The Role of Substance P in Inflammatory Disease

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The diffuse neuroendocrine system consists of specialised endocrine cells and peptidergic nerves and is present in all organs of the body. Substance P (SP) is secreted by nerves and inflammatory cells such as macrophages, eosinophils, lymphocytes, and dendritic cells and acts by binding to the neurokinin-1 receptor (NK-1R). SP has proinflammatory effects in immune and epithelial cells and participates in inflammatory diseases of the respiratory, gastrointestinal, and musculoskeletal systems. Many substances induce neuropeptide release from sensory nerves in the lung, including allergen, histamine, prostaglandins, and leukotrienes. Patients with asthma are hyperresponsive to SP and NK-1R expression is increased in their bronchi. Neurogenic inflammation also participates in virus-associated respiratory infection, non-productive cough, allergic rhinitis, and sarcoidosis. SP regulates smooth muscle contractility, epithelial ion transport, vascular permeability, and immune function in the gastrointestinal tract. Elevated levels of SP and upregulated NK-1R expression have been reported in the rectum and colon of patients with inflammatory bowel disease (IBD), and correlate with disease activity. Increased levels of SP are found in the synovial fluid and serum of patients with rheumatoid arthritis (RA) and NK-1R mRNA is upregulated in RA synoviocytes. Glucocorticoids may attenuate neurogenic inflammation by decreasing NK-1R expression in epithelial and inflammatory cells and increasing production of neutral endopeptidase (NEP), an enzyme that degrades SP. Preventing the proinflammatory effects of SP using tachykinin receptor antagonists may have therapeutic potential in inflammatory diseases such as asthma, sarcoidosis, chronic bronchitis, IBD, and RA. In this paper, we review the role that SP plays in inflammatory disease. J. Cell. Physiol. 201: 167–180, 2004. © 2004 Wiley-Liss, Inc.

The neuro-immune axis is a bidirectional pathway of intersystem communication. Immune responses alter neural function, and in turn, neural activity modifies immunologic function. Inter-system cross-talk is mediated via a common biochemical language of shared ligands such as cytokines and neuropeptides, and their receptors. Mediators classically thought to be synthesized exclusively by the nervous system, are now known to be produced by immunocytes, and vice versa. The diffuse neuroendocrine system consists of specialised endocrine cells and peptidergic nerves and is present in all organs of the body, including the respiratory tract.

Tachykinins are a family of neuropeptides that share the carboxy-terminal sequence Phe-X-Gly-Leu-Met-NH₂, where X is an aromatic (Tyr or Phe) or hydrophobic (Val or Ile) amino acid (Uddman et al., 1997). This common sequence is essential for the tachykinin’s receptor interaction and activation, whereas the distinct amino-terminal sequences of the tachykinins provide their receptor subtype specificity. Five tachykinin peptides have been identified in mammals: substance P (SP), neuropekin A, neuropeptide K, neuropeptide-γ, and neuropepin B.

In mammals, two separate genes encode the tachykinins designated preprotachykinin I (PPT-I) and preprotachykinin II (PPT-II) (Table 1) (Nawa et al., 1984). The PPT-I gene can express four distinct forms of mRNA through alternative splicing, two of which (the β and γ forms) encode synthesis of both SP and NKA, whilst the other two, the α and δ forms, encode SP only (Nawa et al., 1984). The β and γ forms of PPT-I mRNA also encode the synthesis of neuropeptide K and neuropeptide-γ, which are amino-terminally extended forms of NKA, although their function has not been fully clarified (Tatemoto et al., 1985; Kage et al., 1988). The PPT-II gene gives rise to neuropepin B (Hokfelt et al., 2001).

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SUBSTANCE P

SP was discovered by von Euler and Gaddum (1931). They reported that extracts of equine brain and intestine contained a hypotensive and spasmogenic factor. The preparation, termed preparation P, was later found to be proteinaceous. The isolation from bovine hypothalamus and characterization of SP was carried out by Leeman’s group in 1970–1971 (Chang et al., 1971). The structure of SP is as follows:

\[
\text{SP} : \text{Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH}_2
\]

SP is synthesized in the ribosome as a larger protein and then enzymatically converted into the active undecapeptide. The peptide is widely distributed in the central and peripheral nervous systems of vertebrates. In the central nervous system, SP is thought to participate in various behavioral responses and in regulating neuronal survival and degeneration. SP also regulates cardiovascular and respiratory function and is involved in activating the emetic reflex. In the spinal cord, SP participates in neurotransmission of pain and noxious stimuli and modulates autonomic reflexes, including the micturition reflex. In the peripheral system, SP is localized in the primary sensory neurons and neurons intrinsic to the gastrointestinal, respiratory tracts, and genitourinary tracts (Maggi, 2000).

Tachykinin receptors

Tachykinin effects on target cells are mediated by at least three specific receptors, the neurokinin-1 receptor (NK-1R), NK-2R, and NK-3R. These receptors are members of the superfamily of guanine nucleotide binding-coupled receptors, which interact with G-proteins to promote high-affinity binding and signal transduction (Hershey and Krause, 1990). These receptors are glycoproteins with seven putative α-helical transmembrane segments, an extracellular amino-terminus and an intracellular carboxyl tail (Fig. 1). G-protein-linked receptors are generally associated with low abundance mRNA. For example, only 15 transcripts per cell have been reported for the β2-adrenergic receptor (Haddock et al., 1989).

