

Substance P in Rheumatic Diseases

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Abstract: Inflammation in many autoimmune syndromes is modulated by the nervous system. This paper reviews important principles of neurogenic inflammation and findings of substance P overexpression in rheumatic diseases. Substance P is a key molecule in activating inflammation after antidromal axoplasmic transport and secretion at the nociceptor. We summarize the results from studies with rheumatoid arthritis, osteoarthritis, psoriatic arthritis, systemic lupus erythematoses, systemic sclerosis, vasculitides, Sjögren syndrome, vasculitides, reflex sympathetic dystrophy, gouty arthritis, fibromyalgia syndrome, chronic fatigue syndrome and degenerative vertebral spine disorders. Although substance P is associated with many of these diseases, selective receptor blockers have not been effective in therapy. In contrast, the nonselective antagonist capsaicin is beneficial in some conditions. We also discuss a novel therapeutic principle with selective serotonin antagonists that may induce a powerful downregulation of neurogenic inflammation.

Keywords: Substance P, neurogenic inflammation, rheumatic diseases, review.

INTRODUCTION

Inflammatory mediators of the immune system regulate autoimmune diseases. For example, synovitis terminates after injury or loss of neuronal function in patients with rheumatoid arthritis [1] or osteoarthritis [2]. Adjuvant-induced arthritis in rats is prevented after ligation of the spinal cord or peripheral nerves [3]. Pharmacological interruption of afferent C-fibres by capsaicin abolishes the inflammatory reflex after electric stimulation of peripheral nerves [4]. These findings suggest an intimate relationship between cells of the immune system and neuron-derived molecules. For example, serotonin (5-hydroxytryptamine, 5-HT) can induce a self-perpetuating local inflammation [5]. Other peptide neurotransmitters, such as substance P (SP) and calcitonin gene-related peptide (CGRP) activate lymphocytes, mast cells, or macrophages [6], and these cells induce the release of further mediators of nociception and inflammation. Under physiological conditions, these transmitters conduct action potentials to the central nervous system after stimulation of A δ - and C-pain fibres. Repetitive stimulation during synovitis induces antidromal transport, and the subsequent release at the nociceptor induces a remarkable inflammation [7].

In this paper we review some of the most important current knowledge of substance P in rheumatic diseases. Many studies show variable results, making a general interpretation difficult. Furthermore, the quantity of data does not permit a detailed interpretation of results here and the interested reader is therefore referred to the individual article. The complex events of chronic pain and inflammation in the central nervous system are reviewed elsewhere [8].

PRINCIPLES OF NEUROGENIC INFLAMMATION

Neuropeptides are expressed by afferent fibres of the spinal ganglion, and are then translocated to dorsal horn synapses. Substance P, CGRP, glutamate, and aspartate are present in high concentrations in the dorsal part of the dorsal horn and are the most important neurotransmitters of nociception [9]. Other transmitters include neurokinin A (NKA), neurokinin B (NKB), somatostatin (SOM), vasoactive intestinal polypeptide (VIP), pituitary adenylate cyclase-activating peptide (PACAP), cholecystokinin (CCK), bombesin, and galanin [10-12].

In case of occasional stimulation of the nociceptor pain fibres conduct afferent impulses to the spinal cord as shown in Fig. 1A. In case of chronic pain, however, neurons transmit neuropeptides into the opposite, antidromal direction. In this case, mediators released from nerve terminals may initiate vasodilation and local inflammation as shown in Fig. 1B. The term antidromal vasodilation was coined on the basis of this observation [13]. The triple response is another mechanism of neurogenic inflammation and is induced by noxious and mechanical irritation of the skin with a dull object [14]. This response is characterised by erythema (flare response), edema caused by increased vasodilation, and hypersensitivity (due to altered excitability of sensory neurons). This phenomenon is termed the axonal reflex and describes the antidromal action potential of a sensory nerve over its collaterals back to the periphery (i.e., blood vessels) without passing a synapse. Numerous mediators, including substance P and CGRP, initiate inflammatory events by local depolarisation of sensory neuron terminals. These mediators activate immune cells, mast cells, and vascular smooth muscle cells necessary for inflammation. Subsequently, however, the term "axonal reflex" was distinguished from neurogenic inflammation. In contrast to the axonal reflex, which occurs only within the area of innervation, other mechanisms contribute to neurogenic inflammation. The dorsal root reflex describes the activation of more peripheral nociceptors that

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conduct action potentials through spinal interneurons back to the periphery using different afferent neurons. Also these neurons release neuropeptides that contribute to the reddening, however, outside the area of innervation [15, 16].

