Volume 44(1-4):21-38, 2000
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

REVIEW ARTICLE

Neurotransmitters and receptors in the dorsal horn of the spinal cord

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ABSTRACT Modulation of the sensory input can occur within the dorsal horn of the spinal cord where the primary afferent fibers synapse with neurons that transmit to the higher centers. The transmission of the sensory information begins with activation of the peripheral receptors of primary afferent neurons whose cell bodies lie within the dorsal root ganglia and whose central terminals project to secondary neurons in the dorsal horn of the spinal cord. Several neurotransmitters and a large variety of receptors have been found in the superficial laminae of the dorsal horn. The present work reviews the major classes of transmitters and receptors that have been implicated in the transmission and modulation of spinal afferent and pain processing. The role of excitatory and inhibitory amino acids, tachykinin and opioid peptides, calcitonin gene-related peptide (CGRP), nociceptin and nocistatin, biogenic amines, acetylcholine, ATP, nitric oxide as well as capsaicin and vanilloid receptors will be discussed along with the most recent developments in the field. It seems probable that transmission of the somatosensory information from the primary afferent fibers to the secondary dorsal horn neurons depends on the balance between the excitatory effects of excitatory amino acids and the inhibitory actions of several other transmitter systems.
Acta Biol Szeged 44(1-4):21-38 (2000)

KEY WORDS

spinal cord
primary afferent fibers
dorsal horn neurons
somatosensory processes
pain
analgesia

The transmission of somatosensory information begins with activation of the peripheral receptors of primary afferent neurons whose cell bodies lie within the dorsal root ganglia (DRG) and whose central terminals project to secondary neurons in the dorsal horn of the spinal cord. In cutaneous and visceral nerves of the rat, the fastest-conducting large myelinated sensory fibers belong to the Aβ class; the slower-conducting, thinly myelinated fibers belong to the Aδ group; the slowest-conducting unmyelinated small fibers are designated as C-fibers. A weak electrical stimulus can selectively activate large myelinated axons. Stronger, and probably painful, electrical stimuli are required to excite unmyelinated C-fibers in addition to myelinated axons. While several compounds have been proposed as endogenous neurotransmitters released from afferent neurons following a nociceptive (painful) stimulus, current evidence implicate the excitatory amino acids (EAAs), aspartate and glutamate, and the peptide substance P (SP). For instance, in many of the convergent nociceptive spinal dorsal horn neurons, repetitive stimulation of the cutaneous receptive field at a constant strength great enough to excite C-fibers, causes a progressive increase in the number of spikes evoked by the successive arrival of volleys along C-fibers over the first 10-16 stimuli (Mendell and Wall 1965; Mendell 1966). This frequency-dependent potentiation is called wind-up and is associated with the activation of the *N*-methyl-D-aspartate (NMDA) receptor complex (Davies and Lodge 1987; Dickenson and Sullivan 1987; Dickenson 1990; Budai 1994). Release of SP in response to noxious stimulation may increase primary afferent C-fiber activity and an accumulation of SP N-terminal metabolites appears to potentiate wind-up *via* positive modulation of EAA activity (Budai and Larson 1996).

The efficiency of synapses in the CNS is not constant, but can be modified by changing the activity in the presynaptic pathways. Long-term potentiation (LTP) in the hippocampus, which may comprise the synaptic basis of learning and memory, has become the dominant model of activity-dependent synaptic plasticity in the mammalian brain. In the spinal cord, modifications of the synaptic transmission that outlast the stimulus or the event triggering them can also occur after various stimulation procedures or injury. Compelling evidence has accumulated over the last several years, indicating that central hyperactive states resulting from neuronal plastic changes within the spinal cord play a critical role in hyperalgesia associated with nerve injury and inflammation (Mayer et al. 1999). Synaptic modifications that occur in the spinal cord as a result of repeated stimulation are habituation, sensitization and post-tetanic potentiation (Mendell 1984). The repetitive, low-frequency stimulation of unmyelinated axons in a peripheral cutaneous nerve can cause a wind-up

Accepted April 13, 2000
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of dorsal horn neuron activity, with a progressive potentiation of responses to each subsequent stimulus. Modifications of the synaptic transmission in the spinal cord that last for prolonged periods can occur after relatively brief injury discharges or afferent conditioning by various stimulation procedures. Many sensory abnormalities in pathologic conditions, including pain, hyperalgesia and allodynia, are presumably due to the plasticity of the dorsal horn circuits. Primary hyperalgesia can be explained by the sensitization of primary afferent fibers whereas secondary hyperalgesia and allodynia are generally thought to depend on a sensitization of neurons in the dorsal horn. An increased pain sensation (hyperalgesia), decreased pain threshold (allodynia) and persistent nociception following peripheral tissue injury depend both on an increase in the sensitivity of primary afferent nociceptors at the site of the injury (peripheral sensitization), and on an increase in the efficiency of the synapses between primary afferent fibers and the dorsal horn neurons (central sensitization) (Yaksh et al. 1999). One of the most important questions relates to the mechanisms by which a peripheral stimulation can produce long-lasting modifications of spinal synapses that are related to acute or chronic pain. The increase in the postsynaptic response generated at potentiated synapses could be due to any combinations of these: (a) presynaptic modifications which result in an increase in the amount of EAAs and other transmitters released per impulse, (b) postsynaptic modifications such as changes in the characteristics of receptor functions, (c) an extrasynaptic change, such as a reduction in uptake or metabolism of the released substances, or (d) morphological modifications.

The aim of this paper is to give an overview of the neurotransmitters and their receptors that play a major role in mediation of the somatosensory information in the dorsal horn of the spinal cord. Given the vastness and clinical relevance of the field, this paper by no means pretends completeness. We also refer to reviews that have previously been published on related subjects such as the induction of pain (Millan 1999), the localization of transmitters and receptors in the mammalian dorsal horn and primary afferent neurons (Todd and Spike 1993; Coggeshall and Carlton 1997), the pharmacology of spinal afferent and pain processing (Wilcox 1991; Coderre 1993; Zieglgansberger and Tolle 1993; Wilcox and Seybold 1997; Fürst 1999), and the descending or central modulation of spinal pain mechanisms (Fields et al. 1991; Rudomin et al. 1993; Stamford 1995; Lima 1996; Sandkühler 1996; Millan 1997; Mason 1999).