Each tachykinin appears to preferentially activate a distinct tachykinin receptor, although at high ligand concentration, each tachykinin can activate each of the tachykinin receptors. The NK-1R is activated preferentially by SP, the NK-2R by NKA, and the NK-3R by NKB (Nakanishi, 1991). The conserved carboxyl terminal domain of tachykinins interacts with the neurokinin receptors, while the unique amino terminal sequences of tachykinins dictate receptor specificity. The relative affinity of NK-1R for neurokinin A and neurokinin B is 100- and 500-fold lower than for SP, respectively (Gerard et al., 1991). Recently, a fourth tachykinin receptor has been cloned (Donaldson et al., 2001).

TABLE 1. Genes encoding synthesis of mammalian tachykinins

<table>
<thead>
<tr>
<th>Preprotachykinin I gene</th>
<th>Preprotachykinin II gene</th>
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<tr>
<td>α-PPT-I mRNA</td>
<td>PPT-II mRNA</td>
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<tr>
<td>Substance P (SP)</td>
<td>Neurokinin B</td>
</tr>
<tr>
<td>β-PPT-I mRNA</td>
<td>SP, neurokinin A, neuropeptide K</td>
</tr>
<tr>
<td>γ-PPT-I mRNA</td>
<td>SP, neurokinin A, neuropeptide-γ</td>
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<tr>
<td>δ-PPT-I mRNA</td>
<td>SP</td>
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Fig. 1. The neurokinin-1 receptor (NK-1R) is a glycoprotein with seven putative α-helical transmembrane segments, an extracellular amino-terminus and an intracellular carboxyl tail. The human NK-1R consists of 407 amino acid residues and has a relative molecular mass of 46 kDa. The second and third membrane-spanning domains are involved in agonist/antagonist binding, the third cytoplasmic loop is responsible for G-protein interaction, while the cytoplasmic carboxyl terminal contains many serine and threonine residues which when phosphorylated, cause desensitization of the receptor in response to repeated application of agonist.
The human NK-1R consists of 407 amino acid residues and has a relative molecular mass of 46 kDa (Hopkins et al., 1991). The second and third membrane-spanning domains are involved in agonist/antagonist binding, the third cytoplasmic loop is responsible for G-protein interaction, while the cytoplasmic carboxy terminal contains many serine and threonine residues which when phosphorylated, cause desensitization of the receptor in response to repeated application of agonist. Binding of SP to NK-1R mediates rapid endocytosis and internalization of the receptor; this also contributes to desensitization of cells to SP signaling. Agonist stimulation of the NK-1R in many tissue and cell types causes activation of phospholipase C, which catalyzes the hydrolysis of phosphoinositides into inositol 1,4,5-trisphosphate and diacylglycerol. These second messengers are then available for the mobilization of calcium from internal reticular stores, and for the activation of protein kinase C. In Chinese hamster ovary cells, rat NK-1R has been shown to activate both phospholipase C and adenylate cyclase, and thus to stimulate both phosphoinositide metabolism and cAMP formation (Li et al., 1997).

The gene for the human NK-1R is located on chromosome 2, spans 45–60 kb and is contained in five exons, with introns interrupting at sites homologous to those in the NK-2R gene (Gerard et al., 1991). The 5′ flanking region of the NK-1R gene has several putative transcriptional regulatory DNA elements, such as a cAMP responsive element, an AP-1, AP-2, AP-4, NF-xB, OCT-2, and a Sp-1 site. Comparison of rat and human NK-1R sequences reveals 94.5% homology (Takeda et al., 1991).

THE ROLE OF SP IN INFLAMMATION

Evidence to support the involvement of SP in the pathophysiology of inflammatory disease stems from observations of aberrant levels of SP and of SP-nerve fibres in diseased tissue, aberrant expression of NK-1R in diseased tissue, and beneficial effect of NK-1R antagonists and NK-1R knockout in animal models of inflammatory disease. Tachykinins are biologically active at extremely low concentrations (Kranzfeld and Nijkamp, 2001).

Although SP has been described as a peptide of neuronal origin, studies in rodents have demonstrated its production by inflammatory cells such as macrophages, eosinophils, lymphocytes, and dendritic cells (Weinstock et al., 1988; Bost et al., 1992; Killingsworth et al., 1997; Joos et al., 2000). SP enhances lymphocyte proliferation and immunoglobulin production, and enhances cytokine secretion from lymphocytes, monocytes, macrophages, and mast cells (Stanisz et al., 1986; Lotz et al., 1988; Scicchitano et al., 1988; Pascual et al., 1991; Bost and Pascual, 1992; Calvo et al., 1992; Ansel et al., 1993; Covas et al., 1994; Ho et al., 1996; Maggi, 1997). SP-induced release of inflammatory mediators such as cytokines, oxygen radicals, arachidonic acid derivatives, and histamine potentiates tissue injury, and stimulates further leukocyte recruitment, thereby amplifying the inflammatory response (Holzer and Holzer-Petsche, 1997).

