Structure, Functions, and Mechanisms of Substance P Receptor Action

James E. Krause, Yasuo Takeda, and Andrew D. Hershey
Department of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, Missouri, U.S.A.

Substance P is a member of a family of structurally related peptides, called tachykinins, that are involved in the regulation of many biologic processes. Diversity in the generation of multiple tachykinin peptides arises due to multiple genes encoding these peptides as well as by mechanisms of alternative RNA processing and differential posttranslational processing. The multiple peptides are neurotransmitters and/or neuromodulator substances, and they bring about their actions mainly by activating three primary types of receptors, NK-1, NK-2, and NK-3. The pharmacology and tissue locations of these receptor sites are discussed, as is their involvement in certain biologic responses. These three receptor sites have been molecularly characterized by cDNA cloning and functional expression, and all are members of the superfam-ily of receptors coupled to G-regulatory proteins. Second messenger systems established to be activated by tachykinin receptor stimulation include the hydrolysis of inositol containing phospholipids by a phospholipase C mechanism. The role of substance P in neurogenic inflammation and plasma extravasation is briefly discussed. The generation of new research tools recently in the tachykinin field should allow for a detailed examination of the mechanisms of peptide action, including a focus on receptor structure-function relations and regulation of receptor sensitivity. J Invest Dermatol 98:2S–7S, 1992

Substance P (SP) is a neuropeptide considered to function as a neurotransmitter or neuromodulator in the central and peripheral nervous systems. It is well characterized in terms of sites and mechanisms of biosynthesis, distribution, sites of release, and biologic actions. SP is a member of a family of structurally related peptides, the tachykinins, due to their rapid contractile activity on gastrointestinal tissue. SP has excitatory effects on central and peripheral neurons, and it also elicits a variety of biologic responses in non-neuronal tissue. These biologic responses include stimulation of smooth muscle contraction, exocrine and endocrine gland secretion, and plasma extravasation, as well as being involved in the regulation of immune and inflammatory responses. This review will focus on recent findings related to an understanding of the diversity of chemical signaling in tachykinin-secreting neurons. Specifically, the biogenesis of mammalian tachykinin peptides and a pharmacologic and molecular characterization of tachykinin receptors will be addressed. The recent molecular characterization of the SP receptor (SPR) will be a focus, and ongoing studies examining the mechanisms of SPR action will be reviewed. Finally, the multiple roles of SP released from sensory neurons will be mentioned, with a discussion of the role of SP in neurogenic plasma extravasation.

DIVERSITY OF MAMMALIAN TACHYKININ PEPTIDES

The currently characterized mammalian tachykinin peptides are shown in Table I. This family of peptides [1–3] is characterized by a conserved carboxyl terminal sequence of Phe-X-Gly-Leu-Met-NH2, whereas the amino terminal sequences are distinctive for each peptide. The carboxyl terminal domain is essential for receptor interaction and activation, whereas the distinct amino terminal domains of these peptides dictate receptor subtype specificity. The three major mammalian tachykinin peptides are SP, neurokinin A (NKA), and neurokinin B (NKB), and two additional recently described tachykinin peptides, neuropeptide K (NPK) and neuropeptide γ (NPγ), are amino terminal extended derivatives of NKA. These mammalian tachykinin peptides are the products of two distinct genes: the SP/NKA gene and the NKB gene [4,5]. The peptides SP and NKA, and the NKA-related peptides NPK and NPγ, are encoded by mRNA generated by SP/NKA gene transcription and splicing. Three mRNA are expressed as a result of alternative RNA splicing, called α-, β-, and γ-preprotachykinin (PPT) mRNA. The β-PPT mRNA contains sequences from all 7 exons of the gene, whereas α-PPT mRNA lacks exon 6 sequences and γ-PPT mRNA lacks exon 4 sequences. Because NKA is encoded on exon 6, the α-PPT mRNA can encode SP as its only tachykinin peptide. Both the β- and γ-PPT mRNA can encode both SP and NKA. After translation, the β- and γ-PPT can be differentially processed such that either NKA or NPK can be produced from β-PPT [6,7], whereas γ-PPT can yield either NKA or NPγ [7,8]. On the other hand, NKB is the only tachykinin peptide derived from the NKB gene. Therefore, multiple genes, alternative precursor RNA splicing, and differential posttranslational processing are the mechanisms responsible for generating diversity in the production of mammalian tachykinin peptides.

