Neurobiology of Substance P and the NK_1 Receptor

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Substance P belongs to a group of neurokinins (NKs), small peptides that are broadly distributed in the central nervous system (CNS) and peripheral nervous system (PNS). The biological effects of substance P in the CNS, namely regulation of affective behavior and emesis in the brain and nociception in the spinal cord, are mediated by its binding to the NK_1 receptor. The substance P–NK_1 (SP-NK_1) receptor system is the most extensively studied NK pathway, and in contrast to receptors for other neurotransmitters, such as glutamate, which have high expression throughout the CNS, only a minority of neurons (5% to 7%) in certain CNS areas express the NK_1 receptor. The NK_1 receptor is distributed in the plasma membrane of cell bodies and dendrites of unstimulated neurons, but upon substance P binding, the NK_1 receptor undergoes rapid internalization, followed by rapid recycling to the plasma membrane. Release of substance P is induced by stressful stimuli, and the magnitude of its release is proportional to the intensity and frequency of stimulation. More potent and more frequent stimuli allow diffusion of substance P farther from the site of release, allowing activation of an approximately 3- to 5-times greater number of NK_1 receptor–expressing neurons. Recent studies employing pharmacologic or genetic inactivation of NK_1 receptors demonstrate the important role of the SP-NK_1 receptor system in the regulation of affective behavior and suggest that inhibition of this pathway may be a useful approach to treatment of depression and associated anxiety.

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Substance P was first identified in 1931 as a component of brain and intestinal extracts that had smooth muscle contractile activities.[1] Early studies have shown that substance P is present primarily in the gray matter of the central nervous system (CNS), with highest levels seen in the hypothalamus and substantia nigra.[2] Substance P was also found to be expressed in the spinal cord, mostly in dorsal areas, as well as in the peripheral nervous system (PNS), particularly in the autonomic nerves, spinal ganglia, and sympathetic trunk.[3] Delineation of the 11-amino-acid structure[4] and in vitro synthesis[5] of substance P in the early 1970s have paved the way for studies aimed at elucidating the biological roles of substance P in greater detail. Subsequent discovery of peptides that have extensive amino-terminal amino acid sequence similarities with substance P (NH_2-methionine [Met]-leucine [Leu]-glycine [Gly]-X-phenylalanine [Phe]) and that are, like substance P, localized in the CNS and PNS has led to their classification as neurokinins (NKs).[6] In addition to substance P, this group includes NKA and NKB.[3,6]

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Comparative analysis of pharmacologic properties of substance P, NKA, and NKB has led to the recognition of 3 distinct NK receptors, each with a preferred ligand: substance P preferentially binds to the NK_1 receptor, whereas NKA and NKB show preference for the NK_2 and NK_3 receptors, respectively.[3,6] However, each NK possesses agonist properties at all 3 receptor types, but the biological activity of these systems appears to be governed by both co-localization and affinity of various NKs for different NK receptors.[6]

NEUROLOGY OF SUBSTANCE P AND THE NK_1 RECEPTOR

The substance P–NK_1 (SP-NK_1) receptor pathway is the most abundant and the most extensively studied neuropeptide system in both the CNS and PNS, regulating the behavioral responses to a range of noxious and stressful stimuli. In the spinal cord, the SP-NK_1 receptor system modulates nociception, and disruption of the NK_1 receptor reduces the response to some forms of moderate/intense pain in adult mice.[7–9] Expression in the brainstem emetic nuclei has implicated substance P in the control of vomiting,[10] and this hypothesis was validated in clinical studies with substance P (NK_1 receptor) antagonists (SPAs), which have been shown to have antiemetic effects.[11,12] In the PNS, abnormal activity of the SP-NK_1 receptor path-

way has been associated with several inflammatory conditions, such as asthma, inflammatory bowel disease, and migraine.[13] On the basis of its localization in limbic regions (e.g., amygdala, hypothalamus) of the brain,[14] including the spatial overlap with neurotransmitter pathways (e.g., serotonergic, noradrenergic) known to be involved in the regulation of mood, the SP-NK_1 receptor system has also been suggested to play an important role in the control of affective behavior.[15] This hypothesis was recently supported by several preclinical and clinical studies, which have shown that the pharmacologic or genetic inactivation of the NK_1 receptor is associated with anxiolytic and antidepressant effects.[15–17] The aims of this review are to describe the localization and mechanism of action of the SP-NK_1 receptor system in the brain and spinal cord and to discuss preclinical evidence for the involvement of this system in response to stress and affective behavior.

