have been further extended by examining the neuropeptide receptor changes that may take place in these diseases. In surgical specimens from patients with inflammatory bowel diseases, autoradiography has been used to detect SP, SK, and NMK receptors. Receptor binding sites for SP, but not SK or NMK, were expressed in high concentrations by arterioles, venules, and regional lymph nodules in surgical specimens obtained from patients with ulcerative colitis and Crohn's disease (65). These data suggest that SP may be involved in the pathophysiology of

inflammatory bowel disease and it might provide a rationale for the development of new therapies employing specific tachykinin antagonists.

CONCLUSIONS

The roles of substance P in tissue responses to diverse challenges are determined in part by the local availability of SP, the composition of the responding population of cells, and the possible range of functional reactions. The ongoing analysis of SP function and the identification of a cDNA for the SP receptor should facilitate novel pharmacological approaches to the modulation of certain aspects of inflammatory diseases.

ACKNOWLEDGEMENTS

This work was supported in part by the grant NS21710 from the National Institute of Health and by the Howard Hughes Medical Institute.

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NOTE ADDED IN PROOF

It has recently been demonstrated that the *mas* oncogene product is a functional angiotensin receptor (66) and that it is located on chromosome 6 (67). These results elucidate the possible role of the angiotensin receptor in essential hypertension, and also for the first time the possibility of pharmacological manipulation of an oncogene product.