Species differences [4,9] have been noted in the types and relative

Research performed in the authors' laboratory has been supported by grants from the NIH (NS21937 and NS29343) and from the Monsanto-Searle/Washington University Biomedical Program.

Reprint requests to: Dr. James E. Krause, Department of Anatomy and Neurobiology, Washington University School of Medicine, 660 South Euclid Ave., St. Louis, MO 63110.

Abbreviations:
CNS: central nervous system
NK: neurokinin
NKA: neurokinin A
NKB: neurokinin B
NPK: neuropeptide K
NPγ: neuropeptide γ
PPT: preprotachykinin
SP: substance P
Table I. Primary Structures of the Mammalian Tachykinin Peptides

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Substance P</td>
<td>Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH₂</td>
</tr>
<tr>
<td>Neurokinin A</td>
<td>His-Lys-Thr-Asp-Ser-Phe-Val-Gly-Leu-Met-NH₂</td>
</tr>
<tr>
<td>Neurokinin B</td>
<td>Asp-Met-His-Asp-Phe-Phe-Val-Gly-Leu-Met-NH₂</td>
</tr>
</tbody>
</table>

amounts of mRNA that encode SP and NKA-related peptides. As such, the cow has significant levels of α-PPT mRNA, whereas the rat and human express primarily β- and γ-PPT mRNA. As the various SP-encoding mRNA are normally unresolved upon denaturing gel electrophoresis, solution hybridization-nucleic acid protection assays have been established to provide a sensitive method for identifying and quantitating the various PPT mRNA species, and recent studies indicate that regulation of SP gene expression appears to be in part transcriptional [9].

The SP-encoding mRNA are translated attached to the rough endoplasmic reticulum, with a concomitant import of the prohormone into the lumen of the endoplasmic reticulum [10]. Signal peptide is cleaved cotranslationally, and because the gene is expressed in cells possessing a regulated secretory pathway, intracellular trafficking routes the SP precursors from the endoplasmic reticulum to Golgi and finally to newly synthesized large dense-core secretory vesicles. Little information is available concerning the details and mechanisms of this routing. The peptide precursors are cleaved by proteolytic mechanisms, followed by additional posttranslational modifications [7,10]. One reaction of interest that is crucial for tachykinin peptide biologic activity and stability is amidation [11]. In this reaction the amide nitrogen is donated by the amino nitrogen of the adjacent glycine residue. This reaction occurs in two steps, which include hydrolyase and lyase activities. It is believed that these processing reactions occur once the newly formed secretory vesicle has acidified, and processing occurs during axonal transport.

Consequently, processed peptides are present in the mature secretory vesicle in the nerve terminal and are secreted by a calcium-dependent mechanism. Whether regulation of post-translational processing occurs within the nerve terminal to regulate peptide demand is a subject that has not been studied to date. Because multiple peptides can be derived from the tachykinin precursors, understanding which receptor types are expressed in neuronal terminal fields is essential to determining which peptides are active upon secretion. In addition to the tachykinins, other protachykinin-derived peptides are processed and presumably secreted from the tachykinin neurons. These peptide sequences are highly conserved [9], and their functional relevance awaits further study. As discussed below, the current data indicate that the five tachykinin peptides expressed in neurons have biologic roles at three primary types of tachykinin receptors.

MUTIPLE MAMMALIAN TACHYKININ RECEPTORS

Research into tachykinin peptide receptor mechanisms has progressed dramatically over the past 10 years. Three pharmacologically distinct receptors, called neurokinin-1 (NK-1), NK-2, and NK-3 receptors, have been classified based on the rank order of potencies of tachykinins in bioassays and in radioligand binding studies [2,3,12,13]. The rank orders differ between SP, NKA, and NKB by some 10–1000 times, depending upon the agonist and the specific receptor site. A summary of the classification of mammalian tachykinin receptors is provided in Table II. The expression cloning of the bovine stomach NK-2 receptor by Nakanishi and co-workers in 1987 [14] opened the door to a structural characterization of the NK-1, NK-2, and NK-3 receptors. Three receptors have now been molecularly characterized by cDNA cloning, sequence analysis, and functional expression [14–17,20–23]. These studies have added a new research perspective to the study of tachykinin receptor action.