SP elicits local vasodilatation and alters vascular permeability, thus enhancing the delivery and accumulation of leukocytes to tissues for the expression of local immune responses (Pernow, 1983). SP can specifically stimulate the chemotaxis of lymphocytes, monocytes, neutrophils, and fibroblasts (Haines et al., 1993; Kahler et al., 1993; Schratzberger et al., 1997). SP has been reported to induce the expression of endothelial-leucocyte adhesion molecule-1 on human microvascular endothelium, to increase the expression of the leukocyte integrin CD11b on human neutrophils, and to enhance the expression of intercellular adhesion molecular-1 and leucocyte function-associated antigen-1 on murine endothelial cells, and lymphocytes (Matis et al., 1990; DeRose et al., 1994; Vishwanath and Mukherjee, 1996). SP induces a rapid influx of neutrophils and eosinophils in human dermis, occurring in parallel with translocation of P-selectin and upregulation of E-selectin (Smith et al., 1993). By promoting vasodilatation, leukocyte chemotaxis, and leukocyte/endothelial cell adhesion, SP ensures the extravasation, migration, and subsequent accumulation of leukocytes at sites of injury.

SP has also been implicated in the resolution of inflammation. Evidence of a role for SP in tissue repair has been primarily derived from studies detailing its proliferative effect on a variety of cells. SP acts as a mitogen for smooth muscle cells, fibroblasts, endothelial cells, and synoviocytes (Nilsson et al., 1986; Lotz et al., 1987; Ziche et al., 1990; Rameshwar et al., 1997). Indeed, a role for SP in angiogenesis has been proposed (Fan et al., 1993). SP has been implicated in inflammatory reactions of such diverse tissues as the lung (Adcock et al., 1993a; Bozic et al., 1996; Colten and Krause, 1997), the gut (Manthy et al., 1988, 1994), the joints (Levine et al., 1984; Krause et al., 1995), the skin (Foreman, 1987), and the eyes (Bill et al., 1979). The proinflammatory effects of SP on various immune cells are summarized in Table 2.

SP and immunoregulation: lymphocytes

A diverse spectrum of immunoregulatory effects on lymphocyte function has been described for SP (Payan and Goetzl, 1985). SP is a chemoattractant for human lymphocytes and increases lymphocyte traffic and lymph flow through the peripheral lymph nodes of sheep (Moore et al., 1989; Schratzberger et al., 1997). SP acts as a B lymphocyte differentiation cofactor and increases immunoglobulin secretion (particularly IgA) by murine Peyer’s patches, splenic lymphocytes, and mesenteric lymph nodes in an isotype-specific manner (Stanzis et al., 1986; Scicchitano et al., 1988; Bost and Pascual, 1992). In experiments using established B cell lymphoma clones, SP directly stimulated IgA, but not IgM secretion. However, in the presence of LPS, SP stimulated a threefold increase in IgM secretion (Pascual et al., 1991). Depletion of SP in rodents by capsaicin administration or treatment with the SP antagonist spantide, reduced the number of antibody secreting cells (Eglezos et al., 1990). SP can also enhance immunoglobulin secretion by human B-cells and IL-2 production by human T cells and murine T cell lines (Bost and Pascual, 1992; Calvo et al., 1992). SP enhances macrophage inflammatory protein-1α expression and natural killer activity in T lymphocytes (Croitoru et al., 1990; Guo et al., 2002). SP can stimulate the proliferation of human T lymphocytes at concentrations as low as...
Inhibitory effects of SP on lymphocyte proliferation have also been documented (Krco et al., 1986). SP is a chemoattractant for human monocytes (Schratzberger et al., 1997). The chemotactic activity of SP resides in its C-terminal amino acid sequence. SP can also stimulate the secretion of cytokines such as IL-1, TNF-α, and IL-6 from monocytes and macrophages (Bill et al., 1979; Lotz et al., 1988; Ho et al., 1996). SP induces oxidative burst, and stimulates the synthesis and release of arachidonic acid metabolites, prostaglandin E2, thromboxane B2, and toxic oxygen radicals in guinea-pig peritoneal macrophages (Murris-Espin et al., 1995). SP enhances the phagocytosis of murine macrophages via its N-terminus (Bar-Shavit et al., 1980). Recently, the expression of SP and the NK-1R by human peripheral monocytes and sputum macrophages has been shown, suggesting that macrophages may be a major source of SP in inflammatory airway diseases (Ho et al., 1997; Germonpre et al., 1999). The expression of SP and NK-1R by human peripheral monocytes and sputum macrophages has been shown, suggesting that macrophages may be a major source of SP in inflammatory airway diseases (Ho et al., 1997; Germonpre et al., 1999).

**SP and immunoregulation: mast cells**

Direct evidence for a close contact between mast cells and nerves has been obtained. A large proportion of rat intestinal mucosal cells are in direct contact with nerves, some of which contain SP or CGRP (Stead et al., 1987). Such an association has also been found in rat lung, human intestine, and skin (Stead et al., 1989; Nilsson et al., 1990; Naukkarinen et al., 1993). SP induces degranulation and histamine and serotonin release from human and rat mast cells by a receptor-independent mechanism (Shanahan et al., 1985; Repke and Bienert, 1987). SP-triggering of mast cells involves insertion of the amphiphilic SP molecule into the cell membrane, thus enabling direct activation of G proteins (Mousli et al., 1990). Stimulation of murine mast cells with SP activates TNF-α gene expression and induces TNF-α secretion (Ansel et al., 1993).