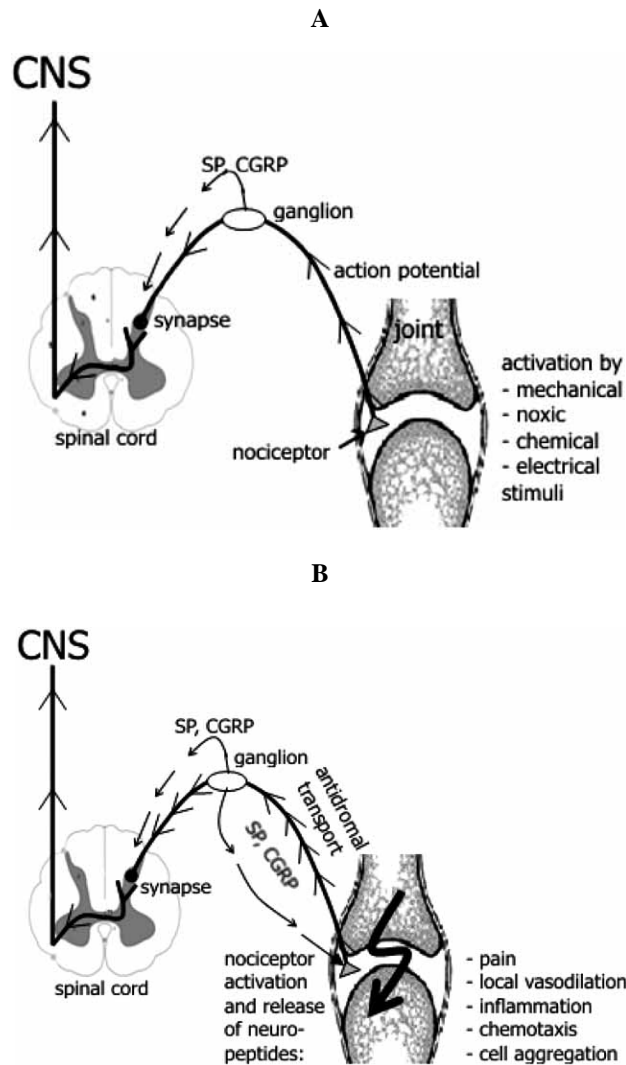


Fig. (1). Principles of pain transmission under physiological and chronic conditions. Neurotransmitters (e. g. SP or CGRP) are transported to the spinal cord and released at the synapse to conduct the action potential to the CNS (Fig. 1A). In case of chronic pain, neuropeptides are transported to and released from the nociceptor. The molecules are powerful inducers of local inflammation (Fig. 1B).

Substance P is a Member of the Tachykinin Family

The tachykinin family includes several neuropeptides, such as substance P [17]. Tachykinins received their name

because they induce tachycardia at low blood pressure [18]. Tachykinins share a common C-terminal sequence, Phe-Xaa-Gly-Leu-Met-NH₂, and include neurokinin A, neurokinin B, neurokinin K, and neuropeptide Y. Alternatively spliced mRNAs of the preprotachykinin I gene (PPT-I) encode substance P, neurokinin A, neuropeptide K, and neuropeptide Y, while the preprotachykinin II gene (PPT-II) encodes neurokinin B [17]. The amino acid sequences of the tachykinins are summarized in Table 1. Tachykinins bind to several neurokinin receptors (NK) that are characterised by a rhodopsin-like trans-membrane structure. Neurokinin receptors are G protein-coupled receptors [19, 20]. These receptors activate G proteins, and subsequently phospholipase C (PLC), phosphoinositol, inositol triphosphate (IP₃), and diacylglycerol [21, 22]. NK-1, NK-2, and NK-3 receptor subtypes show affinities for substance P, neurokinin A, and neurokinin B [23]. Substance P is the primary ligand for the NK-1 receptor, although other tachykinins also bind to this receptor with lower affinity. Monocytes express another mitogen-activated protein kinase (MAP kinase) substance P receptor that is not coupled to G protein [24].

Structure and Function of Substance P

Von Euler and Gaddum (1931) [25] extracted a substance from whole horse brain and small intestine that stimulated the smooth muscle system. This oligopeptide (Arg-Pro-Lys-Pro-Gln-Gln-Phe-Gly-Leu-Met-NH₂) was then shown to be present in the dorsal horn and the dorsal roots and to act as a mediator of vasodilation and plasma extravasation [26]. After synthesis in the spinal ganglion, substance P is translocated to serve as a neurotransmitter in the dorsal horn in response to stimulation of primarily afferent nociceptors. Under pathological conditions, substance P is released at the nociceptor as a result of antidromal transport. In the peripheral tissues, substance P activates vascular smooth muscle cells to induce vasodilation and edema. It also stimulates the release of histamine from mast cells [27, 28] and mediates many other functions of the cardiovascular, respiratory, gastrointestinal, and urogenital systems [39]. Substance P seems to be involved in the pathogenesis of many chronic disorders, such as migraine, bronchial asthma, chronic inflammatory bowel diseases [7, 30], Alzheimer's, and Parkinson's disease [31].

Substance P is expressed in both the central nervous system (CNS) and the peripheral nervous system (PNS). Immunoreactivity and NK-1 receptors are found in the rhinencephalon, telencephalon, basal ganglia, hippocampus, amygdala, diencephalon, hypothalamus, mesencephalon, pons, myelencephalon, and the spinal cord [32]. Other locations include the trigeminal ganglion [33], the spinal ganglion [34], and intrinsic intestinal neurons [35].

Table 1. Tachykinin Family in Mammals

Substance P	Arg-Pro-Lys-Pro-Gln-Gln-Phe-Gly-Leu-Met-NH ₂
Neurokinin A	Hys-Lys-Thr-Asp-Ser-Phe-Val-Gly-Leu-Met-NH ₂
Neurokinin B	Asp-Met-His-Asp-Phe-Phe-Val-Gly-Leu-Met-NH ₂
Neurokinin K	Asp-Ala-Asp-Ser-Ser-Ile-Glu-Lys-Gln-Val-Ala-Leu-Leu-Lys-Ala-Leu-Tyr-Gly-His-Gly-Gln-Ile-Ser-His-Lys-Arg-His-Lys-Thr-Asp-Ser-Phe-Val-Gly-Leu-Met-NH ₂
Neuropeptid γ	Asp-Ala-Gly-His-Gly-Gln-Ile-Ser-His-Lys-Arg-His-Lys-Thr-Asp-Ser-Phe-Val-Gly-Leu-Met-NH ₂

Substance P and CGRP Mediate Similar Effects

CGRP is a 37-amino acid neurotransmitter and is expressed after alternative splicing of the mRNA encoding calcitonin [36]. CGRP activates CGRP1 and CGRP2 receptors [37]. Immunoreactive nerve fibres are found in the skin, muscular system, synovial membrane, periosteum, periarthicular tissue, and the meninges [38-40]. CGRP-positive afferent fibres are found mainly in perivascular tissue, as well as in the epithelium [39, 41], and act as strong vasodilators [42, 43]. CGRP triggers the formation of wheals in human skin and flare responses that can be prevented by pretreatment with histamine (H1) antagonists. CGRP mediates a slow but long-lasting, intense vasodilation (more than 4 hours) that is not mediated by histamine (H1) receptors or nitric oxide [44]. Substance P, on the other hand, mediates a profound flare response, stronger wheals, and increased cutaneous nociception [45].