Excitatory amino acids (EAAs)

L-Glutamate (Glu) and L-aspartate (Asp) are the major excitatory neurotransmitters in the central nervous system (CNS). Glu fulfills several of the criteria for a transmitter involved in neurotransmission from primary afferent fibers to secondary spinal dorsal horn neurons. Glu-like immunoreactivity has been localized in small DRG neurons and in terminals in the superficial dorsal horn. Glu is released after electrical stimulation in a Ca^{2+}-dependent manner. Further, iontophoretically applied EAA agonists activate nociceptive projection neurons, and this activation is inhibited by EAA antagonists such as MK-801, 7-chlorokynurenate (7-Cl-KYNA) or GYKI 53466 (Budai and Larson 1994). In behavioral studies, EAA agonists elicit thermal hyperalgesia, and EAA antagonists block the vocalization elicited by noxious stimulation. These data implicate a contribution of EAAs to neurotransmission between primary afferent fibers and secondary dorsal horn neurons (for reviews see Wilcox 1991; Coderre 1993; Todd and Spike 1993; Urban et al. 1994; Salt and Eaton 1966; Bennett 2000). EAAs produce their effects through two broad categories of receptors; ionotropic and metabotropic Glu receptors. The ionotropic Glu receptors have been traditionally classified into three subtypes upon the basis of pharmacological and electrophysiological data: *N*-methyl-D-aspartate (NMDA) receptors, (*R,S*)-α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA) receptors and kainic acid (KA) receptors. Ionotropic Glu receptors directly regulate the opening of ion channels to Na^+ and K^+, while NMDA receptors do so to Ca^{2+} as well. A family of G-protein-coupled Glu receptors, called metabotropic Glu receptors, has recently been identified. Their activation causes an increase in the turnover of polyphosphoinositides and in the release of Ca^{2+} from intracellular stores.

The application of molecular cloning technology to study of the Glu receptor system has led to an explosion of knowledge about the structure, expression and function of ionotropic and metabotropic Glu receptors. When sequence homologies as well as pharmacological properties are taken into account, the 28 Glu receptor genes that have so far been characterized on a molecular basis can be grouped into 13 subfamilies, 10 for the ionotropic receptor class and 3 for the metabotropic receptor class. Interestingly, on the basis of phylogenetic tree of ionotropic and metabotropic Glu receptors, it appears unprobable that the two families of receptors share a common ancestor gene. Any sequence similarity may reflect evolutionary convergence rather than evolutionary relatedness. Glu receptor subfamilies can also be grouped according to their pharmacological properties as follows (for reviews see Hollmann and Heinemann 1994; Bettler and Mulle 1995; Pin and Duvoisin 1995; Roberts 1995):

AMPA receptors: GluR1-GluR4

These subunits each form homomeric ion channels when expressed in oocytes or in transfected cells. Agonist potencies follow the sequence quisqualate>domoate~AMPA>Glu>KA. Although AMPA is the most potent specific agonist, KA elicits the largest responses because it does not desensitize

the receptors. The co-expression of two or more subunits does not alter the order of agonist potency, although potencies are generally lower in heteromeric receptors. Both homomeric and heteromeric channels show cooperativity, which suggests a multimeric structure of the native channel. The potent antagonists of native non-NMDA receptors, CNQX and NBQX, exhibit no or only low selectivity for different subunits. *In situ* hybridization studies have revealed a widespread, but differential distribution of the GluR1-GluR4 mRNAs.

Low-affinity KA receptors: GluR5-GluR7

When expressed in transfected cells, GluR5 can be activated (in sequence of agonist potencies) by domoate>KA>Glu> AMPA with rapid desensitization. The sequence of potency for GluR6 is domoate>KA>quisqualate>Glu. The absence of responses of GluR6 to AMPA demonstrates that AMPA is an agonist at only a subset of ionotropic non-NMDA subunits. In contrast with GluR1-GluR4, both GLU and KA desensitize GluR5 and GluR6 receptors. The expression patterns of GluR6 and GluR7 transcripts are clearly different from the distribution pattern of [^{3}H]AMPA in the brain, but they overlap very well with most of the [^{3}H]KA binding sites. GluR6 responses can be increased by 50% by phosphorylation with protein kinase A (PKA). This modulatory effect of PKA does not alter the affinity for Glu or the desensitization properties. It has been shown that both AMPA and kainate GluR5 receptors play an enhanced role in spinal nociceptive processing following the development of peripheral inflammation, as antagonists at both receptors are more effective against nociceptive responses, including wind-up, under these inflammatory conditions (Stanfa and Dickenson 1999).

High-affinity KA receptors: KA1 and KA2

The affinities of KA1 and KA2 for KA (binding which is inhibited by CNQX) are significantly higher than those of GluR5-GluR7, suggesting that KA1 and KA2 may represent the high-affinity [^{3}H]KA receptor seen in ligand-binding studies of synaptic membranes and in autoradiographic studies of brain sections. The sequence of agonist potencies, KA>quisqualate>domoate>Glu>>AMPA, also demonstrates that KA1 and KA2 are pharmacologically distinct from GluR5-GluR7 and are high-affinity KA receptors. When the KA2 subunit is co-expressed with GluR6, the AMPA response is nondesensitizing, which is quite different from the AMPA currents of GluR1-GluR4. KA1 and KA2 RNAs are distributed differently in the brain. Whereas KA1 RNA is generally expressed at low levels and is abundant in only two cell types, the CA3 pyramidal cells and the dentate granule cells of the hippocampus, KA2 RNA is widely expressed in most parts of the brain.

NMDA receptors: NMDAR1 and NMDAR2

When expressed in oocytes, homomeric NMDAR1 receptors respond in the presence of glycine (Gly) to Glu, NMDA, quisqualate, ibotenate, Asp, and L-homocysteic acid,but they do not respond to KA, AMPA or trans-ACPD, or to Gly itself. The competitive NMDA receptor antagonists D-APV, CPP, CGS 19755 and 7-Cl-KYNA all inhibit NMDAR1 responses. The channel blocker MK-801, Zn^{2+} and phencyclidine also inhibit the receptor (Kovács and Larson 1994, 1997). These data show that the functional properties that reported for the NMDA receptors from studies on other systems can all be found in NMDAR1. NMDAR1 RNA is expressed in almost all neuronal cells, with particularly high levels in the cerebellum, hippocampus, cerebral cortex, and olfactory bulb. Activators of protein kinase C (PKC) such as PMA produce a 3- to 20-fold stimulation of the receptor response. PKA activators such as 8-bromo-cAMP or forskolin, on the other hand, do not alter the NMDAR1 receptor responses to agonists. In contrast with NMDAR1, none of the NMDAR2 subunits assembles into functional ion channels when expressed as homomeric receptors. However, when co-expressed with NMDAR1, each of the four NMDAR2 subunits co-assemble with NMDAR1 into functional heteromeric receptor that have properties different from those of the homomeric NMDAR1 receptor. The properties of the different heteromeric channels are not equivalent. PKC activators potentiate the responses of the NMDAR1/NMDAR2A and NMDAR1/NMDAR2B, but not those of NMDAR1/NMDAR2C receptors. PKC potentiation is sensitive to the PKC inhibitor staurosporine.