**SP and immunoregulation: eosinophils**

Eosinophil activation by SP is reported to cause their degranulation, release of O₂⁻ and thromboxane B₂. The stimulatory effect of SP on the degranulation of guinea pig eosinophils is mediated via its N-terminus, and is thought to be receptor-independent (Kroegel et al., 1990). Eosinophils from allergic and normal subjects differ in their chemotactic response to SP. SP alone was not chemotactic for eosinophils, whereas the chemotactic response to platelet-activating factor and leukotriene B₄ of eosinophils derived from asthmatic but not normal subjects was enhanced by pre-treatment with low concentrations of SP (Numao and Agrawal, 1992).
continuous pathway of sensory nerves containing SP from the epithelium to arterioles in bronchial mucosa, providing structural support for a local axon reflex (Lamb and Sparrow, 2002). Neuropeptides and capsaicin stimulate the release of inflammatory cytokines in a human bronchial epithelial cell line. Exposure to SP resulted in immediate increases in intracellular calcium, followed by the synthesis of the transcripts for and the release of the proteins of the inflammatory cytokines IL-6, IL-8, and TNF-α (Veronesi et al., 1999). Neuropeptides may also stimulate proliferation of human airway epithelial cells, suggesting a contrasting role of repair after epithelial injury (Kim et al., 1995).

**SP and immunoregulation: hematopoiesis**

SP-immunoreactive nerve fibers have been detected in bone marrow (Weihe et al., 1991). Electron microscopic studies have demonstrated direct synapse-like contacts between nerve endings and the cytoplasmic processes of reticular and fibroblastoid cells of bone marrow. SP stimulates bone marrow progenitors of both erythroid and myeloid lineages, and has, therefore, been implicated in hematopoiesis (Rameshwar et al., 1993). The hematopoietic effect of SP is primarily due to its stimulatory influence on bone marrow stroma (macrophages, reticular adventitial cells, adipocytes, endothelial cells, and fibroblasts). SP induces production of essential hematopoietic growth factors such as IL-1 and stem cell factor by murine bone marrow stroma, and these cytokines in turn regulate stromal expression of NK-1R (Rameshwar and Gascon, 1995). SP also induces IL-3 and granulocyte-macrophage colony stimulating factor by human bone marrow mononuclear cells, and the expression of NK-1R mRNA in human bone marrow fibroblasts, which in turn, proliferate in response to SP stimulation (Rameshwar et al., 1997).

**SP and apoptosis**

SP has been shown to have antiapoptotic effects in many cells, including macrophages, neutrophils, and thymocytes (DeFea et al., 2000; Dimri et al., 2000; Bockmann et al., 2001; Kang et al., 2001). Glucocorticoids and SP appear to have opposing effects in thymocytes, with glucocorticoids acting as strong inducers of apoptosis and SP counteracting this effect (Dimri et al., 2000). Lung epithelial apoptosis contributes to the pathophysiology of asthma, idiopathic pulmonary fibrosis (IPF), and acute lung injury (Kuwano et al., 1999; Matute-Bello et al., 1999; Trautmann et al., 2002). The antiapoptotic effect of SP may protect against epithelial cell injury in these diseases. Tachykinins may stimulate apoptosis in small cell lung cancer, and blocking the effects of SP increases apoptosis in cancer cells (Bepler et al., 1988).

In contrast, SP may promote apoptosis in other biological systems, either directly or indirectly through its ability to induce secretion of proinflammatory cytokines which may promote apoptosis. SP potentiates and NK-1R antagonists protect mice from CD95 and TNF-α-mediated apoptotic liver damage (Bang et al., 2003). SP can also induce a non-apoptotic form of programmed cell death that is independent of caspase activation (Castro-Obregon et al., 2002). Therefore, the effects of SP on cell death and survival are tissue specific and may also depend on the inflammatory microenvironment of the tissue.

**SP in the lung**

In the human respiratory tract, SP-immunoreactive nerves are located beneath and within the epithelium, around submucosal bronchial glands, bronchial blood vessels, and to a lesser extent within airway smooth muscle (Helke et al., 1990; Barnes et al., 1991; Solway and Leff, 1991). Significant amounts of SP are found in central and peripheral airway tissues, as well as bronchoalveolar lavage (BAL) fluid and sputum (Nieber et al., 1992; Lilly et al., 1995a; Tomaki et al., 1995). The content of SP in human lung may decrease with age and after denervation (Hislop et al., 1990).

Neural regulation of the airways consists of cholinergic excitatory, adrenergic inhibitory nerves, and non-adrenergic, non-cholinergic (NANC) nerves. Cholinergic nerves form the predominant bronchoconstrictor neural pathway in human airways. Acetylcholine controls neuronal and non-neuronal target cells via a short-lived action at nicotinic and muscarinic receptors. NANC nerves can be either inhibitory or excitatory. Non-cholinergic excitatory nerves generate antidromic pulses and a local axon reflex, which leads to non-cholinergic bronchoconstriction, plasma extravasation, and vasodilatation (Joos et al., 1987b). SP and neurokinin A are thought to mediate the excitatory part of the NANC nervous system. NK-2Rs are present on smooth muscle of both large and small airways and mediate part of the bronchoconstrictor effect of tachykinins. Most of the proinflammatory effects of SP are mediated by the NK-1R. An extensive cross-talk exists between nerves and the immune system. The complexity of the picture has increased further as it has become clear that classical neurotransmitters, such as acetylcholine and neuropeptides, are produced by non-neuronal cells (Joos, 2001).

