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NEUROKININ$_1$ RECEPTOR ANTAGONISTS AS POTENTIAL ANTIDEPRESSANTS

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■ **Abstract** Selective, nonpeptide antagonists for tachykinin receptors first became available ten years ago. Of the three known tachykinin receptors, drug development has focused most intensively on the substance P-preferring receptor, neurokinin$_1$ (NK_1). Although originally studied as potential analgesic compounds, recent evidence suggests that NK_1 receptor antagonists may possess antidepressant and anxiolytic properties. If confirmed by further controlled clinical studies, this will represent a mechanism of action distinct from all existing antidepressant agents. As reviewed in this chapter, the existing preclinical and clinical literature is suggestive of, but not conclusive, concerning a role of substance P and NK_1 receptors in the pathophysiology of depression and/or anxiety disorders. The ongoing clinical trials with NK_1 receptor antagonists have served as an impetus for much needed, basic research in this field.

INTRODUCTION

To date, all drugs that are FDA-approved for the treatment of major depression are believed to act principally, via one or another mechanism, on monoaminergic neurotransmitter systems in the central nervous system (CNS). Though clearly effective, even the newest of these agents suffer significant drawbacks: A delay period of several weeks is typically required for the drugs to produce a significant improvement in symptoms, a variety of side effects lead to noncompliance, and there is a sizeable rate of nonresponse of approximately 30% and an even higher partial response rate (1). For these and other reasons, active investigation continues in the search for antidepressant agents with novel mechanisms of action.

Because the neurobiology of depression remains poorly understood, and because monoaminergic systems have manifold functional interactions with other neurotransmitter systems, a widely divergent group of compounds has been examined as potential antidepressant agents. Recently, it was reported that an antagonist of a receptor subtype, neurokinin$_1$ (NK_1) for the neuropeptide, substance

P (SP), exhibited antidepressant activity comparable to one of the selective serotonin [5-hydroxytryptamine (5-HT)] reuptake inhibitors in a placebo-controlled, double-blind trial (2). As reviewed here, the behavioral pharmacology of SP-active compounds as putative antidepressants has been an understudied field, compared for example to the extensive literature on SP involvement in nociception. The relevant preclinical and clinical research concerning the involvement of this peptide and its receptors in mood and anxiety disorders is reviewed. The prospect of NK_1 receptor antagonists as novel antidepressant agents remains promising but clearly requires further study.

PRECLINICAL STUDIES

Tachykinins and Tachykinin Receptors

In 1931, Von Euler and Gaddum discovered that an extract from horse intestine and brain caused intestinal smooth muscle contractions; they named the active compound "substance P," for "preparation" (3). In subsequent decades, related nonmammalian peptides such as eledoisin and physalaemin were discovered, and more recently a group of SP-like mammalian peptides were found in the nervous system, including neurokinin A (previously termed substance K) and neurokinin B (previously termed neuromedin K). After a long search, the 11 amino acid sequence of SP was finally elucidated from bovine hypothalamus in 1970 by Chang and Leeman (4), who ironically were searching instead for the structure of the long-elusive corticotropin-releasing factor (CRF). SP is a member of the family of peptides sharing the carboxy-terminal sequence Phe-X-Gly-Leu-Met-amide, collectively termed "tachykinins" for their ability to produce fast smooth muscle contraction (in contrast to bradykinin).

SP can be synthesized from three alternatively spliced forms of the *preprotachykinin-A* (*PPT-A*) gene (5). The β and γ splice variants also contain the coding sequence for neurokinin A, and are more prevalent in rat brain than the α form. Neurokinin B is formed from a separate gene, *PPT-B* (6) (see Figure 1).

Multiple tachykinin receptor subtypes have been proposed on the basis of the varying rank-order of potencies of SP and other tachykinins, e.g. eledoisin, in bioassays using various tissues (7), and the contrasting distribution patterns of different radiolabeled tachykinins (8, 9). Distinct binding sites for neurokinin A (10) and neurokinin B (11) have also been clearly demonstrated. At the 1986 tachykinin symposium in Montreal, the following nomenclature was adapted: the SP-preferring, neurokinin A- and neurokinin B-preferring receptors were termed tachykinin NK_1, tachykinin NK_2, and tachykinin NK_3 receptors, respectively. For brevity the word tachykinin is commonly omitted. Tachykinin receptors is the preferred, general term for this group of receptors, though many authors use the term neurokinin receptors (12, 12a). The selectivity of the ligands for their respective receptors is not absolute (reviewed in 13). The three receptor subtypes have been cloned in rats (14–17) and in humans (18–20).

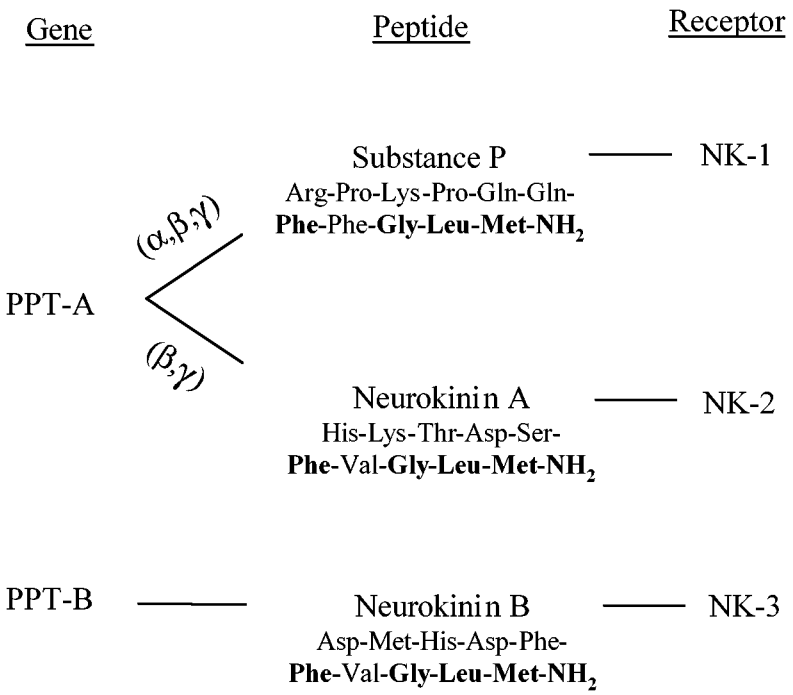

Figure 1 Tachykinin ligand and receptor family (common tachykinin amino acid sequence indicated in bold).

Neuroanatomy

Among the tachykinins, SP is by far the most concentrated in the CNS (21). The densest immunoreactivity is localized in the spinal cord and within the substantia nigra; many other brain regions contain high concentrations of SP, including the striatum, medial and central amygdaloid nuclei, hypothalamus, ventral tegmental area, parabrachial nucleus, and locus coeruleus (21, 22). Neurokinins A and B, and the genes encoding each of the tachykinins are also distributed widely in the CNS (21, 23, 24), but space constraints preclude their discussion here.

[^{125}I] and [^{3}H]-labeled SP autoradiography to identify SP receptors in the rat has also revealed a widespread distribution, in many cases overlapping with the distribution of SP immunoreactivity. Particularly high receptor densities are found in the rat olfactory bulb, lateral septum, amygdalo-hippocampal area, anterior nucleus of the hypothalamus, medial habenular nucleus, superior colliculus, locus coeruleus, and nucleus of the solitary tract (25). Brain regions in which direct SP administration reliably produces behavioral or physiologic responses, such as the cerebral cortex (26), parabrachial nucleus (27), interpeduncular nucleus (28), and periaqueductal grey (29, 30) also contain substantial SP receptor density.

Although SP acts upon neurons in the substantia nigra (31) and ventral tegmental area (32), [^{3}H]-SP binding in these brain regions is very sparse. Selective NK_1 receptor binding and NK_1 receptor mRNA distribution generally correlate well with that of SP receptor binding (33, 34). Intracerebroventricular (ICV) NK_1 agonist administration produces widespread neuronal activation in guinea pigs, as measured by quantitative determination of Fos immunoreactivity in brain regions displaying NK_1 receptors (35). NK_3 receptor binding and receptor mRNA distribution in the rat are also quite abundant, including substantial localization in the accessory olfactory bulb, cerebral cortex, medial septum, bed nucleus of the stria terminalis, basolateral nucleus of the amygdala, many hypothalamic nuclei, medial habenular nucleus, substantia nigra, ventral tegmental area, and nucleus of the solitary tract (36–38). In contrast to the NK_1 and NK_3 receptors, the NK_2 receptor is expressed in the CNS to a considerably lesser extent. Using selective radioligands, two groups have independently demonstrated the presence of NK_2 receptors in neonatal rat brain, but little or no expression within the same region(s) of adult rats (39, 40). The paucity of NK_2 receptors in the CNS remains somewhat an enigma, in view of the high neurokinin A concentrations in many brain nuclei (21).

In humans, widespread distribution of SP and SP receptor binding has also been reported (41–44). Both neurokinin A and B have been demonstrated in human cerebral cortex and striatum (45), as has PPT-B mRNA expression in basal forebrain structures including the hypothalamus, amygdala, and bed nucleus of the stria terminalis (46). Whether NK_2 and NK_3 receptors are present in human brain has been somewhat more controversial. Receptor autoradiography using radiolabeled peptides detects NK_1, but not NK_2 or NK_3 receptors (47). However, NK_3 mRNA expression and receptor immunoreactivity have been found in human brain tissue (20, 48). Considering the predominance of SP in the CNS, the relative lack or equivocal presence of NK_2 and NK_3 receptors in the human CNS, and the preclinical data reviewed below, it is not surprising that NK_1, rather than NK_2 or NK_3, receptor antagonists have been most intensively investigated as potential antidepressants.

The patterns of tachykinin and tachykinin receptor expression in the brain deserve comment. First, the patterns of expression are quite diffuse, and therefore it is difficult to predict the CNS function of SP or other tachykinins based on this neuroanatomy. Moreover, the brain circuits involved in major depression themselves are only now being elucidated, and our current database is not particularly instructive regarding the potential role of particular SP-positive projections. Certainly the potential exists for modulation by SP of emotion and arousal, based on the presence of the peptide and receptors in the hypothalamus and amygdala, other limbic system loci such as the septum and bed nucleus of the stria terminalis, and brainstem sites including the locus coeruleus and raphe nuclei. The effects of SP on these latter two monoamine-containing cell groups so often implicated in the pathophysiology of mood and anxiety disorders is considered in the following section.

Monoamine-Substance P Interactions

Although the actual therapeutic mechanism(s) of action of currently available antidepressants remain obscure, these agents acutely increase synaptic concentrations of norepinephrine and/or serotonin (5-HT), in most cases through inhibition of their respective transporter proteins (49). Therefore, it is of interest in this discussion to consider interactions between SP and these monoamine systems. Given the extensive CNS distribution of both SP and monoaminergic cell bodies and their projections, it would be surprising if these systems did not interact at various loci. Several specific types of interactions can be envisioned. First, neurons may cosynthesize and release SP and a monoamine; traditional antidepressants and NK_1 antagonists could share similar functional effects on targets of these neurons. Second, effects of SP on monoaminergic neurons, particularly those within the serotonergic raphe nuclei and the noradrenergic locus ceruleus, could potentially be responsible for antidepressant-like effects of SP receptor antagonists on monoaminergic neurotransmission. Finally, other monoamine/SP interactions such as monoaminergic innervation of SP neurons and convergence of monoaminergic and SP-ergic neurons on shared target neurons are worthy of consideration.