Group I metabotropic Glu receptors: mGluR1 and mGluR5

When expressed in oocytes, both mGluR1 and mGluR5 respond (in sequence of potency) to quisqualate>ibotenate~ Glu>trans-ACPD, producing large, long-lasting oscillating currents. These currents are typical for receptors coupled to phospholipase C (PLC), which releases Ca^{2+} from internal stores *via* inositol phosphates (IP). EGTA injection selectively inhibits mGluR1 responses, thereby demonstrating that mere membrane depolarization is not sufficient to trigger mGluR signal transduction pathways. Activation of mGluR1 or mGluR5 does not inhibit forskolin-induced cAMP formation, and is therefore not coupled to the inhibitory cAMP cascade. Both mGluR1 and mGluR5 are widely expressed in the brain, overlapping in most regions but also showing some clear differences. The high level of mGluR1 mRNA expression in the hippocampal CA3 pyramidal neurons and cerebellar Purkinje cells suggests that mGluR1 may be involved in LTP at the mossy fiber-CA3 synapse and in long term depression (LTD) at the parallel fiber-Purkinje cells synapses, both of which are pertussis toxin (PTX)-sensitive phenomena.

Group II metabotropic Glu receptors: mGluR2-mGluR4 and mGluR6

Upon expression in transfected cell lines, mGluR2 has been shown to affect IP formation only slightly, but to inhibit forskolin-induced cAMP formation markedly, an effect that is inhibited by PTX in a dose-dependent manner. Thus, mGluR2 appears to be coupled to the inhibitory cAMP cascade *via* a PTX-sensitive G-protein. The rank order of agonist potency for mGluR2 and mGluR3 is GLU~tACPD> IBO>QA, whereas the rank order for mGluR4 is quite different, AP4>GLU~IBO>tACPD. mGluR2 RNA is prominently expressed *e.g.* in the Golgi cells of the cerebellum, cortical neurons and intrinsic neurons of the olfactory bulb.

Increasing evidence also suggests an involvement of mGluRs in nociception and pain behavior, although the contribution of individual mGluR subtypes is not yet clear (Budai and Larson 1998). It has been suggested that the mGluR1 subtype is activated endogenously during brief high-intensity cutaneous stimuli and is critically involved in capsaicin-induced central sensitization (Neugebauer et al. 1999). Also, group I mGluRs (mGluR1 and mGluR5) may act to enhance ionotropic Glu responses but the two types of mGluRs may have different intracellular mechanisms of action (Ugolini et al. 1999). Group I mGluR antagonists have a significant antinociceptive efficacy in a mouse visceral pain model (Chen et al. 2000). In addition to NMDA receptor, metabotropic Glu receptor activation appears to be involved in the generation of the segmental spinal reflex evoked by high-intensity stimulation in the neonatal rat spinal cord *in vitro* (Boxall et al. 1996).

Inhibitory Amino Acids (IAAs)

γ-Aminobutyric acid (GABA) and glycine

GABA and Gly along with their synthesizing enzymes, are distributed broadly in the spinal cord. Both are candidate inhibitory neurotransmitters in the dorsal horn as they increase Cl^- conductance through neuronal cell membranes and hence produce inhibitory postsynaptic potentials.

GABAergic neurons are found throughout the gray matter of the spinal cord, with a higher frequency in the superficial laminae (laminae I-III) (Magoul et al. 1987; Todd and Sullivan 1990; Powell and Todd 1992; Spike and Todd 1992; Todd et al. 1992). Three GABA receptor subtypes have been identified: the $GABA_A$ receptor subtype, which mediates rapid ionotropic transmission; the $GABA_B$ receptor subtype, which mediates a variety of metabotropic responses; and the $GABA_C$ subtype, which has not yet been found in the dorsal horn. There is significantly more $GABA_B$ than $GABA_A$ receptor ligand binding in the dorsal horn. Both receptor subtypes can be found in great numbers on the primary afferent terminals. There is a heavy concentration of GABA receptors exists in lamina II, and much thoughts has therefore been given to the presynaptic modulation of the fine-caliber, presumably nociceptive, primary afferent input. $GABA_B$ receptors are found in abundance in laminae I, III and IV, and accordingly are thought to be involved in the presynaptic control of Aδ and Aβ primary afferent fibers. Almost all cell bodies in the DRG are positively stained with an antibody to the subunits of the $GABA_A$ receptors. Moreover, intrinsic dorsal horn neurons contribute significantly to the $GABA_A$ receptor population in the dorsal horn. Baclofen, a chlorophenyl derivative of GABA and selective ligand for $GABA_B$ receptors, depresses both monosynaptic and polysynaptic transmission in the dorsal horn possibly through a decrease in transmitter release rather than by any antagonism at postsynaptic receptors. It has been reported that $GABA_B$ sites, unlike $GABA_A$ sites, are present in high concentrations in laminae I, II, III and IV of the dorsal horn and that after the neonatal administration of capsaicin this binding is reduced by 40-50% (Price et al. 1984).