Substance P is a Potent Mediator of the Immune System

Substance P is found both in the nervous system and in lymphatic tissues such as the thymus, spleen, lymph nodes, and bone marrow [46]. The innervation of these organs is also mediated by other neurotransmitters, including somatostatin, CGRP, neuropeptide Y, and dopamine [47]. B-lymphocytes and T-lymphocytes, macrophages, mast cells, and astrocytes express NK-1 receptors [46]. In monocytes, substance P stimulates phagocytosis [48]. Monocytes express a substance P MAP-kinase receptor [49]. Substance P shows a strong chemotactic effect [50]. In macrophages, substance P activates the expression of IL-1, IL-6, and TNF- α [51-53]. In neutrophils, substance P activates chemotaxis, aggregation, and the expression of superoxides [54, 48]. NK-1 receptors activate protein kinase C and mediate adhesion of neutrophils to umbilical cord endothelial cells [55]. Conversely, substance P also induces expression of the intercellular adhesion molecule-1 (ICAM-1) on vascular endothelial cells for transendothelial migration of granulocytes [56]. The vascular cell adhesion molecule-1 (VCAM-1) is unaffected. Substance P induces the expression of IL-8 in polymorphonuclear leucocytes [57]. Cultured human lymphocytes express high-affinity substance P receptors [58]. Substance P stimulates lymphocyte proliferation and increases the response to T-cell mitogens such as phytohaemagglutinin and concanavalin A [59], and induces natural killer cell migration [60].

Substance P (NK-1) Receptor Agonists and Antagonists

Several agonists and antagonists have been used to characterise NK receptor subtypes [61]. Because of their subordinate clinical significance, selective NK-1 agonists were not further investigated [30]. NK-1 antagonists, on the other hand, have received much attention because of their potential therapeutic effects in experimental and clinical conditions [7]. Peptide tachykinin antagonists such as spantide I and spantide II are relatively nonselective, show partial agonistic effects and neurotoxicity, and induce the release of histamine from mast cells [61]. These molecules were used to develop more selective NK-1 antagonists with less toxicity. Molecules such as SDZNK134311 and LY303875 significantly decreased neuropathic pain after oral or intrathecal application in guinea pigs [62]. CGP49823 has a significant anxiolytic effect [63]. SR140333 decreases the activity of ex-

perimental colitis [64] and focal cerebral ischemia [65]. GR205171 and MK-0869 showed anti-emetic activity after experimental chemotherapy in the ferret [66, 67]. GR205171 reduced experimental arthritis in rats [68]. CP99994 reduces postoperative pain after tooth extraction, with few side effects [69]. L7540303 (MK-0869) shows a strong antiemetic effect after cisplatin treatment [70], and is now approved for treatment of chemotherapy-induced nausea.

Capsaicin Depletes Neuronal Substance P Deposits

Capsaicin (8-methyl-N-vanillyl-6-nonenamide) is derived from homovanillic acid and is found in peppers (*Capsicum*). Capsaicin dose-dependently activates the vanilloid receptor VR1 on unmyelinated C fibres [7, 71]. Stimulation of these fibres induces the release of substance P and other neurotransmitters, including CGRP and somatostatin [72], with initial pain and vasodilation. Other types of C-fibres such as C-mechanoreceptors and thermoreceptors are not activated [7]. Continuous application induces a reversible depletion of neurotransmitter deposits with subsequent desensitization of the nociceptor [72]. Animal experiments showed that systemic application of capsaicin induced degeneration of afferent C fibres [73-75]. This effect is dose-dependent and more effective in newborn rats than in adult animals [73, 74].

SUBSTANCE P IN INFLAMMATORY AND DEGENERATIVE RHEUMATIC DISEASES

Rheumatoid Arthritis and Osteoarthritis

Most studies of rheumatoid arthritis (RA) use osteoarthritis (OA) as a control and they are thus discussed together in this chapter. Clinical observations suggest an involvement of the nervous system in both diseases [76, 77]: synovitis is frequently symmetrical. Neuronal injury after apoplectic insult [78-80] or poliomyelitis [81] halts synovitis in the paretic limb. Physical injury or psychological stress frequently initiates arthritis.

In RA, synovitis is triggered by an inflammatory infiltration consisting of T helper cells, B-lymphocytes, plasma cells, and dendritic cells. Lymphocytes and macrophages express inflammatory cytokines such as TNF- α , IL-1, IL-6, immunoglobulins, and rheumatoid factors. Complement activation and the release of matrix metalloproteinases initiate cartilage erosion and osteodestruction mediated by fibroblast-like synoviocytes [82, 83]. These cells express mRNA for NK-1 receptors in RA patients [84]. In cultured fibroblast-like synoviocytes, substance P significantly enhanced the proinflammatory activity of TNF- α and IL-1 β on the expression of VCAM-1 [84] whereas healthy controls did not express NK-1 receptors and did not show this effect [85].

By contrast, the term OA was originally coined to discriminate inflammatory joint disorders from degenerative events induced by joint mal-alignment. However, more recently an inflammatory character has also been shown in OA [86]. This inflammation might occur as a consequence of degenerative cartilage debris or intrinsic synovial activation. OA of the finger joints usually is not a consequence of pre-existing significant joint mal-alignment, thus suggesting an autoimmune pathogenesis.