More than two decades ago, several investigators determined that a neuron can synthesize and secrete multiple neurotransmitters; early proof of this concept was the demonstration of 5-HT and SP colocalization in brainstem nuclei of the rat (50). Lesioning studies (51) and direct, immunocytochemical evidence (52) soon revealed that medullary raphe neurons that cosynthesize 5-HT and SP project to the spinal cord. Rostral projections to higher brain centers in rodents, however, do not appear to contain both 5-HT and SP. Selective lesioning of 5-HT neurons with the neurotoxin 5,7-dihydroxytryptamine (with desipramine coadministered to protect noradrenergic neurons) depleted forebrain 5-HT but not SP in the periaqueductal grey, hypothalamus and cingulate cortex (53). Similarly, treatment with another 5-HT neurotoxin, p-chloroamphetamine, either did not affect or increased SP concentrations in forebrain regions studied, raising the possibility of 5-HT/SP interactions other than colocalization (54). In primates, it is possible that SP and 5-HT coexist within both descending and ascending projections, based on the extensive colocalization of both neurotransmitters throughout neurons of the various dorsal raphe subdivisions (55–57), and to a lesser extent within the median raphe nucleus (57). We are unaware of any studies specifically examining colocalization of SP in CNS noradrenergic pathways.

Intracerebroventricular (ICV) administration of SP to rats (58) or guinea pigs (59) produces an increase in plasma catecholamine concentrations; the brain loci involved in mediating SP-induced sympathetic outflow have not been directly identified. One candidate region is the lateral spinal nucleus, which is SP receptor-positive (60) and which sends projections to the intermediolateral cell column (61). Brainstem nuclei including the locus coeruleus may also contribute to SP-induced sympathetic activation. The effect of SP on CNS noradrenergic projections from locus coeruleus neurons is uncertain. SP applied directly to the locus coeruleus is

excitatory (62) via an action on NK_1 receptors (63), producing increased inward cation current and decreased potassium current (64). ICV administration of the rat NK_1 receptor antagonist, RP 67580 results in attenuated responsiveness of locus coeruleus neurons to acute restraint stress as assessed by determination of the number of c-fos immunopositive cells (65), whereas systemic administration of an NK_1 receptor antagonist enhances locus coeruleus firing in response to stress (66). Consistent with the latter finding is the recent report that acute, intravenous administration of NK_1 receptor antagonists produces an attenuation of the suppressant effect of the α_2-adrenergic agonist clonidine on locus coeruleus neuronal activity (66a). It remains to be determined what effect chronic NK_1 receptor blockade has on locus coeruleus neurons; diminished noradrenergic responsiveness to stress has been shown to occur following chronic administration of many antidepressant agents (49).

Studies are lacking regarding the electrophysiologic effect of SP on raphe nucleus neurons, or on serotonin release and turnover by these neurons following local SP administration. The raphe nucleus is innervated by SP-ergic neurons, most likely projecting from outside the brain stem (67). Microinfusion of the SP analog dimethyl-C7 into the median raphe nucleus produced increased locomotor activity (68); another group demonstrated hyperactivity after infusion of peptide agonists at NK_2 and NK_3, but not NK_1 receptors (69). Increased heart rate and blood pressure were demonstrated following SP microinfusion in the dorsal raphe nucleus (70). It is postulated, therefore, that NK_1 receptor activation in raphe neurons produces an increase in serotonergic neuron firing. Studies are lacking to determine whether all subregions of the raphe complex respond similarly to SP. Very recently, NK_1 receptor knockout mice displayed a marked reduction of anxiety and stress-related behaviors (70a). This was associated with a selective desensitization of somatodendritic 5-HT_{1A} autoreceptors. Acute pharmacological blockade of NK_1 receptors with RP 67580 increases 5-HT firing rate, presumably via desensitization of 5-HT_{1A} autoreceptors. The effects of chronic NK_1 receptor antagonist administration upon serotonergic neurotransmission are not known. It is commonly, though not universally, accepted that chronic antidepressant drug treatment results in a net increase in serotonergic neurotransmission in the CNS (49).

SP appears to play an important role in stress-activation of mesocortical dopamine (DA) neurons, as shown by increased cortical DA turnover following injection of dimethyl-C7 into the ventral tegmental area (32) and the failure of footshock to induce cortical DA turnover following injection of anti-SP antibody into the ventral tegmental area (71). In the absence of anti-SP antibody, footshock stress causes SP depletion in the ventral tegmental area (72).

Other types of SP/monoaminergic interactions have not been investigated in any systematic fashion. In electrophysiologic experiments, the excitatory effect of SP on rat cingulate cortex neurons was reduced by norepinephrine application, and less consistently by 5-HT (53). Striatal PPT mRNA expression is apparently regulated positively by 5-HT; e.g. systemic p-chlorophenylalanine (a 5-HT synthesis inhibitor) administration decreased PPT mRNA, whereas 5-HT_2 agonist

administration increased PPT mRNA (73). In summary, the extant database regarding monoamine-SP interactions supports the possibility of antidepressant-like actions of NK_1 receptor antagonists.

Behavioral Activity of Centrally Administered Tachykinin Receptor Agonists

The most extensive research on SP has been in relation to its role in nociception. This peptide was shown in the late 1970s to be concentrated in primary nociceptive fibers (74), to excite spinal cord units that respond to noxious stimuli (75), and to be depleted following intrathecal capsaicin administration (which also increases the pain threshold of these rats) (76). Unfortunately, research in this area has failed to generate any novel analgesic compounds in clinical practice (76a). In this section we review the understudied topic of behavioral responses of laboratory animals after CNS administration of SP and SP-like compounds, particularly those relevant to the potential antidepressant efficacy of SP receptor blockade. Active investigation has also been conducted regarding the potential use of SP antagonists as antimigraine and antiemetic agents, comprehensively reviewed elsewhere (77, 78).

Because no well-established pattern of behaviors in laboratory animals leads to the designation of a particular compound as "depressogenic," behavioral experiments after agonist administration provide at best indirect evidence of the involvement of SP receptor activation in depression. By contrast, several readily observed behaviors correlate well with a drug's (e.g. benzodiazepine partial and full inverse agonists) anxiogenic potential. These behaviors include inhibition of social interaction, defensive behavior, and reduced exploration of open, unprotected spaces. Results of several studies suggest that tachykinin receptor activation in one or another brain region may produce anxiety-like states. A larger body of literature has documented behavioral effects such as repetitive foot-drumming and grooming, which are of less clear relevance to mood or anxiety disorders in humans. Studies in which tachykinins were administered peripherally are difficult to interpret owing to the possibility of side effects on pain sensation and blood pressure, as well as to limited (if any) blood-brain barrier permeability; these studies are not included in this discussion.

Several studies have demonstrated aversion and/or anxiety-like behavior following central administration of neurokinin receptor agonists. The place conditioning paradigm, in which rats are allowed to choose or avoid spending time in a compartment that has previously been paired with drug administration, has been used to demonstrate the aversive quality of central SP administration. For example, subchronic ICV adminstration of the dimethyl-C7 analog resulted in conditioned place aversion (79). Intracerebroventricularly administered SP, neurokinin A, and NK_1- or NK_2-selective agonists all produced an anxiogenic effect in the mouse elevated plus-maze test (80). In this behavioral test, which typically utilizes rats or mice, reduction of entries into or time spent within open, unprotected arms of the maze is interpreted as anxiety behavior. Of note, in most cases the SP agonists

produced dose-response curves with a biphasic profile in which high doses were ineffective, suggesting effects on multiple receptor subtypes, diffusion to additional brain regions, or non-specific effects of high-dose drug administration.

SP has been microinjected into a number of brain regions, in an effort to ascertain potential neuroanatomical pathways involved in the production of anxiety or specific behaviors. Microinjection into the rat periaqueductal grey causes both anxiogenesis, as assessed in the elevated plus-maze (29) and conditioned place aversion (81). High doses were less effective than intermediate doses tested, perhaps secondary to diffusion to neighboring brain regions in which SP exerts opposite effects. Conversely, microinjection of SP into the rat nucleus basalis magnocellularis, a cholinergic nucleus involved in memory and reinforcement, produced anxiolytic rather than anxiogenic effects in the elevated plus-maze and social interaction tests (82). Moreover, SP, via NK_1 receptors, induced place preference rather than aversion when injected into this region (83, 84). These studies indicate further that tachykinin receptor activation can exert multiple behavioral effects depending on the specific brain region(s) involved.

In guinea pigs, ICV administration of the peptide NK_1 agonist GR resulted in prolonged vocalizations (2). It is interesting that acute pretreatment with the tricyclic antidepressant imipramine or the selective serotonin reuptake inhibitor (SSRI) fluoxetine, but not anxiolytics, greatly attenuated the vocalizations. Not surprisingly, NK_1 receptor antagonist pretreatment also blocked the acute effect of the SP agonist. Although this behavioral assay is not generally used to screen for novel antidepressants, the results of these studies suggest that SP is "depressogenic." The interpretation of these findings is, however, complex. The antidepressant efficacy of imipramine and fluoxetine requires prolonged treatment, not acute administration as was performed in this study. Moreover, it remains to be demonstrated whether chronic NK_1 agonist administration is active in animal models of depression such as learned helplessness.

In a variety of animals, CNS administration of SP induces one or another pattern of repetitive motor activity that is not clearly related to any specific, human neuropsychiatric disorder. Like many other neuropeptides [eg., ACTH, bombesin, oxytocin, prolactin, CRF and urocortin (85–88)], SP produces grooming and scratching behavior in rats after ICV administration (89). Similarly, central SP or NK_1-selective SP analog administration (90, 91), and to a lesser degree neurokinin A analog administration (91), produces grooming and scratching in mice. The substantia nigra is likely a major anatomic site mediating this grooming behavior (31), and it is dopamine D_1 receptor-dependent (92). In contrast, ICV SP-induced grooming is blocked by the more D_2-selective antagonist, haloperidol (89). Intrathecal SP administration in mice causes a scratching response that resembles the response to a noxious, painful stimulus (93) and is distinct from the grooming behavior in ICV-injected rodents. As noted previously, clinical trials of NK_1 receptor antagonists have failed to show analgesic properties (76a).

In gerbils, ICV administration of NK_1 receptor agonists produces repetitive hind paw tapping, which is suggested to resemble a warning response in this

species, without overall locomotor activation (94). Guinea pigs respond to SP administration with locomotor hyperactivity, wet-dog shakes and face-washing behavior (59); selective NK_1, but not NK_2 or NK_3 receptor agonist administration, mimics SP- induced locomotor activation (95).

In general, most of the behavioral effects of SP described above can be attributed to NK_1 receptor activation. However, as noted earlier, NK_2 receptor activation is reportedly anxiogenic (80). The NK_3 agonist senktide was recently shown to produce anxiolysis in the mouse elevated plus-maze paradigm (96). Senktide administration also produces wet-dog shakes in guinea pigs similarly to NK_1 agonist administration (95). It is unclear whether NK_2 or NK_3 agonist administration would produce behavioral effects in humans.

Effects of Stress on Substance P Systems

Stressful life experience is now believed to play a major role in precipitating episodes of many neuropsychiatric disorders including major depression, and laboratory stressors can produce behavioral and physiological changes resembling depression or other disorders (97). For these reasons, it is of particular interest to examine the influence of experimental stressors on SP neurotransmission.