Glycine immunoreactivity within the dorsal horn of the rat spinal cord is relatively sparse in laminae I and II, but is present in higher concentrations in lamina III and deeper parts (Todd and Sullivan 1990). Gly-containing axons in laminae I-III may be derived from various sources, such as local interneurons, distant spinal cells or supraspinal neurons. Glycinergic neurons in the dorsal horn may receive a significant synaptic input from myelinated low-threshold mechanoreceptors (Todd 1991; Todd et al. 1991). Two types of Gly receptors exist in the CNS, strychnine-sensitive and -insensitive ones. In general, the strychnine-sensitive receptors predominate in the dorsal horn. Gly receptors and $GABA_A$ receptors are often colocalized and the former are regarded as being postsynaptic to primary afferent fibers. The strychnine-insensitive Gly modulatory binding site of the NMDA receptor/channel complex may play a major role in the regulation of NMDA receptor-mediated synaptic events (Thomson 1990; Huettner 1991). Electrophysiological studies on isolated systems have shown that Gly increases the current evoked by NMDA, and it is absolutely required as a co-agonist for NMDA receptor/channel activation (Johnson and Ascher 1987; Kleckner and Dingledine 1988; Kushner et al. 1988). Intrathecal application of 7-chlorokynurenate, an antagonist at the allosteric Gly site associated with the NMDA receptor, decreases the frequency-dependent potentiation (wind-up) elicited by peripheral stimulation (Dickenson and Aydar 1991). In behavioral experiments, the blockade of strychnine-sensitive Gly receptors in the spinal cord unmasks a facilitatory effect of Gly on NMDA-induced convulsions (Larson and Beitz 1988). In intact animals, low doses of iontophoretically administered Gly increase whereas high doses of Gly inhibit the firing of single nociceptive neurons in the dorsal horn of the spinal cord evoked by iontophoretically applied NMDA. When inhibitory effects of Gly

dominate, iontophoretic application of strychnine reveals a facilitation of the NMDA responses by Gly (Budai et al. 1992a). In behavioral studies, NMDA, strychnine and bicuculline (a $GABA_A$-receptor antagonist) produce similar touch-evoked allodynia. It has been hypothesized that $GABA_A$ sites regulate presynaptic Glu release, while Gly regulates the excitability of neurons postsynaptic to the glutamatergic terminals (Ishikawa et al. 2000).

Peptides

Tachykinins: Substance P (SP) and Neurokinin A (NKA)

The preprotachykinin A gene that encodes SP produces three different messenger RNAs. They encode both SP and NKA as they are synthesized from some of the same precursor proteins (Helke et al. 1990). The mRNA for preprotachykinin A gene occurs in small and medium-sized DRG nerve cells (Boehmer et al. 1989). It is probable that the two tachykinins coexist in many DRG neurons.

The interactions of EAAs and tachykinins, such as SP, in spinal afferent and nociceptive processing are suggested by several findings. SP and EAAs are found to be co-localized in the central terminals of primary afferent neurons. SP produces a long-lasting enhancement of the responses of dorsal horn neurons to iontophoretically applied EAA agonists. Combined treatment with SP and NMDA produces a profound enhancement of the responses of the dorsal horn neurons to nonnoxious and noxious mechanical stimulation, as well as the behavioral responses. These effects probably depend on both presynaptic and postsynaptic actions of SP on EAA neurotransmission, since SP has been found to enhance the release of Glu and Asp from the spinal dorsal horn, whereas SP produces a potentiation of Glu and NMDA-induced currents in spinal dorsal horn neurons *in vitro*. SP is neither necessary nor sufficient to elicit pain at the spinal level. On the basis of a detailed analysis of behavioral and electrophysiological studies, a modulatory role of SP in EAA-related nociceptive transmission has been postulated. SP has also been proposed as the agent, which mediates the slow temporal summation (wind-up) of C-afferent, evoked responses in dorsal horn nociceptive neurons (Budai and Larson 1996). It has been shown that SP enhances while the SP antagonist DPDT-SP attenuates the prolonged discharge of dorsal horn neurons evoked by repetitive stimulation of C-afferents. Neither compound affects the A-fiber-evoked firing or spontaneous cell activity, indicating that the effects of SP are highly selective to C-fiber-mediated nociceptive transmission (Kellstein et al. 1990). NKA is released from the primary afferent terminals in response to the same mechanical, thermal and chemical stimuli that release SP (Diez Guerra et al. 1988; Duggan et al. 1990).

Neurokinin (NK) receptors consist of three categories: NK-1, NK-2 and NK-3. The most widely studied of these is the NK-1 receptor, often referred to as the SP receptor (Polgár et al. 1999). SP exerts its effects on spinal cord neurons primarily via NK-1 receptors and secondarily *via* NK-2 receptors. These receptors have been cloned, sequenced and expressed in oocytes. The mechanism of action of SP at these receptors involves the production of IP_3 and diacylglycerol (DAG) by activation of the enzyme phospholipase C *via* PTX-insensitive G-proteins. This results in an elevated intracellular Ca^{2+} level by promoting the release of Ca^{2+} from intracellular stores. The activation of NK and/or EAA receptors, may therefore lead to the activation of various intracellular second messengers. These include activation of (a) NO synthase and consequently NO synthesis, which in turn activates soluble guanylate cyclase and increases cGMP, (b) phospholipase A_2, which triggers the production of arachidonic acid, and (c) PKC *via* generation of DAG. By immunohistochemistry, the heaviest immunoreactivity for NK-1 receptors is observed in the middle part and lateral fourth of lamina I where the great majority of immunoreactive perikarya represents fusiform and multipolar cells. In lamina II, the middle and medial part shows moderate immunoreactivity, most of the cells resembles stalked cells. In lamina III, the labeled perikarya are evenly distributed, while those in lamina IV accumulates mainly in the lateral part (Polgár et al. 1999). The activation of NK-1 receptors is dependent on the C-terminal domain of SP and may be responsible for most of the SP effects. The C-terminal sequence of SP is also responsible for a series of immunological events. By contrast, the N-terminal domain of SP is involved in the modulation of catecholamine release from adrenal chromaffin cells and in promoting histamine release from peritoneal mast cells. Effects of SP that are independent of NK receptors have also been reported. In rat peritoneal mast cells, G-proteins may be activated through their direct interaction with the cationic cluster of SP, which is located at its N-terminus. These effects of SP are sensitive to PTX.