Tachykinins released from sensory C-fibres induce neurogenic inflammation, characterized by vasodilatation, increased postcapillary venule permeability, and neutrophil adherence to blood vessels (Umeno et al., 1990; Piedimonte et al., 1993). Neuropeptides can be released from sensory nerves by a range of substances, including allergen, ozone, or bronchoactive agonists such as histamine, prostaglandins, and leukotrienes (Kaufman et al., 1980; Martins et al., 1991b; Takebayashi et al., 1998). Tachykinin-mediated inflammatory responses may be enhanced in endotoxaemia and implicated in endotoxin-induced lung injury. LPS enhances SP-mediated neutrophil accumulation in the lungs and vascular permeability in guinea pig airways (Kuo et al., 1998). Tachykinins, especially SP through the NK-1 receptor, induce a series of leukocyte responses to trigger and amplify the inflammatory processes, including upregulation of ICAM-1 expression on vascular endothelial cells and enhancement of neutrophil transendothelial migration, mediating leukocyte adhesion to the endothelial or epithelial cells in the airways (DeRose et al., 1994; Baluk et al., 1995; Nakagawa et al., 1995; Bhatia et al., 1998). SP also stimulates human monocytes to release IL-1, IL-6, and TNF (Lotz et al., 1988).
Animal studies using NK-1R antagonists and NK-1R gene knockout mice further support the theory that the NK-1R may be involved in the pathogenesis of airway inflammation (Bozic et al., 1996; Kaltreider et al., 1997). Increased expression of NK-1R mRNA by alveolar macrophages was observed in a murine model of antigen-induced airway inflammation (Kaltreider et al., 1997).

**Neurokinin receptors in the lung**

NK-1Rs and NK-2Rs are present in several structures of human central airways, including smooth muscle, glands, vessels, and pulmonary arteries. NK-1R and NK-2R mRNA are found in equal abundance in bronchi and subpleural lung, suggesting multiple sites of action of neuropeptides (Solway and Leff, 1991). Different tachykinin receptors are involved in the direct and indirect bronchoconstrictor effect of tachykinins in the rat (Joos et al., 1994). In other words, tachykinins may cause bronchoconstriction directly at high concentrations, mediated by direct interaction with NK-1Rs, and indirectly through mast cells and cholinergic nerves.

Tachykinin-induced pulmonary stretch receptor activation and airway effects of capsaicin are mediated by the activation of NK-2Rs (Matsumoto et al., 1997; Vieira et al., 1997). Stimulation of NK-1 receptors causes relaxation of human pulmonary arteries which is mediated largely by nitric oxide and prostacyclin released from the endothelium (Corboz et al., 1998).

**Neutral endopeptidase**

Tachykinins are degraded by the enzymes neutral endopeptidase (NEP) and angiotensin converting enzyme (ACE) (Joos et al., 2000). NEP is more important than ACE in airway neuropeptide metabolism (Martins et al., 1991a). NEP maintains low levels of SP in the extra-cellular fluid under basal conditions and terminates its proinflammatory effects. NEP has been localized in the lungs of different animal species (Nadel and Borson, 1991). NEP is present in the basal cells of the epithelium, type II alveolar cells, neutrophils, submucosal glands, airway smooth muscle, and postcapillary venules and nerves. Inhibition of NEP enhances various airway effects of exogenously administered SP and tachykinins, including airway smooth muscle contraction, plasma extravasation, and airway mast cell activation (Nadel and Borson, 1991; Roques et al., 1993). Moreover, the airway effects of endogenous sensory neuropeptides are enhanced in the presence of a NEP inhibitor (Cheung et al., 1993). NEP and ACE participate in the metabolism of SP when administered intravascularly, whilst NEP degrades tachykinins administered by aerosol (Shore et al., 1988).

A variety of environmental irritants and sensitizers, such as toluene diisocyanate, cigarette smoke, and hypochlorous acid, and pathogens, such as the human influenza virus A/Taiwan, the Sendai virus and Mycoplasma, decrease airway NEP and increase the response of airways to SP and NKA (Borson, 1991; Nadel, 1991). Glucocorticoids can reduce the magnitude of plasma extravasation produced in the rat trachea, mediated, in part, by an upregulation in NEP synthesis (Piedimonte et al., 1990). Intestinal inflammation results in down-regulation of NEP, which may contribute to uncontrolled inflammation (Sturiale et al., 1999).

**Asthma**

SP-immunoreactive nerves are increased in the submucosa of patients with severe or fatal asthma and NEP in tissue may be reduced (Nadel and Borson, 1991; Ollerenshaw et al., 1991). Airway epithelial damage may stimulate sub-epithelial sensory nerves, with subsequent release of neuropeptides (Laitinen et al., 1985). Increased amounts of SP are found in sputum and BAL fluid of asthmatics and levels in BAL fluid increase after intra-segmental allergen challenge in atopics (Nieber et al., 1992; Tomaki et al., 1995). SP causes many of the typical changes observed in asthmatic airways (Fig. 2), including bronchoconstriction, increased mucus secretion, facilitation of cholinergic neurotransmission, vasodilatation, and plasma leakage (Lundberg et al., 1983; Laitinen et al., 1985; Martling et al., 1987; Helke et al., 1990; Kuo et al., 1990; Barnes et al., 1991; Mantyh, 1991; Solway and Leff, 1991). Tachykinins cause macrophage chemotaxis, mast-cell degranulation, T-cell recruitment, and B-cell immunoglobulin production in respiratory tissues (Goetzl and Sreedharan, 1992; Tiberio et al., 1997). Furthermore, SP and NKA are potent mitogens of smooth muscle cells, endothelial cells, epithelial cells, and fibroblasts and hence are potential mediators of the thickened airways found in asthma (Nilsson et al., 1985; Kuwano et al., 1993; Harrison et al., 1995; Kim et al., 1995).