Experimental stressors of various types (acute and chronic, physical and psychological) are known to alter the synthesis and/or secretion of various neuropeptides, including CRF, vasopressin, neurotensin, opioid peptides and somatostatin in various brain regions (98). SP neurons are also responsive to aversive stimuli. Intermittent footshock in rats reduces SP content in the ventral tegmental area (72, 99), olfactory tubercle (100), and several hypothalamic nuclei (101). Increased SP concentrations were observed in the medial septum and dentate gyrus (100). Acute decreases in peptide content are believed to result from depletion following bursts of synaptic release, diffusion, and/or peptidase activity. Explanations for acute increases in peptide concentration during a time frame too brief to be accounted for by new peptide synthesis and transport are obscure but could reflect diffusion from nerve terminals adjacent to the dissection or perhaps greater efficiency of peptide extraction from extracellular than intracellular compartments. Other reported stress-induced changes in SP concentrations include the following: (*a*) decreases in the septum, striatum and hippocampus following one hour of immobilization (102), (*b*) increases in periaqueductal grey, and (*c*) decreases in nucleus accumbens following saline injection (103). Isolation (one day or one week) caused a modest increase in SP content in the dorsal periaqueductal grey but not in the hippocampus or amygdala (104). SP content was decreased in the frontal cortex and increased in the nucleus accumbens and amygdala following whole-body vibration (105). Finally, chronic (14-day) adjuvant-induction of arthritis was associated with an increase in SP concentration in the median eminence/arcuate nucleus and in the paraventricular nucleus of the hypothalamus (106). It is worth noting that NK_1 receptor activation in the hypothalamus inhibits synthesis and secretion of CRF (reviewed in 107). Because patients with depression exhibit

hypersecretion of CRF and hypothalamic-pituitary-adrenal (HPA) axis hyperactivity (97), it is not initially intuitive from this standpoint that NK_1 receptor antagonists would be beneficial in depression. However, extrahypothalamic, not hypothalamic, CRF systems likely play a seminal role in the pathogenesis of depression.

The NK_1 receptor undergoes rapid endocytosis following ligand binding and activation (108). Internalization of NK_1 receptors (109), as in the case of β-adrenergic receptors (110), is necessary for dephosporylation of desensitized receptors and recycling of receptors to the membrane surface. Decreases in SP receptor binding in the septum and amygdala/piriform cortex of immobilized rats (102) likely reflect stress-induced receptor activation and internalization, though SP concentration itself was unaltered in the amygdala/piriform cortex in this study. Using immunocytochemistry, Rupniak and colleagues have demonstrated NK_1 receptor internalization in the basolateral amygdala following maternal separation in guinea pig pups (2) and after immobilization in gerbils (111); the latter response is prevented by systemic administration of the NK_1 receptor antagonist, L-760,735.

Several stress regimens have been used as a basis for the development of animal models of depression. To our knowledge, no studies of SP peptide content or gene expression in these animal models have been published. We have conducted preliminary studies of PPT-A gene expression in adult rats subjected as neonates to repeated maternal separation, a model which has been used to model anxiety and/or depression (112, 113). No differences in PPT-A mRNA levels in the medial amygdaloid nucleus, medial habenula, or nucleus of the solitary tract were observed relative to controls (S Stout, unpublished data).

In summary, numerous alterations in SP synthesis and/or release and NK_1 receptor activation have been reported following exposure to acute or chronic stressors. In most cases, these changes are suggestive of SP neuronal activation by stress. The pattern of activation, or in some cases inhibition, is likely dependent on the timing and precise nature of the stressor. Brain regions of particular interest include the amygdala, hypothalamus and other limbic sites, cerebral cortex, ventral tegmental area, nucleus accumbens, and periaqueuductal grey. Collectively these nuclei take part in the processing of motivation, reward, and physiologic and cognitive responses to fear, and as such are of great relevance to mood and anxiety disorders.

Transgenic Animals

Transgenic mice lacking the NK_1 receptor have been reported to undergo essentially normal development and display normal withdrawal responses to acute, painful stimuli (114). However, these mice did not display a normal analgesic response to swim stress, and they displayed very little aggression when confronted with an unfamiliar, intruder mouse. There was no effect of the gene deletion on behavior in the open-field test of anxiety. Although another signaling pathway (e.g. NK_3 receptor-mediated) may have compensated by adapting a novel function in these animals compared with wild-type mice, the results appear to implicate

NK_1 receptor activation in the adaptive more than in the anxious response to threat. Ultrasound vocalizations of neonatal mice subjected to maternal separation are significantly less frequent in the NK_1 receptor knockout as compared with wild-type mice, a reduction similar to the one observed in anxiolytic- or antidepressant-treated mice (115). Transgenic mice in which the PPT-A gene encoding SP and neurokinin A is deleted have also been generated (116). These animals do display a diminished responsiveness to acute, intense pain, in contrast to the data reported in NK_1 knockout mice. To our knowledge, behavioral analysis pertinent to the neurobiology of depression or anxiety disorders has not been conducted in the PPT-A knockout mice.

Effects of Antidepressant Drugs and Electroconvulsive Stimuli on SP Systems

Contrasting findings have been reported with respect to the effect of antidepressant treatment in SP tissue concentrations. In the study with the highest internal consistency, 40-day treatment with any of five different antidepressant agents resulted in a modest reduction of SP concentration in the striatum, substantia nigra, and amygdala (117). By contrast, 14-day treatment with chlorimipramine, nortriptyline, or amitriptyline did not change SP concentrations in the amygdala, cingulate cortex, nucleus accumbens or hypothalamus (118). In another study, repeated injections with desipramine for 14 days did not alter substantia nigra SP concentrations (119). Finally, 14-day treatment with amoxapine or amitriptyline did not change SP concentration in the hypothalamus or cerebral cortex (120). Thus, these studies collectively argue that chronic antidepressant administration does not likely reduce SP concentrations in the rat CNS. Some of the differences between these studies may reflect different durations of treatment [i.e. more than 14 days may be required for some effects on SP concentration, though neurochemical effects of antidepressants in rodents generally require fewer than 14 days of treatment (121–124)]. The drugs chosen, doses, routes of administration, and antisera used in the SP assays also varied between these studies.

There is some evidence that antidepressant treatment may actually increase SP concentration in the periaqueductal grey. Two-week administration of the tricyclic antidepressant imipramine, the SSRIs zimelidine, or alaproclate increased SP and neurokinin A/B concentrations in the periaqueductal grey (125), though these effects were not reproduced in each experiment reported in this series. Mixed effects in the frontal cortex and no effect in the hypothalamus or raphe nucleus were found. A second study by this group, however, failed to demonstrate any effect of tricyclic antidepressant or alaproclate treatment on SP concentration in the periaqueductal grey (118). A third study did show increased SP and neurokinin A concentrations in the periaqueductal grey and increased extracellular SP concentration in the periaqueductal grey region as sampled by microdialysis, following acute alaproclate administration (126). Because SP administration in the periaqueductal grey is aversive and anxiogenic, it is counterintuitive that antidepressant agents would

increase the concentration of this peptide. More in accordance with prediction, acute treatment with the anxiolytic compound diazepam reduced SP concentration in the rostral hippocampus and dorsal periaqueductal grey (104). Obviously tissue peptide concentration is a relatively crude measure of peptidergic circuit activity.

A few studies have examined PPT-A gene expression following antidepressant drug treatment. Transient decreases in medullary raphe PPT-A mRNA expression following subchronic chlorgyline, a monoamine oxidase inhibitor, and zimelidine treatment have been reported (127). Neurons in this region project to the spinal cord, where decreases in SP concentration were detected following antidepressant treatment (125), though not in this study. As noted previously, ascending projections from rodent raphe neurons have not been shown to contain SP; perhaps of more relevance to the pathobiology of depression is determining if ascending pathways in nonhuman primates are affected. In a second study, treatment with zimelidine for 5 but not 14 days, or chronic chlorgyline treatment significantly increased striatal γPPT mRNA expression (128). Finally, our group did not observe a change in PPT-A gene expression following 26-day treatment with the antidepressants fluoxetine, reboxetine, venlafaxine, or tranycypromine in various basal forebrain structures, including the striatum (129); brainstem nuclei such as the medullary raphe were not examined in this study. In summary, conflicting evidence exists for either SP concentration or PPT-A gene expression changes in response to antidepressant drug treatment.

Electroconvulsive shock (ECS) in rats, used as a model for human electroconvulsive therapy, resulted in a 43% decrease in preprotachykinin-A mRNA in the dorsolateral caudate-putamen following daily administration for six days (130), a report opposite to the effects of subchronic zimelidine or chronic chlorgyline administration noted above. Conversely, SP concentrations were unaffected in the striatum and other brain regions following ECS (131). Other reports from this group have demonstrated positive dopaminergic regulation of striatal PPT-A mRNA expression (132); for example, chronic administration of the D_2 receptor antagonist and antipsychotic halperidol, but not atypical antipsychotic drugs, resulted in a reduction in PPT-A mRNA expression in the dorsolateral caudate-putamen and nucleus accumbens (133). Electroconvulsive therapy is known not only for its efficacy in treatment of major depression but particularly for depression with psychotic features and the manic phase of bipolar illness (134). Therefore, it is possible that effects of ECS on striatal SP neurotransmission may be related to antimanic or antipsychotic potential, rather than an antidepressant effect per se. Differences between the aforementioned study results may also merely reflect methodologic differences such as the time courses of treatment, splice variants that were probed, and the striatal subdivisions chosen for image analysis.

Preclinical Evaluation of Neurokinin Receptor Antagonists

Selective, nonpeptide antagonists of the various tachykinin receptor subtypes were not available prior to 1991 (135). These antagonists are highly species-dependent

in terms of their relative potencies (136–138) (see Table, Figure 2). In general, antagonists that bind potently to human NK_1 receptors bind similarly to guinea pig and gerbil NK_1 receptors, and these compounds have lower affinity for rat and murine NK_1 receptors. By contrast, the antagonist RP 67,580 preferentially binds to rat and mouse NK_1 receptors rather than guinea pig receptors. For this reason, behaviors produced in guinea pigs and gerbils by tachykinin agonist administration have proved useful for the identification of potential NK_1 receptor antagonists with favorable qualities for evaluation in humans, such as oral bioavailability and brain penetrance. However, models of anxiety and depression are much better established in rodent species, and therefore many neuropharmacologic studies have been carried out in rats and mice.