Localization of Substance P and NK_1 Receptors in the Brain and Spinal Cord

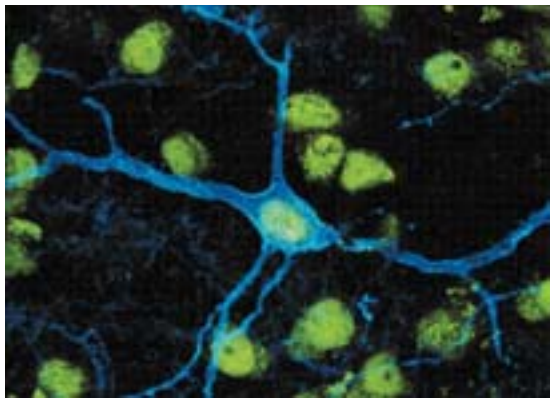
Autoradiographic, immunohistochemical, and messenger RNA (mRNA) expression studies have documented broad distribution of substance P and NK_1 receptors in the CNS. Intense substance P staining was observed in amygdala, locus ceruleus, hypothalamus, substantia nigra, and peduncular nuclei, whereas moderate labeling was detected in caudate putamen, nucleus accumbens, and raphe nuclei, as well as in the lamina I of the spinal cord.[18–21] On the other hand, relatively low levels of substance P staining were identified in the cerebral cortex, cerebellum, and hippocampus.[18–21] Substance P was shown to localize to synaptic vesicles,[22] to be expressed in both the neuronal cell bodies and dendrites,[19] and to be released in a calcium-dependent manner.[23,24] Taken together, these findings suggested that substance P may be a neurotransmitter involved in regulation of emotional and stress responses.[20]

The distribution of the NK_1 receptor in the CNS generally corresponds to that of substance P, as shown by electrophysiologic studies involving localized application of substance P[25–28] and autoradiographic, immunohistochemical, and mRNA expression experiments.[14,21,29] These studies demonstrate high levels of NK_1 receptor expression in brain areas crucial for the regulation of affective behavior and response to stress, such as the amygdala, hypothalamus, hippocampus, frontal cortex, raphe nucleus of the brainstem, and the locus ceruleus. In some regions (e.g., substantia nigra, lateral interpeduncular nuclei), though, intense substance P staining does not always appear to be accompanied by coexpression of NK_1 receptors.[6,13,21] It has been suggested that, in these areas, substance P may bind to closely related NK_2 or NK_3 receptors,[6,13] although experimental evidence for this hypothesis is lacking. Alternatively, the apparent "mismatch" may be a consequence of technical limitations.[6,13] In contrast to the receptors for known neurotransmitters (e.g., serotonin, norepinephrine), which are expressed by virtually all neurons in a given CNS region, NK_1 receptors are expressed by the minority (5% to 7%) of neurons in both the brain and the spinal cord (Figure 1).[14,31–33] Selective expression of NK_1 receptors suggests that treatment with SPAs may be associated with a more favorable tolerability profile than therapy with agents targeting the serotonergic or noradrenergic pathway. This concept is supported by the findings from the phase 2 clinical trial with the SPA aprepitant (MK-0869), which showed a significantly lower incidence of sexual dysfunction in patients receiving aprepitant (300 mg q.d.) than in those treated with the selective serotonin reuptake inhibitor paroxetine (20 mg q.d.) (3% vs. 26%, respectively; $p \leq .001$).[15]

Activation of substance P–containing neurons in response to acute noxious stimuli or stress results in the rapid synaptic release of substance P, which then binds to the closely apposed NK_1 receptors in the immediate vicinity. On unstimulated neurons, NK_1 receptors are localized on the plasma membrane of both the cell body and dendrites (Figure 2A), but after stimulation and substance P binding, they are rapidly internalized (within 5 minutes) into the cytoplasm via endosomes (Figure 2B).[30,34] This NK_1 receptor internalization in response to substance P binding is readily reversible, with complete return of internalized receptors to the surface after 30 minutes. Preclinical studies have demonstrated an increased number of neurons with NK_1 receptor internalization in the anterior basolateral amygdala in response to maternal separation, further supporting the involvement of the SP-NK_1 receptor system in response to stress.[15]