In RA synovial tissue, immunohistochemical staining of substance P and CGRP positive fibres was reduced and less

intense, compared with OA controls [87, 88]. Substance P and CGRP positive neurons were only found in the deep cell layer and not in the intimal layer, suggesting that inflammation-induced depletion of neuropeptides occurs in RA. Substance P positive neurons showed a higher density in RA (255 fibres/mm²) when compared with OA (187 fibres/mm², $p < 0.009$) [89]. In OA synovial tissue, substance P was found in the free nerve terminals and free nerve fibres, in particular in the medial part of the knee (88 %), and to a lesser extent in the lateral (47 %) and the suprapatellar (20 %) parts [90]. Some of these nerve terminals showed axonal branches that were surrounded by clustered monocytes. Stronger expression of NK-1 receptor mRNA was found within the synovial layer and the interstitial layer in RA patients when compared with OA patients. The identity of these cells was unclear.

In synovial tissue culture, fibroblasts express low quantities of substance P (19 ± 2 pg/ml) and this effect can be enhanced by human recombinant transforming growth factor beta (TGF β) and bFGF [91]. Other cytokines, such as IL-1 β , TNF- α , NGF, vascular endothelial growth factor (VEGF), and epidermal growth factor (EGF) had no effect. Substance P induced the overexpression of prostaglandin E2 (PGE2) and collagenase. In addition, the proliferation of synovio-cytes increases in the presence of substance P, potentially because of its amino acid sequence homology to acidic fibroblast growth factor [52, 92].

Peripheral blood monocytes overexpress TNF- α in response to substance P [53], and display increased transcription of TNF- α mRNA after stimulation of NK-1 and NK-2 receptors in both RA patients and healthy controls [93]. Animal studies further suggest that IL-1 α and TNF- α induce the overexpression of substance P [94]. In addition, substance P stimulates peripheral blood lymphocytes to proliferate in response to concanavalin A or phytohaemagglutinin. Although some RA patients showed a much stronger proliferation, the findings were not significantly different from healthy controls [95]. In serum, substance P median values were more elevated in RA than in healthy controls [96].

In synovial fluid, analyses of neuropeptide concentrations have been contradictory. Substance P could not be demonstrated in RA patients and controls with early meniscus degenerations or ligamentous lesions [97, 98]. NKA, CGRP, and NPY results were also variable. In contrast, substance P levels in the synovial fluid showed no differences in patients with RA (946.6 ± 82.8 pg/ml, $n=24$), OA (1034.0 ± 116.6 pg/ml, $n=15$), and Reiter's syndrome (887.5 ± 179.9 pg/ml, $n=6$). In posttraumatic arthritis, however, substance P levels (1883.1 ± 368.9 pg/ml, $n=15$, $p < 0.005$) were more elevated [99]. Plasma concentrations were the same in RA (676.4 ± 58.1 pg/ml, $n=7$) and OA (540.0 ± 68.0 pg/ml, $n=10$), but were elevated in Reiter's syndrome (931.6 ± 103.6 pg/ml, $n=8$). Substance P levels in these chronic conditions were significantly elevated when compared with those in acute posttraumatic arthritis (435.3 ± 58.1 pg/ml, $n=14$) and healthy controls (446.1 ± 23.8 pg/ml, $n=13$, $p < 0.01$). Other studies demonstrated elevated synovial fluid substance P concentrations in RA when compared to OA or healthy controls [100-105]. Apart from elevated substance P concentrations, the turnover of neuropeptides also appears to be

upregulated in synovial inflammation. Neuronal endopeptidase (NEP), but not angiotensin-converting enzyme (ACE), was elevated both in plasma and synovial fluid in RA compared with OA and controls [101].

Substance P concentrations were significantly elevated in the cerebrospinal fluid of preoperative OA patients (23 ± 6 fmol/ml) undergoing total joint replacement when compared with healthy controls (12 ± 3 fmol/ml, $p < 0.001$) [106]. The findings correlated with pain intensity and decreased to 18 ± 5 fmol/ml ($p=0.05$) 4 to 6 months after the intervention, but still remained elevated when compared with the healthy controls ($p < 0.01$). In conclusion, substance P is expressed in RA and OA and it appears to mediate inflammation. However, it is not clear whether substance P is always more elevated in RA than in OA. Results of substance P measurements in arthritides are summarized in Table 2.

Substance P in Psoriatic Arthritis (PsA)

Psoriatic synovitis is characterised by CD8⁺ positive T-cells [111-113]. Trauma as a stimulatory event appears to be more important in PsA than in other arthritides [114-116]. Neurogenic inflammation is exemplified in psoriasis by the Köbner phenomenon [115]: psoriatic skin lesions can be induced by trauma with stimulation of nociceptive fibres of the papillary layer. Similarly, joint trauma can precipitate synovial inflammation and is termed "deep Köbner phenomenon" [117]. Substance P is present in psoriatic skin and synovitis and might induce angiogenesis by mediators such as vascular endothelial growth factor (VEGF), TGF β or angiopoietin. Accumulating T-cells appear to enhance the local autoimmune inflammation [118]. Synovial fluid in PsA had less elevated substance P concentrations (24.7 ± 1.8 pmol/ml; $n=8$) when compared with RA (43.1 ± 9.8 pmol/ml; $n=18$) but higher titers than seen in OA (12.0 ± 1.3 pmol/ml; $n=12$). These findings were correlated to systemic inflammation [109].

