# Substance P and the Tachykinins

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# 20 Substance P and the Tachykinins

*M. R. Howard · K. Haddley · T. Thippeswamy · S. Vasiliou · J. P. Quinn*

**Abstract:** The tachykinins are part of an important neurotransmitter pathway involving several neuropeptides and receptors, all of which are discussed in this chapter. The major pathway analyzed has been that of substance P (SP) and its high-affinity receptor, NK1. Tachykinins are widely distributed throughout the mammalian body in both the central nervous system (CNS) and the peripheral nervous system (PNS) with numerous functions being attributed to them in each of these systems. Tachykinins are predominantly synthesized in neurons of the CNS and the PNS and stored in large dense vesicles. Upon excitation of these neurons, tachykinins are released and act on their appropriate receptors on the target cells to evoke various responses. Although SP is predominantly thought of as a neurotransmitter, there is a growing appreciation of it as an inflammatory molecule acting analogous to a cytokine; its expression has been found in a variety of nonneuronal cells. We have also summarized this role, as expression of the tachykinins in the CNS in cells other than neurons may be important for its function in both normal physiology and pathological conditions. SP is implicated in a range of disorders ranging from itch and migraine through epilepsy, Parkinson's disease and psychiatric and cognitive disorders. In many of these disorders not only the level but also the duration and tissue specificity of expression contributes to overall function.

**List of Abbreviations:** AHR, airway hyperactivity; AMPA, alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid; AP1, activator protein one; AVP, arginine vasopressin; BDNF, brain-derived neurotrophic factor; bHLH, basic helix–loop–helix; $Ca^{2+}$, calcium ion; cAMP, cyclic adenosine monophosphate; cGMP, cyclic guanosine monophosphate; CNS, central nervous system; CRE, cAMP responsive element; CREB, cAMP response element binding; CGRP, calcitonin gene-related peptide; CVLM, caudal ventrolateral medulla; DAG, diacylglycerol; DRG, dorsal root ganglia; EC, extracellular region; EEG, electroencephalogram; EKA,B,C,D, endokininA, B, C, D; EL, extracellular loop; EPSP, excitatory postsynaptic potential; GABA, gamma-aminobutyric acid; GFR, growth factor receptor; GPCRs, G-protein-coupled receptors; HK1, hemokinin-1; 5-HT, serotonin; 5-HTT, serotonin transporter; IB-4, isolectin B-4; IL, interleukin; IC, intracellular region; IP3, inositol 1,4,5-triphosphate; $K^{+}$, potassium ion; kb, kilobase; K/O, knockout; $Mg^{2+}$, magnesium ion; μM, micromolar; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; mRNA, messenger RNA; $Na^{+}$, sodium ion; NF-H, neurofilament H; NGF, nerve growth factor; NK1, neurokinin1; NKA, neurokinin A; NKB, neurokinin B; NMDA, *N*-methyl-D-aspartate; NMR, nuclear magnetic resonance; NO, nitric oxide; NOS, nitric oxide synthase; NPK, neuropeptide K; NPγ, neuropeptide γ; NRSE, neuronal restrictive silencer element; NRSF/REST, neuronal restrictive silencer factor; NT-3, neurotrophic factor; PAG, periaqueductal gray matter; PKG, protein kinase gamma; PLC, phospholipase C; PNS, peripheral nervous system; POU, octamer binding protein of the Pit/Oct/Unc family; PPS, perforant path stimulation; QPCR, quantitative polymerase chain reaction; RT-PCR, reverse transcriptase-polymerase chain reaction; sGC, soluble guanylyl cyclase; SP, substance P; SSSE, self-sustaining status epilepticus; TAC1/PPT-A, tachykinin1/preprotachykinin-A; TAC3/PPT-B, tachykinin3/preprotachykinin-B; TAC4/PPT-C, tachykinin4/preprotachykinin-C; TACR1,2,3, tachykinin receptor1,2,3; TF, transcription factor; TLE, temporal lobe epilepsy; TNF, tumor necrosis factor; Trk, tyrosine kinase

## 1 Introduction

Ulf von Euler initially observed substance P (SP) while analyzing the distribution of acetylcholine in the rabbit gastrointestinal tract. A crude extract from horse brain and gut caused transient hypotension and contraction of the intestine. However, upon addition of atropine contractile activity was still observed, demonstrating that acetylcholine was not responsible. A paper soon followed describing an atropine-resistant "unidentified depressor substance" found in both brain and gut that stimulated smooth muscle and lowered blood pressure (von Euler and Gaddum, 1931). The term SP did not appear until Gaddum and Schild (1934) described the purification of a stable active proteinaceous powder previously termed "preparation P." In the 1950s, Bengt Pernow working with von Euler and Fred Lembeck made significant progress in determining the distribution of so-called preparation P in the brain and in the periphery, and subsequently, its association with Hirschsprung's disease (Ehrenpreis and Pernow, 1953; Pernow, 1953),

which led to the first suggestion that "preparation P" is indeed SP (see below) and could be a neurotransmitter (Lembeck, 1953).

Thirty years after the initial observation, Susan Leeman and Michael Chang purified a sialogogic peptide, which demonstrated properties and composition similar to that of the partially purified SP by Lembeck (Lembeck and Starke, 1968). Chang and Leeman called the unknown protein "substance P" and went on to sequence and synthesize the 11-amino-acid peptide as Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met from bovine hypothalamus (Chang et al., 1971; Tregear et al., 1971). The pharmacological properties of SP suggested it was part of a larger family of peptides. The amino acid sequence revealed homology with a group of nonmammalian molecules that were sequenced in the 1960s. Subsequently, in 1966 Erspamer coined the term "tachykinins" for the group of peptides that shared similar structural characteristics and exhibited activities such as rapid and potent smooth muscle contraction (Bernardi et al., 1966). The prefix "tachy" is from the Greek "Tachys" meaning quick, and kinin was defined as a "general name indicating a hypotensive polypeptide" that contracts most isolated smooth muscles, but relaxes duodenum. In addition, the name may be applied to any polypeptide that is related to bradykinin (quoted from Khawaja and Rogers, 1996). However, it is worthy to note that the tachykinins do not conform to the definition of kinin as they display little structural similarities to bradykinin and do not relax the duodenal smooth muscles.

The discovery of SP and its putative role as a neurotransmitter sparked the explosion of tachykinin research resulting in the discovery, albeit much later on, of other tachykinin family members, neurokinin A (NKA) (Kangawa et al., 1983; Nawa et al., 1984; Krause et al., 1987), neurokinin B (NKB) (Kanazawa et al., 1984; Kimura et al., 1984), neuropeptide γ (Kage et al., 1988), neuropeptide K (Tatemoto et al., 1985), and more recently hemokinin-1 (HK1) (Zhang et al., 2000).

Tachykinins are widely distributed throughout the mammalian body in both the central nervous system (CNS) and the peripheral nervous system (PNS), with numerous functions being attributed to them in each of these systems. Tachykinins are predominantly synthesized in neurons of the CNS and the PNS and stored in large dense vesicles. Upon excitation of these neurons, tachykinins are released and act on their appropriate receptors on the target cells to evoke various responses. In addition, tachykinins have been found to be expressed in a variety of nonneuronal cells (Quinn et al., 1995; Pennefather et al., 2004).

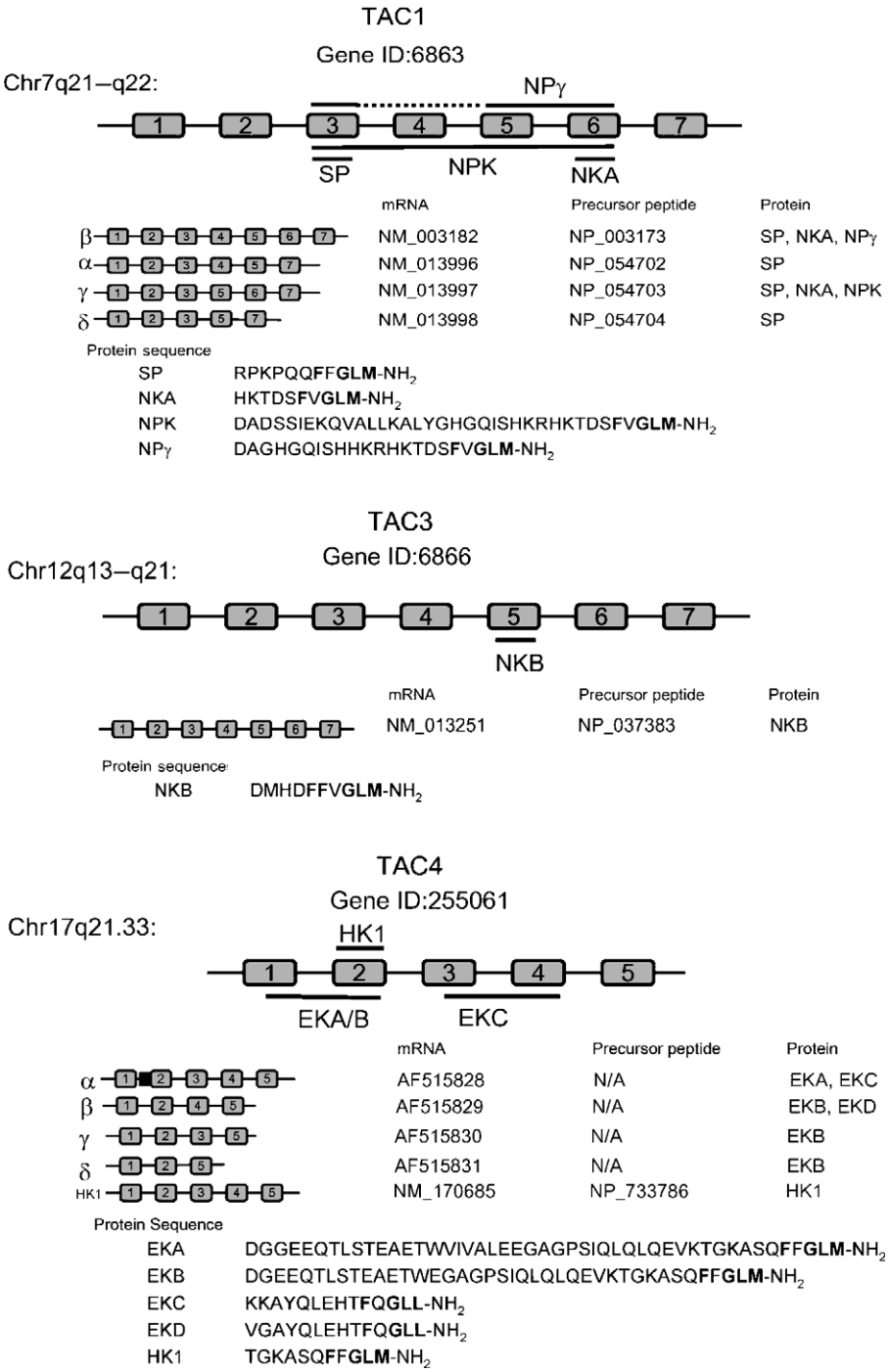
## 1.1 Gene Structure of Tachykinins and Their Receptors

### 1.1.1 Preprotachykinin-A

Mammalian SP is derived from the preprotachykinin-A (PPT-A) gene. The more recent discovery of the presence of two other related genes, preprotachykinin-B (PPT-B) and preprotachykinin-C (PPT-C), suggests that they along with PPT-A appear to originate from a common ancestral gene (Carter and Krause, 1990; Zhang et al., 2000). The human PPT-A gene is 8 kb long and consists of seven exons, which give rise to four alternatively spliced messenger (mRNA) transcripts termed α, β, γ, and δ (❯ *Figure 20-1*). Each isoform differs only in its exon usage, the β isoform utilizing all seven exons of the PPT-A gene, α lacking the sixth exon, γ lacking the fourth, and the most recently discovered δ isoform lacking both the fourth and the sixth exon. Polypeptides produced from these splice variants confer cells with the ability to generate SP, being encoded by exon 3; however, NKA can only be produced by β and γ PPT-A mRNAs since they contain exon six that encodes NKA. Neuropeptide K (NPK) and neuropeptide γ (NPγ) are N-terminal extended forms of NKA produced from β and γ PPT-A mRNA, respectively (❯ *Figure 20-1*). Nuclear magnetic resonance (NMR) analysis of SP suggests an α-helical core from Pro4 to Phe8 stabilized by two hydrogen bonds, one between Phe7-NH and Lys3-CO, and the other between Phe8-NH and Pro4-CO; an extended highly flexible $NH_2$ terminal Arg1-Pro2-Lys3; and a central turn on Gly9, thus bringing the COOH-terminal amide in contact with the γ-carbonyl oxygen atom of both glutamines (Lavielle et al., 1988; Regoli et al., 1994). SP is synthesized in the ribosomes as part of a larger protein and then enzymatically converted into an active peptide. SP is widely distributed in the CNS and the PNS of

**Figure 20-1**

**Schematic representation of the biosynthesis of the tachykinin genes. Genes TAC1, TAC3, and TAC4 are shown along with the transcription and translation products and relevant associated information, which can be accessed at http://www.ncbi.nlm.nih.gov/**



vertebrates. In the PNS, SP is expressed predominantly in the primary sensory neurons and the postganglionic neurons of the gut.