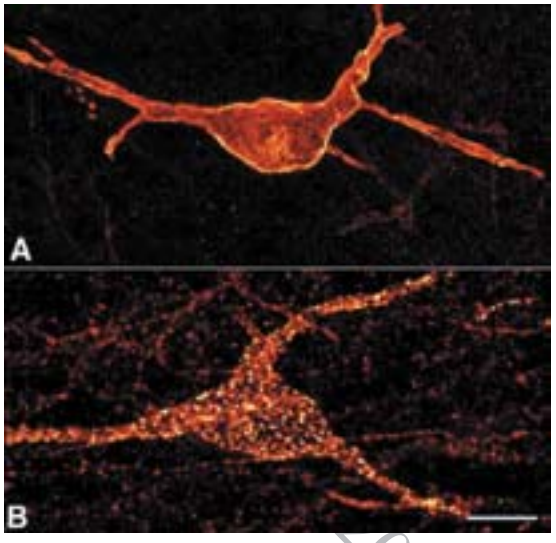
The degree of substance P release and NK_1 receptor internalization is proportional to the intensity and frequency of stressful stimuli. More potent stimuli (e.g., thermal) result in a substantially greater release of substance P and in-

Figure 1. Selective Expression of the Neurokinin-1 (NK_1) Receptor in the Central Nervous System[a]



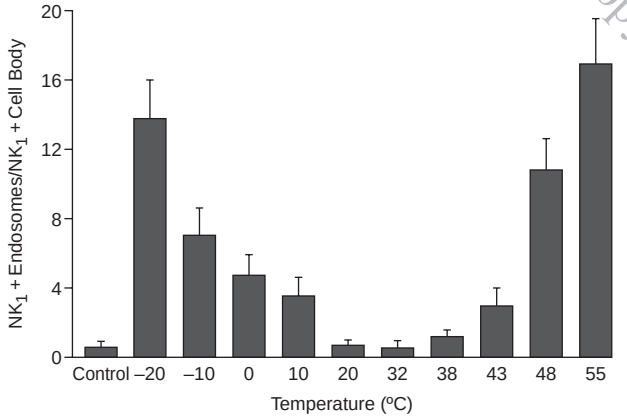
[a]Reprinted with permission from Mantyh et al.[30] Confocal photomicrograph shows that only a small proportion of central nervous system neurons (green) express the NK_1 receptor (blue).

Figure 2. Internalization of Neurokinin-1 (NK_1) Receptors in Stimulated Neurons[a]



[a]Reprinted with permission from Mantyh et al.[30] On unstimulated neurons, the NK_1 receptor is uniformly distributed in the plasma membrane of both the cell bodies and dendrites (A). Five minutes after stimulation and substance P release, NK_1 receptors are internalized into the cytoplasm via endosomes (B).

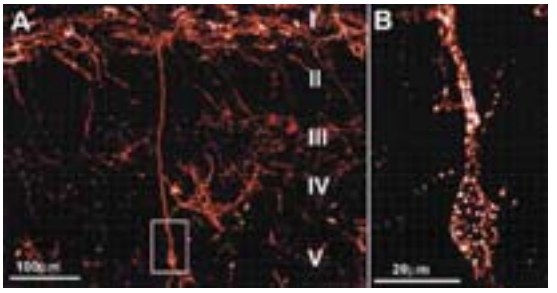
Figure 3. Greater Excitation of Substance P–Expressing Neurons Promotes Higher Neurokinin-1 (NK_1) Receptor Internalization[a]



[a]Adapted with permission from Allen et al.[35]

ternalization of the NK_1 receptor (Figure 3). Similarly, previous exposure to stress amplifies the activity of the SP-NK_1 receptor system, as documented in the rat model of irritant-induced hindpaw inflammation, in which the initial decrease in NK_1 receptor activity is followed by a prolonged period (≥ 8 days) of increased expression.[36] Greater substance P release in response to higher intensity or repeated administration of stressful stimuli not only leads to more potent activation of adjacent NK_1 receptor–expressing neurons, but also permits diffusion of substance P away from the site of release and therefore stimulation of more distant neurons. It has been estimated that approximately 3 to 5 times more neurons are activated in response to more potent or more frequent stress stimuli (Figure 4).[35]