Several case reports [119-121] show that damage to upper or lower motor neurons terminates synovitis and skin lesions in PsA. In these patients, bilateral analysis of the synovial membrane and synovial fluid showed that inflammatory infiltrates persisted despite the obliteration of clinical synovitis. Furthermore, substance P positive fibres were present in both synovial membranes, but the clinically involved joint showed less intense staining. The uninflamed joint showed CD4⁺ cells but no CD8⁺ cells. Synovial fluid from the inflamed joint contained mediators, substance P and IL-1 β , which were absent in the unaffected joint, suggesting that axonal damage induces defective neuropeptide secretion. Thus, immobilization has a protective and anti-inflammatory effect similar to RA [122]. In summary, overexpression of substance P also occurs in PsA and appears to be reversed if the synovitis is terminated.

Systemic Lupus Erythematosus (SLE)

Data on human substance P in SLE are limited. In the mouse model of lupus nephritis, acute renal lesions displayed increased concentrations of substance P and CGRP, while chronic kidney disease was characterised by NPY overexpression [123]. Hybrid mice of this strain with central nervous system vasculitis and neurological impairments such

Table 2. Summary of Studies of Substance P in Various Arthritides

Study	Ref.	Assay	Specimen	Rheumatoid Arthritis	Osteoarthritis	Psoriatic Arthritis	Gouty Arthritis	Others	Controls
Miller	[89]	IHC	synovia	density of SP-containing nerve fibres	density of SP-containing nerve fibres				
			human	255 fibres/mm ²	187 fibres/mm ²				
Anichini	[96]	ELISA	serum	528 ± 273 pg/ml					
			human	seropositive, n=31					361 ± 232 pg/ml
			serum	721 ± 418 pg/ml					healthy controls, n=61
			human	seronegative, n=10					
Larsson	[97]	RIA	SF	not detected					not detected, n=5
			human	n=5					knee lesion
Larsson	[98]	RIA	SF	not detected					not detected, n=13
			human	n=18					knee lesion
Marshall	[99]	RIA	SF	946.6 ± 82.8 pg/ml	1034.0 ± 116.6 pg/ml			1883.1 + 368.9 pg/ml	
			human	n=24	n=15			posttraumatic arthritis, n=15	
			SF					887.5 + 179.9 pg/ml	
			human					Reiter's syndrome, n=6	
		RIA	plasma	676.4 ± 58.1 pg/ml	540.0 ± 68.0 pg/ml			453. + 58.1 pg/ml	
			human	n=7	n=10			posttraumatic arthritis, n=14	446.1 + 23.8 pg/ml
			plasma					931.6 + 103.6 pg/ml	healthy controls, n=13
			human					Reiter's syndrome, n=8	
Matucci-Cerinic	[100]	RIA	SF	41.2 ± 9.8 pg/ml	11.4 ± 10.2 pg/ml				
			human	n=26	n=10				
Matucci-Cerinic	[101]	RIA	SF	43.1 ± 16.6 pg/ml	12 + 13.1 pg/ml				
			human	n=30	n=14				
		RIA	plasma	14.4 + 10.2 pg/ml	13.6 + 10.6 pg/ml				11.3 + 3.9 pg/ml
			human						healthy controls
Hernanz	[103]	RIA	SF	89 ± 51 pmol/l	52 ± 15 pmol/l		80 ± 46 pmol/l		
			human	n=40	n=20		n=20		
Menkes	[104]	RIA	SF	15.68 + 1.54 pg/100µl	5.20 + 0.85 pg/100µl				
			human	n=19	n=9				
		RIA	synovial tissue	4.96 + 1.01 pg/mg protein	17.14 + 7.54 pg/mg protein				
			human	n=16	n=7				

(Table 2) contd.....

Study	Ref.	Assay	Specimen	Rheumatoid Arthritis	Osteoarthritis	Psoriatic Arthritis	Gouty Arthritis	Others	Controls
Agro	[105]	RIA	SF	18 nmol/l	1.5 nmol/l. n=5				
			human	n=10					
		RIA	serum	12.2 nmol/l	0.7 nmol/l				not shown
			human						
Lindh	[106]	RIA	cerebrospinal fluid		23 ± 6 fmol/ml				
			human		preoperative				12 ± 3 fmol/ml
					n=13				healthy controls, n=9
					18 ± 5 fmol/ml				
Devallier	[107]	EIA*	SF	3.94 + 1.21 ng/ml	1.91 + 0.56 ng/ml				
			human	n=16**	n=8				
Westermark	[108]	RIA	SF	48.4 pg/ml					
			human	RA < 12 months, n=9				1.25 pg/ml	
			SF	56.2 pg/ml				healthy controls, n=10	
			human	RA > 12 Mo., n=32					
Marabini	[109]	RIA	SF knee	43.1 + 9.8 pmol/ml	12.0 + 1.3 pmol/ml	24.7 + 1.8 pmol/ml			
			human	n=18	n=12	n=8			
Lunam	[110]	IHC	synovial tissue				length of SP-containing nerve fibres		length of SP-containing nerve fibres
			elbow				median 202, mean 819		median 579, mean 660
			chicken				SE + 89.5		SE ± 92

*Antibodies used in this study detect all tachykinins. ** this group included the following diseases: rheumatoid arthritis n=12; oligoarthritis n=2; systemic lupus erythematosus n=1; sarcoidosis n=1.

SP: substance P; SF: synovial fluid; NF: nerve fibres; posttr. arthritis: posttraumatic arthritis; IHC: immunohistochemistry.

as persistent tremor or cognitive dysfunctions did not show altered neuropeptide levels in the hippocampus at the age of 5 months. After 8 months, however, more chronic lesions in the hippocampus showed increased immunohistochemical staining for substance P, CGRP, NPY, and VIP. In the cortex, substance P, CGRP, and VIP were unchanged in both age groups [124]. Neuropeptides appeared to regulate cognitive and learning abilities, because animals with hippocampal lesions significantly improve mental function after substance P gene transfer [125]. In human lupus skin lesions, the density of sensory fibres expressing CGRP, NPY, and tyrosine hydroxylase was increased in the epidermis and upper dermis [126]. Substance P fibres were not analysed in further detail.