Behavioral models for testing compounds with antidepressant potential include those that are strictly drug screening tests based on empirically observed properties of antidepressants in animals unrelated to depressive symptomatology and those designed to resemble one or more symptom of depression. An example of the former type is the rat forced swim test, as originally formulated by Porsolt (139), in which antidepressant agents increase struggling and decrease passive floating. Tests that model depressive symptoms include chronic

TABLE 1 Preclinical Evaluation of Nonpeptide NK_1 Receptor Antagonists

Compound	Species selectivity	Anxiolytic activity	Antidepressant activity	Reference(s)
CP-96345	h,[a] gp,[b] g[c] > r,[d] m[e]	no	[f]	141
RP 67,580	r, m > gp			151
WIN 51708	r > h			152
FK 888	h > r	yes		80, 153
FK 224	h ∼ r			153
CGP 49823	g > r	yes		143, 154
SR 140333	r ∼ h ∼ gp			155
GR 203040	h, g > r			156
SZT NKT 343	h, g, gp > r			157
CP 122,721	h, gp > r			158
LY 303870	h, gp > r, m			159
L-733,060	h, g, gp > r	see text	see text	2

[a]h = human

[b]gp = guinea pig

[c]g = gerbil

[d]r = rat

[e]m = mouse

[f]blank = not tested; ; ; ;

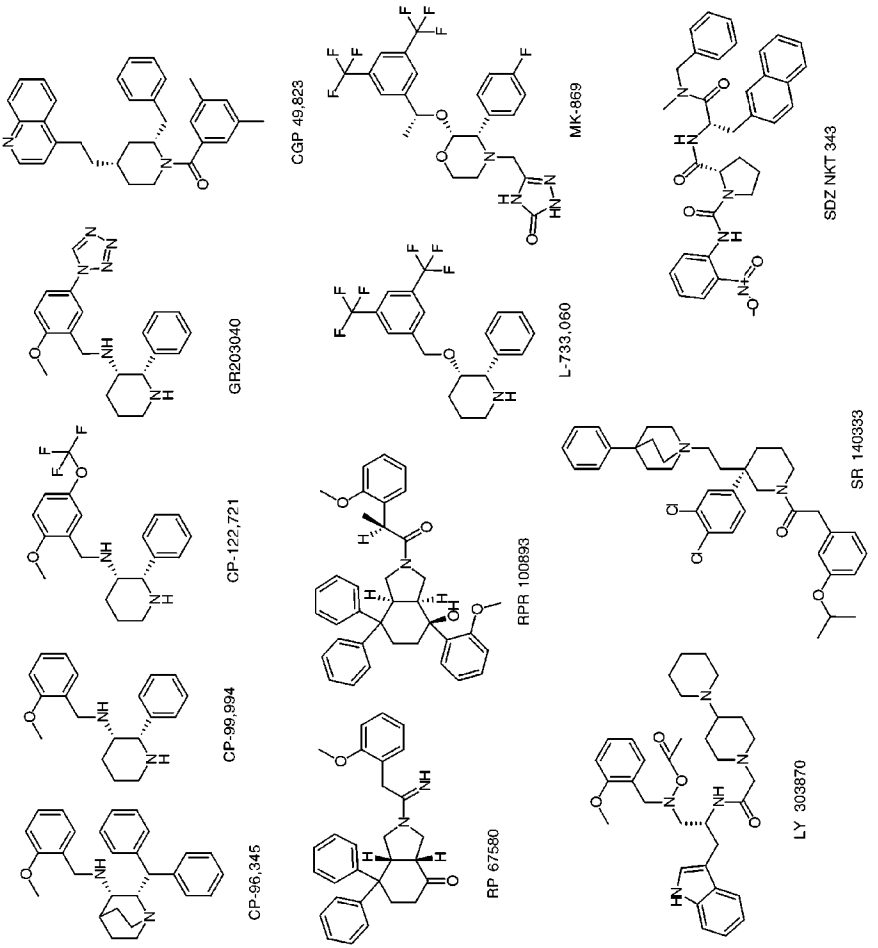

Figure 2 Chemical structures of neurokinin$_1$ antagonists.

mild stress-induced anhedonia, in which rats subjected to various stressors develop an increased threshold for rewarding stimuli such as intra-accumbens electrical stimulation. Unlike tests of anxiety, behavior modification in these models is generally unidirectional; i.e. "depressogenic" compounds are not commonly identified in this manner. Aside from equivocal results in the swim test (140), published studies are lacking regarding activity of tachykinin receptor antagonists in these models.

As noted previously, maternal separation of neonatal animals has been used as a model of anxiety or depression (112, 113). Relatively brief manipulation of these animals produces long-term endocrine and behavioral sequellae, which are responsive to antidepressant administration. Although the effects of NK_1 receptor antagonists on the long-term consequences of maternal deprivation have not been reported, Rupniak and colleagues did report that systemic pretreatment with L 733,060 or other NK_1 receptor antagonists reduced the vocalizations of guinea pig pups (2) or neonatal mice (115) subjected to maternal separation. Most importantly, antidepressant and anxiolytic agents reduced guinea pig vocalizations as well. Clearly, more preclinical studies are required in order to evaluate the antidepressant activity of NK_1 receptor antagonists.

Evidence in laboratory animals is equivocal regarding the anxiolytic potential of NK_1 receptor antagonists. In the mouse black-and-white box test (similar in principle to the elevated plus-maze), systemic administration of CP 96,345 produced overall motor impairment but no anxiogenic or anxiolytic effect per se (141). Newer NK_1 receptor antagonists have not been reported to produce motor impairment; in general the more recent compounds have higher selectivity for the NK_1 receptor with lower affinity for other sites such as sodium and calcium channels (142). ICV injection of the NK_1 receptor antagonist, FK 888, produced anxiolytic effects in the mouse elevated plus-maze test (statistical significance depended largely on analysis of percent time in open arms of the maze rather than on analysis of percent of entries) without altering overall locomotor activity or motor coordination (80). The antagonist compound CGP 49823 reportedly produced anxiolysis in rats placed in an unfamiliar environment (143) and in the rat social interaction test (140), but according to the latter abstract was ineffective in the elevated plus-maze test (140).

The behavioral and physiologic effects of tachykinin receptor antagonists have been further described in other experiments not directly related to antidepressant or anxiolytic potential. ICV SP administration increased heart rate and blood pressure, effects that were reversed by CP 96,345 (144); neurokinin A and NK_2 receptor antagonists produced effects similar to SP and CP 96,345, respectively. Subsequently, many brain regions have been identified that elicit pressor responses to SP microinjections, including the locus coeruleus, periaqueductal grey, parabrachial nucleus, rostral ventrolateral medulla, central nucleus of the amygdala, paraventricular nucleus, and other hypothalamic nuclei (27). In a related study, ICV NK_1, but not NK_2 receptor blockade, reduced the cardiovascular response (increase in heart rate and mean arterial pressure) of rats exposed to acute stress induced by

s.c. injection of formalin (145). The doses required to produce this effect were reportedly less than those required to produce antinociception following intrathecal administration. However, the interpretation of the NK_1 receptor antagonist effect as a disruption of a general, physiologic stress response would have been clearer if a nonpainful stressor had been chosen in this study.

Microinjection of NK_1 receptor antagonists, CP 96,345 or CP 99,994 into the caudal pontine reticular nucleus, a relay nucleus involved in processing acoustic stimuli, blocked footshock-induced sensitization of the acoustic startle response (146). One source of SP neurons projecting to this region is the laterodorsal tegmental nucleus; there are no known direct SP-ergic projections from the central nucleus of the amygdala (147), a region necessary for acoustic startle response sensitization (148).

Systemic CP 96,345 administration, or microinjection directly into the medial hypothalamus of cats results in blockade of the facilitation of hypothalamic-stimulated defensive rage behavior by medial amygdaloid electrical stimulation (149). These results, along with the demonstration of a direct medial amygdaloid-medial hypothalamus pathway, suggest that when activated, neurons in the medial amygdaloid nucleus (a region of peak PPT-A expression within the brain) release SP from nerve terminals in the hypothalamus, resulting in potentiation of certain fear- or stress-induced behaviors.

At least two studies have indicated a possible anxiolytic profile of NK_2 receptor antagonists, despite the reportedly sparse distribution of this receptor in the CNS. Acute peripheral injection of the NK_2 receptor antagonists SR 48968 and GR 159897 produced an increase in the amount of time mice or marmosets spent in exposed or potentially threatening locations, in light-dark box and human confrontation tests, respectively (150). ICV administration of SR 48968 was also anxiolytic in the mouse elevated plus-maze test (80). The antidepressant activity of NK_2 receptor antagonists in animal models has not been studied.

CLINICAL STUDIES

Cerebrospinal Fluid (CSF) and Postmortem Measurements of Substance P

In the absence of a direct method for obtaining neurochemical measurements of CNS activity in humans, neurotransmitters and metabolites are frequently measured in cerebrospinal fluid (CSF) samples obtained by lumbar puncture in psychiatric patients and controls. Although there are certainly limitations to this method, such as the lack of anatomic resolution and the preferential sampling of neurotransmitters released from brain regions in proximity to the ventricular system, the blood-brain barrier ensures that a change in neurotransmitter concentration in a particular patient population can at least be assumed to represent some sort of change in CNS activity. CSF findings in studies of depressed patients include

increased CRF (160), decreased somatostatin (161), and decreases in the serotonin metabolite 5-HIAA, particularly within impulsive and/or suicidal subgroups of patients (162).

The measurement of CSF SP concentrations in depressed patients is an understudied area. Rimón et al (163) reported that depressed patients exhibited a fourfold mean elevation in SP-like immunoreactivity. Electrophoresis patterns also indicated an increase in SP degradation in the depressed group. A second study from the same investigators was not sufficiently powered to examine changes in depression, but it was noted that the three patients with depression had a substantially higher mean CSF SP concentration than did controls or patients with personality disorders, though the differences were not statistically significant (164). In neither study was SP concentration significantly altered in schizophrenic patients relative to controls. The findings of increased CSF SP in depression were not replicated by a second group (165), though the patient populations in the two studies differed widely. Furthermore, lower rather than higher CSF SP concentrations correlated with psychic anxiety and self-reported symptoms of sadness and inner tension in a group of nondepressed patients, most of whom suffered from chronic pain syndromes (166). Obviously, the relevance of the latter study to patients with mood disorders is unclear. In a study that unfortunately did not include healthy controls, successful treatment of depression with fluoxetine for six weeks did not significantly alter CSF concentration of (N-terminally extended) SP in these patients (167). Treatment effects on CSF SP concentrations have not been examined in any subsequent, published studies. To our knowledge, there have in fact been no studies of CSF SP in depression in the past decade. Without further data, it is impossible to draw conclusions from the extant, contradictory literature.

A second method commonly employed to examine the involvement of a neurotransmitter system in human disorders is postmortem examination of human tissue. In most of these studies, patterns of gene expression or membrane receptor density rather than neuropeptide concentration have been studied owing to a variety of technical reasons. In a study of NK_1 receptor autoradiography in cingulate cortex of controls, and patients with schizophrenia, unipolar and bipolar depression collected by the Stanley Foundation, total receptor binding was unchanged, but a decrease in superficial/deep cortical layer NK_1 receptor density ratio was noted in the unipolar depression group (168). Clearly, further work is required to characterize more fully the regional pattern of SP and NK_1 receptor expression in depression.

Clinical Trials

The preclinical studies and CSF and postmortem data reviewed above do not establish a clear link between SP neurotransmission and depression. The reported antidepressant efficacy of the NK_1 receptor antagonist, MK-869 (2) was therefore a surprise to the field. MK-869 was selected from among various NK_1 receptor

antagonists under development based on its affinity for human receptors, oral bioavailability, selectivity, brain penetrance, and favorable pharmacokinetics. Regarding selectivity, it is important to note that MK-869 has little or no affinity for serotonin, norepinephrine and dopamine transporters, or for monoamine oxidase A and B. Therefore, any efficacy of the drug may be assumed to result from its action on NK_1 receptors.

In a double-blind study of 213 patients randomly assigned to once-daily placebo, the SSRI paroxetine (20 mg) or MK-869 (300 mg), clinical improvement as measured with the 21-item Hamilton rating scale for depression (HAM-D) was virtually identical over a 6-week period in the paroxetine and MK-869 groups (Figure 3). The therapeutic delay period was also similar in the two groups. In addition, the NK_1 receptor antagonist reduced symptoms of anxiety, as measured by the Hamilton anxiety scale (HAM-A) (Figure 3). Thus far, results of NK_1 receptor antagonists in patients with anxiety disorders have not been reported.