The N-terminal metabolic fragments of SP are reportedly also active in spinal neurotransmission. A decreased behavioral responsivity to repeated intrathecal (i.t.) injections of SP appears to be brought about, at least in part, by the rapid accumulation of N-terminal metabolites of SP (Larson 1988). The major metabolite of SP appears to be the N-terminal heptapeptide SP_{1-7}. Exogenously administered SP_{1-7} inhibits the excitatory behavioral effects of SP in the spinal cord (Igwe et al. 1990a, 1990b, 1990c), and there is a strong correlation between the accumulation of N-terminal fragments of SP and the development of desensitization to the behavioral effects of SP. Two binding sites for $[^3H]SP_{1-7}$ recently characterized in the mouse spinal cord may account for the activity of SP N-terminal fragments. N-terminal fragments of SP have also proven capable of altering high-

affinity, ß-FNA-insensitive, μ-type opioid binding (Krumins et al. 1989; Krumins et al. 1990). Iontophoretic application of SP causes an increase in the response of the cells to mechanical stimulation of the cutaneous receptive field, which is generally accompanied by increases in the responses to the EAAs. However, reciprocal changes caused by SP or its N-terminal fragment SP_{1-7} in EAA responses have also been reported (Budai et al. 1992b; Dougherty et al. 1993). This type of change is characterized by decreases in the responses to one class of EAA agonist (NMDA *versus* non-NMDA), despite increases in the responses to the other type of EAA (non-NMDA) agonist. Overall inhibitory effects of SP on EAA responses are observed on occasion (Dougherty et al. 1993). The reciprocal changes in EAA responses following SP or SP_{1-7} application may represent a switching of the dominant input to a particular cell from one type of EAA receptor to another. It has been proposed that the increases caused in the EAA responses by SP are due to the activation of appropriately inter-linked receptor-mediated events in particular dorsal horn neurons and that the second messenger systems activated by the combination of NMDA and SP are different from those activated by the combination of non-NMDA EAAs and SP (Dougherty et al. 1993). An alternative second messenger system could provide a mechanism for the reciprocal changes observed following co-application of SP or SP_{1-7} and non-NMDA agonist, since interregulatory effects between the EAA receptor subtypes involve Ca^{2+} release.

The hypothesis that a SP antagonist may possess analgesic activity in man remains to be proven. Antagonists that are based on modifications of one or more of the 11 amino acids that comprise SP possess poor pharmacokinetic properties and limited *in vivo* activities. However, during the past decade, several nonpeptide, metabolically stable antagonists have become available (Watling 1992). Behavioral studies have shown that CP 96,345, a potent nonpeptide NK-1 antagonist, blocks the slow EPSP induced by noxious cutaneous stimulation and repetitive C-fiber stimulation, and reduces noxious heat neuronal responses without effect on innocuous inputs (Nagahisa et al. 1993). Unfortunately, CP 96,345 has been demonstrated to have nonspecific activity in the formalin and carrageenan-induced models of nociception, which is probably due to its affinity for the L-type Ca^{2+} channels. More recently, a new piperidine derivative, CP-99,994, has been introduced and reported to have significantly reduced affinity for the L-type Ca^{2+} channel. Another potent, nonpeptide NK-1 receptor antagonist, RP 67580, inhibits the first and second phases of the formalin response in a dose-related manner. RP 67580 attenuates the facilitation, but not the baseline, of a spinal flexor reflex by noxious conditioning stimuli in the rat (Parsons et al. 1996). These results support the hypothesis that NK-1 receptors play a role in prolonged nociceptive transmission in the spinal cord.

Calcitonin gene-related peptide (CGRP)

CGRP occurs in highest concentration in the small DRG nerve cells. Its two forms are encoded in two variants of messenger RNA that are spliced from the same gene that encodes calcitonin. Primary afferent neurons seem to be the only source of CGRP in the dorsal horn (Tuchscherer and Seybold 1989). The majority of primary afferent neurons associated with pain transmission contains CGRP (Nasu 1999; Pezet et al. 1999). Immunoreactive CGRP is released from the central processes of primary afferent neurons following peripheral application of noxious thermal, mechanical and chemical stimuli (Morton and Hutchison 1989; Garry and Hargreaves 1992). The discharge frequency of wide dynamic range neurons in the dorsal horn decreases significantly upon activation of CGRP receptors which may play an important role in the transmission of the presumed nociceptive information (Yu et al. 1999). Pronase-induced deafferentation significantly increases CGRP binding in the superficial (I-II) and deeper (II-IV) laminae of the dorsal horn (Helgren et al. 1999). In mutant mice that lack αCGRP mRNA, CGRP immunoreactivity is almost completely absent from the spinal cord and is not observed at all in the spinal ganglia. The antinociceptive behavior tested by the tail-flick and hot-plate tests does not differ significantly in mutant and wild-type mice, except when challenged by morphine (Salmon et al. 1999).

Opioid peptides: enkephalins and dynorphins

Two classes of opioid peptides are present within the spinal dorsal horn: the enkephalins: Met- and Leu-enkephalin and Enk-8 (Met-enkephalin-Arg-Gly-Leu); and the dynorphins: dynorphin A and dynorphin B. Enkephalin molecules are derived from the precursor protein preproenkephalin (PPE), whereas dynorphins are derived from a different precursor, preprodynorphin (PPD) (Todd and Spike 1992, 1993). The enkaphalins are the putative endogenous ligands for delta (δ) opioid receptors. Dynorphin A is the endogenous ligand for the kappa (κ) opioid receptors.

The importance of opioid receptors in the control of pain and somatosensory processes is widely accepted and is of significant clinical relevance. Their three major subtypes, called mu (μ), delta (δ) and kappa (κ), are transmembrane G-protein-coupled receptors. In the spinal dorsal horn, the most prevalent type seems to be the μ (70% or more), with considerably fewer δ (23% or less) and κ (7% or less) opioid receptors (Gulya et al. 1986; Besse et al. 1990a, 1990b; Stevens et al. 1991; Dado et al. 1993; Coggeshall and Carlton 1997). In ligand-binding experiments, each type is preferentially located in the superficial laminae, laminae I-III. The majority of the opioid receptors are located on fine primary afferent fibers and terminals since dorsal rhizotomy causes a significant loss in opioid receptor binding in the superficial

dorsal horn (Stevens and Seybold 1995). The receptor loss parallels the disappearance of the fine primary afferent fibers after axotomy. However, much opioid ligand binding still remains after dorsal rhizotomy and this residual binding is regarded as being postsynaptic to the fine primary afferent input (Gouarderes et al. 1985, 1986; Faull and Villiger 1987). Two isoforms of the μ opioid receptor (MOR1 and MOR1B) have recently been cloned (Schulz et al. 1998).