Figure 4. Substance P Released From Sensitized Neurons Diffuses and Activates Relatively Distant Neurokinin-1 (NK_1) Receptor–Expressing Neurons[a]



[a]Adapted with permission from Abbadie et al.[37] Sensitization induces greater release of substance P, which in turn induces activation of a greater number of NK_1 receptor–expressing neurons.

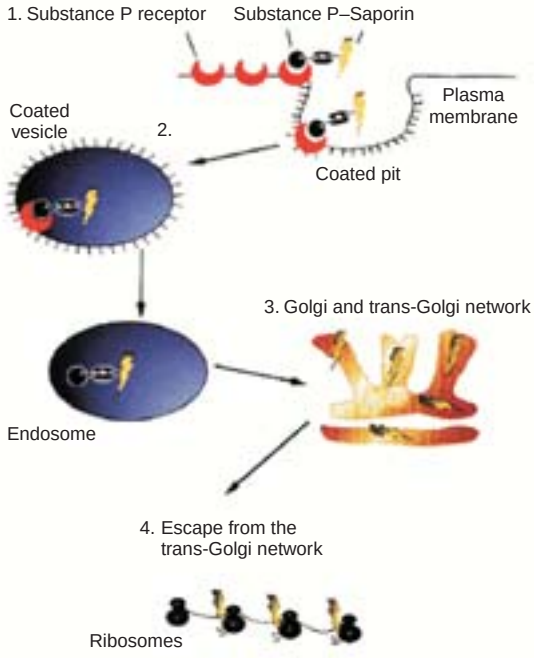
Role of SP-NK_1 Receptor Pathway in Affective Behavior

Several preclinical studies have evaluated physiologic and behavioral responses to changes in the activity of the SP-NK_1 receptor pathway, providing strong evidence for the important role of this system in the regulation of emotional behavior. Early experiments with localized application of substance P or NK_1 receptor agonists demonstrated potentiation of anxiety-like behavior in several model systems.[15,38–40]

A separate line of research focused on identifying the consequences of inactivation of the NK_1 receptor by either pharmacologic (SPAs, cytotoxic substance P–saporin [SP-SAP] conjugates) or genetic (NK_1 receptor knockout mice) means. The results of these studies consistently demonstrate that the blockade of SP-NK_1 receptor signaling results in antidepressant/anxiolytic effects and increased noradrenergic and serotonergic neurotransmission in the locus ceruleus and dorsal raphe, respectively.[15–17,41,42] In 1 study, intra-amygdala administration of SPA L760,735 in guinea pig pups significantly reduced the duration of vocalizations in response to maternal separation,[42] a model of anxiety-like behavior.[43] The review by Santarelli and Hen in this supplement[44] describes the preclinical studies with SPAs and NK_1 receptor knockout mice in more detail; the following is a review of preliminary experiments with SP-SAP conjugates.

SP-SAP conjugate exploits the internalization of the NK_1 receptor to achieve selective killing of NK_1 receptor–expressing neurons by SAP, which inhibits ribosomal protein synthesis (Figure 5).[45,46] Localized application of SP-SAP promotes selective pharmacologic ablation

Figure 5. Mechanism of Cytotoxic Action of Substance P–Saporin (SP-SAP) Complex[a]

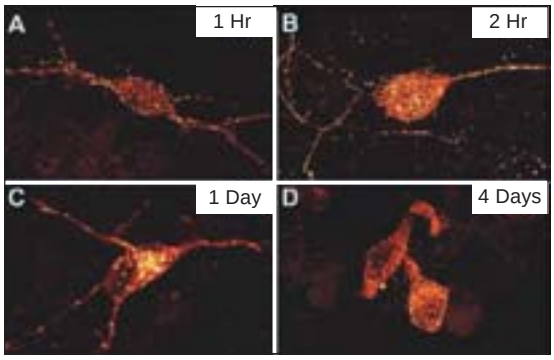


[a]SP-SAP conjugate is internalized via the neurokinin-1 (NK_1) receptor endosomal pathway, and saporin subsequently escapes from trans-Golgi network to inhibit ribosomal protein synthesis and thereby induce cell death. Localized application of SP-SAP effectively leads to ablation of neurons containing the NK_1 receptor.