Of note, MK-869 did not produce significantly higher rates of any side effect reported, when compared with placebo or paroxetine, and was associated with a substantially lower incidence of sexual dysfunction when compared to paroxetine (3% vs. 26%). This side effect is one of the principal liabilities of paroxetine and the other SSRIs; in fact, rates much higher than 26% have been reported (169, 170) when patients are specifically asked about this side effect. In summary, the report indicated that NK_1 receptor antagonists may possibly offer similar efficacy in certain patient populations to established antidepressant agents with a more favorable side effect profile.

Such a dramatic finding begs for confirmation and several clinical trials are currently underway to test the hypothesis that NK_1 receptor antagonists are effective antidepressants and/or anxiolytics. In a second trial, MK-869 apparently did not demonstrate higher efficacy than placebo (171). A more potent NK_1 receptor antagonist has subsequently been brought into clinical trials; no data have been published regarding this second Merck compound at the time of this writing.

CONCLUSIONS

With the exception of the report from the Merck group detailing preclinical development of NK_1 receptor antagonists and the antidepressant efficacy of the compound, MK-869, a systematic literature review failed to provide strong evidence for the antidepressant efficacy of this class of compounds. In most cases, insufficient and/or contradictory evidence exists to establish particular arguments for or against the involvement of SP in depression. Alterations in SP synthesis and secretion in animal models of depression, and the effects of NK_1 receptor antagonists in these models are essentially untested. Measurement of SP in human tissue is a neglected field: To date it is equivocal whether CSF SP is altered in depression, and studies of PPT-A gene expression in postmortem brain tissue of depressed patients are lacking.

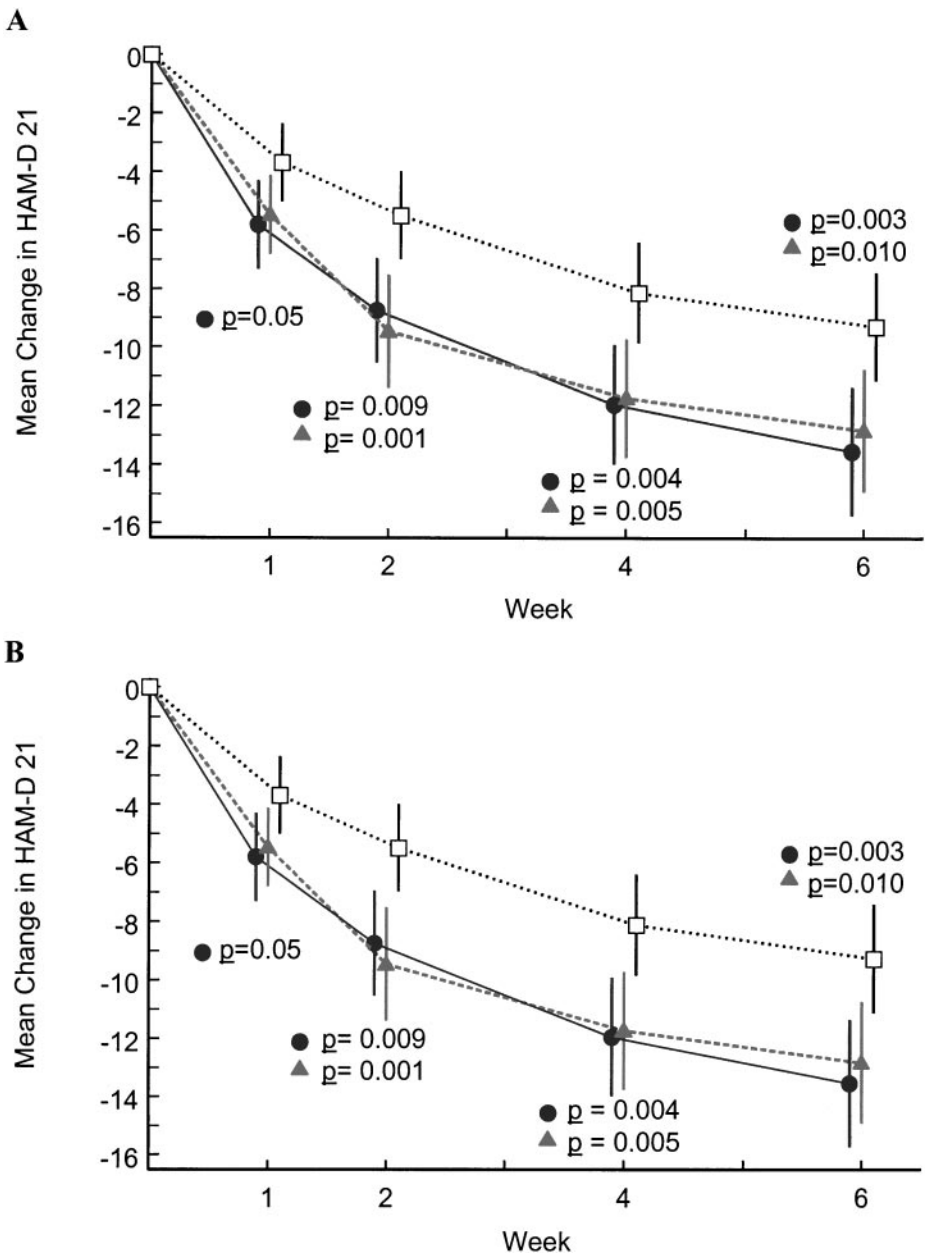

Figure 3 Effect of treatment with MK-869 (300 mg/day) or paroxetine (20 mg/day) on mean change from baseline on the Hamilton Depression Scale (HAM-D21) (*A*) and the Hamilton Anxiety Scale (14 items), (*B*) in patients with major depressive disorder. Comparisons are of MK-869 (dark circles, n = 66) or paroxetine (grey triangles, n = 68) versus placebo (open squares, n = 64). Error bars show 95% confidence intervals. Reprinted with minor modifications, with permission (2). Copyright 1998, American Association for the Advancement of Science.

The rich anatomic distribution of SP and NK_1 receptor-expressing neurons in limbic and monoaminergic regions of the brain is certainly compatible with the involvement of SP-ergic neurotransmission in depression and/or antidepressant efficacy. Studies to date have identified several regions and pathways of particular interest, including the following: (*a*) a medial amygdala projection to the hypothalamus involved in potentiation of defensive rage behavior, (*b*) the locus coeruleus, in which NK_1 receptor activation may stimulate noradrenergic neuronal firing in response to stress, (*c*) the periaqueductal grey, which is heavily innervated by SP neurons and which has long been shown to be involved in behavioral and physiologic fear responses. These and other regions in the brain serve as plausible loci for mediating antidepressant responses to NK_1 receptor antagonist administration. Considerable, additional basic science efforts to determine the neurochemical and physiologic role of SP in these various brain regions may yield further insight into the psychopharmacologic potential of NK_1 receptor antagonists.

Based on the limited research in this field, the role of SP neuronal pathways in depression is unclear. However, the findings of the first clinical trial of an NK_1 receptor antagonist in depression are encouraging and should stimulate further preclinical and clinical evaluation of these compounds. The limitations of current antidepressant medications, including the delay for a full therapeutic response, a substantial rate of nonresponders, and bothersome side effect profiles, merit the full exploration of all plausible agents with novel antidepressant mechanisms of action.

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LITERATURE CITED

1. Charney DS, Berman RM, Miller HL. 1998. Treatment of depression. See Ref. 172, pp. 705–731
2. Kramer MS, Cutler N, Feighner J, Shrivastava R, Carman J, et al. 1998. Distinct mechanism for antidepressant activity by blockade of central substance P receptors. *Science* 281:1640–45
3. Maggio JE. 1988. Tachykinins. *Annu. Rev. Neurosci.* 11:13–28
4. Chang MM, Leeman SE. 1970. Isolation of a sialogogic peptide from bovine hypothalamic tissue and its characterization as substance P. *J. Biol. Chem.* 245:4784–90
5. Krause JE, Chirgwin JM, Carter MS, Xu ZS, Hershey AD. 1987. Three rat preprotachykinin mRNAs encode the neuropeptides substance P and neurokinin A. *Proc. Natl. Acad. Sci. USA* 84:881–85
6. Kotani H, Hoshimaru M, Nawa H, Nakanishi S. 1986. Structure and gene organization of bovine neuromedin K precursor. *Proc. Natl. Acad. Sci. USA* 83:7074–78
7. Lee CM, Iversen LL, Hanley MR, Sandberg BE. 1982. The possible existence of multiple receptors for substance P. *Naunyn-Schmiedebergs Arch. Pharmacol.* 318:281–87

8. Mantyh PW, Maggio JE, Hunt SP. 1984. The autoradiographic distribution of kassinin and substance K binding sites is different from the distribution of substance P binding sites in rat brain. *Eur. J. Pharmacol.* 102:361–64
9. Danks JA, Rothman RB, Cascieri MA, Chicchi GG, Liang T, et al. 1986. A comparative autoradiographic study of the distributions of substance P and eledoisin binding sites in rat brain. *Brain Research* 385:273–81
10. Buck SH, Burcher E, Shults CW, Lovenberg W, O'Donohue TL. 1984. Novel pharmacology of substance K–binding sites: a third type of tachykinin receptor. *Science* 226:987–89
11. Laufer R, Gilon C, Chorev M, Selinger Z. 1986. Characterization of a neurokinin B receptor site in rat brain using a highly selective radioligand. *J. Biol. Chem.* 261:10257–63
12. Maggi CA. 2000. The troubled story of tachykinins and neurokinins. *Trends Pharmacol. Sci.* 21:173–75

12a. Watling KJ. 1988. Tachykinin receptors. In *The RBI Handbook of Receptor Classification and Signal Transduction, 3rd ed*. pp. 112–113. Natick, MA: RBI