### 1.1.2 Preprotachykinin-B

NKB is the only tachykinin derived from the PPT-B gene. The human gene consists of seven exons and the sequence that encodes NKB is located on exon 5 (*Figure 20-1*). Human NKB mRNA expressed in the placenta appears to be encoded by seven exons interrupted by six introns spanning a region of 5.4 kb. Exons 2 to 6 encode the precursor, while exons 1 and 7 are untranslated regions. The human PPT-B gene generates only one mRNA that produces NKB, while in the bovine, PPT-B generates two mRNA transcripts, the difference being at the 5′ extremity of their untranslated regions (Kotani et al., 1986; Page et al., 2001).

Human placenta contains higher levels of the NKB mRNA transcript than those found individually in the human whole brain and spinal cord. However, it is undetectable in peripheral tissues from nonpregnant animals even though its endogenous receptor, NK3, has been found in a number of locations throughout the human body (Donaldson et al., 1996; Krause et al., 1997). Page et al. (2000, 2001) suggest that NKB has a possible role in placental physiology and preeclampsia. Most recently, NKB and NK3 mRNA have been found to be expressed in human airways and pulmonary arteries and veins, suggesting the involvement of NKB in lung physiopathology (Pinto et al., 2004).

### 1.1.3 Preprotachykinin-C (Hemokinin)

During the study of mouse B cell development an mRNA differential display screen revealed a predicted peptide sequence that contained the tachykinin signature motif (FXGLM) (Zhang et al., 2000). Subsequent characterization led to the discovery of a third PPT gene called PPT-C or TAC4; mouse TAC4 contains four exons while the rat homolog contains five, with both giving rise to a peptide called HK1 that displays a preference for the NK1 receptor in an SP-like manner. HK1 is expressed in mouse hematopoietic cells and so far has not been observed in neuronal tissue (Zhang et al., 2000; Pennefather et al., 2004). However, HK1 does display characteristics similar to SP in terms of its receptor binding, being an NK1, NK2, and NK3 agonist with the highest selectivity for NK1 (Kurtz et al., 2002). Furthermore, in humans, at least four other tachykinins have been found to be expressed (Page, 2004). Four alternatively spliced mRNAs, $\alpha$, $\beta$, $\gamma$, and $\delta$, give rise to four proteins named endokinin A (EKA), B, C, and D, respectively (Page, 2004; Patacchini et al., 2004). $\alpha$TAC4 encodes both EKA and EKC; $\beta$TAC4 encodes EKC and EKD; both $\gamma$TAC4 and $\delta$TAC4 encode EKB only (*Figure 20-1*). There is tissue-specific regulation of these transcripts; $\alpha$TAC4 is expressed in the adrenal gland, liver, and spleen and $\beta$TAC4 in the heart, liver, bone marrow, prostate, adrenal gland, and testis. Both $\gamma$ and $\delta$ isoforms showed similar expression patterns in the adrenal gland and the placenta (Page, 2004; Patacchini et al., 2004).

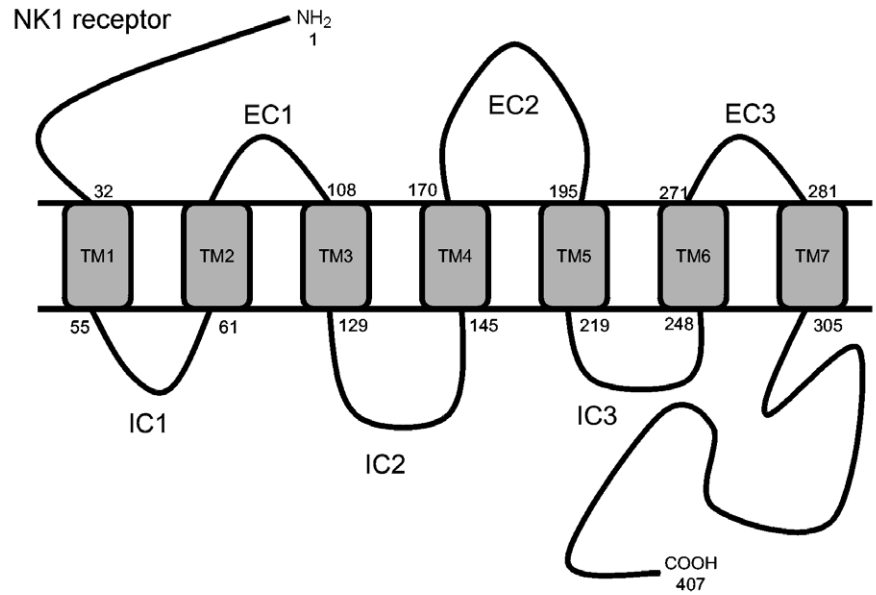
### 1.1.4 Tachykinin Receptors

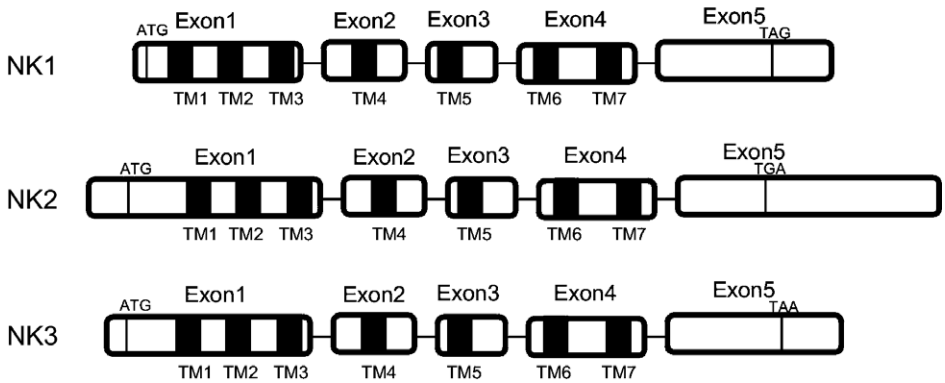
In parallel with the identification of a number of endogenous tachykinins, several classes of tachykinin receptors have been discovered. From an evolutionary perspective the vertebrate tachykinin receptors are highly conserved and have evolved from one common gene or one ancestral receptor (Pennefather et al., 2004). The tachykinin receptors belong to a family of G-protein-coupled receptors (GPCRs) and show a high incidence of homology between mammalian species (Gerard et al., 1993). Like all GPCRs they are composed of seven transmembrane domains (TMI–VII), three extracellular loops (EC1–3), three intracellular loops (IC1–3), an extracellular amino terminus, and an intracellular carboxy terminus (*Figure 20-2*) (Maggi, 1995).

Ligand binding and receptor chimera studies have identified three tachykinin receptors. These tachykinin receptors have also been designated as NK1, NK2, and NK3 and display preferential selectivity

**Figure 20-2**

**Schematic representation of the human NK1 receptor protein and the gene structure of NK1, NK2, and NK3. SP binds to extracellular (EC) regions 1 and 2, G proteins bind at transmembrane region (TM) 5, 6, and intracellular region (EC3). The human NK1, NK2, and NK3 genes contain five exons and four introns. The regions within exons encoding TMs are shown as *black boxes*. The *thin black lines* show the start and stop codons and highlight the difference in both 5′ and 3′ untranslated regions between the genes. Access numbers for each can be utilized for further information at http://www.ncbi.nlm.nih.gov/**




| | Gene | Gene ID | Location | mRNA | Peptide |
|---|---|---|---|---|---|
| NK1 | TACR1 | 6869 | Chr2p12 | NM_001058 | NP_001049 |
| NK2 | TACR2 | 6865 | Chr10q11-q21 | NM_001057 | NP_001048 |
| NK3 | TACR3 | 6870 | Chr4q25 | NM_001059 | NP_001050 |

to SP, NKA, and NKB, respectively. The term "neurokinin" is used to denote tachykinins expressed in the nervous system (Buck et al., 1984; Lee et al., 1986; Harada et al., 1987; Maggi, 1995). NK1 is also the preferred receptor for the recently identified tachykinins HK1, EKA, and EKB (Zhang et al., 2000). It has

been demonstrated that all tachykinins exhibit a limited selectivity for a particular receptor and that there is crosstalk between tachykinin ligands and the receptors (Mussap et al., 1993). NK1 function has been addressed not only by the interactions with SP and other tachykinins but also by specific receptor antagonists such as WIN-51, 708, L-733, 060, RPR100893 and GR205171, MK-0869 (L-754,030), L-758, 298, and MEN 11467 and MEN 11149 (Cirillo et al., 1998; Tattersall et al., 2000; Santarelli et al., 2002). The NK1 receptor is also the preferred target for HK1 and endokinins in the periphery, where the ligand binding produces effects analogous to SP binding (Morteau et al., 2001; Kurtz et al., 2002; Page et al., 2003). Although the receptor-binding model eloquently highlights the preferential affinities of the tachykinin ligands to their receptor species, tissue anomalies in this relationship have raised questions about the existence of more tachykinin receptors for SP, NKA, and NKB (reviewed in Maggi, 2000).

Tachykinin GPCRs may be regulated in a conformational manner and ligand binding, membrane lipid composition, and extracellular medium may all act to stabilize the receptor favoring one ligand binding/effector conformation over the other (Berthold and Bartfai, 1997). Ligand binding to the tachykinin receptors initiates internalization of the ligand–receptor complex, resulting in phospholipase $C_\beta$ ($PLC_\beta$) activation. PLC utilizes membrane lipids increasing the intracellular inositol-1,4,5-triphosphate (IP3) and diacylglycerol (DAG). IP3 induces intracellular calcium release from the endoplasmic reticulum, leading to the activation of a number of signal transduction cascades. DAG activates protein kinase C (PKC), nitric oxide synthase (NOS), and arachidonic acid production, thus stimulating further regulatory pathways resulting in upregulation of cyclic nucleotides (cyclic guanosine monophosphate, cGMP; cyclic adenosine monophosphate, cAMP) (Krause et al., 1992; Garcia et al., 1994; Radhakrishnan et al., 1995).

SP binding to NK1 receptor initiates signal transduction upon NK1-SP internalization. GPCR redistribution induced by agonist binding determines the subsequent responsiveness of the cell to particular agonists and plays a major role in the regulation of signal transduction pathways (Quartara and Maggi, 1997). Receptor endocytosis/internalization is responsible for both desensitization by reducing the number of cell surface receptors available to interact with agonists, and resensitization following receptor processing (i.e., dissociation of ligand, dephosphorylation) and recycling to the plasma membrane.

NK1-GPCR endocytosis pathway involves the formation of clathrin-coated pits and is mediated by a family of cytosolic proteins termed arrestins (McConalogue et al., 1998). Binding of SP to the NK1 receptor causes a rapid translocation of β-arrestins from the cytosol to the plasma membrane, followed by the redistribution of both NK1 and β-arrestins to endosomes, where they remain associated until gradually (after 4–6 h) NK1 is recycled to the plasma membrane and β-arrestins return to the cytosol (McConalogue et al., 1999).

The NK1 receptor has been studied more extensively than NK2 and NK3. NK1 has two isoforms, one with a shorter C-terminal tail denoted as the short isoform and the other, the long isoform. The longer isoform is found predominantly in the brain, while the shorter isoform is associated with peripheral tissues (Caberlotto et al., 2003). The existence of these isoforms may account for discrepancies arising in the potency and preferential binding of tachykinins to their receptors in different tissues. Additionally, the recent discovery of human NK2 receptor isoforms may also account for the differences reported in the tissue specificity of NKB (Naline et al., 1989; Croci et al., 1998; Candenas et al., 2002). The existence of multiple induced conformations of NK1 and NK2 has been confirmed, with each study showing distinct binding affinities for antagonists and agonists and in some cases the ability to activate different effector systems (Maggi and Schwartz, 1997; Palanche et al., 2001; Patacchini and Maggi, 2001; Lecat et al., 2002). With the discovery of additional tachykinins and tachykinin-related peptides such as hemokinins and endokinins, the debate on whether additional tachykinin receptors exist has been readdressed (Pennefather et al., 2004). NK1 is the most abundantly distributed neurokinin receptor in the CNS, and it is also present in a number of target organs that are innervated by SP-expressing small-diameter primary afferent neurons. Clinical and experimental animal model studies have suggested the involvement of NK1 in a number of functions such as nociception, cognition, and basal ganglia functions as well as psychiatric and neurological disorders (Quartara and Maggi, 1997; Kramer et al., 1998; Quartara and Maggi, 1998).