of neurons expressing the NK_1 receptor (Figure 6) and thereby allows the examination of the role of the SP-NK_1 receptor pathway in various regions of the brain and spinal cord. Injection of the SP-SAP complex into the dorsal horn of the spinal cord resulted in a marked attenuation of responses to highly noxious stimuli, mechanical and thermal hyperalgesia,[45] and chronic neuropathy and inflammation,[47] suggesting that the SP-NK_1 receptor pathway in the spinal cord may be involved in nociception.

The SP-SAP complex has also been used to evaluate the role of the SP-NK_1 receptor pathway in affective behaviors, using the elevated plus maze (EPM) assay. In EPM, animals face a choice between active exploration of a novel environment and fear of heights and open spaces; several measurements, such as the time spent in the open arms and the number of entries in the open arms, are considered indicators of anxiety-like behavior.[17] In preliminary studies (P.W.M., unpublished studies), injection of SP-SAP into the rat amygdala was associated with a significant prolongation of the time spent in the open arms and a significantly higher number of entries in the open arms, whereas no effect was observed on the overall locomotory activity. These anxiolytic effects of SP-SAP are highly reminiscent of the findings reported in NK_1 receptor knockout mice and with the use of SPAs (see review by Santarelli and Hen in this supplement[44]).[15–17]

Figure 6. Stages of Neuronal Death Following Internalization of Substance P–Saporin Into Cultured Neurons Expressing Neurokinin-1 Receptors[a]



[a]Adapted with permission from Mantyh et al.[45]

SUMMARY

The SP-NK_1 receptor pathway is involved in the neural processing of a range of noxious and stressful stimuli, and it represents the most abundant and the most thoroughly studied neuropeptide system in both the CNS and PNS. In the brain, substance P and the NK_1 receptor are co-localized in emetic nuclei and regions involved in the regulation of stress and emotional responses (amygdala, hypothalamus, hippocampus, frontal cortex, the micronuclei), suggesting that this pathway may be an important regulator of emesis and affective behavior. This idea is supported by results of preclinical and clinical studies with SPAs, which have been shown to have both antiemetic and antidepressant/anxiolytic properties. In the spinal cord, the SP-NK_1 receptor pathway has been implicated in nociception. Importantly, the proportion of neurons expressing the NK_1 receptor in various regions of the brain, such as the amygdala, and spinal cord is low (5% to 7%). This selectivity contrasts with ubiquitous expression of glutamatergic, serotonergic, and noradrenergic receptors.

Acute stress stimuli lead to the release of substance P, binding of substance P to the NK_1 receptors in the immediate vicinity, and rapid internalization of the activated NK_1 receptors. The extent of substance P release and NK_1 receptor internalization corresponds to the intensity and frequency of afferent stimulation, with more intense or repeated stimuli producing greater receptor internalization. Additionally, greater release of substance P in response to more intense stimulation allows substance P diffusion away from the site of release, leading to activation of up to 5 times more NK_1 receptor–expressing neurons.

Recent studies involving pharmacologic or genetic inactivation of the NK_1 receptor corroborate the regulatory role of the SP-NK_1 receptor pathway in affective behavior. Behavioral assays consistently demonstrate that inhibition of NK_1 receptor activity results in anxiolytic and antidepressant effects.

Drug name: paroxetine (Paxil).