13. Regoli D, Boudon A, Fauchere J-L. 1994. Receptors and antagonists for substance P and related peptides. *Pharmacol. Rev.* 46:551–99
14. Yokota Y, Sasai Y, Tanaka K, Fujiwara T, Tsuchida K, et al. 1989. Molecular characterization of a functional cDNA for rat substance P receptor. *J. Biol. Chem.* 264:17649–52
15. Hershey AD, Krause JE. 1990. Molecular characterization of a functional cDNA encoding the rat substance P receptor. *Science* 247:958–62
16. Nakanishi S. 1991. Mammalian tachykinin receptors. *Annu. Rev. Neurosci.* 14: 123–36
17. Shigemoto R, Yokota Y, Tsuchida K, Nakanishi S. 1990. Cloning and expression of a rat neuromedin K receptor cDNA. *J. Biol. Chem.* 265:623–28
18. Takeda Y, Chou KB, Takeda J, Sachais BS, Krause JE. 1991. Molecular cloning, structural characterization and functional expression of the human substance P receptor. *Biochem. Biophys. Research Commun.* 179:1232–40
19. Gerard NP, Eddy RL, Shows TB, Gerard C. 1990. The human Neurokinin A (Substance K) receptor, molecular cloning of the gene, chromosome localization and isolation of cDNA for tracheal and gastric tissues. *J. Biol. Chem.* 20455–62
20. Buell G, Schulz MF, Arkinstall SJ, Maury K, Missotten M, et al. 1992. Molecular characterisation, expression and localisation of human neurokinin–3 receptor. *FEBS Lett.* 299:90–95
21. Arai H, Emson PC. 1986. Regional distribution of neuropeptide K and other tachykinins (neurokinin A, neurokinin B and substance P) in rat central nervous system. *Brain Research* 399:240–49
22. Shults CW, Quirion R, Chronwall B, Chase TN, O'Donohue TL. 1984. A comparison of the anatomical distribution of substance P and substance P receptors in the rat central nervous system. *Peptides* 5:1097–1128
23. Warden MK, Young WS, III. 1988. Distribution of cells containing mRNAs encoding substance P and neurokinin B in the rat central nervous system. *J. Comp. Neurol.* 272:90–113
24. Marksteiner J, Sperk G, Krause JE. 1992. Distribution of neurons expressing neurokinin B in the rat brain: immunohistochemistry and in situ hybridization. *J. Comp. Neurol.* 317:341–56
25. Mantyh PW, Hunt SP, Maggio JE. 1984. Substance P receptors: localization by light microscopic autoradiography in rat brain using [^{3}H]SP as the radioligand. *Brain Research* 307:147–65
26. Jones RS, Olpe HR. 1982. A structure–activity profile of substance P and some

of its fragments on supraspinal neurones in the rat. *Neurosci. Lett.* 33:67–71
27. Ku Y-H, Tan L, Li L-S, Ding X. 1998. Role of corticotropin-releasing factor and substance P in pressor responses of nuclei controlling emotion and stress. *Peptides* 19:677–82
28. Ogata N. 1979. Effects of substance P on neurons of various brain regions in vitro. *Brain Research* 176:395–400
29. Aguiar MS, Brandão ML. 1996. Effects of microinjections of the neuropeptide substance P in the dorsal periaqueductal gray on the behaviour of rats in the plus-maze test. *Physiol. Behavior* 60:1183–86
30. de Araujo JE, Huston JP, Brandão ML. 1998. Aversive effects of the C-fragment of Substance P in the dorsal periaqueductal gray matter. *Exp. Brain Research* 123:84–89
31. Kelley AE, Iversen SD. 1979. Substance P infusion into substantia nigra of the rat: behavioural analysis and involvement of striatal dopamine. *Eur. J. Pharmacol.* 60:171–79
32. Elliott PJ, Alpert JE, Bannon MJ, Iversen SD. 1986. Selective activation of mesolimbic and mesocortical dopamine metabolism in rat brain by infusion of a stable substance P analogue into the ventral tegmental area. *Brain Research* 363:145–47
33. Dam TV, Martinelli B, Quirion R. 1990. Autoradiographic distribution of brain neurokinin–1/substance P receptors using a highly selective ligand [3H]-[Sar9, Met(O2)11]-substance P. *Brain Research* 531:333–37
34. Maeno H, Kiyama H, Tohyama M. 1993. Distribution of the substance P receptor (NK-1 receptor) in the central nervous system. *Brain Research. Mol. Brain Research* 18:43–58
35. Yip J, Chahl LA. 1999. Distribution of Fos-like immunoreactivity in guinea-pig brain following administration of the neurokinin-1 receptor agonist, [SAR9,MET-(O2)11]substance P. *Neuroscience* 94: 663–73
36. Dam T-V, Escher E, Quirion R. 1990. Visualization of neurokinin-3 receptor sites in rat brain using the highly selective ligand [3H]senktide. *Brain Research* 506:175–79
37. Ding YQ, Shigemoto R, Takada M, Ohishi H, Nakanishi S, et al. 1996. Localization of the neuromedin K receptor (NK3) in the central nervous system of the rat. *J. Comp. Neurol.* 364:290–310
38. Shughrue PJ, Lane MV, Merchenthaler I. 1996. In situ hybridization analysis of the distribution of neurokinin-3 mRNA in the rat central nervous system. *J. Comp. Neurol.* 372:395–414
39. Dam TV, Escher E, Quirion R. 1988. Evidence for the existence of three classes of neurokinin receptors in brain. Differential ontogeny of neurokinin-1, neurokinin-2 and neurokinin-3 binding sites in rat cerebral cortex. *Brain Research* 453:372–76
40. Hagan RM, Beresford IJ, Stables J, Dupere J, Stubbs CM, et al. 1993. Characterisation, CNS distribution and function of NK2 receptors studied using potent NK2 receptor antagonists. *Regul. Pept.* 46:9–19
41. Mai JK, Stephens PH, Hopf A, Cuello AC. 1986. Substance P in the human brain. *Neuroscience* 17:709–39
42. Nomura H, Shiosaka S, Tohyama M. 1987. Distribution of substance P-like immunoreactive structures in the brainstem of the adult human brain: animmunocytochemical study. *Brain Research* 404:365–70
43. Tiberi M, Lemay J, Magnan J. 1987. Specific binding of [3H]-substance P to various regions of the human foetal brain. *Peptides* 8:947–49
44. Jordan D, Kermadi I, Rambaud C, Gilly R, Bouvier R, et al. 1995. Regional distribution of substance P binding sites in the brainstem of the human newborn. *Brain Research* 695:117–24
45. Arai H, Emson PC, Carrasco LH.

1987. Huntington's disease: changes in tachykinin content in postmortem brains. *Ann. Neurol.* 22:587–94
46. Chawla MK, Gutierrez GM, Young W. Sr., McMullen NT, Rance NE. 1997. Localization of neurons expressing substance P and neurokinin B gene transcripts in the human hypothalamus and basal forebrain. *J. Comp. Neurol.* 384:429–42
47. Dietl MM, Palacios JM. 1991. Phylogeny of tachykinin receptor localization in the vertebrate central nervous system: apparent absence of neurokinin-2 and neurokinin-3 binding sites in the human brain. *Brain Research* 539:211–22
48. Mileusnic D, Lee JM, Magnuson DJ, Hejna MJ, Krause JE, et al. 1999. Neurokinin-3 receptor distribution in rat and human brain: an immunohistochemical study. *Neuroscience* 89:1269–90
49. Owens MJ, Stout SC, Mulchahey JJ, Plotsky PM. 1996. Molecular and neurobiological mechanisms in the treatment of psychiatric disorders. In *Textbook of Psychiatry*, ed. A Tasman, J Kay, JA Lieberman. Philadelphia: WB Saunders
50. Chan-Palay V, Jonsson G, Palay SL. 1978. Serotonin and substance P coexist in neurons of the rat's central nervous system. *Proc. Natl. Acad. Sci. USA* 75:1582–86
51. Björklund AJ, Emson PC, Gilbert RF, Skagerberg G. 1979. Further evidence for the possible coexistence of 5-hydroxytryptamine and substance P in medullary raphe neurones of rat brain. *Brit. J. Pharmacol.* 66:112P–13P
52. Hokfelt T, Ljungdahl A, Steinbusch H, Verhofstad A, Nilsson G, et al. 1978. Immunohistochemical evidence of substance P-like immunoreactivity in some 5-hydroxytryptamine-containing neurons in the rat central nervous system. *Neuroscience* 3:517–38
53. Jones RS, Olpe HR. 1984. Monoaminergic modulation of the sensitivity of neurones in the cingulate cortex to iontophoretically applied substance P. *Brain Research* 311:297–305
54. Barden N, Daigle M, Picard V, Di Paolo T. 1983. Perturbation of rat brain serotonergic systems results in an inverse relation between substance P and serotonin concentrations measured in discrete nuclei. *J. Neurochem.* 41:834–40
55. Baker KG, Halliday GM, Hornung JP, Geffen LB, Cotton RG, et al. 1991. Distribution, morphology and number of monoamine-synthesizing and substance P-containing neurons in the human dorsal raphe nucleus. *Neuroscience* 42:757–75
56. Charara A, Parent A. 1998. Chemoarchitecture of the primate dorsal raphe nucleus. *J. Chem. Neuroanat.* 15:111–27
57. Sergeyev V, Hökfelt T, Hurd Y. 1999. Serotonin and substance P co-exist in dorsal raphe neurons of the human brain. *NeuroReport* 10:3967–70
58. Unger T, Becker H, Petty M, Demmert G, Schneider B, et al. 1985. Differential effects of central angiotensin II and substance P on sympathetic nerve activity in conscious rats. Implications for cardiovascular adaptation to behavioral responses. *Circ. Research* 56:563–75
59. Brent PJ, Johnston PA, Chahl LA. 1988. Increased plasma catecholamines and locomotor activity induced by centrally administered substance P in guinea-pigs. *Neuropharmacology* 27:743–48
60. Guan ZL, Ding YQ, Li JL, Lu BZ. 1998. Substance P receptor-expressing neurons in the medullary and spinal dorsal horns projecting to the nucleus of the solitary tract in the rat. *Neurosci. Research* 30: 213–18
61. Jansen AS, Loewy AD. 1997. Neurons lying in the white matter of the upper cervical spinal cord project to the intermediolateral cell column. *Neuroscience* 77:889–98
62. Guyenet PG, Aghajanian GK. 1977. Excitation of neurons in the nucleus locus coeruleus by substance P and related peptides. *Brain Research* 136:178–84

63. McLean S, Ganong AH, Seeger TF, Bryce DK, Pratt KG, et al. 1991. Activity and distribution of binding sites in brain of a nonpeptide substance P (NK1) receptor antagonist. *Science* 251:437–39
64. Shen KZ, North RA. 1992. Substance P opens cation channels and closes potassium channels in rat locus coeruleus neurons. *Neuroscience* 50:345–53
65. Hahn MK, Bannon MJ. 1999. Stress-induced C-fos expression in the rat locus coeruleus is dependent on neurokinin 1 receptor activation. *Neuroscience* 94: 11183–88
66. Hahn MK, Bannon MJ. 1998. Tachykinin NK_1 receptor antagonists enhance stress-induced c-fos in rat locus coeruleus. *Eur. J. Pharmacol.* 348:155–60
66a. Haddjeri N, Blier P. 2000. Effect of neurokinin-1 receptor antagonists on the function of 5-HT and noradrenaline neurons. *Neuroreport* 11:1323–27.
67. Lorens SA, Paris JM, Brodin E. 1991. Neurokinin innervation of the rat median raphe nucleus does not originate in the brain stem. *Ann. NY Acad. Sci.* 632:431–34
68. Paris JM, Lorens SA. 1987. Intra–median raphe infusions of muscimol and the substance P analogue DiMe-C7 produce hyperactivity: role of serotonin neurons. *Behav. Brain Research* 26:139–51
69. Mason GS, Elliott PJ. 1992. Behavioural consequences following infusion of selective neurokinin agonists into the median raphe nucleus of the rat. *Neuropharmacology* 31:757–60
70. Gradin K, Qadri F, Nomikos GG, Hillegaart V, Svensson TH. 1992. Substance P injection into the dorsal raphe increases blood pressure and serotonin release in hippocampus of conscious rats. *Eur. J. Pharmacol.* 218:363–67
70a. Santarelli L, Gobbi G, Debs PC, Sibille EL, Blier P, et al. 2001. Genetic and pharmacological disruption of NK1 receptor function decreases anxiety-related behaviors and increases serotonergic function. *Proc. Natl. Acad. Sci. USA* In press
71. Bannon MJ, Elliott PJ, Alpert JE, Goedert M, Iversen SD, et al. 1983. Role of endogenous substance P in stress-induced activation of mesocortical dopamine neurones. *Nature* 306:791–92
72. Bannon MJ, Deutch AY, Tam SY, Zamir N, Eskay RL, et al. 1986. Mild footshock stress dissociates substance P from substance K and dynorphin from Met- and Leu-enkephalin. *Brain Research* 381:1986
73. Walker PD, Riley LA, Hart RP, Jonakait GM. 1991. Serotonin regulation of tachykinin biosynthesis in the rat neostriatum. *Brain Research* 546:33–39
74. Chan-Palay V, Palay SL. 1977. Ultrastructural identification of substance P cells and their processes in rat sensory ganglia and their terminals in the spinal cord by immunocytochemistry. *Proc. Natl. Acad. Sci. USA* 74:4050–54
75. Randic M, Miletic V. 1977. Effect of substance P in cat dorsal horn neurons activated by noxious stimuli. *Brain Research* 128:164–69
76. Yaksh TL, Farb DH, Leeman SE, Jessell TM. 1979. Intrathecal capsaicin depletes substance P in the rat spinal cord and produces prolonged thermal analgesia. *Science* 206:481–83
76a. Hill R. 2000. NK1 (substance P) receptor antagonists—why are they not analgesic in humans? *Trends Pharmacol. Sci.* 21:244–6
77. Beattie DT, Connor HE, Hagan RM. 1995. Recent developments in tachykinin NK1 receptor antagonists: prospects for the treatment of migraine headache. *Can. J. Physiol. Pharmacol.* 73:871–77
78. Rupniak NM, Kramer MS. 1999. Discovery of the antidepressant and anti-emetic efficacy of substance P receptor (NK1) antagonists. *Trends Pharmacol. Sci.* 20: 485–90
79. Elliott PJ. 1988. Place aversion induced