### 1.2 Tissue-Specific and Stimulus-Inducible Tachykinin Gene Expression

The expression of neuropeptide genes is extremely plastic in that the PPT-A gene can be induced by a number of different stimuli and this induction is dependent, in part, upon transcription factors binding to regulatory consensus sequence in the promoter of the gene. Induction of the PPT-A gene not only is crucial for normal physiological signaling but also accounts for modulation of gene expression in pathological states. A fragment of the PPT-A gene promoter spanning base pairs −865 to +445 has been extensively studied and contains a number of regulatory DNA elements upstream and downstream of the transcriptional start site and is capable of supporting marker/reporter gene expression in a number of cell types including dorsal root ganglion (DRG) and hippocampal neurons in culture (Harmar et al., 1993; Morrison et al., 1994; Mendelson and Quinn, 1995; Mendelson et al., 1995; Paterson et al., 1995a–c; Walker et al., 2000). Nerve growth factor (NGF) is a known endogenous inducer of the PPT-A gene in states of chronic pain and inflammation (Ma and Bisby, 1998). This promoter fragment also supports NGF regulation of marker gene, determining in part that the region could mediate differential PPT-A expression to this stimulus (Harrison et al., 1999). The PPT-A promoter contains many transcription factors that have been defined as mediating a response to NGF including binding sites for activator protein one (AP1), cAMP responsive element (CRE), basic helix–loop–helix (bHLH) proteins, and members of the POU domain family of proteins (Fiskerstrand and Quinn, 1996). Both AP1 and CRE can mediate inducible PPT-A expression in response to NGF. Members of the cAMP response element binding (CREB) TF family are activated via phosphorylation by a Ras-dependent protein kinase and are capable of upregulating gene expression following NGF stimulation (Hawley et al., 1992; Ginty et al., 1994). In addition, the cellular composition of AP1-binding transcription factors (TF such as fos and jun) is modulated in response to NGF stimulation (Quinn et al., 1989; Gizang-Ginsberg and Ziff, 1990) and may vary in a tissue-specific manner (Andrews et al., 1993). Octamer binding sites show functional inducibility to NGF with increases in the concentration of N-oct-2 binding protein in DRG following treatment with NGF (Wood et al., 1992; Mendelson et al., 1998).

In nonneuronal cells, neuronal genes are in part dominantly repressed by the binding of neuronal restrictive silencer factor (NRSF) at the neuronal restrictive silencer element (NRSE) located in the gene (Palm et al., 1998). NRSF binding at the transcriptional start site of the PPT-A gene results in dominant repression of transcription supported by the proximal promoter fragment and accounts for the lack of gene expression from this fragment in PC12 and HeLa cells (Mendelson et al., 1995). However, more recently it has been suggested that PPT-A expression could be enhanced by expression and function of REST isoforms (Quinn et al., 2002) as observed for other neuropeptide genes such as arginine vasopressin (AVP) (Coulson et al., 1999; Quinn et al., 2002). A role has also been demonstrated for the regulation of the PPT-A promoter by members of the bHLH family of transcription factors (Paterson et al., 1995a–c; MacKenzie et al., 2000). bHLH and REST isoforms are differentially regulated during a CNS challenge such as epilepsy (Palm et al., 1998). It will be interesting to directly correlate whether the modulation of specific transcription factors will affect PPT-A plasticity at the level of transcription in the CNS. It is likely that synergistic action of these TFs at a number of regulatory DNA sites mediate the modulation of the PPT-A promoter, in neurons, e.g., in response to NGF during states of chronic pain and inflammation and in CNS neurons.

## 2 Distribution of Tachykinins and Their Receptors

Tachykinins are widely distributed in both the nervous system and the other peripheral tissues, and numerous functions have been attributed to tachykinins in each of these tissues. A number of methods have been used to study the distribution of the tachykinin-encoding genes and their receptors in the CNS and peripheral tissues, including:

1. in situ hybridization, using probes directed against the mRNA for the receptor;
2. immunocytochemistry with antibodies raised against synthetic peptide sequences corresponding to different parts of the receptor. Initial studies of SP localization were described using antibodies

raised against the carboxyl terminus, which has now been shown to be common among all tachykinins; and
3. reverse transcriptase-polymerase chain reaction (RT-PCR) and quantitative PCR (QPCR).

SP and NKA are mostly synthesized and stored in large dense-core vesicles of primary afferents of PNS (McCarthy and Lawson, 1989) and CNS neurons (Pickel et al., 1983; Maley, 1996). In addition, the majority of SP-containing neurons also contain NKA, particularly the capsaicin-sensitive afferent neurons (Carter and Krause, 1990). Both peptides have been implicated centrally in numerous and diverse processes such as neurotransmission (Otsuka and Yoshioka, 1993; Patacchini et al., 1998), inflammation (Barnes, 1991, 1992; Donaldson et al., 1992; Leslie et al., 1995), neurological pain/neuralgia (Noguchi et al., 1988; Cao et al., 1998; Basbaum, 1999a, b; Honor et al., 1999), memory and learning, depression and anxiety (Kramer et al., 1998; Rupniak and Kramer, 1999), and epilepsy (Liu et al., 1999a, b; Liu et al., 2000; Fetissov et al., 2003). Peripheral SP functions are diverse and are involved in immune system stimulation (Iwamoto et al., 1993; Maggi, 1997), fibroblast and smooth muscle growth (Nilsson et al., 1985; Katayama and Nishioka, 1997), hypotension, smooth muscle contraction, and cellular proliferation (Lecci et al., 2000). NKB is present in the brain and the spinal cord (Patacchini et al., 2000) and has recently been detected in the reproductive system of both humans and rodents (Page et al., 2000; Pinto et al., 2001; Patak et al., 2003). The newly discovered PPT-C/TAC4 gene that encodes HK1 is widely expressed in nonneuronal tissue in humans and mice (Page et al., 2003). The function of HK1 and its isoforms are not yet clear but they are believed to function in a manner similar to SP via the activation of the NK1 receptor (Kurtz et al., 2002; Page et al., 2003; Patacchini et al., 2004).

## 2.1 Distribution of PPT-A and Tachykinin Receptors in the Primary Sensory Afferents

The bulk of the SP present in the PNS is synthesized in the primary sensory neurons, which are located within the DRG (Hokfelt et al., 1975a, b). SP along with other neuropeptides such as NKA is stored in dense-core vesicles within the DRG neurons (McCarthy and Lawson, 1989). Not all sensory afferent neurons express PPT-A mRNA. There are different subpopulations of DRG neurons and only a small proportion of these neurons support PPT-A expression and SP synthesis. DRG neurons consist of two main populations; the first group (15–20%) are large-diameter (>30 μm), light, nonpeptidergic neurons giving rise to myelinated Aδ fibers, and the second group (75–80%) are small-diameter (<30 μm), dark, mostly peptidergic neurons giving rise to unmyelinated C-fibers (Lawson et al., 1993, 1997).

The large-diameter DRG neurons can also be distinguished on the basis of protein expression profile such as the expression of high-molecular-weight neurofilament protein, NF-H (Averill et al., 1995; Molliver et al., 1995), neurotrophin receptors, and tyrosine kinase C (*trkC*) (Wright and Snider, 1995). These neurons are largely glutamatergic and do not express PPT-A under basal conditions. Upon low-frequency stimulation they release glutamate from their central terminals that bind to postsynaptic *N*-methyl-D-aspartate (NMDA) receptors on the second-order projection neurons and islet cell excitatory interneurons in lamina II of the dorsal horn and to a lesser extent in lamina V of the spinal cord. Under normal physiological conditions, myelinated Aδ fibers transmit impulses generated by nonnoxious stimuli such as pressure (mechanoceptors) and heat (proprioceptors) from the periphery.

The peptidergic small-diameter DRG neurons can be further subdivided into two groups: the capsaicin-sensitive peptidergic (majority) and the nonpeptidergic neurons. Peptidergic neurons can also be distinguished immunohistochemically based on the expression of the high-affinity NGF receptor, *trkA* (Basbaum and Woolf, 1999). The capsaicin-sensitive peptidergic neurons support PPT-A mRNA expression and thus express SP and NKA. Under normal physiological conditions, C-fibers transmit impulses generated by noxious (peptidergic neurons) and thermal stimuli (nonpeptidergic neurons) (Basbaum, 1999a, b). The C-fibers also release glutamate-like Aδ fibers in the dorsal horn of the spinal cord and, in addition, they release SP, calcitonin gene-related peptide (CGRP), brain-derived neurotrophic factor (BDNF), and other neuromodulators that bind to their appropriate receptors pre- and postsynaptically, thus potentiating

further glutamate release. The majority of C-fibers terminate in lamina I and the outer region of lamina II (substantia gelatinosa) of the dorsal horn and to a lesser extent in laminae III, VI, and X of the spinal cord and also as projection fibers in the trigeminal ganglion of the CNS, suggesting the role of SP in trigeminal neuralgia (Basbaum and Woolf, 1999).

The nonpeptidergic small-diameter neurons do not support PPT-A expression under basal conditions. These neurons can be distinguished from the peptidergic neurons as they do not express CGRP, and they exhibit surface proteins not found in the peptidergic population such as isolectin B-4 (IB-4) and the purinergic P2X3 receptor. In addition, these neurons express c-Ret growth factor receptor (GFR-α) for GDNF (Basbaum and Woolf, 1999; Caterina and Julius, 1999).

The three types of DRG neurons described above broadly define the DRG populations although some crossover in marker molecule expression is evident between them. This is particularly apparent in neurotrophin receptor expression; some populations express only one trk receptor (Mu et al., 1993; Kashiba et al., 1995; Wright and Snider, 1995), whereas the others express more than one trk receptor (McMahon et al., 1994; Karchewski et al., 1999), suggesting complex neurotrophin-dependent plasticity in DRG. Differential expression of growth factor receptors on sensory neurons has major implications for regulatory control and modulation of gene expression during pathological states.

Whether or not sensory fibers express functional NK1 receptors is a topic for debate. A number of studies propose the presence of the NK1 receptor on DRG neurons and their fibers (McCarson, 1999), while others do not (Andoh et al., 1996; von Banchet and Schaible, 1999). These studies suggest that the putative NK1 receptors present in DRG may be involved in a negative/positive feedback mechanism(s) in which SP acts in an autocrine or paracrine manner to modulate its own release. The controversies with respect to NK1 receptors' expression in DRG is due to the methods used for detection, with some showing more sensitivity than others, which could be due to, for example, the source of antibodies in case of immunocytochemistry and Western blotting. The expression of PPT-A/SP and its functions in DRG, described in this chapter, conform to the observations determined under basal conditions. It should be noted that PPT-A/SP expression, like the expression of all other neuropeptides, is highly dynamic and under certain physiological and pathological conditions the expression profiles can be modulated (discussed in Section 1.2). However, under pathological conditions SP has been demonstrated to increase its own release via presynaptic IP3-mediated release of calcium from the internal stores leading to depolarization and increased neuronal excitability (Xie et al., 1995).

## 2.2 Distribution of SP and NK1 in the Spinal Cord

The axons of primary afferents from the DRG terminate in the lamina of the dorsal horn of the spinal cord. These sensory neurons synthesize, store, and release a variety of neurotransmitters (mostly glutamate) and neuropeptides (SP, NKA, and CGRP) from their terminals. The anatomical location of the presynaptic release sites in relation to the postsynaptic receptor sites in the dorsal horn of the spinal cord is discussed in this section.

The NK1 receptor is highly expressed in lamina I (the marginal), lamina III, and to a lesser extent in laminae II, IV, and V of the dorsal horn of the spinal cord (Brown et al., 1995; Marvizon et al., 1999). SP is found in laminae I and II (Hokfelt et al., 1975a, b; Cuello and Kanazawa, 1978) and to a lesser extent in lamina V (Ruda et al., 1986).

NK1 is expressed by 80% of lamina I neurons that project to the thalamus, periaqueductal gray matter (PAG), parabrachial area, and caudal ventrolateral medulla (CVLM), as demonstrated by retrograde tracing combined with immunocytochemistry (Marshall et al., 1996; Todd et al., 2000). NK1 immunoreactivity has also been detected in lamina II, albeit at lower levels than in lamina I and also in moderate levels in laminae III–VI. This immunoreactivity is associated with the surface membranes of cell bodies and dendritic processes. The majority of NK1-expressing neurons in the dorsal horn do not contain either gamma-aminobutyric acid (GABA) or glycine. It is therefore likely that SP released in the superficial laminae of the dorsal horn acts almost exclusively on NK1 receptors of the excitatory neurons (Littlewood et al., 1995). Acute noxious peripheral stimulation causes excitation of dorsal horn neurons that express the NK1

receptor. In the rat, it has been observed that following acute noxious stimulation, the majority of NK1-immunoreactive neurons in lamina I show internalization of the NK1 receptor (upon SP binding) and upregulation of *c-fos* expression (Mantyh et al., 1995; Doyle and Hunt, 1999).