NK-1Rs are predominantly localized to bronchial vessels, epithelial cells, submucosal glands, and vascular endothelium, whereas NK-2Rs are predominantly localized to airway smooth muscle (Bai et al., 1995; Strigas and Burcher, 1996). SP is more potent than NKA in stimulating airway mucus secretion, microvascular leakage, and vasodilation whereas NKA is a more potent constrictor of human bronchi than SP (Sheldrick et al., 1995; Van Rensen et al., 2002). Conflicting data exist with regard to NK-1R expression in asthma. Some studies have shown upregulated NK-1R expression in asthmatic lung compared to normal controls, whereas others have shown no differences (Solway and Leff, 1991; Adcock et al., 1990a; Chu et al., 2000).

Asthmatics are hyperresponsive to SP and NKA (Joos et al., 1987a; Cheung et al., 1994). Incubating human bronchi with serum from asthmatic patients atopic to *Dermatophagoides pteronyssinus* caused an enhanced sensitivity and contractile response to SP and NKA (Ben-Jebara et al., 1993). Normal airway smooth muscle becomes hyperresponsive to acetylcholine and tachykinins following exposure to IL-1β and TNF-α. IL-1β-enhanced cholinergic airway smooth muscle contractile responses are mediated by the actions of SP released from intrinsic airway neurons (Wu et al., 2002). Animal and human studies have suggested that tachykinins may cause bronchoconstriction both directly and indirectly, by activation of postganglionic cholinergic nerves and mast cells (Lundberg et al., 1983; Joos et al., 1988; Mantyh, 1991). SP can liberate histamine from airway and BAL fluid mast cells, but the lack of effect of specific H1-antagonists on tachykinin-induced bronchoconstriction in asthmatics suggests that histamine does not play a major role (Crimi et al., 1990; Lilly et al., 1995b).
Genetic factors appear to determine the magnitude of the airway response to tachykinins (Pauwels et al., 1993). Corticosteroids may attenuate neurogenic inflammation in asthmatic lung. The NK-1R gene has glucocorticoid-responsive elements, indicating that steroids may modulate transcription (Ihara and Nakanishi, 1990). A comparison of the NK-1R gene with known promoter elements has indicated a region at −52 to −45 from the transcription start site that resembles a consensus activation protein-1-binding site (Sheng et al., 1988). Binding at these sites is stimulated by long-term mediators of inflammation, such as cytokines, and reduced by glucocorticoids (Adcock et al., 1993b). This suggests increased NK-1R gene expression due to the chronic inflammatory process that can be down-regulated by steroid therapy. Experiments in rat pancreatic acinar cells and human IM-9 lymphoblasts indicate a decrease in NK-1R mRNA after glucocorticoid treatment and NK-1R mRNA is reduced in asthmatic lung specimens after incubation with dexamethasone (Ihara and Nakanishi, 1990; Gerard et al., 1991; Adcock et al., 1993a). Recent studies have shown that inhaled steroid reduces bronchial responsiveness to tachykinins in patients with asthma (Van Schoor et al., 2002).

NK-1R antagonists may have therapeutic potential in patients with asthma. Pretreatment of antigen-sensitized guinea pigs with NK-1R antagonists prevented the development of bronchial hyperreactivity. Tachykinin antagonists prevent the bronchoconstriction and increased permeability in an animal model of exercise-induced asthma, and a combined NK1/NK2 antagonist prevents bradykinin-induced bronchoconstriction in asthmatics (Ichinose et al., 1992; Solway et al., 1993).

The role of SP in other diseases of the respiratory tract

Neurogenic inflammation participates in virus-associated respiratory infection. Respiratory syncitial virus (RSV) makes the airway susceptible to the
proinflammatory effects of SP by upregulating SP NK-1R expression on airway cells (King et al., 2001). This effect may contribute to the inflammatory reaction to the virus and could be a target for the therapy of RSV disease (Piedimonte et al., 1999). Upregulation of lymphocyte NK-1R expression may participate in the development of primary and secondary immune responses to respiratory virus infections (Tripp et al., 2002). Parainfluenza virus-induced M2 receptor dysfunction and hyperresponsiveness are prevented by a NK-1R antagonist, but not by a NK-2R antagonist, whereas both antagonists had similar anti-inflammatory effects (Jacoby et al., 2000). The acute airway inflammation observed in patients after exposure to adenoviral vectors may also exhibit a neurogenic component (Piedimonte et al., 1997).

Cigarette smoking releases SP from sensory nerves, induces adhesion of leukocytes to tracheal mucosa, decreases NEP activity, and exaggerates neurogenic inflammatory responses (Lundberg and Saria, 1983; Dusser et al., 1989; Baluk et al., 1996). Chronic smoke-induced airway hyperresponsiveness is related to an increase in SP synthesis and release in neurons innervating the lungs and airways (Kwong et al., 2001). Although some studies have shown no differences, others have shown that NK-1R and NK-2R expression are increased in the lungs of smokers (Solway and Leff, 1991; Mapp et al., 2000). Smoke-induced bronchoconstriction in guinea pigs consists of an early phase induced by both a cholinergic reflex and tachykinin release, probably evoked by the activation of broncho-pulmonary C fibres, and a late phase caused by the action of arachidonic acid metabolites (Hong et al., 1995). These findings may help explain the increased incidence of airway hyperresponsiveness and cough in people exposed to tobacco smoke.