Systemic Sclerosis

NPY is a strong vasoconstrictor, while substance P and CGRP induce vasodilation. Sensory C-fibres expressing the latter neuropeptides mediate the dysesthesia and itching that

characterise the early phase of scleroderma. In contrast, NPY and VIP belong to the sympathetic and parasympathetic nervous system in deeper skin layers. In plasma, substance P is increased in scleroderma patients (7.1 ± 3.2 pmol/l) when compared with healthy controls (1.6 ± 1.6 pmol/ml, n=42, $p < 0.01$) [127].

In the skin of the forearms and the back, substance P positive fibres are found mainly as free sub-epidermal nerve endings, with a significantly elevated density in scleroderma patients when compared with healthy controls. However, the distribution of substance P positive fibres in the forearms did not differ from that in the skin of the back within the scleroderma patient group and in healthy controls [128].

The effect of substance P on vascular tone in scleroderma is controversial, probably because different experimental techniques have been used to analyse it. In early scleroderma, intravenously administered substance P alone was unable to induce the same vasodilation as in healthy controls, suggesting a functional endothelial dysfunction

[129]. Glyceryl trinitrate-induced vasodilation and thus smooth muscle relaxation were unimpaired. However, another study showed that substance P induced vasodilatory effects in primary Raynaud and scleroderma patients and in healthy controls after intracutaneous injection [130]. In contrast, intra-arterial injection of substance P induced vasoconstriction of digital arteries in scleroderma patients with Raynaud phenomenon, while healthy controls showed vasodilation [131], again suggesting that endothelial signal transduction was impaired in the scleroderma patients. In scleroderma patients with pulmonary hypertension, adenosine caused the expected vasodilation in the pulmonary artery, but substance P-induced vasoconstriction also suggested a strong dysregulation of the vascular endothelium [132]. In addition, substance P appears to be differentially upregulated depending on the phase of the disease [133].

Animal experiments demonstrate that substance P regulates parasympathetic miosis, hyperaemia, and disturbance of the blood-water barrier of the eye [134]. These effects could not be blocked by denervation of the trigeminal nerve or tetrodotoxin, suggesting a local NK receptor-mediated mechanism. In humans, infrared pupillometry confirmed these results and showed that, compared to healthy controls, increased substance P-induced miosis occurred more effectively in limited scleroderma than in the diffuse form [135]. In summary, several mechanisms have been demonstrated and substance P-induced endothelial dysfunction appears to play an important role in Raynaud syndrome.

Sjögren Syndrome

Large salivary glands and many labial glands are controlled by the sympathetic and parasympathetic nervous systems. Stimulation of postganglionic parasympathetic fibres activates the release of acetylcholine together with other transmitters such as substance P, VIP, and ATP. Sympathetic fibres release norepinephrine, NPY, and ATP [136]. Substance P and CGRP co-localize in the ganglia [137]. In animal experiments, ligation of parasympathetic fibres of the parotid gland reduced the substance P concentration by almost 90 % [137], while the CGRP content decreased by only 20 % and the amount of CGRP-positive fibres did not change. On the contrary, sympathectomy induced an increase of both substance P and CGRP, suggesting that sensory, parasympathetic fibres of the auriculotemporal nerve express substance P, whereas the facial nerve and the dorsal root contain CGRP. Intravenous CGRP induced increased secretion of amylase but not saliva, whereas in combination with substance P it stimulated the production of saliva. In humans, immunohistochemistry did not show different staining of substance P, VIP, NPY, and nitric oxide synthase (NOS) in patients with Sjögren syndrome and healthy controls [138]. However, the number of nerve terminals in contact with acini, blood vessels, and lymphocytes was decreased, and these appeared to be degenerated in patients with Sjögren syndrome. Thus, degeneration of substance P positive fibres may contribute to the loss of salivary secretion. Furthermore, immunohistochemistry revealed that salivary glands of patients with Sjögren syndrome have a decreased number of nerve fibres expressing substance P and NPY, however, the axons had a larger diameter [139]. Another study confirmed that substance P regulates saliva secretion

with increased intracellular calcium and ATP concentrations, with excretion of water, potassium, and chloride [140].

Immunohistochemistry showed that expression of substance P and CGRP was not localized to the centres of focal lymphocyte infiltrates, peritubular, or periacinar areas [136, 141, 142]. Perivascular areas of the large salivary glands predominantly showed positive staining for both neuropeptides [140]. The inflamed areas of the secretory parenchyma showed acinar atrophy [141]. Neutral endopeptidase (NEP), which is involved in the degradation of substance P, was stained in a similar pattern as substance P itself in the periphery of inflammatory infiltrates [141]. Electron microscopy demonstrated immunoreactive fibres directly in the area of secretory cells, together with vascular smooth muscle cells and lymphocytes, suggesting that these neurons also control the blood circulation [136]. The differential expression of inflammatory and anti-inflammatory neuropeptides may thus be dysregulated and induce acinar atrophy, apoptosis, and necrosis.