As mentioned previously, NK1 is the preferential target for SP released from primary afferents. The distribution of the NK1 receptor in relation to the release sites for SP is crucial for modulating impulse summation and filtering. This suggests the juxtaposition of SP-releasing sensory afferents and NK1 receptors in the dorsal horn of the spinal cord under normal physiological conditions. However, under certain pathological conditions such as nociception and inflammation, the SP/NK1 organization alters. In contrast to the PNS, SP-containing sensory afferent terminals in the CNS are mostly located in close proximity to NK1-expressing postsynaptic neurons (Ribeiro-Da-Silva and Hokfelt, 2000). However, mismatches can be seen in lamina III of the dorsal horn where NK1 expression is detected although SP release sites are absent. This suggests that SP released in the lamina I might diffuse to the deeper lamina of the dorsal horn to exert its action on NK1 receptors. This further highlights the importance of other signaling pathways in which the response of central neurons in the brain region to SP release at the level of the spinal cord would be both delayed and sustained, the mechanism of which is shown to be of particular importance in the modulation of pain signaling. In addition to the SP released from sensory afferents of the DRG, the intrinsic neurons of the dorsal horn of the spinal cord express SP and the terminals of the descending serotonergic pathways from higher centers in the brain also release SP (Hokfelt et al., 1978; Gilbert et al., 1982). Of the DRG neuron-derived SP, only 25% is released centrally and the rest is released from peripheral terminals to modulate the excitability of the contractile tissues and control endocrine function and the response to inflammatory agents (Harrison and Geppetti, 2001)

Immunostaining for *c-fos* and NK1 receptor was carried out to examine whether the nociceptive signals from specific peripheral tissues (e.g., skin, muscle, and knee joint) or activity generated by a particular insult (nerve injury or formalin-induced inflammation) was preferentially modulated by SP in the spinal cord (Doyle and Hunt, 1999). Although the number of *c-fos*/NK1-positive neurons was correlated with the intensity of the noxious stimulus in lamina I, no such correlation was observed in the deeper laminae (V–X). It was also noted that *c-fos*/NK1 immunoreactivity in the superficial laminae was unrelated to any particular peripheral target, which was not the case for deeper laminae. In the deeper layers of the dorsal horn, the greatest colocalization of *c-fos*/NK1 immunoreactivity was observed following stimulation of knee joint nociceptors and formalin-induced inflammation, suggesting the direct role for SP in the regulation of joint pain and inflammatory hyperalgesia (Doyle and Hunt, 1999). Therefore, NK1-expressing neurons in lamina I may be involved in discrimination of the intensity of pain-inducing stimuli, whereas NK1 receptors in deeper laminae are concerned with special localization or the detection of particular nociceptive stimuli.

In addition to being involved in the response of NK1 neurons in the dorsal horn to noxious stimulation, NK1 receptors may also be involved in the maintenance of hyperalgesia (Mantyh et al., 1997). The intrathecal administration of SP conjugated to cytotoxin saporin resulted in the ablation of NK1-immunoreactive neurons and was accompanied by reduction in the capsaicin-induced hyperalgesia in rats. Studies from NK1 K/O mice have also revealed that the mice were resistant to inflammatory hyperalgesia induced by injection of complete Freund's adjuvant in the ipsilateral paw (De Felipe et al., 1998a, b). Overall, these observations suggest that lamina I projection neurons expressing NK1 may control dorsal horn excitability via reciprocal projections with the brainstem. Ablation of lamina I NK1-expressing neurons disrupts the ascending pathway to the brainstem and the descending pathways that control spinal cord excitability, thus reducing behavioral hypersensitivity due to peripheral injury.

The blocking effect of analgesics such as opioids on SP release from primary afferents has been demonstrated both in vivo and in vitro (Trafton et al., 1999). To investigate the functional implications of opioid regulation of the tachykinin pathway, Trafton et al. (1999) used NK1 receptor internalization as a measure of the postsynaptic response to morphine administration. They reported a slight reduction in NK1 receptor signaling following morphine administration at a dosage that was sufficient to produce opioid analgesia in awake animals. However, a combination of morphine with a low (ineffective) dose of the NK1 antagonist (GR205171) was able to decrease NK1 receptor internalization to a degree that was greater than that of either drug on its own. The possible explanation for this is that the SP released from primary

afferents may either diffuse into the extracellular space (extracellular pool) or bind to NK1 receptors and become internalized (intracellular pool). Only the intracellular pool is directly measurable because it depends on the saturation of NK1 receptor binding. The extracellular SP is unable to mediate postsynaptic signaling due to lack of NK1 binding sites on the plasma membrane. Opioids may be able to reduce the total amount of SP released by primary afferents; however, this reduction has no effect on the already saturated NK1 binding sites. It is evident that a high proportion of tachykinin signaling remains intact following opioid administration.

Many NK1 neurons in lamina I receive high-density contacts from serotonergic fibers from the raphe nuclei of the medulla. Serotonergic function has been found to increase following pharmacological blockade or genetic disruption of the NK1 receptors in mice, suggesting crosstalk between tachykinin and 5-HT (Gross et al., 2000). This might also suggest the role of tachykinins and 5-HT in anxiety-related behaviors (Ranga and Krishnan, 2002; Adell, 2004; Blier et al., 2004) (see Section 4.2).

## 2.3 Distribution of SP and NK1 in the Brain

Many techniques have been utilized by numerous groups to determine the NK1 and SP content in mammalian brains, including the brains of humans and rats. These techniques include QPCR, immunohistochemistry, in situ hybridization, and radioimmunoassay. Most of these studies agree with each other concerning the distribution and density of SP or NK1 in specific regions of the brain, although slight variations in the intensity are reported between studies, which are most likely due to the sensitivity of the techniques used and the source of antibodies (❯ *Table 20-1*).

In both humans and rats, increased SP content is observed in the caudate putamen of the forebrain, the nucleus accumbens, the globus pallidus, the medial amygdaloid nucleus, the medial habenular nucleus, the lateral habenular nucleus, the substantia nigra, the superior colliculus, the periaqueductal gray, the parabrachial nuclei, the locus coeruleus, the medullary raphe nuclei (project to the spinal cord), the lamina I and outer lamina II of the trigeminal subnucleus caudalis, and the dorsal motor nucleus of the vagus as compared with the content in the spinal cord) (Warden and Young, 1988; Ribeiro-Da-Silva and Hokfelt, 2000; Ribeiro-Da-Silva et al., 2000, for extensive review). In the rodent, moderate levels of SP are detected in the hypothalamus and low levels in the thalamic nuclei, the cortex, the hippocampal areas, and the cerebellum. In contrast, these areas show high levels of SP expression in humans (Mai et al., 1986; Pioro and Cuello, 1990).

In rodents (guinea pigs and rats), intense NK1 expression is detected in the caudate putamen and superior colliculus, while there is moderate to low concentration in the inferior colliculus, the olfactory bulb, the hypothalamus, the hippocampus, the substantia nigra, the cerebral cortex, the septum, the striatum, and in various regions of the mesencephalon (Shults et al., 1984; Dam and Quirion, 1986; Mantyh et al., 1989). In humans, NK1 expression is intense in the caudate putamen, the nucleus accumbens (ventral striatum), the superior colliculus, the cortex, the amygdala, and the locus coeruleus (very high level expression). Moderate NK1 expression is detected in the human superficial cortical regions, the visual cortex, the hippocampus (CA regions including dentate gyrus), and the hypothalamus, while low NK1 mRNA expression is detected in the thalamus (central medial, mammillary body), the globus pallidus, the cerebellum, and the dorsal raphe nucleus (Caberlotto et al., 2003).

The collective data from the above studies suggest that there is a mismatch between the concentration/innervation of SP and the density of NK1 receptors in a number of regions within the brain. This mismatch is particularly evident in the rodent hippocampus. NK1 occurs throughout the hippocampal formation and is strong in the hilus of the dentate gyrus, while there is little SP or NKA immunoreactivity in this region (Mantyh et al., 1989; Ribeiro-Da-Silva and Hokfelt, 2000). The mismatch between SP/NKA and NK1 in these areas may indicate that NKB or NK3 may play a major role in these regions; however, one should consider that the levels of mRNA are not static and that SP can also diffuse from other areas. It is interesting to note that the human hippocampus does not show the mismatch between SP and NK1. However, the above studies do not distinguish between SP and NKA. Overall, this may also indicate that despite the high sequence homology of the rat and human PPT-A and NK1 genes, the mechanisms involved in modulation of neurotransmission via tachykinergic signaling may differ considerably between humans and rats.

**Table 20-1**
**SP/PPT-A and the NK1 receptor distribution in rat and human brain**

| Brain Regions | Rats | | Humans | |
|---|---|---|---|---|
| | SP | NK1 | SP | NK1 |
| Cerebral cortex | | | | |
| Neocortex | + | | ++/+++ | ++/+++ |
| Cingulate cortex | ++ | | | ++/+++ |
| Hippocampal formation | | | | |
| CA layers | | | | |
| CA1 | + | | | |
| CA2 | + | | | |
| CA3 | + | +++ | +++ | ++ |
| Dentate gyrus | + | +++ | +++ | ++ |
| Basal ganglia | | | | |
| Caudate putamen | +++ | +/+++ | +++ | +++ |
| Nucleus accumbens | +++ | ++/+++ | +++ | +++ |
| Globus pallidus | +++ | | +++ | ++ |
| Amygdala | | | | |
| Medial amygdaloid nucleus | +++ | — | | ++ |
| Basolateral amygdaloid nucleus | | ++ | | ++ |
| Diencephelon | | | | |
| Hypothalamus | +/++ | +/+++ | | ++ |
| Thalamus | + | +/+++ | | + |
| Habenular nucleus | +++ | +/++ | +++ | |
| Mesencephelon | | | | |
| Substantia nigra | +++ | + | +++ | + |
| Interpeduncular nucleus | +++ | ++ | | |
| Superior colliculus | ++/+++ | +++ | | +++ |
| Inferior colliculus | ++ | +/++ | | |
| Periaquaductal gray | +++ | ++ | | |
| Raphe nuclei | + | + | | |
| Pons | | | | |
| Parabrachial nucleus | +++ | ++/+++ | | |
| Locus coeruleus | +++ | +++ | | +++ |
| Dorsal raphe nucleus | +++ | +++ | +++ | + |
| Trigeminal sensory neurones | + | | | |

Data are based on a range of studies and represent the SP or PPT-A mRNA and NK1 or NK1 mRNA expression based on in situ hybridization (Warden and Young, 1988; Hurd et al., 1999; Caberlotto et al., 2003), receptor-binding studies (Mantyh et al., 1989), and immunohistochemical studies. Intensity ranges from none (−) to high (+++). In some cases, detailed information is not available for NK1 in the rat brain or for SP/NK1 in the human brain. The detection methods used in the above studies did not distinguish between SP or NKA. There are some striking differences between rats and humans, and mismatches between SP concentration and NK1 receptor content in specific brain regions, for example, the rat hippocampus and basal amygdaloid nucleus. The type of staining observed for each region is not always disclosed and for this reason the table represents areas of the brain in which SP and NK1 can be seen, rather than their precise structural locations. Note that the NK1- or SP-expressing cells represent the complexity of tachykinergic signaling and the role of the tachykinins in a plethora of neuronal pathways

## 2.4 Peripheral Tachykinins

Although local nerves have been believed to be the major source of tachykinins in the peripheral tissues, recently PPT-A has been shown to be induced and expressed in other cell types such as monocytes, macrophages, pancreatic islet cells, and various tumors (McGregor et al., 1995; Ho et al., 1997; Germonpre et al., 1999; Lambrecht et al., 1999; Singh et al., 2000). This has led to the hypothesis that SP not only acts as a mediator of the neuroimmune system but is also involved in direct interaction between immune cells either in a paracrine or in an autocrine fashion independent of sensory nerves, i.e., "neurogenic inflammation" (Ho et al., 1997; Lai et al., 1998). Expression of NK1 receptors in nonneuronal tissues and cells, such as in osteoclasts and human mucosal mononuclear cells, is increasingly recognized (Ho et al., 1997; Lambrecht et al., 1999). Through these receptors, SP has been shown to regulate production of a number of cytokines including IL-1, IL-6, IL-8, and TNF-$\alpha$ to mediate inflammatory and cell proliferative responses (Lotz et al., 1988; Palma and Manzini, 1998). It has been suggested that SP is a key molecule in the neuroimmune axis. In addition to the classical peptides SP and NKA, the recently discovered HK1 and the EKs have been found in nonneuronal cells/tissues such as pulmonary, cardiovascular, and articular cartilages, and cells of the immune system. The role of tachykinins in respiration, joint function, and gut mobility is briefly discussed in this section when expressed both in neuronal and in nonneuronal cells. The interaction of PPT-A expression in the periphery may mimic regulation in the CNS under various stresses.