NK-1 tachykinin receptors are expressed by neurons and glia in the CNS, neurons within the myenteric plexus, smooth muscle cells, acinar cells, endothelial cells, fibroblasts, and various types of circulating immune and inflammation-activated immune cells [2,3,12]. SP is the most potent ligand at this site, with a Kd of 0.5–1 nM, whereas NKA and NKB have a 100–1000 times lower affinity. Receptor activation at some sites result in inositol phospholipid hydrolysis [2,3]; however, this has not been shown to be a universal response. The cloning of rat [15,16] and human [17] NK-1 receptor cDNA has established them to be members of the G-protein–coupled receptor superfamily, and the cloning and characterization of the gene has recently been accomplished [18]. The recent discovery of a SP-receptor selective nonpeptide antagonist [19] has also placed a new perspective on the study of NK-1 receptor structure and function, and these advances now allow for a much better understanding of the molecular basis of SP function.

Table II. Classification of Mammalian Tachykinin Receptors

<table>
<thead>
<tr>
<th>Type</th>
<th>Ligand</th>
<th>Model Tissue</th>
<th>Second Messenger</th>
<th>Biologic Correlate</th>
</tr>
</thead>
<tbody>
<tr>
<td>NK-1</td>
<td>SP &gt; NKA &gt; NKB</td>
<td>Rat salivary gland, Dog carotid artery, Rat cerebral cortex (layers II, III)</td>
<td>PLC/PI-Ca²⁺</td>
<td>Capillary permeability</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rat vas deferens, Rabbit pulmonary artery, Rat cerebral cortex (layer VI during P1-P14)</td>
<td>PLC/PI-Ca²⁺</td>
<td>Hyperalgesia</td>
</tr>
<tr>
<td>NK-2</td>
<td>SP &gt; NKA &gt; NKB</td>
<td>PLC/PI-Ca²⁺</td>
<td>Tachycardia</td>
<td></td>
</tr>
<tr>
<td>Subtypes?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NK-3</td>
<td>NKB &gt; NKA &gt; SP</td>
<td>Rat portal vein, Guinea pig myenteric plexus, Rat cerebral cortex (layers IV, V)</td>
<td>PLC/PI-Ca²⁺</td>
<td>Analgesia, Capillary permeability</td>
</tr>
</tbody>
</table>

* Shown is the natural agonist potency profile, examples of model tissues where each receptor type is selectively present, and the established second messenger system utilized for each. Certain biologic correlates related to peripheral and central actions of the tachykinin systems are also noted.
The NK-2 receptor is primarily expressed in peripheral tissues (smooth muscle) with little overlap with NK-1 receptor sites [2,3,12]. This site has the highest affinity for NKA, NPK, and NPy, and 10–100 times lower affinity for NKB and SP. The NK-2 receptor was cloned from bovine stomach in 1987 using an oocyte expression system [14]. Sequence analysis and hydropathy analysis indicated a glycoprotein possessing seven putative α-helical transmembrane domains with sequence similarity to other G-protein-coupled receptors. Recently, the rat and human NK-2 receptors were cloned by homology approaches [20–22], and were shown to be some 90% identical to the bovine sequence. The overall length ranged from 384 residues for the bovine to 398 residues for the human receptor. Areas of diversity in these sequences are located primarily in the amino terminal extracellular domain and in the carboxyl terminal intracellular domains. Activation of the NK-2 receptor also results in inositol phospholipid hydrolysis. The gene encoding the human NK-2 receptor was also recently isolated [20].

The NK-3 receptor is expressed primarily in the CNS and in certain peripheral tissues, e.g., smooth muscle cells and the rat portal vein, with little overlap with the NK-1 receptor [2,3,12]. The affinity for NKB at this site is approximately 1 nM, with NKA and NKB having 10–100 lower times affinity. This has been the least studied of three tachykinin receptor types; however, it was recently characterized molecularly by cDNA cloning and expression by Shigemoto and co-workers [23]. The deduced protein sequence consists of 452 residues with homology to other members of the G-protein-coupled receptor superfamily. The expression of NK-3 receptor mRNA in the CNS is more restricted than that of the NK-1 receptor mRNA [18,24]. One system that involves the NK-3 receptor appears to be spinal systems related to antinociception.