Tachykinin-containing capsaicin-sensitive nerves may play a role in the generation of non-productive cough (Karlsson, 1993). Capsaicin stimulates airway C-fibres, and is one of the most potent tussigenic stimuli known. Airway rapidly adapting afferent nerves also participate in the cough reflex. These nerves do not normally contain tachykinins, but begin to produce them in inflamed airways of allergic inflammation and viral infection (Hunter et al., 2000; Carr et al., 2002). Patients with non-asthmatic chronic cough have high levels of SP and interleukin-8 in sputum, as well as mild neutrophilia (Pizzichini et al., 1999). SP is released during allergic reactions in the nose and causes an increase in nasal microvascular leakage in patients with allergic rhinitis (Braunstein et al., 1991).

Increased levels of SP have been found in BAL fluid recovered from patients with IPF and sarcoidosis, and SP activates monocytes recovered from BAL fluid more in patients with IPF and sarcoidosis than healthy volunteers (Takeyama et al., 1996; Brunelleschi et al., 2000). We recently demonstrated upregulated NK-1R expression in BAL cells, bronchial epithelium, and granulomas of patients with sarcoidosis compared with normal controls (O’Connor et al., 2003). SP, by activating the NK-1R, stimulates the secretion of TNF-α, a critical cytokine in the pathogenesis of granulomatous inflammation in sarcoidosis, from alveolar macrophages and epithelial cells (Ho et al., 1996; Kuo et al., 2000). SP, acting through upregulated NK-1R expression, may increase proinflammatory cytokine production in the lungs of patients with sarcoidosis and thus amplify localized pulmonary inflammatory responses. In contrast to untreated patients with sarcoidosis, we were unable to detect NK-1R expression in BAL cells and endobronchial biopsies of a patient with sarcoidosis who was taking high dose corticosteroids (O’Connor et al., 2003). Furthermore, culture of alveolar and bronchial epithelial cells in dexamethasone downregulated NK-1R expression on these cells (O’Connor et al., 2003). We suggest that the downregulatory effect of corticosteroids on NK-1R expression may explain, in part, the disease modifying effect of corticosteroids in sarcoidosis.

**SP IN THE GASTROINTESTINAL TRACT**

The enteric nervous system of the gut is comprised of approximately 10^9 neurons, which contain a plethora of peptidergic neurotransmitters. The gastrointestinal mucosa, rich in peptidergic innervation and immune content, therefore, provides the ideal milieu for neuro-immune interactions to occur. SP regulates smooth muscle contractility, epithelial ion transport, vascular permeability, and immune function in the gastrointestinal tract (Pernow, 1983; Lordal et al., 1996). In the human colon, SP nerve fibres ramify throughout the lamina propria and to form dense networks beneath the epithelium (Keast et al., 1985). SP-containing subepithelial nerves are in intimate contact with intestinal mucosal mast cells of the rat (Stead et al., 1989). However, neurons are not the exclusive source of SP. Enteroeocendocrine cells, human colonic eosinophils, rat ileal macrophages, and mouse colonic glia express SP (Keast et al., 1985; Bernstein and Vidrich, 1994; Metwali et al., 1994; Castagliuolo et al., 1997).

Repeated oesophageal acidification caused by relaxation of the lower oesophageal sphincter occurs in gastro-oesophageal reflux disease. Intra-oesophageal SP causes SP release from extrinsic afferent nerve endings which activates local inhibitory pathways to the lower oesophageal sphincter via NK-1Rs (Blackshaw and Dent, 1997). SP acts on NK-1R cholinergic vagal neurons in the dorsal motor nucleus of the vagus nerve, which control enteric NANC motor inhibition of the gastric fundus (Chang et al., 1999). Therefore, an antiemetic site of action of NK-1R antagonists may be in the dorsal motor nucleus to prevent excitation of neurons controlling fundic relaxation (Ladabaum and Hasler, 1999). Tachykinins stimulate duodenal contraction and bicarbonate secretion and are potent pancreatic circulatory stimulants and secretagogues (Pawlik et al., 1992). SP regulates the severity of acute pancreatitis and pancreatitis-associated lung injury (Bhattia et al., 1998; Grady et al., 2000). Localization of IL-8 positive immune cells around pancreatic nerves in chronic pancreatitis supports the existence of a neuroimmune interaction (Di Sebastiano et al., 2005). SP and NKA are synthesized by enteric cholinergic motor neurons that project to the longitudinal and circular muscle of the intestine (Maggi, 1990). NK-1R is the primary tachykinin receptor involved in NANC transmission (Saban et al., 1999). NK-1R stimulation evokes a myogenic excitatory and a neurogenic inhibitory motor
response (Lecci et al., 1999). Interstitial cells of Cajal function as pacemakers of rhythmic activity and intermediaries in neural input from the enteric nervous system to the muscle, and express NK-1R (Epperson et al., 2000). Increased NK-1R expression is seen in human colonic mucosal mononuclear cells when compared to peripheral blood mononuclear cells, suggesting a direct role for SP in mucosal immunomodulation (Goode et al., 1998, 2000b).