by the substance P analogue, dimethyl-C7, is not state dependent: implication of substance P in aversion. *Exp. Brain Research* 73:354–56
80. Teixeira RM, Santos AR, Ribeiro SJ, Calixto JB, Rae GA, et al. 1996. Effects of central administration of tachykinin receptor agonists and antagonists on plus-maze behavior in mice. *Eur. J. Pharmacol.* 311:7–14
81. Aguiar MS, Brandão ML. 1994. Conditioned place aversion produced by microinjections of substance P into the periaqueductal gray of rats. *Behav. Pharmacol.* 5:369–73
82. Hasenöhrl RU, Jentjens O, De Souza Silva MA, Tomaz C, Huston JP. 1998. Anxiolytic-like action of neurokinin substance P administered systemically or into the nucleus basalis magnocellularis region. *Eur. J. Pharmacol.* 354:123–33
83. Hasenöhrl RU, Gerhardt P, Huston JP. 1992. Positively reinforcing effects of the neurokinin substance P in the basal forebrain: mediation by its C-terminal sequence. *Exp. Neurol.* 115:282–91
84. Nikolaus S, Huston JP, Hasenöhrl RU. 1999. Reinforcing effects of neurokinin substance P in the ventral pallidum: mediation by the tachykinin NK1 receptor. *Eur. J. Pharmacol.* 370:93–99
85. Isaacson RL, Hannigan JHJ, Brakkee JH, Gispen WH. 1983. The time course of excessive grooming after neuropeptide administration. *Brain Research Bulletin* 11:289–293
86. Kulkosky PJ. 1988. Bombesin and ceruletide-induced grooming and inhibition of ingestion in the rat. *Ann. NY Acad. Sci.* 525:201–18
87. Drago F, Contarino A, Busa L. 1999. The expression of neuropeptide-induced excessive grooming behavior in dopamine D1 and D2 receptor-deficient mice. *Eur. J. Pharmacol.* 365:125–31
88. Jones DN, Kortekaas R, Slade PD, Middlemiss DN, Hagan JJ. 1999. The behavioural effects of corticotropin-releasing factor-related peptides in rats. *Psychopharmacology* 138:124–32
89. Van Wimersma Greidanus TB, Maigret C. 1988. Grooming behavior induced by substance P. *Eur. J. Pharmacol.* 154:217–20
90. Katz RJ. 1979. Central injection of substance P elicits grooming behavior and motor inhibition in mice. *Neurosci. Lett.* 12:133–36
91. Ravard S, Betschart J, Fardin V, Flamand O, Blanchard JC. 1994. Differential ability of tachykinin NK-1 and NK-2 agonists to produce scratching and grooming behaviours in mice. *Brain Research* 651:199–208
92. Stoessl AJ, Brackstone M, Rajakumar N, Gibson CJ. 1995. Pharmacological characterization of grooming induced by a selective NK-1 tachykinin receptor agonist. *Brain Research* 700:115–20
93. Hylden JL, Wilcox GL. 1981. Intrathecal substance P elicits a caudally-directed biting and scratching behavior in mice. *Brain Research* 217:212–15
94. Bristow LJ, Young L. 1994. Chromodacryorrhea and repetitive hind paw tapping: models of peripheral and central tachykinin NK1 receptor activation in gerbils. *Eur. J. Pharmacol.* 253:245–52
95. Piot O, Betschart J, Grall I, Ravard S, Garret C, et al. 1995. Comparative behavioural profile of centrally administered tachykinin NK1, NK2 and NK3 receptor agonists in the guinea-pig. *Brit. J. Pharmacol.* 116:2496–2502
96. Ribeiro SJ, De Lima TC. 1998. Naloxone-induced changes in tachykinin NK3 receptor modulation of experimental anxiety in mice. *Neurosci. Lett.* 258:155–58
97. Stout SC, Nemeroff CB. 1994. Stress and psychiatric disorders. *Seminars in the Neurosciences* 6:271–80
98. Stout SC, Kilts CD, Nemeroff CB. 1995. Neuropeptides and stress: preclinical findings and implications for pathophysiology.

In *Neurobiological and Clinical Consequences of Stress: From Normal Adaptation to PTSD*, ed. A. Deutch. New York: Raven
99. Lisoprawski A, Blanc G, Glowinski J. 1981. Activation by stress of the habenulo-interpeduncular substance P neurons in the rat. *Neurosci. Lett.* 25:47–51
100. Siegel RA, Düker E-M, Fuchs E, Pahnke U, Wuttke W. 1984. Responsiveness of mesolimbic, mesocortical, septal and hippocampal cholecystokinin and substance P neuronal systems to stress, in the male rat. *Neurochem. Int.* 6:783–89
101. Siegel RA, Duker EM, Pahnke U, Wuttke W. 1987. Stress-induced changes in cholecystokinin and substance P concentrations in discrete regions of the rathypothalamus. *Neuroendocrinology* 46:75–81
102. Takayama H, Ota Z, Ogawa N. 1986. Effect of immobilization stress on neuropeptides and their receptors in rat central nervous system. *Regul. Pept.* 15:239–248
103. Rosen A, Brodin K, Eneroth P, Brodin E. 1992. Short-term restraint stress and s.c. saline injection alter the tissue levels of substance P and cholecystokinin in the peri-aqueductal grey and limbic regions of rat brain. *Acta Physiol. Scand.* 146:341–48
104. Brodin E, Rosen A, Schott E, Brodin K. 1994. Effects of sequential removal of rats from a group cage, and of individual housing of rats, on substance P, cholecystokinin and somatostatin levels in the periaqueductal grey and limbic regions. *Neuropeptides* 26:253–60
105. Nakamura H, Moroji T, Nohara S, Nakamura H, Okada A. 1990. Effects of whole-body vibration stress on substance P- and neurotensin-like immunoreactivity in the rat brain. *Environ. Research* 52:155–63
106. Chowdrey HS, Larsen PJ, Harbuz MS, Lightman SL. 1995. Endogenous substance P inhibits the expression of corticotropin-releasing hormone during a chronic inflammatory stress. *Life Sci.* 57:2021–29
107. Nussdorfer GG, Malendowicz LK. 1998. Role of tachykinins in the regulation of the hypothalamo-pituitary-adrenal axis. *Peptides* 19:949–68
108. Mantyh PW, Allen CJ, Ghilardi JR, Rogers SD, Mantyh CR, et al. 1995. 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. USA* 92:2622–26
109. Garland AM, Grady EF, Lovett M, Vigna SR, Frucht MM, et al. 1996. Mechanisms of desensitization and resensitization of G protein-coupled neurokinin1 and neurokinin2 receptors. *Mol. Pharmacol.* 49:438–46
110. Yu SS, Lefkowitz RJ, Hausdorff WP. 1993. Beta-adrenergic receptor sequestration: a potential mechanism of receptor sensitization. *J. Biol. Chem.* 268:337–41
111. Smith DW, Hewson L, Fuller P, Williams AR, Wheeldon A, et al. 1999. The substance P antagonist L-760,735 inhibits stress-induced NK(1) receptor internalisation in the basolateral amygdala. *Brain Research* 848:90–95
112. Winslow JT, Insel TR. 1991. The infant rat separation paradigm: a novel test for novel anxiolytics. *Trends Pharmacol. Sci.* 12:402–04
113. Ladd CO, Huot RL, Thrivikraman KV, Nemeroff CB, Meaney MJ, et al. 2000. Long-term behavioral and neuroendocrine adaptations to adverse early experience. *Prog. Brain Research* 122:81–103
114. De Felipe C, Herrero JF, O'Brien JA, Palmer JA, Doyle CA, et al. 1998. Altered nociception, analgesia and aggression in mice lacking the receptor for substance P. *Nature* 392:394–97
115. Rupniak NMJ, Carlson EC, Harrison T, Oates B, Seward E, et al. 2000. Pharmacological blockade or genetic deletion of