Vasculitides

Data are limited to a few studies. Substance P is a strong vasodilator and thus may be involved in the pathogenesis of vasculitides. In some diseases associated with vasculitis, such as scleroderma, substance P is able to induce potent vasoconstriction [131]. Substance P also stimulates the degranulation of mast cells and the release of proinflammatory cytokines such as TNF- α , and it activates polymorphonuclear leucocytes. Other important substance P-induced events in vasculitis include the activation of phagocytic macrophages that overexpress PGE2 and the adhesion of granulocytes to vascular endothelial cells. In leukocytoclastic vasculitis, the pattern of substance P and CGRP immunoreactive fibres corresponded to that of healthy controls [126]. These fibres were rarely detected within the epidermis and were found more frequently around blood vessels adjacent to perivascular nerves. Despite the observations above, the data suggest that substance P is not involved in the pathogenesis of vasculitides.

Reflex Sympathetic Dystrophy

Typical features of reflex sympathetic dystrophy are hyperalgesia, allodynia, and finally atrophic degeneration. These findings strongly suggest an involvement of the nervous system. Skin biopsies revealed substance P- and CGRP-expressing fibres with strong inflammation and Langerhans cells in the epidermis [143]. Although control biopsies were not described and thus hamper interpretation, these results are consistent with the clinical benefit of topical capsaicin: a case study describes a 31-year-old female patient with reflex sympathetic dystrophy after contusion of the hand [144]. Conventional treatments included blocking of the ganglion stellatum, oral amitriptyline, carbamazepine, lioresal, ibuprofen, opioids, and physical therapy, without significant improvement. The patient was then treated with topical capsaicin and showed significant pain reduction after 3 weeks. Two months later the pain had diminished from 10 to 2 on the visual analogue pain scale (0 to 10), and the function of the hand was considerably improved. Unfortunately, the disease relapsed three months later and capsaicin was no longer effective. Nevertheless, this case demonstrates the potential of capsaicin as a treatment option in chronic pain syndromes

and strongly suggests the involvement of neurogenic inflammatory mechanisms.

Gouty Arthritis

In urate-crystal animal models, substance P enhanced synovial inflammation while somatostatin was ineffective [145]. Substance P-immunoreactive fibres were significantly shorter in sodium urate-induced synovitis (median 202, mean 272, SE \pm 89.5) in comparison with healthy tissue (median 579, mean 660, SE \pm 92), suggesting that substance P is rapidly depleted in peripheral nerves [110]. In humans, synovial fluid substance P immunoreactivity was significantly increased in gout (80 ± 46 pmol/l, n=20, $p < 0.05$) and RA (89 ± 51 pmol/l, n=40, $p < 0.01$) when compared with OA (52 ± 15 pmol/l, n=20). Serum levels did not differ significantly among the three groups [103].

Fibromyalgia Syndrome and Chronic Fatigue Syndrome

Patients with fibromyalgia syndrome (FMS) have a reduced mechanical and capsaicin-induced pain threshold and a significantly larger area of pain perception [146]. Immunohistochemistry demonstrated significantly more IgG deposits, collagen type III, and mast cells in the dermis and vascular wall of FMS patients [147]. The data showed a correlation between degranulated mast cell infiltrates with connective tissue destruction and IgG deposits, suggesting that vascular permeability might be a consequence of increased pain and substance P release from nociceptive fibres.

Substance P in the cerebrospinal fluid might be an indicator of chronic pain. Not surprisingly, substance P concentrations were more elevated than in controls [148-150], and

Caucasians showed higher levels than Hispanics [151]. Patients with chronic fatigue syndrome, on the other hand, did not show significantly elevated substance P concentrations when compared to patients with cerebrovascular diseases and healthy controls [152]. Different pathologies of the two diseases may explain these discordant findings, although more data would be required to better define the pathologic mechanism of chronic fatigue syndrome.

In serum, substance P concentrations were not significantly elevated in patients with FMS [153, 154]. In muscle tissue, Sprott *et al.* (1998) [155] did not find substance P expression in patients with FMS. Similarly, patients with FMS, myofascial pain syndrome, and healthy controls did not show significant differences in positive staining for substance P [156]. However, the mean optical density of the immunostaining for SP was statistically greater in patients with myofascial pain syndrome, suggesting that substance P is overexpressed in this condition of chronic pain. In contrast, another study used immunofluorescence and Western blots to demonstrate that substance P binds to the connective tissue of skeletal muscle with a perivascular pattern, but not to skeletal muscle alone [157]. In conclusion, findings of substance P expression in FMS are somewhat controversial although this neuropeptide appears to be an indicator of chronic pain. The results of some of the most important studies are summarized in Table 3.

Degenerative Vertebral Spine Disorders

Lower back pain is characterised by a recurrent local irritation of nociceptive pain fibres. Degenerative facet joints

Table 3. Summary of Studies on Substance P in Fibromyalgia

Study	Ref.	Assay	Specimen	Fibromyalgia	Controls	Remarks
Vaeroy	[148]	RIA	csf	36.1 ± 2.7 fmol/ml n=30	9.6 ± 3.2 fmol/ml n=35	1. Control findings were used from a study by Almay <i>et al.</i> , 1988
						2. Patients with Raynaud's phenomenon probably had secondary fibromyalgia
Bradley	[149]	RIA	csf	19.26 ± 1.58 fmol/ml with premedication 19.21 ± 2.00 fmol/ml without medication n=21	12.83 ± 1.92 fmol/ml n=10	
Russel	[151]	RIA	csf	42.8 ± 14.9 fmol/ml n=32	16.3 ± 6.0 fmol/ml n=30	
Reynolds	[153]	RIA	plasma	371 ± 91 pg/ml n=32	397 ± 84 pg/ml n=26	
Samborski	[154]	EIA	serum	28.3 ± 11.3 pg/ml n=37	25.8 ± 8.7 pg/ml n=10	
Welin	[158]	unknown	csf	significantly elevated n=26	significantly decreased n=15	Published as abstract, no statistical data shown

csf: cerebrospinal fluid.

with chondral damage and exposed subchondral bone displayed substance P positive nerve fibres in erosion channels that extended to the subchondral bone [159]. Control facet joints had only mild irregularities. The presence of substance P positive fibres suggests that facet joints participate in the etiology of low back pain.