**Inflammatory bowel disease**

The pathogenesis of IBD represents an interaction between genetic predisposing factors, exogenous, and endogenous triggers, and modifying factors, resulting in a spontaneously relapsing and remitting inflammatory process (Shanahan, 1993). Elevated levels of SP have been reported in the rectum and colon of UC patients, and correlate with disease activity (Koch et al., 1987; Mazumdar and Das, 1992; Bernstein et al., 1993). Other studies show an increase in SP nerves in hypervascular lesions, but a decrease in severe inflammatory lesions of UC colon (Kimura et al., 1994). Conflicting results also exist regarding SP in Crohn's disease (CD). Mucosal levels of SP were found to be significantly decreased in the rectum of patients with CD (Bernstein et al., 1993). Others have reported no significant difference between mucosal levels of SP from CD patients and those of controls (Koch et al., 1987). An increased density of SP-immunoreactive fibres has been demonstrated in hypervascular lesions of CD and in CD colon (Mazumdar and Das, 1992; Kimura et al., 1994). Autoradiographic studies demonstrated a 1,000-fold upregulation of SP binding sites in the lymphoid follicles and submucosal vasculature of patients with IBD (Mantyh et al., 1988, 1994). We have recently shown that proinflammatory cytokines induce NK-1R expression in colonic epithelial cells, suggesting that colonic inflammation may potentiate further SP-induced inflammatory and proliferative effects (Goode et al., 2003). Furthermore, we have shown marked upregulation of NK-1R mRNA levels in IBD colonic mucosal biopsies compared with non-inflamed mucosal expression levels (Goode et al., 2000a).

Considerable evidence has implicated SP in the pathophysiology of experimental models of IBD. Elevated levels of SP have been associated with *Trichinella spiralis*-induced enteritis (Swain et al., 1992; Agro and Stanisz, 1993). Increased SP levels were dependent on the presence of lymphocytes, as the effect was abolished in athymic rats (Swain et al., 1992). Furthermore, blockade of SP with either SP antibodies, or with the NK-1R antagonist CP 96,345, reduced jejunal inflammation (Agro and Stanisz, 1993; Kataeva et al., 1994).

SP plays an important role in *Clostridium difficile* toxin A-induced enterocolitis. Intraluminal administration of toxin A induced release of SP from primary afferent neurons, while capsaicin pre-treatment inhibited toxin A-mediated fluid secretion, neutrophil infiltration, myeloperoxidase activity, and rat mast cell protease II release in the rat ileum (Mantyh et al., 1996b; Wershil et al., 1998). Administration of specific SP antagonists inhibited toxin A-mediated TNF-α release from isolated intestinal macrophages (Pothoulakis et al., 1994; Castagliuolo et al., 1997). Mice genetically deficient in the NK-1R were protected from the secretary and inflammatory changes (Castagliuolo et al., 1998). Increased SP binding sites have been demonstrated in the lymphoid aggregates and vasculature of a patient with *C. difficile* toxin A-induced pseudomembranous colitis (Mantyh et al., 1996a). In contrast, NK-1R mRNA expression is significantly reduced in patients with HIV infection. This may contribute to the mucosal abnormality, altered intestinal motility and GI symptoms associated with HIV infection (McGowan et al., 1997).

**SP AND ARTHRITIS**

Substantial evidence indicates that SP contributes to the pathophysiology of joint inflammation. Studies demonstrate the involvement of neurogenic inflammation in adjuvant-induced experimental arthritis in rats (Levine et al., 1984, 1985). They found that joints which developed severe arthritis had a dense innervation of SP-containing sensory neurons, and a higher SP content, than joints that developed mild arthritis. They also reported that infusion of SP into the knee exacerbated the severity of experimental arthritis, whereas infusion of a SP antagonist had no effect. Neural depletion of SP by capsaicin administration, eliminates paw swelling and tenderness within the inflamed joint (Colpaert et al., 1983). Furthermore, elevated SP levels and accelerated cartilage degradation has been reported in rabbit knees injected with IL-1β or TNF-α (O’Byrne et al., 1990).

Increased levels of SP in the synovial fluid and serum of patients with rheumatoid arthritis (RA) have been documented (Marshall et al., 1990; Menkes et al., 1993). Others demonstrated tachykinin-immunoreactivity in the stromal nerves of normal tissues, but not of RA tissues (Gronblad et al., 1988). SP stimulates prostaglandin E₂, and collagenase release from RA synoviocytes and increases proliferation of RA synoviocytes (Lotz et al., 1987). These findings support a role for SP in the cartilage destruction, bone lesion development, and pannus formation of arthritis. NK-1R mRNA is expressed by RA synoviocytes, but not by normal synoviocytes (Krause et al., 1995).

**CONCLUSIONS**

The wide range of inflammatory diseases in which SP participates suggests a major future role for neurokinin receptor antagonists in the management of these diseases. Studies with neurokinin receptor antagonists suggest that blocking the binding of SP to the NK-1R interrupts the inflammatory cascade that triggers and maintains intestinal lesions of IBD (Sonea et al., 2002). Tachykinins may participate in the gastrointestinal dysmotility associated with infection, inflammation, stress, and pain. Tachykinin agonists and antagonists may become adjuncts to the treatment of motor disorders that involve pathological disturbances of the gastrointestinal tachykinin system and may ultimately have a role as spasmylic, antiinflammatory, antiemetic, and antinociceptive drugs (Holzer and Holzer-Petsche, 1997). SP derivatives cause apoptosis in small cell lung cancer cells, and block calcium mobilization induced by neuropeptides, and thus are potential therapeutic compounds for the treatment of small cell lung cancer (Rosati et al., 1998). At present, trials with neurokinin receptor antagonists in patients...
with asthma, IBD, and depression are under way. Ultimately, tachykinin receptor antagonists may have therapeutic potential in other inflammatory diseases such as sarcoidosis, chronic bronchitis and chronic cough and RA (Chung and Chang, 2002).

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