MOLECULAR CHARACTERIZATION OF THE SUBSTANCE P (NK-1) RECEPTOR

The rat SP or NK-1 receptor was cloned from brain and submandibular gland cDNA libraries [15,16] and the deduced amino acid sequence demonstrated a 407-residue glycoprotein that appeared to be a member of the G-protein-coupled receptor superfamily. Expression of the NK-1 receptor in cell lines resulted in the appearance of high-affinity SP binding sites with Kd values ranging from 0.5–3 nM. The ligand displacement profile of naturally occurring tachykinin peptides was SP >> NKA > NKB, and activation of the receptor was shown to result in the rapid and transient appearance of inositol 1,4,5 trisphosphate (IP3) [17,25]. The production of cellular IP3 due to receptor stimulation was dependent on the number of receptors expressed per cell, and ligand responses and loss of receptor responsiveness appeared to be directly related to receptor occupancy. When NK-1 receptor mRNA was transcribed from the cloned cDNA and expressed in Xenopus oocytes, sensitivity to SP was induced as detected by sensitive electrical assays [26]. At a holding potential of −60 mV, SP evoked an oscillatory inward membrane current ranging from 50 nA to greater than 2 μA. The current appeared with a latency of seconds after peptide administration, and subsequent responses to ligand were much reduced in amplitude. Recovery from this apparent desensitization occurred after washout periods of 30–50 min. Consequently, this system may be convenient for examining mechanisms regulating receptor sensitivity.
Figure 2. A model for a mechanism of action of substance P acting on the NK-1 receptor. Ligand stimulation of the receptor activates a G-protein cascade in mammalian cells. See text for discussion. Dissociation of $G_a$ from the G-protein complex due to ligand stimulation results in the activation of PLC by a pertussis-toxin–insensitive mechanism. PLC catalyzes the breakdown of inositol-containing phospholipids and generates two second messengers, the calcium mobilizing agent inositol 1,4,5-triphosphate and an activator of protein kinase C, diacylglycerol.

Taken together, these studies clearly demonstrate that the isolated cDNA expresses a receptor that binds the ligand with high affinity and expresses the pharmacologic profile of the NK-1 tachykinin receptor class. In transfected cell lines, the expressed protein functions by activating phospholipase C (PLC), which results in the production of IP$_3$. In the Xenopus system, the expressed protein functions by activating a receptor-channel coupling mechanism that involves the production of IP$_3$, which releases Ca$^{2+}$ from internal stores and generates an oscillatory chloride current.

Figure 1 shows a comparison of the primary structure of the rat NK-1 receptor and human NK-1 receptor. Of the 407 amino acid residues present in each structure, 94.5% of these are identical, with the differences distributed throughout the primary structure. Two N-linked glycosylation sites are present in the extracellular amino terminal domain, and a relatively long serine and threonine-rich carboxyl terminal domain exists intracellularly. This domain, as well as the third intracellular domain, which is also serine and threonine rich, may be phosphorylated and consequently involved in desensitization mechanisms, based upon studies performed with the $\beta$-adrenergic receptor [27]. It is interesting to note that the NK-1 receptor has been shown to most potently desensitize, compared to other tachykinin receptors, i.e., NK-2 and NK-3, and this correlates with the number of potential phosphorylation sites present in these two domains [14–17,20–23,26,28]. The structural similarities and differences noted between the cloned tachykinin receptors presumably have relevance with regard to ligand binding and receptor regulation, as well as possible to second-message system activation as a result of coupling to specific G-proteins.

A model of SPR action is shown in Fig 2, based on results obtained with both the cloned receptor expressed in cell lines and that normally expressed in certain tissues. Ligand activation of the receptor results in the stimulation of a G-protein cycle in which a GDP → GTP interchange occurs on the $G_a$ subunit, with an accompanying dissociation of the $\alpha$ from the $\beta\gamma$ subunits. The $G_a$ subunit (presumably from the $G_{ag}$ branch) then activates a specific isoform of PLC in a pertussis-toxin–insensitive manner. PLC then catalyzes the conversion of phosphatidylinositol bisphosphate to IP$_3$ and diacylglycerol. The former releases Ca$^{2+}$ from internal stores, whereas diacylglycerol has been shown to potentiate activate protein kinase C [29,30]. The intrinsic GTPase activity associated with the $G_a$ subunit inactivates the effector function of $G_a$, thus terminating the response and promoting the reassociation of $G_a$ and $G_b$ subunits. The second messge systems activated by ligand interaction with receptor appear to play some role in receptor regulation, though these studies are still in their infancy. Presumably additional signalling pathways of tachykinin peptides and receptors will be described, and the relationships between receptor responses and receptor regulation will be a future focus of investigation.