The dorsal horn regions that receive descending inputs from the rostral ventromedial medulla (RVM) contain μ, δ and κ opioid receptors, as well as enkephalinergic interneurons and terminal fields (Besse et al. 1991; Fields et al. 1991; Arvidsson et al. 1995a, 1995b; Mansour et al. 1995; Chieng et al. 1996). RVM axon terminals contact enkephalinergic dorsal horn neurons (Glazer and Basbaum 1984; Cho and Basbaum 1989). Release of enkephalin at spinal levels has been demonstrated (Bourgoin et al. 1988; Tang et al. 1989; Yaksh and Chipkin 1989; Collin et al. 1992; Dado et al. 1993). Opioids directly inhibit primary afferent nociceptors (Werz et al. 1987; Moises et al. 1994; Taddese et al. 1995) and nociceptive dorsal horn neurons (Omote et al. 1990; Glaum et al. 1994; Grudt and Williams 1994; Randic et al. 1995). Endogenous opioids acting *via* spinal μ opioid receptors contribute to brainstem control of nociceptive spinal dorsal horn neurons. The inhibition appears to result in part from presynaptic inhibition of afferents to dorsal horn neurons (Budai and Fields 1998).

Recently, two endogenous peptides, endomorphin-1 and endomorphin-2 (Zadina et al. 1997), specific for μ-opioid receptors have been identified. Endomorphin-2-like immunoreactivity was found to be colocalized in a subset of SP- and μ opioid receptor-containing fibers in the superficial laminae of the spinal cord and spinal trigeminal nuclei. Disruption of primary sensory afferents by mechanical (deafferentation by dorsal rhizotomy) or chemical (exposure to the primary afferent neurotoxin, capsaicin) methods virtually abolishes endomorphin-2-like immunoreactivity in the dorsal horn. These results indicate that endomorphin-2 is present in primary afferent fibers where it can serve as the endogenous ligand for pre- and postsynaptic μ receptors and as a major modulator of pain perception (Martin-Schild et al. 1998; Martin-Schild et al. 1999). Intrathecal application of endomorphin-1 and endomorphin-2 increases the tail-flick latency and, to the lesser extent, the paw pressure latency (Przewlocka et al. 1999). The formalin-induced behavior is attenuated by endomorphins, and endomorphins antagonize allodynia in a dose-dependent manner (Przewlocki et al. 1999).

Nociceptin (orphanin FQ) and nocistatin

Nociceptin (orphanin FQ), the natural agonist of orphan opioid receptor-like receptor (ORL-1), is a heptadecapeptide that is endowed with supraspinal pronociceptive activity *in vivo*. *Via* ORL-1 receptors, nociceptin FQ triggers the same G-protein-mediated signaling pathways, as do opioids, however, to produce pharmacological effects that sometimes differ from, and even oppose those of opioids (for reviews see Darland et al. 1998; Yamamoto et al. 1999). Nociceptin stimulates an outward K^+ current and/or inhibits voltage-gated Ca^{2+} channels, thereby reduces synaptic efficacy, *i.e.* neuronal activity. Nociceptin is derived from a larger precursor, prepronociceptin (PPNOC). The entire coding sequence of the precursor protein has been cloned for nociceptin. The deduced nociceptin precursor shows sequence similarity to the opioid peptide precursors and shares characteristic structural features particularly with preprodynorphin. *In situ* hybridization analysis of nociceptin precursor mRNA in the mouse central nervous system reveals that it is highly expressed in discrete neuronal sites with the pattern distinct from those of opioid peptides (Houtani et al. 1996). Nociceptin and its precursor mRNA are present in a number of brain regions, less abundant in the spinal cord, and negligible in the cerebellum. *In situ* hybridization analysis reveals that hybridization-positive neurons are distributed in the superficial layer (lamina I) of the dorsal horn and are also interspersed between the tract of Lissauer in the spinal cord (Okuda-Ashitaka et al. 1996). Combined *in situ* hybridization and immunohistochemistry have shown a correlation between nociceptin immunoreactivity and PPNOC mRNA. In the spinal cord, nociceptin is observed throughout the dorsal and ventral horns (Neal et al. 1999). By using double label immunohistochemistry and confocal microscopy, the co-localization of μ opioid receptors and ORL-1 receptors is not observed in either perikarya or neuropil in the DRG, the Lissauer's tract or in the superficial laminae of the spinal cord. Likewise, there is no evidence for co-localization of these receptors within the periaqueductal gray, the nucleus raphe magnus, the gigantocellular reticular nucleus, and the nucleus of the solitary tract. These observations indicate that μ opioid and ORL-1 receptors are expressed predominantly on different fiber systems in these regions (Monteillet-Agius et al. 1998).

Antinociceptive effects for nociceptin have been reported in spinal applications. Nociceptin dose-relatedly inhibits the C-fiber evoked wind-up and post-discharge of dorsal horn neurons, but not the baseline C-fiber-evoked responses. The antinociceptive role of nociceptin in the spinal cord differs from that of classical opioids (Stanfa et al. 1996). Intrathecal nociceptin produces dose-dependent depression of a spinal nociceptive flexor reflex and behavioral antinociception in the tail flick test with no signs of sedation or motor impairment (Xu et al. 1996). Intrathecally administered nociceptin depresses both the phase 1 and phase 2 flinching behavior in the formalin test and depresses the level of thermal hyperalgesia in a dose-dependent manner (Yamamoto et al. 1997). It is of interest to note that intracerebroventricular application

of nociceptin, in contrast with intrathecal administrations, enhances the formalin-induced pain behavior (Wang et al. 1999). Nociceptin elicits a rapidly appearing, naltrexone-reversible, dose-dependent analgesia in the tailflick assay without any indication of hyperalgesia. Blockade of sigma receptors with haloperidol enhances the analgesic potency of spinal nociceptin, but not as dramatically as supraspinal nociceptin (King et al. 1997). Intrathecal nociceptin dose-dependently alleviates mechanical and cold allodynia-like behavior in two models of neuropathic pain (Hao et al. 1998). Whole-cell recordings from substantia gelatinosa neurons in transverse lumbar spinal cord slices show that exogenous nociceptin, at low concentrations, depresses excitatory postsynaptic potentials evoked by stimulation of dorsal rootlets or, at high concentrations, it hyperpolarizes substantia gelatinosa neurons and suppresses spike discharges. The latter actions are not reversed by the known opioid receptor antagonist, naloxone. It is concluded that nociceptin-like peptide is concentrated in nerve fibers of the rat dorsal horn and that it may serve as an inhibitory transmitter within the substantia gelatinosa (Lai et al. 1997). It has also been shown that nociceptin inhibits excitatory synaptic transmission in the superficial layers of the rat dorsal horn by acting on presynaptic, presumably ORL-1 receptors (Liebel et al. 1997). Nociceptin primarily inhibits EAA (Glu, KA and quisqualate)-induced currents in isolated rat spinal dorsal horn neurons *via* non-opioid mechanisms (Shu et al. 1998). Recently, the introduction of two selective and competitive nociceptin receptor antagonists has been reported: one peptide analog (Calo et al. 2000) and one nonpeptidyl in nature (Ozaki et al. 2000). However, the peptide ORL-1 receptor antagonist [Phe_1 psi(CH_2-NH)Gly_2]nociceptin-(1-13)-NH_2, may act as a nociceptin agonist in the rat spinal cord (Carpenter and Dickenson 1998).