### 2.4.1 Respiration

TKs are synthesized and released in a subset of sensory neurons innervating the mammalian respiratory tract; the neuroeffector role of these neurons is attributed to the release of TKs from their peripheral nerve terminals. TKs induce smooth muscle contraction, glandular secretion, plasma protein extravasation, cough, and other effects.

NK1 receptors located on the endothelial cells lining the microvessels play a role in plasma protein extravasation elicited by a variety of irritant stimuli and by antigen challenge in the mammalian airways (Lagente and Advenier, 1998). The inhalation of irritants or hyperpneic conditions in normal animals or antigen administration in sensitized animals also induces acute episodes of bronchoconstriction leading to respiratory distress that are at least in part mediated by TKs (Yasumitsu et al., 1996; Yoshihara et al., 1996a, b; Tramontana et al., 1998; Lai and Lee, 1999; Lai et al., 1999). A similar mechanism of bronchoconstriction operates in asthmatic patients. Both NK1 and NK2 receptors located on smooth muscle cells lining the bronchioles are involved in the bronchoconstriction response that is predominantly induced by endogenous NK2 released from the DRG neurons (Lai and Lee, 1999). This also results in atropine-resistant bronchoconstriction-mediated (Yuan et al., 1996) acetylcholine release (Hey et al., 1996). Capsaicin, a derivative of capsicum, causes respiratory distress and lethality at a very high dose. Pretreatment of guinea pigs with NK1 and NK2 receptor antagonists offered complete protection against the lethal dose of capsaicin, whereas NK2 receptor antagonists alone protect $\sim$80% of animals (Patacchini et al., 1999).

An acute inflammatory challenge, either allergic or nonallergic, induces both the early and the delayed airway hyperactivity (AHR) to bronchoconstrictor agents such as acetylcholine or histamine accompanied by infiltration of immune cells. The vagal stimulation releases acetylcholine and in turn leads to plasma protein extravasation in small bronchi and distal airways (Savoie et al., 1995). NK2 receptor antagonists block the AHR and reduce the infiltration of neutrophils and lymphocytes (Schuiling et al., 1999). The latter effect could be due to NK2 receptor antagonists binding on to the TK receptors, which reduces or prevents endogenous TK binding to NK1 receptor, thus blocking both the early and the delayed hyperresponsiveness to histamine induced by antigen in sensitized guinea pigs. NK1 K/O mice failed to recruit neutrophils upon challenge with allergens (Bozic et al., 1996). The proinflammatory effect induced by IL-17 or IL-1$\beta$ in the rat airways is mediated by endogenous TKs acting through NK1 receptors (Hoshino et al., 1999).

It could be hypothesized that the inhibition of plasma protein extravasation by TK receptor antagonists accounts in part for a reduced infiltration of immune cells. However, if we take into account the profiles of the effects induced by NK1 or NK2 receptor antagonists on immune cell infiltration, it is clear that the

blockade of plasma extravasation cannot solely explain the reduction of inflammatory response by these antagonists. For example, NK2 receptor blockade does not affect eosinophil infiltration. In this respect, it is worth noting that TKs can activate resident immune cells such as alveolar macrophages (Brunelleschi et al., 1990) and that these cells can release chemokines that attract other immune cells (Newton and Vaddi, 1997). The clinical testing of NK2 antagonists in asthma and obstructive pulmonary diseases is of particular interest in view of the fact that the expression of NK2 receptor mRNA is greatly increased in these pathologies compared with that in controls (Bai et al., 1995). Saredutant (SR 48968), a nonpeptide NK2 receptor antagonist, has been found to reduce the bronchoconstriction induced by inhaled NKA in asthmatic subjects (Van Schoor et al., 1998), thus making this drug a likely candidate for clinical trials in asthma.

Another possible clinical application of TK receptor antagonists is as antitussive agents. Inhalation of NKA and SP induces cough in guinea pigs (Takahama et al., 1993). Both the NK1 and the NK2 receptor antagonists reduce capsaicin- and citric-acid-induced cough although in the latter case the role of NK2 predominates (Yasumitsu et al., 1996). Cough is a reflex due to the direct activation of sensory neurons by irritants; therefore it is difficult to explain an antitussive activity via the blockade of NK1 or NK2 receptors on endothelial, smooth muscle, or immune cells. This indicates the possibility of a central action for the antitussive effect of TK receptor antagonists (Bolser et al., 1997). However, peripheral NK2 receptors or central NK2 receptors located in areas where access via the blood–brain barrier is facilitated are also likely to be involved since peptide NK2 receptor antagonists also display antitussive activity (Yasumitsu et al., 1996).

### 2.4.2 Chondrocyte Mechanotransduction

SP has an osteogenic stimulating effect that is probably caused by stimulating stem cell mitosis, osteoprogenitor cell differentiation, or osteoblastic activity (Shih and Bernard, 1997) possibly via regulation of intracellular calcium levels (Mori et al., 1999). SP regulation of chondrocyte behavior is complex. It has been suggested that autocrine/paracrine signaling via SP and NK1 is important in the signaling pathway through which the chondrocytes respond to mechanical stimulation. Mechanical stimulation of human articular chondrocytes in monolayer culture results in the activation of an integrin-dependent IL-4 signaling loop (Millward-Sadler et al., 2000). This signaling is associated with cell membrane hyperpolarization and alteration in the relative levels of aggrecan and matrix metalloproteinase (Millward-Sadler et al., 2000). Further, PPT-A K/O mice and specific receptor antagonist studies confirmed that SP is necessary for both hyperpolarization and gene expression plasticity following mechanical stimulation (Millward-Sadler et al., 2003). Interestingly, the NK1 receptor antagonist had no effect on IL-4-induced hyperpolarization, whereas IL-4 receptor antibodies inhibited the hyperpolarization response of chondrocytes to SP. This suggests that SP activity is upstream of IL-4 release in the mechanotransduction pathway (Millward-Sadler et al., 2003). Blockade of the hyperpolarization response to SP but not IL-4 by inhibiting adenylate cyclase activity implies cAMP in NK1-mediated signaling and cytokine release (Laniyonu et al., 1988; Lacour et al., 1994).

Increased SP levels have been reported in synovial fluid and cerebrospinal fluid from patients with rheumatoid arthritis and osteoarthritis (Lindh et al., 1997). Immunohistochemical analysis of the joint capsules from patients with anterior knee pain syndrome revealed increased SP-immunoreactive nerve fibers (Witonski and Wagrowska-Danielewicz, 1999). Release of SP from chondrocytes, either by mechanical stimulation or by other means, influences the activity of a wide range of cell types in the joint and periarticular structures, including macrophages, osteocytes, and nociceptive fibers. These observations suggest that PPT-A gene expression plasticity plays a vital role in the pathophysiology of remodeling and regeneration of bone and cartilage in joint diseases.

### 2.4.3 Gut

In the gastrointestinal tract, SP-containing nerve fibers and their cell bodies are present along the entire length; they are least prominent in the esophagus and upper part of the stomach (Polak and Bloom, 1981).

According to Nilsson et al. (1975) the highest concentration of SP occurs in the duodenum. SP-positive postganglionic parasympathetic neurons are located in the myenteric plexuses, and the postganglionic nerve terminals of these neurons innervate chiefly the inner circular muscles, although the outer longitudinal muscles contain few SP-positive fibers. SP-containing nerve fibers are also in close contact with the blood vessels (Polak and Bloom, 1981).

In the human gastric mucosa, Ferri et al. (l984) have demonstrated SP immunoreactivity in the oxyntic (parietal) zone of the gastric glands. Fibers containing this peptide are numerous and interconnecting in the "antrum" 3 cm above the pyloric aperture, suggesting the role of SP in pyloric sphincter regulation. In the duodenum, SP-positive fibers were present in large numbers in the base and core of the villi as well as in the muscularis mucosae and around blood vessels, suggesting the role for SP in absorption of nutrients and peristalsis. SP was also present in nerve fibers in the submucosa, neuronal perikarya between the lobules of Brunner's glands, and in Meissner's plexus.

## 3 SP Function in Pain

### 3.1 Role of SP in the Perception and Transmission of Pain and Inflammation

Nociception is the detection of pain and can be divided into two distinct categories depending on the duration: acute and chronic pain. Acute pain is severe/sharp short-term and provides an important "warning system" that all is not well within the body/environment, e.g., pain felt when treading on a track. Chronic pain is pain persisting for the long term that may be associated with nonnoxious (non-painful) stimuli (allodynia) or may be due to increased sensitivity to noxious (painful) stimuli (hyperalgesia). Unlike acute pain, chronic pain serves no useful physiological function and is therefore the target of much pharmaceutical research.

Pain can be further subdivided into three more categories depending on its location or point of origin:

1. Viscerosomatic pain, or pain detected internally, e.g., abdominal pains. Viscerosomatic pains share many pathways, with spreadout of receptors leading to poor definition of the point of origin of the pain.
2. Inflammatory pain due to irritants or nonspecific stimuli, mediated by the immune system and usually associated with the release of chemical mediators, including histamine and SP.
3. Neurological pain or pain induced following prolonged peripheral nerve activation or peripheral/central nerve lesion (axotomy/nerve crush). Inflammatory and neurological pains are often chronic pain with associated allodynia (nonnoxious stimuli results in nociception) or hyperalgesia (noxious stimuli are perceived with a greater intensity). SP is thought to be a major modulator of the neurotransmission of pain, being in part, along with the glutamatergic activation of NMDA receptors, the major cause of allodynia and hyperalgesia.

Tachykinergic signaling, in particular NK1-SP-mediated, has been previously implicated as the neurotransmitter for pain. Thermal (Duggan et al., 1987), mechanical (Duggan et al., 1988), and chemical (capsaicin) (Duggan et al., 1988; Takano et al., 1993) stimulation of the skin all elicit SP release from sensory afferents, increasing the SP concentration in the dorsal horn. Furthermore, direct electrical stimulation of C-fibers (Brodin et al., 1987; Klein et al., 1992) also increases SP concentration in the dorsal horn. In addition, intrathecal injection of SP induces pain-like behavior in rodents (Piercey et al., 1981; Hylden and Wilcox, 1983; Matsumura et al., 1985). Most strikingly, NK1 antagonists block the response of dorsal horn neurons to noxious stimuli (Radhakrishnan and Henry, 1991; Snider et al., 1991), suggesting that NK1 receptors mediate the pain perception. Collectively, these data provide a strong argument for SP as a candidate neurotransmitter for pain signaling; however, more recent data suggest SP is not the neurotransmitter of noxious stimuli, rather it is the neuromodulator of painful stimuli as outlined below.

SP is synthesized by small-diameter nociceptive neurons whose central terminals release the peptide in the dorsal horn of the spinal cord following intense peripheral stimulation. This is thought to promote

central hyperexcitability and increased pain sensitivity (Fitzgerald and Gibson, 1984; De Felipe et al., 1998). However, the function of SP in pain and nociception remains unclear. Human clinical trials with NK1 antagonists have proved to be largely ineffective but there remains the possibility that the wrong pain conditions or time of administration could have been targeted. This possibility was raised again by the analysis of NK1 K/O mice where a marked reduction in various types of visceral nociception as well as a reduced response to noxious chemical stimulation from somatic tissues was recorded (Bester et al., 2001). Recent data suggest that many of the peripheral inflammations release SP within the spinal cord (Bueno and Fioramonti, 1999). The "silent" nociceptors are recruited into action following inflammatory lesions of the skin or deep tissues or following partial nerve ligation. The development of pain behaviors was unaffected, initially by NK1 receptor or PPT-A deletion (Cao et al., 1998; Basbaum, 1999a, b; Dery et al., 2001). This suggests a possible role for SP in chronic inflammatory diseases. This role for SP as a neuromodulator of painful stimuli is supported by the demonstration that short-duration noxious thermal stimuli do not cause SP release in the dorsal horn. Only intense and prolonged thermal stimuli result in SP release (Duggan et al., 1987, 1988, 1992). Iontophoretical application of SP to dorsal horn neurons produces a slow response characterized by delayed onset (20–40 s) but sustained (30–90 s) response was observed as a slow excitatory postsynaptic potential (EPSP) (Nowak and Macdonald, 1982; Urban and Randic, 1984). Further, SP-mediated slow EPSP in response to noxious stimuli is the generation of an initially fast response (glutamate-mediated) followed by a slow and delayed afterdischarge that lasts for the duration of the stimulus. This initial fast response is not abolished by application of NK1 antagonists, while the slow prolonged response is abolished either by NK1 receptor antagonists (Radhakrishnan and Henry, 1991) or by depletion of SP by capsaicin treatment (Hey et al., 1996).

## 3.2 Generation of the Pain Signal by Primary Sensory Afferents or "First-Order Neurons"

Primary sensory afferent receptors detect noxious and nonnoxious stimuli and relay impulses from the point of origin in the periphery to the thalamus via lamina I and interneurons in the dorsal horn of the spinal cord. As mentioned previously, primary sensory afferents consist of two fiber types, myelinated, nonpeptidergic Aδ fibers and unmyelinated, mostly peptidergic C-fibers, whose cell bodies are found in the DRG of the PNS and whose central axons terminate in the dorsal horn of the spinal cord and trigeminal ganglion of the CNS.