REFERENCES

1. Von Euler US, Gaddum JH. An unidentified depressor substance in certain tissue extracts. J Physiol 1931;72:74–87
2. Pernow B. Studies on substance P purification, occurrence, and biological actions. Acta Physiol Scand 1953;29:1–90
3. Hokfelt T, Pernow B, Wahren J. Substance P: a pioneer amongst neuropeptides. J Intern Med 2001;249:27–40
4. Chang MM, Leeman SE, Niall HD. Amino-acid sequence of substance P. Nat New Biol 1971;232:86–87
5. Tregear GW, Niall HD, Potts JT Jr, et al. Synthesis of substance P. Nat New Biol 1971;232:87–89
6. Saria A. The tachykinin NK1 receptor in the brain: pharmacology and putative functions. Eur J Pharmacol 1999;375:51–60
7. Cao YQ, Mantyh PW, Carlson EJ, et al. Primary afferent tachykinins are required to experience moderate to intense pain. Nature 1998;392: 390–394
8. De Felipe C, Herrero JF, O'Brien JA, et al. Altered nociception, analgesia and aggression in mice lacking the receptor for substance P. Nature 1998; 392:394–397
9. King TE, Heath MJ, Debs P, et al. The development of the nociceptive responses in neurokinin-1 receptor knockout mice. Neuroreport 2000;11: 587–591
10. Armstrong DM, Pickel VM, Joh TH, et al. Immunocytochemical localization of catecholamine synthesizing enzymes and neuropeptides in area postrema and medial nucleus tractus solitarius of rat brain. J Comp Neurol 1981;196:505–517
11. Campos D, Pereira JR, Reinhardt RR, et al. Prevention of cisplatin-induced emesis by the oral neurokinin-1 antagonist, MK-869, in combination with granisetron and dexamethasone or with dexamethasone alone. J Clin Oncol 2001;19:1759–1767
12. Van Belle S, Lichinitser MR, Navari RM, et al. Prevention of cisplatin-induced acute and delayed emesis by the selective neurokinin-1 antagonists, L-758,298 and MK-869. Cancer 2002;94:3032–3041
13. Quartara L, Maggi CA. The tachykinin NK1 receptor, pt 2: distribution and pathophysiological roles. Neuropeptides 1998;32:1–49
14. Mantyh PW, Hunt SP, Maggio JE. Substance P receptors: localization by light microscopic autoradiography in rat brain using [3H]SP as the radioligand. Brain Res 1984;307:147–165
15. Kramer MS, Cutler N, Feighner J, et al. Distinct mechanism for antidepressant activity by blockade of central substance P receptors. Science 1998;281:1640–1645
16. Rupniak NM, Carlson EC, Harrison T, et al. Pharmacological blockade or genetic deletion of substance P (NK(1)) receptors attenuates neonatal vocalisation in guinea-pigs and mice. Neuropharmacology 2000;39: 1413–1421
17. Santarelli L, Gobbi G, Debs PC, et al. Genetic and pharmacological disruption of neurokinin 1 receptor function decreases anxiety-related behaviors and increases serotonergic function. Proc Natl Acad Sci U S A 2001; 98:1912–1917
18. Brownstein MJ, Mroz EA, Kizer JS, et al. Regional distribution of substance P in the brain of the rat. Brain Res 1976;116:299–305
19. Ljungdahl A, Hokfelt T, Nilsson G. Distribution of substance P-like immunoreactivity in the central nervous system of the rat, 1: cell bodies and nerve terminals. Neuroscience 1978;3:861–943
20. Nicoll RA, Schenker C, Leeman SE. Substance P as a transmitter candidate. Annu Rev Neurosci 1980;3:227–268
21. Nakaya Y, Kaneko T, Shigemoto R, et al. Immunohistochemical localization of substance P receptor in the central nervous system of the adult rat. J Comp Neurol 1994;347:249–274
22. Pickel VM, Joh TH, Reis DJ, et al. Electron microscopic localization of substance P and enkephalin in axon terminals related to dendrites of catecholaminergic neurons. Brain Res 1979;160:387–400
23. Iversen LL, Jessell T, Kanazawa I. Release and metabolism of substance P in rat hypothalamus. Nature 1976;264:81–83
24. Otsuka M, Konishi S. Release of substance P-like immunoreactivity from isolated spinal cord of newborn rat. Nature 1976;264:83–84
25. Cheeseman HJ, Pinnock RD, Henderson G. Substance P excitation of rat locus coeruleus neurones. Eur J Pharmacol 1983;94:93–99
