Neurobiology of Substance P and the NK₁ Receptor

Patrick W. Mantyh, Ph.D., J.D.

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 structure⁴ and in vitro synthesis⁵ 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 (NH₂-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.⁶

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 NK₁ receptor, whereas NKA and NKB show preference for the NK₂ and NK₃ 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 NK₁ RECEPTOR**

The substance P–NK₁ (SP-NK₁) 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-NK₁ receptor system modulates nociception, and disruption of the NK₁ 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 (NK₁ receptor) antagonists (SPAs), which have been shown to have antiemetic effects.¹¹,¹² In the PNS, abnormal activity of the SP-NK₁ receptor path-
way has been associated with several inflammatory conditions, such as asthma, inflammatory bowel disease, and migraine. On the basis of its localization in limbic regions (e.g., amygdala, hypothalamus) of the brain, including the spatial overlap with neurotransmitter pathways (e.g., serotonergic, noradrenergic) known to be involved in the regulation of mood, the SP-NK\textsubscript{1} receptor system has also been suggested to play an important role in the control of affective behavior. This hypothesis was recently supported by several preclinical and clinical studies, which have shown that the pharmacologic or genetic inactivation of the NK\textsubscript{1} receptor is associated with anxiolytic and antidepressant effects. The aims of this review are to describe the localization and mechanism of action of the SP-NK\textsubscript{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\textsubscript{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\textsubscript{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. On the other hand, relatively low levels of substance P staining were identified in the cerebral cortex, cerebellum, and hippocampus, 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. Substance P was shown to localize to synaptic vesicles, to be expressed in both the neuronal cell bodies and dendrites, and to be released in a calcium-dependent manner. Taken together, these findings suggested that substance P may be a neurotransmitter involved in regulation of emotional and stress responses.

The distribution of the NK\textsubscript{1} receptor in the CNS generally corresponds to that of substance P, as shown by electrophysiologic studies involving localized application of substance P and autoradiographic, immunohistochemical, and mRNA expression experiments. These studies demonstrate high levels of NK\textsubscript{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 nucleus), though, intense substance P staining does not always appear to be accompanied by coexpression of NK\textsubscript{1} receptors. It has been suggested that, in these areas, substance P may bind to closely related NK\textsubscript{1} or NK\textsubscript{2} receptors, lacking experimental evidence for this hypothesis. Alternatively, the apparent “mismatch” may be a consequence of technical limitations. 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\textsubscript{1} receptors are expressed by the minority (5% to 7%) of neurons in both the brain and the spinal cord (Figure 1). Selective expression of NK\textsubscript{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; \( p \leq .001 \)).

![Figure 1. Selective Expression of the Neurokinin-1 (NK\textsubscript{1}) Receptor in the Central Nervous System](image)

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Activation of substance P-containing neurons in response to acute nociceptive stimuli or stress results in the rapid synaptic release of substance P, which then binds to the closely apposed NK\textsubscript{1} receptors in the immediate vicinity. On unstimulated neurons, NK\textsubscript{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). This NK\textsubscript{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\textsubscript{1} receptor internalization in the anterior basolateral amygdala in response to maternal separation, further supporting the involvement of the SP-NK\textsubscript{1} receptor system in response to stress. The degree of substance P release and NK\textsubscript{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...
ternalization of the NK₁ receptor (Figure 3). Similarly, previous exposure to stress amplifies the activity of the SP-NK₁ receptor system, as documented in the rat model of irritant-induced hindpaw inflammation, in which the initial decrease in NK₁ 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 NK₁ 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

## Role of SP-NK₁ Receptor Pathway in Affective Behavior

Several preclinical studies have evaluated physiologic and behavioral responses to changes in the activity of the SP-NK₁ 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 NK₁ 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 NK₁ receptor by either pharmacologic (SPAs, cytotoxic substance P–saporin [SP-SAP] conjugates) or genetic (NK₁ receptor knockout mice) means. The results of these studies consistently demonstrate that the blockade of SP-NK₁ 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 supplement44 describes the preclinical studies with SPAs and NK₁ 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 NK₁ receptor to achieve selective killing of NK₁ receptor-expressing neurons by SAP, which inhibits ribosomal protein synthesis (Figure 5).45,46 Localized application of SP-SAP promotes selective pharmacologic ablation

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**Figure 2. Internalization of Neurokinin-1 (NK₁) Receptors in Stimulated Neurons**

Reprinted with permission from Mantyh et al.30 On unstimulated neurons, the NK₁ receptor is uniformly distributed in the plasma membrane of both the cell bodies and dendrites (A). Five minutes after stimulation and substance P release, NK₁ receptors are internalized into the cytoplasm via endosomes (B).

**Figure 3. Greater Excitation of Substance P–Expressing Neurons Promotes Higher Neurokinin-1 (NK₁) Receptor Internalization**

Adapted with permission from Allen et al.35

**Figure 4. Substance P Released From Sensitized Neurons Diffuses and Activates Relatively Distant Neurokinin-1 (NK₁) Receptor–Expressing Neurons**

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 NK₁ receptor–expressing neurons.
of neurons expressing the NK₁ receptor (Figure 6) and thereby allows the examination of the role of the SP-NK₁ 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, and chronic neuropathy and inflammation, suggesting that the SP-NK₁ 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-NK₁ 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. 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 locomotor activity. These anxiolytic effects of SP-SAP are highly reminiscent of the findings reported in NK₁ receptor knockout mice and with the use of SPAs (see review by Santarelli and Hen in this supplement).

**SUMMARY**

The SP-NK₁ 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 NK₁ receptor are co-localized in emetic nuclei and regions involved in the regulation of stress and emotional responses (amygdala, hypothalamus, hippocampus, frontal cortex, the microneu- clei), 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-NK₁ receptor pathway has been implicated in nociception. Importantly, the proportion of neurons expressing the NK₁ 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 NK₁ receptors in the immediate vicinity, and rapid internalization of the activated NK₁ receptors. The extent of substance P release and NK₁ 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 NK₁ receptor–expressing neurons.

Recent studies involving pharmacologic or genetic inactivation of the NK₁ receptor corroborate the regulatory role of the SP-NK₁ receptor pathway in affective behavior. Behavioral assays consistently demonstrate that inhibition of NK₁ receptor activity results in anxiolytic and antidepressant effects.
Drug name: paroxetine (Paxil).

REFERENCES

38. Elliott PJ. Place aversion induced by the substance P analogue, dimethyl-