The majority of Aδ afferents (proprioceptive) terminate in the inner region of lamina II of the dorsal horn and to a lesser extent in lamina V. Conversely, the majority of C-fibers (nociceptors) terminate in lamina I and the outer region of lamina II (substantia gelatinosa) of the dorsal horn and to a lesser extent in laminae III, VI, and X and also in the trigeminal ganglion of the CNS (Basbaum, 1999a, b). Under normal physiological conditions, Aδ fibers transmit impulses generated by nonnoxious stimuli such as pressure and heat, while C-fibers transmit impulses generated by both noxious and thermal stimuli (peptidergic and nonpeptidergic, respectively) (Basbaum and Woolf, 1999).

## 3.3 Relaying the Pain Message from Sensory Neurons to the Dorsal Horn

Wide-dynamic-range neurons respond to nonnoxious stimuli of differing intensity (mediated initially mostly via Aδ fibers), while the nociceptive neurons respond to noxious stimuli (mediated initially mostly by C-fibers) (Guirimand and Le Bars, 1996). The Aδ fibers synapse directly with interneurons (islet cells) in lamina II of the dorsal horn. However, those of C-fibers not juxtaposed to the interneurons in lamina II form close connections with the projection neurons of lamina I (Marshall et al., 1996). On low-threshold mechanical or thermal (nonnoxious) stimulation of sensory afferents, glutamate is released from Aδ terminals where it binds to postsynaptic alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) and NMDA receptors in the dorsal horn interneurons or projection neurons. Glutamate binding to AMPA receptors facilitates influx of $Na^+$ ions into the postsynaptic terminal, resulting in depolarization.

The NMDA receptor is a ligand-dependent voltage-gated $Ca^{2+}$ ion channel. On initial AMPA-mediated depolarization and binding of glutamate, $Mg^{2+}$ blockade of the NMDA channel pore is removed, allowing influx of $Ca^{2+}$ into the second-order neurons resulting in depolarization and propagation of the impulse.

Upon high-intensity noxious stimulation, both glutamate and SP are released from C-fibers. As mentioned above, the binding of glutamate to its receptors elicits a fast EPSP; however, the concomitant release of SP also elicits a second-phase slow EPSP. SP binding preferentially to the NK1 receptor on the postsynaptic membrane of projection neurons results in the activation of PLC$\gamma$, which in turn generates DAG and IP3, initiating calcium release from internal stores leading to further depolarization and propagation of impulse to the higher centers of the brain.

DAG activates PKC that indirectly modulates the response of the postsynaptic neuron to glutamate via phosphorylation of the NMDA receptor. Phosphorylation of the NMDA receptor increases the opening time of the channel leading to prolonged influx of $Ca^{2+}$ and $Na^{+}$, thereby prolonging depolarization of second-order neurons. DAG also contributes to the formation of arachidonic acid, an event in the formation of prostagladins, PGE2 and PI2 that sensitize the cell membrane to further depolarization. IP3 triggers intracellular calcium release from the internal stores (endoplasmic reticulum) resulting in a further increase in intracellular calcium levels, thus further prolonging depolarization of the second-order neurons.

In summary, the release of glutamate from Aδ fibers following mechanical or thermal stimulation results in depolarization of the second-order interneurons in lamina II of the dorsal horn. However, the intensity of a noxious stimulus could result in the corelease of glutamate and SP from C-fiber terminals. SP diffuses from its release site into the interneurons (islet cells) of lamina II and projection neurons of laminae I and III, thus propagating the nociception. Following high-potency noxious stimulation or during inflammation, SP release is increased and NK1 receptor binding is extended beyond laminae I and III to the deeper layers of laminae IV and VI (Brown et al., 1995). This plasticity of SP release results in recruitment and prolonged activation of second-order neurons. It is this action of SP that may be responsible for the development of allodynia and hyperalgesia. The depolarization of second-order neurons is prolonged in response to the lower-intensity noxious stimuli (hyperalgesia) or mechanical and thermal stimuli (allodynia), resulting in the perception of intense pain (chronic pain) since the neuronal pathways have become hypersensitive. NK1 receptor internalization studies have supported the role of SP in hyperalgesia/allodynia. Ablation of the NK1 neurons of the dorsal horn using SP conjugated to a potent neurotoxin (saporin) results in the augmentation of hyperalgesia/allodynia in rodents. However, the behavior of NK1-ablated mice and control mice was unaltered in response to mild noxious stimuli, suggesting that SP plays a role in modulating the response to chronic intense pain and in supplementing persistent pain states, while acute pain is mediated by the NMDA cascade (Liu et al., 1997).

Both glutamate and SP mediate excitatory responses to sensory stimuli; however, it is also possible that both SP and glutamate can function as positive feedback loops directly or indirectly to modulate their own release. Increased intracellular $Ca^{2+}$ following NMDA receptor or NK1 receptor binding can activate the enzyme NOS in postsynaptic neurons. NOS catalyzes the production of a gaseous free radical NO from the substrate L-arginine. NO diffuses from the postsynaptic neuron and activates soluble guanylyl cyclase (sGC) in the presynaptic neuron to increase intracellular cyclic GMP (cGMP). Downstream of cGMP, PKG is activated, which may lead to modification of gene expression and modulation of transmitter release or may modulate ion channels. Furthermore, glutamate binding to presynaptic NMDA receptors (Liu et al., 1996) and kainate receptors (Chizh et al., 1997) on the central terminals of DRG neurons may enhance depolarization resulting in further release of both glutamate and SP. The existence of presynaptic NK1 receptors indicates that SP itself may directly enhance its own release by increasing depolarization of primary sensory afferents.

## 3.4 Neuronal Pathways of Pain Processing: Delivering the Message

The detection of painful stimuli, pressure, and temperature are coordinated by three main pain pathways: the spinoreticular, the spinothalamic, and the parabrachial pathways (Gauriau and Bernard, 2002).

This section describes these pathways in brief. The spinoreticular pathway, as its name suggests, is centered on the deep lamina of the dorsal horn of the spinal cord and the reticular system in the brainstem. The spinoreticular system is implicated in mediating somatic motor responses and the emotional behaviors associated with pain. Furthermore, this pathway offers a feedback regulation of nociception through a descending pathway, the reticulospinal loop. The second pathway, the spinothalamic pathway, originates in the superficial layers of lamina I of the dorsal horn and projects to thalamic areas and is most likely responsible for pain sensation of tactile origin. The spinobrachial pathway also originates in the superficial layer of lamina I of the dorsal horn but the axons terminate in the parabrachial area among others. This pathway is concerned with emotional, autonomic, and neuroendocrine aspects of the pain experience. In fact, the majority of nociceptive messages converge on the parabrachial area and are then connected to higher brain regions responsible for emotions (amygdala), emotional behavior (periaqueductal gray), and autonomic homeostatic adaptation (hypothalamus and ventrolateral medulla) in response to pain (Gauriau and Bernard, 2002). It is likely that neuromodulation of pain pathways by SP occurs not only at the levels of the spinal cord but also in the brain region. For example, NK1-expressing projection neurons of the dorsal horn relay nociceptive information, directly or indirectly, to brain areas such as the amygdala, the ventromedial nucleus of the hypothalamus, and PAG. These brain areas have been implicated in the mediation of antinociception caused by opioids, electrical brain stimulation, or stress-induced analgesia (Fields, 2000). It is possible that SP and the NK1 receptor play an important role in regulating the endogenous antinociception mediated by release of opioids at the synapses.

## 4 Neurodegenerative Diseases and Other CNS Disorders

PPT-A mRNA has been identified in the normal and the pathological state in various regions of the CNS in rats (Warden and Young, 1988; Harlan et al., 1989; Brene et al., 1990) and in humans (Hurd et al., 1999), suggesting a role for tachykinins in the pathophysiology of a variety of etiologies or diseases (Kramer et al., 1998; Maubach et al., 1998; Liu et al., 1999a, b). For example, SP can enhance neural or neurite growth in vitro (Iwasaki et al., 1989) and counteract the effects of neurotoxins administered to animals, and has mnemogenic and anxiolytic properties in vivo (Hasenohrl et al., 1989). SP/PPT-A expression has also been studied in several neurodegenerative disorders, including Parkinson's disease (Gresch and Walker, 1999), Alzheimer's disease (Bouras et al., 1990), and Huntington's disease (Richfield et al., 2002). All of these conditions are associated with a progressive loss of SP and PPT-A expression in the brain. Additionally, SP/NK1 antagonist MK-869 had antidepressant effects in patients with moderate to severe major depression, suggesting that SP may play an important role in psychiatric disorders (Kramer et al., 1998; Maubach et al., 1999). It is likely that inappropriate expression of the PPT-A gene is correlated with the disease profiles in which tachykinin gene products are implicated.

### 4.1 Epilepsy

Recent studies have shown a strong correlation between the incidence of epilepsy, SP, and the integrity of the dentate gyrus in a rodent model (Liu et al., 1999a, b). Epilepsy is a chronic medical condition produced by temporary and maladaptive alterations in the electrical function of the brain, causing seizures that affect awareness, movement, and/or sensation. Epilepsy affects more than 50 million people worldwide. Epilepsy is a biphasic disorder in which an initial seizure may lead to the generation of spontaneous, continuous, and long-lasting seizure activity. The process by which an initial seizure can lead to the development of epilepsy is termed epileptogenesis. The process of epileptogenesis itself is poorly understood, but it is believed that the initial imbalances in inhibitory and excitatory inputs during seizure lead to long-term plastic changes in glutamatergic and GABAergic signaling pathways, novel expression of neuropeptides in brain regions, and at a later stage synaptic reorganization and mossy fibers sprouting. Although many new antiepileptic drugs have been invented and tested in the last decade, about one-third of epileptic patients still suffer from

inadequately controlled seizures or significant side effects. Novel PPT-A expression has been widely implicated in both the initiation and maintenance phases of epilepsy.

### 4.1.1 Neuronal Cell Excitability Is Regulated via the Interplay Between Excitatory Glutamatergic and Inhibitory GABAergic Neurotransmission

There are many types of seizure showing a variety of symptoms and severity, whose presence can be determined by the pattern of burst firing activity as seen on an electroencephalogram (EEG). There are a number of animal models that show similarity to etiology and symptoms of human temporal lobe epilepsy (TLE), one of the most extensively studied forms of epilepsy. All seizures, however, have common features, including spontaneous, increased frequency and sustained, irregular neuronal firing patterns, i.e., all show signs of excessive excitability.

The control of cell excitability is important in maintaining normal physiological control of any neuronal cell. This control is manifested by the interplay between the excitatory and inhibitory inputs into this system. If oversimplified, it could be said that the balance between the glutamatergic signaling pathway (excitatory) and the GABAergic signaling pathway (inhibitory) controls neuronal excitability. Seizure-like activity and associated cell death has been attributed to increased excitability of neurons due to increased glutamate-mediated NMDA receptor activation (Wasterlain et al., 2000). In addition, the excessive release of presynaptic glutamate in vivo in kainic acid-induced seizures further confirms NMDA-receptor-mediated excitability during seizures (Bruhn et al., 1997).

### 4.1.2 The Biphasic Nature of Epilepsy: Initiation and Maintenance of Seizure

Epilepsy can be divided into two stages: the initiation phase, in which a single seizure may increase the propensity of a cell toward developing and maintaining continuous seizures, and the maintenance phase, in which a cell can be said to be epileptic due to the presence of spontaneous and continuous long-lasting firing activity.

Experimental initiation of epilepsy via perforant path stimulation (PPS) can be blocked by the application of agonists of inhibitory pathways ($GABA_A$ agonists) or antagonists of excitatory pathways (NMDA/AMPA/kainate), electrical stimulation of GABAergic pathways, NK1 receptor antagonists, galanin (an inhibitory neuropeptide) and its receptor agonists, opiate receptor agonists (delta) and antagonists (kappa), and finally by ionic imbalances across the neuronal membrane (elevated intracellular $Na^{2+}$ with low extracellular $K^{+}$, or high extracellular $Mg^{2+}$). Conversely, experimental initiation of epilepsy can be elicited by $GABA_A$ antagonists, glutamate receptor agonists (NMDA/AMPA/kainate), electrical stimulation of glutamatergic pathways, NK1 receptor agonists, SP or NKB (excitatory neuropeptides), galanin receptor antagonists, opiate receptor antagonists (delta), and agonists (kappa), and finally by ionic imbalances across the neuronal membrane (elevated extracellular $K^{+}$ with low extracellular $Na^{2+}$ or low extracellular $Mg^{2+}$) (Liu et al., 1999a, b; Wasterlain et al., 2002). So it would seem that the initiation of seizure has many entry points and is responsive to drug treatments, e.g., benzodiazepines are currently used in the treatment of seizure as agents that stimulate GABAergic pathways.