26. Ogata N. Effects of substance P on neurons of various brain regions in vitro. Brain Res 1979;176:395–400
27. Ogata N, Abe H. Substance P decreases membrane conductance in neurons of the guinea pig hypothalamus in vitro. Neuropharmacology 1982;21: 187–189
28. Murase K, Nedeljkov V, Randic M. The actions of neuropeptides on dorsal horn neurons in the rat spinal cord slice preparation: an intracellular study. Brain Res 1982;234:170–176
29. Maeno H, Kiyama H, Tohyama M. Distribution of the substance P receptor (NK-1 receptor) in the central nervous system. Brain Res Mol Brain Res 1993;18:43–58
30. Mantyh PW, DeMaster E, Malhotra A, et al. Receptor endocytosis and dendrite reshaping in spinal neurons after somatosensory stimulation. Science 1995;268:1629–1632
31. Mantyh PW, Hunt SP. Changes in 3H-substance P receptor binding in the rat brain after kainic acid lesion of the corpus striatum. J Neurosci 1986;6: 1537–1544
32. Mantyh PW, Gates T, Mantyh CR, et al. Autoradiographic localization and characterization of tachykinin receptor binding sites in the rat brain and peripheral tissues. J Neurosci 1989;9:258–279
33. Mantyh PW, Pinnock RD, Downes CP, et al. Correlation between inositol phospholipid hydrolysis and substance P receptors in rat CNS. Nature 1984;309:795–797
34. Mantyh PW, Allen CJ, Ghilardi JR, et al. Rapid endocytosis of a G protein-coupled receptor: substance P evoked internalization of its receptor in the rat striatum in vivo. Proc Natl Acad Sci U S A 1995;92:2622–2626
35. Allen BJ, Rogers SD, Ghilardi JR, et al. Noxious cutaneous thermal stimuli induce a graded release of endogenous substance P in the spinal cord: imaging peptide action in vivo. J Neurosci 1997;17:5921–5927
36. Stucky CL, Galeazza MT, Seybold VS. Time-dependent changes in Bolton-Hunter-labeled 125I-substance P binding in rat spinal cord following unilateral adjuvant-induced peripheral inflammation. Neuroscience 1993;57:397–409
37. Abbadie C, Trafton J, Liu H, et al. Inflammation increases the distribution of dorsal horn neurons that internalize the neurokinin-1 receptor in response to noxious and non-noxious stimulation. J Neurosci 1997;17: 8049–8060
38. Elliott PJ. Place aversion induced by the substance P analogue, dimethyl-C7, is not state dependent: implication of substance P in aversion. Exp Brain Res 1988;73:354–356
39. Aguiar MS, Brandao ML. Effects of microinjections of the neuropeptide substance P in the dorsal periaqueductal gray on the behaviour of rats in the plus-maze test. Physiol Behav 1996;60:1183–1186
40. Krase W, Koch M, Schnitzler HU. Substance P is involved in the sensitization of the acoustic startle response by footshocks in rats. Behav Brain Res 1994;63:81–88
41. Rupniak NM, Kramer MS. Discovery of the antidepressant and anti-emetic efficacy of substance P receptor (NK1) antagonists. Trends Pharmacol Sci 1999;20:485–490
42. Boyce S, Smith D, Carlson E, et al. Intra-amygdala injection of the substance P [NK(1) receptor] antagonist L-760735 inhibits neonatal vocalisations in guinea-pigs. Neuropharmacology 2001;41:130–137
43. Hofer MA. Multiple regulators of ultrasonic vocalization in the infant rat. Psychoneuroendocrinology 1996;21:203–217
44. Santarelli L, Gobbi G, Blier P, et al. Behavioral and physiologic effects of genetic or pharmacologic inactivation of the substance P receptor (NK_1). J Clin Psychiatry 2002;63(suppl 11):11–17
45. Mantyh PW, Rogers SD, Honore P, et al. Inhibition of hyperalgesia by ablation of lamina 1 spinal neurons expressing the substance P receptor. Science 1997;278:275–279
46. Wiley RG, Lappi DA. Destruction of neurokinin-1 receptor expressing cells in vitro and in vivo using substance P-saporin in rats. Neurosci Lett 1997;230:97–100
47. Nichols ML, Allen BJ, Rogers SD, et al. Transmission of chronic nociception by spinal neurons expressing the substance P receptor. Science 1999;286:1558–1561