The nociceptin precursor protein contains another biologically active peptide, which is termed nocistatin and is isolated from bovine brain. Nocistatin blocks nociceptin-induced allodynia and hyperalgesia, and attenuates pain evoked by prostaglandin E_2. Intrathecal pretreatment with antinocistatin antibody decreases the threshold for nociceptin-induced allodynia. Although nocistatin does not bind to the nociceptin receptor, it binds to the membrane of mouse brain and of spinal cord with high affinity. Nociceptin and nocistatin peptides may play opposite roles in pain transmission (Okuda-Ashitaka et al. 1998). It is suggested that nociceptin may be involved in the second phase of the mouse formalin test and, under such pathophysiological conditions, nocistatin can exhibit antagonism against nociceptin at the spinal level (Nakano et al. 2000). Nocistatin induces a moderate facilitation of the flexor reflex without producing reflex depression. It interacts with nociceptin in a complex fashion, increasing excitation and reducing inhibition (Xu et al. 1999).

Biogenic amines

Serotonin (5-HT)

5-HT is present in the axons and terminals of raphe-spinal neurons in the dorsal horn, especially in the superficial laminae, laminae I-III. The origin of serotonergic projection to the dorsal horn is mainly the nucleus raphe magnus (Dahlström and Fuxe 1965; Basbaum et al. 1978; Miletic et al. 1984). 5-HT and several peptides, including SP, CGRP, enkephalins and somatostatin, may be co-localized in the same raphe neurons and in their terminals. 5-HT may also be co-localized with GABA (Millhorn et al. 1987a, 1987b). Molecular cloning has identified seven distinct families of 5-HT receptors (5-HT_{1-7}). The 5-HT_3 family consists of ligand-gated ion channel receptors. The other 6 families interact with G-proteins and are coupled to second messengers. Three 5-HT receptor subtypes influence the dorsal horn somatosensory processing: 5-HT_1, 5-HT_2, and 5-HT_3. There are three major sources of 5-HT receptors to the spinal cord dorsal horn: the DRG cells, the intrinsic spinal neurons and the descending systems. Neonatal capsaicin treatment or dorsal rhizotomy decrease 5-HT_{1A} and 5-HT_3 receptor binding in laminae I and II, but some still remains, indicating both pre- and postsynaptic localizations. A large majority of the 5-HT receptors in the dorsal horn do not participate in classic synapses, but are found in extrasynaptic sites along the dendrites and somas.

The activation of 5-HT receptors can produce multiple physiological events, since 5-HT receptor families can either promote or inhibit different second messenger systems. Intrathecally administered 5-HT can either inhibit (Hylden and Wilcox 1983) or stimulate (Hylden and Wilcox 1983; Clatworthy et al. 1988) nociceptive reflexes. Iontophoretic application in the vicinity of dorsal horn neurons generally causes inhibition (Griersmith and Duggan 1980), although excitatory effects have also been reported (Todd and Millar 1983). It has been suggested that the 5-HT_{1B} and 5-HT_{1D} receptor subtypes mediate selective inhibition of nociceptive neurons, whereas 5-HT_{1A} agonists facilitate nociceptive responses (El-Yassir et al. 1988; Alhaider and Wilcox 1993). Spinal 5-HT_3-mediated analgesia involves GABA receptors, probably through the excitation of GABAergic interneurons (Alhaider et al. 1991).

Norepinephrine (NE)

The activation of α_2-adrenergic receptors in the dorsal horn of the spinal cord produces powerful analgesia in humans and animal models. There is a dense concentration of both noradrenaline (Dahlström and Fuxe 1965) and α_2-adrenergic receptors in the dorsal horn (Young and Kuhar 1980; Jones et al. 1982; Unnerstal et al. 1984; Nicholas et al. 1993; Roudet et al. 1993). Although there are no noradrenergic

neurons in the rostral-ventromedial medulla (RVM) or periaqueductal gray (PAG), there is evidence that brainstem noradrenergic neurons contribute to the PAG-RVM inhibition of spinal nociceptive transmission. The adrenergic innervation of the spinal cord dorsal horn arises from the A5, the locus coeruleus and the A7 noradrenergic cell groups in the pons (Westlund et al. 1982; Schroder and Skagerberg 1985; Proudfit 1988; Clark and Proudfit 1993). There is a projection from the rostral PAG to A5 (Kwiat and Basbaum 1990), A7 and to locus coeruleus (Cameron et al. 1995), and a dense projection from the RVM to the A7 noradrenergic cell group (Clark and Proudfit 1993; Holden and Proudfit 1998). There is behavioral and electrophysiological evidence that brainstem NE neurons contribute to the pain-modulating action of the PAG-RVM-dorsal horn pathway. Direct spinal application of adrenergic agonists produces behavioral analgesia (Reddy et al. 1980; Barbaro et al. 1985) and inhibition of dorsal horn neurons through α_2-adrenergic receptors. Dorsal horn microiontophoresis of selective α_2-adrenoceptor antagonists, but not the selective α_1 antagonists, significantly reverses the descending inhibition originating from the PAG. At low ejection currents, clonidine, an α_2 adrenoceptor agonist markedly reduces noxious heat-evoked responses but has no consistent action on the responses to iontophoresed EAAs (NMDA or KA). At ejection currents higher than those required to block descending inhibition, idazoxan potentiates responses to both heat and EAA iontophoresis. At higher ejection currents, EAA responses are inhibited by clonidine. This indicates that both presynaptic and postsynaptic α_2 receptors are involved in the inhibition of the recorded neurons (Budai et al. 1998).