Experimental inhibition of the maintenance phase of self-sustaining status epilepticus (SSSE) is difficult to manage. Many of the above inhibitors of the initiation phase are ineffective in halting or preventing the sustained seizures associated with the maintenance phase. Pharmacological treatment with benzodiazepines is ineffective as are other drugs such as sodium channel blockers and non-NMDA receptor blockers (Wasterlain et al., 2002). Glutamatergic antagonists, NK1 antagonists, and inhibitory neuropeptides such as galanin, however, are effective in ameliorating SSSE, suggesting that the maintenance phase of epilepsy is strongly dependent on NMDA and tachykinin receptor activation. This implies that SP may play a modulatory role in sensitizing neuronal cells in glutamatergic pathways that is similar to that proposed for its role in modulating cell excitability in models of pain and inflammation.

### 4.1.3 Epileptic Pathways: the Hippocampus in the Limbic System Is a Major Brain Region Associated with Seizure

Metabolic studies using 2-deoxyglucose autoradiography have shown a number of regions of the brain with high metabolic activity during SSSE produced via perforant path stimulation (Wasterlain et al., 2002). These areas include the hippocampus, amygdala, the caudate putamen, the substantia nigra, the nucleus accumbens, and the medial thalamus. Interestingly, all of these areas express medium to high levels of SP.

Wasterlain et al. (2002) proposed a hippocampal model of initiation and maintenance of seizure/epilepsy based on these metabolic studies and those involving pharmacological agonists and antagonists of principal pathways. ❯ *Figure 20-3* depicts the excitatory and inhibitory pathways involved in the control of hippocampal excitability in nonepileptic brain models and their modification in epileptic brain models. PPS by electrodes generates SSSE by intense activation of GABAergic signaling in the dentate gyrus of the hippocampus, resulting in excessive GABA release. On binding of GABA to its receptors, they are internalized, thus effectively removing the GABA inhibitory control from the hippocampus. Removing inhibition of the GABAergic pathway in this manner indirectly increases neuronal excitability, increasing the likelihood of seizure. The pathological changes in neurotransmitter-mediated excitability lead to decreased anticonvulsant (galanin/dynorphin) and increased proconvulsant (SP/NKB) neuropeptide expression, producing long-lasting changes in excitability by further potentiating glutamate release. In particular, decreased galanin or dynorphin and increased SP mediate removal of inhibition and increase excitability on mossy fibers of the dentate gyrus, thus increasing glutamate release in the CA3 pyramidal layer. The CA3 neurons respond to glutamate release from the mossy fibers by increasing the firing activity. CA3 nerve terminals synapse with the frontal cortex, which is concerned with the regulation of motor activity. Additionally, excitatory connection to the cortex from the hippocampus usually results in negative feedback to the dentate gyrus to inhibit further glutamate release but on depletion of inhibitory neuropeptides (galanin/opioids), this negative feedback is lost; hence the glutamatergic input to cortical areas further increases seizure activity.

Seizures may last from anywhere between a few minutes to a few hours and in some cases recurrent episodic seizures may go on for days. Late synthesis and expression of galanin, depletion of excitatory neurotransmitters, and neuronal death all contribute to the cessation of seizure without pharmacological intervention. Unfortunately, massive cell death induced during seizure is not fully investigated and in some cases epilepsy could be fatal. However, recent research on adult neurogenesis suggests that the dentate gyrus granular zone is a promising source for the generation of new neurons as a replacement for dead neurons (Alvarez-Buylla and Lim, 2004).

### 4.1.4 SP Is a Proconvulsant: the Modulation of the Transcriptional Regulation of PPT-A Gene Expression in Seizure and Epilepsy

Neuropeptide expression is modified during seizure, which in turn leads to increased excitability in the hippocampus and other brain regions. SP has been widely implicated in modifying excitability and mediating both the initiation and the maintenance phases of epilepsy (Liu et al., 1999a, b, 2000; Wasterlain et al., 2000, 2002). SP is detected at low levels in the rodent hippocampus. However, production of neuronal proteins is subject to "plasticity" at the level of gene regulation; thus the concentration of SP within tissues can be altered in response to specific stimuli, e.g., growth factors, stress, and drugs.

SP demonstrates highly proconvulsive properties. High levels of SP in hippocampal neurons can induce SSSE at electrical stimuli below the threshold stimulation normally required to induce such activity (Liu et al., 1999a, b). Furthermore, elevated levels of SP and PPT-A in the dentate gyrus subregions of the hippocampus (CA1 and CA3) have been implicated in neuronal damage following SSSE induced by PPS, or chemoconvulsant seizure induction with kainic acid, or lithium–pilocarpine induction (❯ *Figure 20-4*) (Brene et al., 1992; Liu et al., 1999a, b, 2000). NK1 receptor antagonists applied before or during the initiation and maintenance phases abolish SSSE (Liu et al., 1999a, b). Moreover, SP application to brain slices induces glutamate release (Liu et al., 1999a, b). In studies employing an in vivo kainic acid epilepsy

**Figure 20-3**

**Excitatory and inhibitory pathways in the rodent hippocampus. (a) shows the excitatory glutamatergic pathways stimulated in normal brain; normally inhibitory input from GABAergic interneurons in the hilus and from inhibitory neuropeptides (galanin, dynorphin, somatostatin, and NPγ) controls excitability of glutamatergic signaling, preventing the generation of seizure. However, in an epileptic model (b), excessive stimulation of inhibitory systems depletes inhibitory neurotransmitters and neuropeptides, allowing unbridled activation of glutamatergic pathways leading to the development of seizure. Additionally, novel excitatory neuropeptide expression (SP) further reinforces seizure by sensitizing glutamatergic pathways and increasing glutamate release. Late expression of galanin in the hilus restores some inhibitory control to dampen seizure**

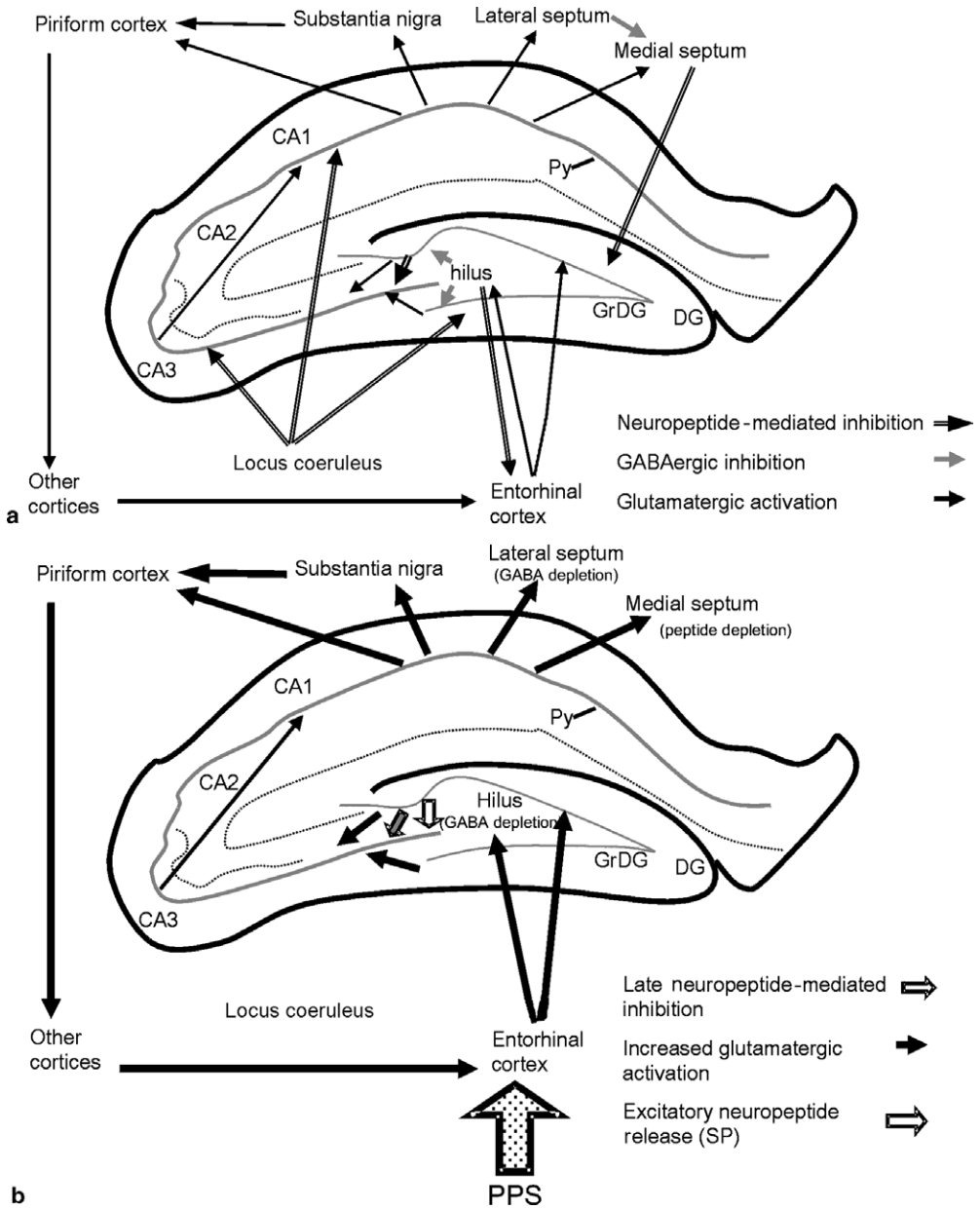
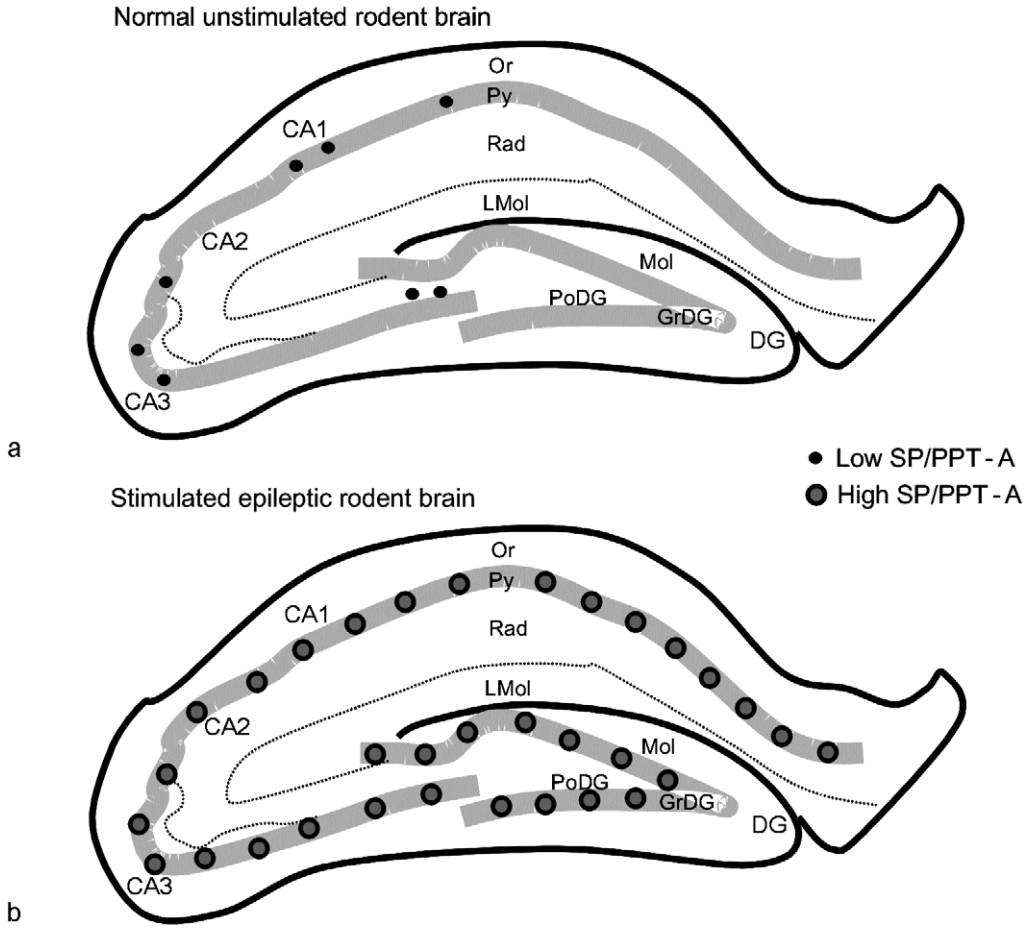


**Figure 20-4**

**Schematic representation of the inducibility of SP/PPT-A in the rodent hippocampus following experimental seizure induction. SP/PPT-A mRNA expression is very low in the normal unstimulated rodent hippocampus (a). Low levels of expression are detected by immunostaining and in situ hybridization in the CA1, CA2, and CA3 pyramidal cell layers (Py), and a few SP-positive cell bodies are found in the hilus (h) of the dentate gyrus (DG). In the epileptic mouse (b) massive induction and de novo synthesis of SP is observed. Marked upregulation of PPT-A and SP expression is seen in all areas that express in the unstimulated mouse. In addition, novel expression is seen in the granule cell layer (GrDG) of the dentate gyrus**




model, PPT-A gene K/O studies have correlated the lack of SP with the inability to induce seizures and associated damage within the hippocampus in mice (Liu et al., 1999a, b). Additionally, these K/O mice do not show induction of caspases and other genes associated with cell death that are normally expressed in seizure. It is not surprising that PPT-A mRNA is increased in the hippocampus during seizure, and de novo synthesis of SP in the dentate gyrus, an area that does not express SP during normal physiological functioning, is observed in a number of in vivo and in vitro epileptic models.