substance P (NK_1) receptors attenuates neonatal vocalisation in guinea–pigs and mice. *Neuropharmacology* 39:1413–21
116. Cao YQ, Mantyh PW, Carlson EJ, Gillespie AM, Epstein CJ, et al. 1998. Primary afferent tachykinins are required to experience moderate to intense pain. *Nature* 392:390–94
117. Shirayama Y, Mitsushio H, Takashima M, Ichikawa H, Takahashi K. 1996. Reduction of substance P after chronic antidepressants treatment in the striatum, substantia nigra and amygdala of the ra. *Brain Research* 739:70–78
118. Brodin K, Ogren SO, Brodin E. 1994. Clomipramine and clonazepam increase cholecystokinin levels in rat ventral tegmental area and limbic regions. *Eur. J. Pharmacol.* 263:175–80
119. Oblin A, Zivkovic B, Bartholini G. 1984. Involvement of the D-2 dopamine receptor in the neuroleptic-induced decrease in nigral substance P. *Eur. J. Pharmacol.* 105:175–77
120. Hamon M, Gozlan H, Bourgoin S, Benoliel JJ, Mauborgne A, et al. 1987. Opioid receptors and neuropeptides in the CNS in rats treated chronically with amoxapine or amitriptyline. *Neuropharmacology* 26:531–39
121. Manier DH, Gillespie DD, Sanders-Bush E, Sulser F. 1987. The serotonin/noradrenaline-link in brain. I. The role of noradrenaline and serotonin in the regulation of density and function of beta adrenoceptors and its alteration by desipramine. *Naunyn–Schmiedebergs Arch. Pharmacol.* 335:109–14
122. Blier P, de Montigny C, Chaput Y. 1987. Modifications of the serotonin system by antidepressant treatments: implications for the therapeutic response in major depression. *J. Clin. Psychopharmacol.* 7:24S–35S
123. Baker GB, Greenshaw AJ. 1989. Effects of long–term administration of antidepressants and neuroleptics on receptors in the central nervous system. *Cell. Mol. Neurobiol.* 9:1–44
124. Nestler EJ, McMahon A, Sabban EL, Tallman JF, Duman RS. 1990. Chronic antidepressant administration decreases the expression of tyrosine hydroxylase in the rat locus coeruleus. *Proc. Natl. Acad. Sci. USA* 87:7522–26
125. Brodin E, Ögren SO, Theodorsson-Norheim E. 1987. Effects of subchronic treatment with imipramine, zimelidine and alaproclate on regional tissue levels of substance P- and neurokinin A/neurokinin B–like immunoreactivity in the brain and spinal cord of the rat. *Neuropharmacology* 26:581–90
126. Rosén A, Franck J, Brodin E. 1995. Effects of acute systemic treatment with the 5 HT-uptake blocker alaproclate on tissue levels and release of substance P in rat periaqueductal grey. *Neuropeptides* 28: 317–24
127. Riley LA, Hart RP, Jonakait GM. 1991. Both zimelidine and clorgyline decrease preprotachykinin mRNA in adult medullary raphe nuclei. *Molecular and Cellular Neurosciences* 2:139–44
128. Walker PD, Riley LA, Hart RP, Jonakait GM. 1991. Serotonin regulation of neostriatal tachykinins following neonatal 6–hydroxydopamine lesions. *Brain Research* 557:31–36
129. Stout SC, Owens MJ, Nemeroff CB. 1999. Preprotachykinin-A and neurokinin 1 receptor mRNA expression are unchanged in rat forebrain after chronic antidepressant administration. *Soc. Neurosci. Abstr.* 25:1066
130. Zachrisson O, Mathé AA, Lindefors N. 1997. Decreased levels of preprotachykinin–A and tachykinin NK1 receptor mRNA in specific region of the rat striatum after electroconvulsive stimuli. *Eur. J. Pharmacol.* 319:191–95
131. Stenfors C, Srinivasan GR, Theodorsson E, Mathé AA. 1992. Electroconvulsive stimuli and brain peptides: effect of

modification of seizure duration on neuropeptide Y, neurokinin A, substance P and neurotensin. *Brain Research* 596: 251–58
132. Brené S, Lindefors N, Herrera–Marschitz M, Persson H. 1993. Differential regulation of preprotachykinin-A mRNA expression in striatum by excitation of hippocampal neurons. *Eur. J. Neurosci.* 5:839–45
133. Marcus MM, Nomikos GG, Malmerfelt A, Zachrisson O, Lindefors N, et al. 1997. Effect of chronic antipsychotic drug treatment on preprosomatostatin and preprotachykinin A mRNA levels in the medial prefrontal cortex, the nucleus accumbens and the caudate putamen of the rat. *Mol. Brain Research* 45:275–82
134. Figiel GS, McDonald WM, McCall WV, Zorumpski C. 1998. Electroconvulsive therapy. See Ref. 172, pp. 523–45
135. Snider RM, Constantine JW, Lowe JAd, Longo KP, Lebel WS, et al. 1991. A potent nonpeptide antagonist of the substance P (NK1) receptor. *Science* 251:435–37
136. Beresford IJ, Birch PJ, Hagan RM, Ireland SJ. 1991. Investigation into species variants in tachykinin NK1 receptors by use of the non-peptide antagonist, CP-96,345. *Brit. J. Pharmacol.* 104:292–93
137. Gitter BD, Waters DC, Bruns RF, Mason NR, Nixon JA, et al. 1991. Species differences in affinities of non-peptide antagonists for substance P receptors. *Eur. J. Pharmacol.* 197:237–38
138. Barr AJ, Watson SP. 1993. Non-peptide antagonists, CP-96,345 and RP 67580, distinguish species variants in tachykinin NK1 receptors. *Brit. J. Pharmacol.* 108: 223–27
139. Porsolt RD, LePichon M, Jalfre M. 1977. Depression: a new animal model sensitive to antidepressant treatments. *Nature* 266:730–32
140. Vassout A, Schlaub M, Gentsch C, Ofner S, Schilling W, et al. 1994. CGP 49823, a novel NK1 receptor antagonist: behavioural effects. *Neuropeptides* 26 suppl 1:38
141. Zernig G, Dietrich H, Maggi CA, Saria A. 1992. The substance P (NK1) receptor antagonist (+/−)-CP-96,345 causes sedation and motor impairment in Swiss albino mice in the black-and-white box behavioral paradigm. *Neurosci. Lett.* 143: 169–72
142. Guard S, Boyle SJ, Tang KW, Watling KJ, McKnight AT, et al. 1993. The interaction of the NK1 receptor antagonist CP-96,345 with L-type calcium channels and its functional consequences. *Brit. J. Pharmacol.* 110:385–91
143. File SE. 1997. Anxiolytic action of a neurokinin1 receptor antagonist in the social interaction test. *Pharmacol. Biochem. Behav.* 58:747–52
144. Tschöpe C, Picard P, Culmann J, Prat A, Itoi K, et al. 1992. Use of selective antagonists to dissociate the central cardiovascular and behavioural effects of tachykinins on NK1 and NK2 receptors in the rat. *Brit. J. Pharmacol.* 107:750–55
145. Culman J, Klee S, Ohlendorf C, Unger T. 1997. Effect of tachykinin receptor inhibition in the brain on cardiovascular and behavioral responses to stress. *J. Pharmacol. Exp. Ther.* 280:238–46
146. Krase W, Koch M, Schnitzler HU. 1994. Substance P is involved in the sensitization of the acoustic startle response by footshocks in rats. *Behav. Brain Research* 63:81–88
147. Kungel M, Ebert U, Herbert H, Ostwald J. 1994. Substance P and other putative transmitters modulate the activity of reticular pontine neurons: an electrophysiological and immunohistochemical study. *Brain Research* 643:29–39
148. Hitchcock JM, Sananes CB, Davis M. 1989. Sensitization of the startle reflex by footshock: blockade by lesions of the central nucleus of the amygdala or its efferent pathway to the brainstem. *Behav. Neurosci.* 103:509–18
149. Shaikh MB, Steinberg A, Siegel A. 1993.

Evidence that substance P is utilized in medial amygdaloid facilitation of defensive rage behavior in the cat. *Brain Research* 625:283–94
150. Walsh DM, Stratton SC, Harvey FJ, Beresford IJ, Hagan RM. 1995. The anxiolytic-like activity of GR159897, a non-peptide NK2 receptor antagonist, in rodent and primate models of anxiety. *Psychopharmacology* 121:186–91
151. Garret C, Carruette A, Fardin V, Moussaoui S, Peyronel JF, et al. 1991. Pharmacological properties of a potent and selective nonpeptide substance P antagonist. *Proc. Natl. Acad. Sci. USA* 88:10208–12
152. Sachais BS, Krause JE. 1994. Both extracellular and transmembrane residues contribute to the species selectivity of the neurokinin-1 receptor antagonist WIN 51708. *Mol. Pharmacol.* 46:122–28
153. Aramori I, Morikawa N, Zenkoh J, O'Donnell N, Iwami M, et al. 1994. Subtype– and species–selectivity of a tachykinin receptor antagonist, FK888, for cloned rat and human tachykinin receptors. *Eur. J. Pharmacol.* 269:277–81
154. Pozza MF, Olpe HR, Ofner S, Schoeffel C, Evans RH. 1998. The potency of the novel tachykinin receptor antagonist CGP49823 at rat and gerbil motoneurones in vitro. *Eur. J. Pharmacol.* 342:203–08
155. Emonds-Alt X, Doutremepuich JD, Heaulme M, Neliat G, Santucci V, et al. 1993. In vitro and in vivo biological activities of SR140333, a novel potent non-peptide tachykinin NK1 receptor antagonist. *Eur. J. Pharmacol.* 250:403–13
156. Beattie DT, Beresford IJ, Connor HE, Marshall FH, Hawcock AB, et al. 1995. The pharmacology of GR203040, a novel, potent and selective non-peptide tachykinin NK1 receptor antagonist. *Brit. J. Pharmacol.* 116:3149–57
157. Walpole CS, Brown MC, James IF, Campbell EA, McIntyre P, et al. 1998. Comparative, general pharmacology of SDZ NKT 343, a novel, selective NK1 receptor antagonist. *Brit. J. Pharmacol.* 124:83–92
158. McLean S, Ganong A, Seymour PA, Bryce DK, Crawford RT, et al. 1996. Characterization of CP-122,721; a nonpeptide antagonist of the neurokinin NK1 receptor. *J. Pharmacol. Exp. Ther.* 277: 900–08
159. Iyengar S, Hipskind PA, Gehlert DR, Schober D, Lobb KL, et al. 1997. LY 303870, a centrally active neurokinin–1 antagonist with a long duration of action. *J. Pharmacol. Exp. Ther.* 280:774–85
160. Nemeroff CB, Widerlöv E, Bissette G, Walleus H, Karlsson I, et al. 1984. Elevated concentrations of corticotropin–releasing factor–like immunoreactivity in depressed patients. *Science* 226:1342–44
161. Widerlov E, Bissette G, Nemeroff CB. 1988. Monoamine metabolites, corticotropin releasing factor and somatostatin as CSF markers in depressed patients. *J. Affect. Disord.* 14:99–107
162. Risch SC, Nemeroff CB. 1992. Neurochemical alterations of serotonergic neuronal systems in depression. *J. Clin. Psychiatry* 53 Suppl:3–7
163. Rimón R, Le Greves P, Nyberg F, Heikkilä L, Salmela L, et al. 1984. Elevation of substance P–like peptides in the CSF of psychiatric patients. *Biol. Psychiatry* 19:509–16
164. Heikkilä L, Rimón R, Terenius L. 1990. Dynorphin A and substance P in the cerebrospinal fluid of schizophrenic patients. *Psychiatry Research* 34:229–36
165. Berrettini WH, Rubinow DR, Nurnberger JIJ, Simmons-Alling S, Post RM, et al. 1985. CSF substance P immunoreactivity in affective disorders. *Biol. Psychiatry* 20:965–70
166. Almay BG, Johansson F, Von Knorring L, Le Greves P, Terenius L. 1988. Substance P in CSF of patients with chronic pain syndromes. *Pain* 33:3–9

167. Mårtensson B, Nyberg S, Toresson G, Brodin E, Bertilsson L. 1989. Fluoxetine treatment of depression. Clinical effects, drug concentrations and monoamine metabolites and N-terminally extended substance P in cerebrospinal fluid. *Acta Psychiatr. Scand.* 79:586–96
168. Burnet PW, Harrison PJ. 1999. Substance P (NK1) receptors in the cingulate cortex in unipolar and bipolar mood disorder and schizophrenia. *Biol. Psychiatry* 47:80–83
169. Rosen RC, Lane RM, Menza M. 1999. Effects of SSRIs on sexual function: a critical review. *J. Clin. Psychopharmacol.* 19:67–85
170. Hirschfeld RM. 1999. Management of sexual side effects of antidepressant therapy. *J. Clin. Psychiatry* 60:27–30
171. Kramer MS, Alexander R, Ball WA, Shedden A, Reines SA. 2000. The therapeutic potential of substance P (NK-1 receptor) antagonists (SPA). *Neuropsychopharmacology* 23:S22
172. AF Schatzberg, CB Nemeroff, eds. 1998. *Textbook of Psychopharmacology*. Washington, D.C.: The American Psychiatric. 2nd ed.