Radiculopathies comprise neuropathic pain that is characterised by damage to CNS or PNS nerves without stimulation of nociceptors. Substance P has been localized on small-blood-vessel endothelium of the annulus fibrosus of human intervertebral discs, where there is also evidence for NK-1 receptors [160]. The vascular effects of substance P, such as vasodilation, plasma extravasation, and angiogenesis, may thus contribute to inflammatory events and discogenic pain. Herniated discs expressed substance P and the C-terminal peptide of NPY in large clusters or in isolated areas close to fibroblasts [161]. These data suggest a dual innervation with both afferent and efferent neurons, as substance P is found in C fibres and NPY is expressed by sympathetic neurons. However, the expression pattern in healthy controls was unclear in this study.

In different spinal cord compression syndromes and pain conditions, CSF substance P concentrations were significantly increased when compared with healthy controls (18.5 ± 3.6 pg/ml) [162]. The maximum concentration occurred in fractures of the lower extremities (57.7 ± 5.5 pg/ml). Levels in patients with lumbar canal stenosis and radicular pain (48.5 ± 3.3 pg/ml) were significantly more elevated than in patients without radicular pain (23.9 ± 7.9 pg/ml, $p < 0.01$, $n=8$). The substance P concentrations also correlated with pain intensity determined on the visual analogue scale. However, no correlation was found between the substance P concentrations in the CSF and in serum. Similar results were obtained in another study [163]. In contrast, one study described no significant differences between the substance P concentrations in the CSF of patients with radicular compression and those of healthy controls [106]. Preoperative substance P concentrations in patients with herniated discs were not significantly different after nucleotomy. Synovial tissue from facet joints displayed occasional substance P positive nerve fibres, with a perivascular pattern and adjacent to fat cells, but lacked CGRP immunostaining [164].

DISCUSSION

This review summarises important findings on substance P overexpression in rheumatic diseases. Many investigations demonstrate in part quite contradictory results. These conflicts might be due to the use of different reagents. Furthermore, many studies are hampered by the limited number of patients that do not share the same history and premedication. Similarities of substance P overexpression are found, for example, in rheumatoid arthritis and osteoarthritis, suggesting that these diseases share a common neurogenic background. Other diseases, such as psoriatic arthritis, are strongly associated with trauma and initiating pain, thus suggesting a strong involvement of the afferent nervous system.

Animal experiments showed potent, reduced nociception induced by NK-1 antagonists [62, 165-167]. NK-1 receptor knockout mice showed no altered acute pain perception, but

pain wind-up was absent [168]. Preprotachykinin knockout mice missing both substance P and NKA showed normal nociception [169]. In contrast to animal studies, the use of selective NK-1 blockers as therapeutic interventions in humans has been disappointing [170, 171]. NK-1 antagonists might be less selective in humans than in animals, resulting in non-selective blocking of several ion channels [7]. Furthermore, if substance P-mediated pathways are blocked, other neurotransmitters such as glutamate or CGRP might bypass pain transmission. In addition, substance P activity is also mediated by NK-2 and NK-3 receptors, and selective NK-1 antagonism does not prevent stimulation of NK-2 or NK-3 receptors. Indeed, only one human study demonstrated significant pain relief, with CP99994, after dental extraction [172]. This condition is characterised by acute pain induced by inflammatory mediators such as prostaglandins, while substance P is upregulated only after 1 to 3 days. CP99994 was only effective at high dose, and was thus potentially blocking ion channels. Nevertheless, NK-1 receptor antagonists have gained clinical significance in other diseases as anxiolytic, antidepressive, or antiemetic drugs [173].

In contrast, topical capsaicin has shown promising results in chronic degenerative cervical spine disease [174], low back pain [175], and RA and OA [176-178]. The clinical observations and findings from neuroscience research suggest that capsaicin is probably the most important therapy available for substance P-mediated neurogenic inflammation. In contrast to NK-1 antagonists, capsaicin blocks several neuropeptides, including substance P, CGRP, and somatostatin [72]. Parenteral gold appeared to suppress substance P expression in the synovial fluid of RA patients, suggesting its mechanism is similar to that of capsaicin [179].

Novel Approaches to Downregulating Neurogenic Inflammation

Serotonin is a neurotransmitter and induces neuronal depolarization with an increase in intracellular Ca^{2+} . Serotonin modulates the release of substance P, GABA, dopamine, cholecystinin, acetylcholine, NKA, and CGRP [180, 181]. Several receptors have been identified, and the 5-HT₃ subtype appears to mediate inflammation [182]. This receptor is expressed by monocytes, chondrocytes, and T-cells, but not by dendritic cells [183]. *In vitro* findings correlate with the results of double-blinded clinical [184, 185] and placebo-controlled trials [186, 187, 188] that show strong pain relief in FMS after treatment with tropisetron, a selective 5-HT₃ antagonist. Furthermore, local injections of tropisetron have shown a potent analgesic effect in RA, OA, tendinopathies, periarthropathies, and myofascial pain syndrome that is similar to but longer lasting than the effect of lidocaine [189-192]. 5-HT₃ blockers appear to inhibit the release of proinflammatory mediators [182, 191]. In contrast to NK-1 receptor antagonists that inhibit substance P and leave other neuropeptides unaffected, 5-HT₃ blockers downregulate a variety of cytokines. This observation may explain their success as potent therapy in many chronic pain conditions. Further basic science and clinical research is needed to more precisely delineate neurogenic inflammation as a new and alternative therapeutic target in autoimmune and chronic pain disorders.

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