There are many unanswered questions as to how SP might be upregulated during seizure, e.g., which classes of regulatory transcription factors are induced during seizure and how do they affect the transcription of PPT-A? Defining the pathways regulating the PPT-A gene will help delineate general changes associated with seizure induction. Recent research suggests that bHLH factors are differentially regulated

following status epilepticus, with some increasing (Mash1, Id2), some decreasing (Hes5), and others remaining mostly unchanged (NeuroD/BETA2, NeuroD2/NDRF, Id3, Rath2/Nex1) (Elliott et al., 2001). The PPT-A gene contains a number of regulatory elements termed E boxes that bind bHLH factors to regulate transcription. It is possible that these bHLH factors are responsible, in part, for the modulation of the PPT-A gene expression during seizure and as such may provide a novel target for therapeutic intervention. Similarly, a major repressor of the proximal rat PPT-A promoter is the transcription factor NRSF, often also called REST (Bubb et al., 2002), which demonstrates differential expression during rodent epilepsy models (Palm, 1998). Interestingly, NRSF/REST has several isoforms, which have been suggested to act as both repressors and activators of transcription (Bubb et al., 2002); these isoforms are also differentially regulated during epilepsy (Palm, 1998).

## 4.2 Anxiety and Depression

Studies on the NK1 K/O mice and clinical studies suggest that SP is involved in emesis, stress responses, aggression, anxiety, depression, and reward (Cao et al., 1998; De Felipe et al., 1998a, b; Kramer et al., 1998; Rupniak and Kramer, 1999; Rupniak et al., 2000). These apparently diverse behavioral manifestations are potentially interlinked and SP could modulate these pathways that are important to the animals in the face of major environmental stressors (Culman and Unger, 1995; Culman et al., 1997; De Felipe et al., 1998a, b). The NK1 receptor is widely distributed within subcortical and brainstem regions and within the spinal cord, as described previously. The receptor is highly but heterogeneously expressed within the amygdala, and by the cholinergic neurons of the striatum, nucleus accumbens, and nucleus basalis. These areas of the brain have been linked to anxiety and reward behaviors. The NK1 receptor is also strongly expressed in the hypothalamus, PAG, and the superficial laminae of the spinal cord. These areas control pain processing and flight/fight responses (autonomic) following environmental challenges such as attack, injury, or invasion of territory (Lumb and Lovick, 1993; Bandler and Shipley, 1994; Lovick, 1996; De Felipe et al., 1998a, b).

Selective deletion of the NK1 receptor gene using homologous recombination in embryonic stem cells resulted in mice that bred and developed normally. However, close examination of these mice revealed a number of remarkable behavioral changes compared with wild-type litter mates; K/O mice were less aggressive as measured by the resident–intruder assay (De Felipe et al., 1998a, b) and had reduced levels of anxiety as judged by their response to brief maternal separation (Rupniak et al., 2000). These measures anticipated the current clinical trials of NK1 antagonists in human subjects suffering from anxiety and depression (Kramer et al., 1998).

The reduction in anxiety and aggressive behavior thought to be coupled with a number of other behavioral changes in NK1 K/O mice suggested that the NK1 receptor might be involved in orchestrating basic survival behaviors. For example, there was also a reduction in stress-induced analgesia that may be due to descending inhibitory control on the spinal processing of nociception. The behavioral monitoring suggested that the unaltered behavior of mutant mice was due to lack of change in the hot-plate threshold following a brief cold-water swim (De Felipe et al., 1998a, b). The behavioral changes were correlated with morphological changes using immunohistochemistry. For example, there was no change in the number of *c-fos*-positive neurons in the lumbar spinal cord following concurrent noxious stimulation of both fore- and hindpaws (Bester et al., 2001).

Remarkably, there was a failure of the NK1 K/O mice to develop a conditioned place preference to morphine (Murtra et al., 2000). The loss was specific to morphine as mice responded when cocaine or food were used as rewards. Moreover, the analgesic response to opiates was largely intact (De Felipe et al., 1998a, b). We conclude that SP plays an important and specific role in mediating the motivational aspects of opiates. While this may represent a major new pharmacological route for the control of drug abuse, there is also the possibility of dissecting away the analgesia from the euphoria-producing properties of the opiates. Morphine given exogenously binds to the μ opiate receptor and by this route it is thought to "hijack" the opiate reward pathways and other pathways that modulate nociception (Robbins and Everitt, 1999).

The data argue strongly that a key synapse in the opiate reward process lies within the area of ventral forebrain and occurs between collaterals of SP-releasing populations of striatal projection neurons and large

cholinergic neurons of the nucleus accumbens and nucleus basalis that express the NK1 receptor. These neurons have also been implicated in associative learning and respond to stimuli that serve to trigger a "learn and reward" task (Graybiel et al., 1994; Boix et al., 1995).

### 4.3 Parkinson's Disease

Parkinson's disease results in reduced levels of dopamine, which is due to loss of dopaminergic neurons (∼50%) in the substantia nigra whose fibers have extensive synapses within the striatum. The decrease in levels of dopamine is reflected in the striatonigral neurons by lower levels of D1 receptors and SP, among others. It was demonstrated that the loss of SP led to further loss of dopaminergic neurons (Barker, 1986). SP striatal neurons also inhibit the neurons of the internal globus pallidus. In addition to dopamine neurotransmission, serotonin neurotransmission regulates striatal PPT-A mRNA levels. It has been suggested that the activation of 5-HT transmission could compensate for the loss of PPT-A in striatal neurons (Gresch and Walker, 1999a, b). Furthermore, it was demonstrated that serotonin 2A/2C receptors mediate PPT-A mRNA expression in the striatum after dopamine depletion produced with 6-hydroxydopamine in adult rats. However, higher levels of SP could be toxic. NK1 antagonists can block the toxic effects of methamphetamine administered to the striatum (Yu et al., 2002). A major role for PPT-A expression in Parkinson's disease was proposed from studies involving 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) treatment of primates. MPTP induces Parkinson-like symptoms, which is due to the destruction of dopaminergic neurons in the substantia nigra. Animals with an acute or chronic parkinsonian phenotype had decreased levels of PPT-A in the striatum while in asymptomatic animals PPT-A levels were unaffected (Wade and Schneider, 2001). The authors suggest that PPT gene expression may be directly related to expression of parkinsonian motor symptomatology regardless of duration of MPTP exposure, duration of the parkinsonism, or extent of dopamine denervation. The plasticity of PPT-A expression in this CNS region is demonstrated in that ciproxifan, the histamine H3 receptor ligand, can reverse the effect of methamphetamine on the PPT-A gene (Pillot et al., 2003). Therefore intervention, which targets genes on the same pathway as PPT-A by increasing neuropeptide gene expression, could be useful therapy.

In addition to NK1 receptor activation by SP, there are reports of NK3 receptor involvement in the rat midbrain neurons in Parkinson's disease. Modulation of NK3 activity in the rat nigrostriatal dopamine neurons has been reported to affect dopamine levels and hence SP could be a potential therapeutic target for Parkinson's disease (Bannon and Whitty, 1995; Whitty et al., 1995).

## 5 Genetic Models Available for In Vivo Analysis

Gene disruption in order to study the role of a specific protein is commonly employed in the generation of preclinical animal models. In the case of the tachykinins, the study of their function(s) has significantly been enhanced by the generation of K/O mice with targeted disruptions of the PPT-A and TACR1 genes, which encode SP, NKA, and NK1, respectively (Cao et al., 1998; De Felipe et al., 1998a, b; Zimmer et al., 1998).

PPT-A K/O animals are deficient in SP and NKA but are able to develop normally, are fertile, and can take care of their offspring (Bilkei-Gorzo et al., 2002). Behavioral studies carried out in these animals have demonstrated a reduction in sensitivity to nociceptive stimulation in acute and chronic pain models, although the response to mildly painful stimuli is unaffected (Cao et al., 1998). Mutant animals do not experience neurogenic inflammation, which normally follows SP release. This is indicative of a role of SP/NKA in the production of moderate to intense pain.

The role of the tachykinins in stress-related behaviors has also been addressed in the PPT-A K/O animals. It has been shown that PPT-A(−/−) mice were more active in the forced-swimming test and tail suspension paradigm (both used as indicators of depression-related behaviors) and were less fearful in models of anxiety (such as the open-field arena and the elevated zero maze). Their behavior was comparable

with that of wild-type animals treated with antidepressant drugs, including tricyclic and selective serotonin re-uptake inhibitors. These observations support the view that the tachykinin system mediates the development of anxiety and depression disorders.

Mice lacking the PPT-A gene have been resistant to kainic acid-induced seizures that mimic hippocampal hyperexcitability seen in cases of status epilepticus. Neuronal cell death in the hippocampus caused by repetitive epileptic seizure activity could be compromised in the absence of SP release (Liu et al., 1999a, b).

Another approach to the study of the tachykinin pathway has been through the inactivation of the tachykinin NK1 receptor. Genetic disruption of the NK1 receptor does not affect the general health or fertility of the mutant mice but has a significant effect in the amplification ("wind up") and intensity coding of nociceptive reflexes, which appear to be absent in these animals (De Felipe et al., 1998a, b). The role of the NK1 receptor in pain and hyperalgesia has also been investigated using the NK1(−/−) model. It has been proposed that the response to noxious mechanical stimuli is modulated by NK1, which is also implicated in hyperalgesia pathway (Laird et al., 2000). Endogenous pain-control mechanisms, such as stress-induced analgesia, were found to be substantially impaired in the NK1(−/−) mice (Bester et al., 2001). In behavioral experiments, mice lacking the NK1 receptor were observed to be less aggressive than their wild-type counterparts in the "resident–intruder" test.

To further explore the function of the human tachykinins and understand their potential role in a variety of pathophysiological processes, the generation of genetic in vivo models of the human PPT-A and NK1 genes has been attempted. MacKenzie et al. (2000) have reported the production of a yeast artificial chromosome (YAC) transgenic model that comprises the human PPT-A gene (hPPT-A) and can drive appropriate expression of β-galactosidase within the adult mouse brain (MacKenzie and Quinn, 2002). YAC constructs have a large cloning capacity (within the range of several hundreds of kilobases) and may contain not only the coding region of a particular gene, but also the majority of regulatory sequences that are required for correct transcription. It has been demonstrated that the hPPT-A YAC transgenic mouse is able to express SP/NKA in appropriate areas of the developing mouse brain, and expression is observed in regions at significantly earlier time points than originally suggested from conventional analysis such in situ hybridization. By crossing the hPPT-A and hNK1 alleles onto the relevant ablated genetic backgrounds, PPT-A and NK1(−/−) are already available (De Felipe et al., 1998a, b; Zimmer et al., 1998), and as such it should be possible to construct an animal that expresses only the human genes. Such a "humanized" animal model would be a valuable tool for preclinical pharmacological and behavioral studies.

The use of transgenic in vivo models to complement biochemical analyses has proven extremely advantageous in the study of the role of the tachykinins in physiological conditions as well as in disease states. Further, exploiting the possibilities that these models can offer will undoubtedly assist future advances in our understanding of the function of the tachykinins. However, one should be careful to extrapolate too much from experiments done on only one mouse strain as dramatic differences have been observed in tachykinin function in the lung in the response to virus infection depending on the strain of mouse used (Payne et al., 2001).

## 6 Summary

The tachykinins are central to normal physiology and disease, both acting individually or in synergy with other neurotransmitters. They are involved in a plethora of disease states or conditions. The tachykinin receptor antagonists have been tested or are currently being tested in clinical trials for a number of conditions including depression, anxiety, pain, and cancer with limited success. Investigation of the molecular and cellular regulation and function of these neuropeptides and their receptors will lead to better strategies for clinical intervention. Further, analysis of emerging genomic information from clinical analysis of polymorphisms associated with disease may dictate those populations at risk that would be more amenable to therapeutic targeting of the tachykinins. We look forward to these research-defining mechanisms central to neural transmission in general and also for better therapeutic strategies.

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