Substance P, Sensory Neurons, and Neurogenic Inflammation Numerous SP-containing cell bodies exist in spinal ganglia at all levels (see [31] for review). These cells are small, and they comprise 20–30% of the ganglia cell population. These cells have projections both centrally and peripherally, and the release of SP from the peripheral terminals of sensory fibers can be brought about by nerve stimulation or tissue irritation. A variety of different functions for the peripheral endings of the sensory SP neurons have been described and previously discussed by Lembek [32] in his 1988 Ulf von Euler lecture, as well as by others [33]. These effects include vasodilation, plasma extravasation, histamine release, contraction of smooth muscles, and activation of a slow excitatory postsynaptic potential in postganglionic neurons. It has been suggested that these responses, which occur to different extents in many of the organs so far investigated, are part of a local defense mechanism that was
studied by Sir Thomas Lewis in the 1920s and 1930s and called the "nocifensor system" [34].

In 1901, Bayliss [35] confirmed earlier reports and extended the observation that antidromic stimulation of sensory nerves leads to skin vasodilation with an increase in vascular permeability. Lewis [34] postulated that a cluster of sensory neuron axon collaterals in the skin mediated the flare response by local axon reflexes via release of neurotransmitter onto small vessels. The flare was one of three parts of the "triple response" to injury for a discrete area of skin. This inflammatory response includes 1) the red (erythema) reaction, due to small vessel dilatation in the skin; 2) a flare response, arteriolar dilatation dependent on a local axon reflex; and 3) a wheal, a transient edematous area of skin accompanied by intense itching. The injury can be evoked by either mechanical, thermal, or inflammatory stimuli, and can be reproduced by an intradermal injection of histamine. Histamine alone, however, cannot explain the full extent of reactions that occur during an inflammatory response. It has been suggested that an axon reflex involving primary afferent neurons of the C-fiber type is responsible for the flare reaction [34,35]. Consequently, effort has been aimed at identifying the neurotransmitter substance that mediates this response. A variety of different lines of evidence provide support for the role of SP in mediating the antidromic vasodilation [31–33]. These include 1) the presence of SP associated with pain sensory fibers, which are known to be involved in antidromic vasodilation and the axon reflex mechanism; 2) release of SP from sensory neuron peripheral nerve endings during antidromic stimulation; 3) mimicry of the vasodilation and plasma extravasation upon application of SP; 4) the effects of capsaicin, which depletes SP and other sensory transmitters from chemosensitive C-fiber sensory neurons and blocks antidromic vasodilation and plasma extravasation; and 5) inhibition of antidromic vasodilation by NK-1 receptor peptide antagonists.

The specific events in the "triple" response can be mediated by SP directly, either alone or in combination with other sensory transmitters, or indirectly via release of histamine from mast cells. Foreman and co-workers [36] have evaluated the role of SP in various aspects of the response in humans, and have produced good evidence for the role of a classical NK-1 receptor involvement, as well as an apparently novel SP receptor mechanism. Results obtained with a series of SP peptide fragments and other agonists indicate that the wheal-and-flare responses involve different receptor mechanisms. SP, physalaemin, and SP [4–11] all produce wheal responses, indicative of classical NK-1 receptor involvement. Similar observations have been made for peptides active on plasma extravasation in rats [37,38]. On the other hand, SP and related peptides that produce flare responses seem to do so via their actions as histamine-releasing agents ([39,40] and references therein). Consequently, the N-terminal sequence of SP is quite active in this regard, whereas SP [4–11] and edelsoin, peptides that lack the amino terminal sequence of SP, do not release histamine and do not produce a flare response. The results with NK-1 receptor peptide antagonists on mast cells are complicated, as these antagonists are more potent than SP as histamine-releasing agents. In summary, wheal appears to be at least partly due to SP action at the NK-1 receptor, whereas flare appears to be mediated indirectly via the release of histamine (and perhaps additional mediators) from skin mast cells. The wheal response presumably is due to endothelial cell contraction stimulated by SP, which results in gap formation in capillary walls [41].

SUMMARY

A variety of biologic roles exist for the tachykinins, and recent studies have focused on a molecular characterization of the receptors mediating these responses. Thus, a new perspective has been placed on tachykinin research, and some studies have already been carried out attempting to relate receptor structure to function and to short-term and long-term mechanisms of regulation. With the recent tools generated and an understanding of the mechanisms of receptor action currently being investigated, it seems likely that this will continue to be a fruitful area of research.

REFERENCES

35. Bayliss WM: On the origin from the spinal cord of vasodilator fibers of the hind limb, and on the nature of these fibers. J Physiol 26:173–209, 1901