Neurons in DRG which give rise to primary afferent fibers contain all three α_2-adrenergic receptor subtypes (α_{2a}, α_{2b} and α_{2c}) mRNAs (Nicholas et al. 1993; Nicholas et al. 1996; Gold et al. 1997). The superficial layers of the spinal dorsal horn, where nociceptive primary afferent fibers terminate most densely, contain dense α_2-adrenoceptor binding, whereas only a small number of dorsal horn cells contain α_2-adrenoceptor mRNA. Noradrenaline or clonidine significantly reduces the evoked release of Glu from spinal cord synaptosomes (Kamisaki et al. 1993) and the release of SP-like material and CGRP from spinal cord slices (Bourgoin et al. 1993). It has been demonstrated that the α_{2a} adrenoceptor subtype is the primary mediator of α_2 adrenergic spinal analgesia and is necessary for analgesic synergy with opioids (Stone et al. 1997; Stone et al. 1998). Immunoreactivity (IR) for both receptor subtypes has been observed in the superficial layers of the dorsal horn of the spinal cord. The primary localization of the α_{2a} adrenoceptor in the rat spinal cord is on the terminals of capsaicin-sensitive, SP-containing primary afferent fibers. α_{2c} Adrenoceptor-IR does not appear to colocalize with the NK-1 receptor, nor is it localized on astrocytes, as evidenced by a lack of co-staining with the glial marker GFAP. However, some co-localization is observed between α_{2c} adrenoceptor-IR and enkephalin-IR, suggesting that the α_{2c} adrenoceptor may be expressed by a subset of spinal interneurons. Interestingly, neither subtype is detected on descending noradrenergic terminals (Stone et al. 1998).

Dopamine (DA)

There is more noradrenaline than dopamine in the spinal cord (Fleetwood-Walker and Coote 1981). Both arise from supraspinal neurons. The dopaminergic projections to the dorsal horn originate from the A11 cell group in the diencephalon (Skagerberg and Lindvall 1985). Dopamine D2 receptor ligand binding is concentrated in the superficial dorsal horn, chiefly in laminae II and III (Bouthenet et al. 1987; Yokoyama et al. 1994). Focal electrical stimulation in the region of the A11 dopamine cell group selectively suppresses the nociceptive responses of spinal, multireceptive neurons in the rat. Iontophoretically applied dopamine D2 receptor agonists cause selective inhibition of the responses to noxious stimuli of the dorsal horn neurons, whilst the spontaneous activity is unaffected (Fleetwood-Walker et al. 1988).

Acetylcholine (ACh)

Choline acetyltransferase, the marker enzyme for cholinergic neurons which synthesizes ACh, is abundant in the spinal dorsal horn, especially in the superficial laminae (Kása and Morris 1972; Kimura et al. 1981; Barber et al. 1984; Kása 1986; Phelps et al. 1988; Houser 1990; Ribeiro-da-Silva and Cuello 1990; Todd 1991). Choline acetyltransferase has not been found in DRG cells or in their axons (Barber et al. 1984; Borges and Iversen 1986). Acetylcholinesterase, the enzyme which removes ACh by hydrolysis, has also been localized in the dorsal horn, with highest concentrations in laminae I-III (Kása 1986). Radioactive nicotinic ACh receptor ligands preferentially bind to the substantia gelatinosa, laminae II-III or IV, with the emphasis on laminae III and IV (Wamsley et al. 1981a; Gillberg and Aquilonius 1985; Gillberg and Wiksten 1986). It is not clear whether nicotinic ACh receptors are restricted to any particular cell type or sensory modality. Muscarinic ACh receptors are concentrated in the superficial layers of the dorsal horn, especially in laminae II and I, and may be associated with fine-caliber afferent inputs. Both nicotinic and muscarinic ligands bind to DRG cells and dorsal rhizotomy significantly reduces their binding sites in the dorsal horn (Wamsley et al. 1981a, 1981b; Seybold and Elde 1984; Seybold 1985). Accordingly, a significant number of dorsal horn ACh receptors are thought to be situated on the primary afferent terminals.

In early studies, iontophoretically applied ACh was found to depress the responses of dorsal horn interneurons to EAAs (Curtis et al. 1966) or to evoke either excitation or depression

(Weight and Salmoiraghi 1966; Curtis 1967). Later, it was shown that cholinergic compounds can depolarize one set of dorsal horn neurons and hyperpolarize others in slice preparations (Urban et al. 1989). These effects are mediated by both nicotinic and muscarinic receptors. Muscarinic antagonists interfere with the descending inhibition of dorsal horn neurons produced by stimulation of the medial medulla (Zhuo and Gebhart 1990a, 1990b). The analgesic effect of intrathecally administered clonidine on neuropathic pain is mediated, in part, by an increased ACh release which activates spinal muscarinic and nicotinic receptors. Spinally released ACh, therefore plays a role in the antiallodynic effect of intrathecally administered clonidine in neuropathic pain (Pan et al. 1999).

Nicotine exerts antinociceptive effects by interacting with one or more of the subtypes of nicotinic ACh receptors. Mice lacking the α4 subunit of the neuronal nicotinic ACh receptors no longer express high-affinity [^{3}H]nicotine and [^{3}H]epibatidine binding. These mutant mice display a reduced antinociceptive effect of nicotine in the hot-plate test, and diminished sensitivity to nicotine in the tail-flick test (Marubio et al. 1999). Epibatidine, a potent agonist for neuronal nicotinic ACh receptors sharing similar structural and functional characteristics with ACh and nicotine, was used in this study. Analgesia through the activation of muscarinic ACh receptors may occur in animal models of acute noxious stimulation and of chronic hypersensitivity pain (Eisenach 1999). It has been shown that presynaptic M3 muscarinic receptors in the spinal cord are involved in the second phase of nociception induced by formalin injection into the paw (Honda et al. 2000).

ATP and adenosine receptors

The role of ATP as a putative neurotransmitter in the mediation of nociceptive has been intensely investigated. ATP has been found to excite some dorsal horn neurons and to potentiate their responses to NMDA (Jahr and Jessell 1983). This action of ATP is likely to take place through activation of the ionotropic P2X receptor family which may be located both on intrinsic dorsal horn neurons and on the central terminals of fine primary afferent fibers. In fact, the activation of presynaptic P2X receptors in the dorsal horn has been shown to evoke the release of Glu from cultured DRG cells (Gu and MacDermott 1997). Further, P2Y receptors, a family of metabotropic receptors (acting through PLC) activated by the endogenous ligand ATP, have been demonstrated to increase the EAA-mediated transmission in the dorsal horn (Li and Perl 1995). The superficial layers of the spinal cord dorsal horn express P2X2, P2X4, and P2X6 subunits entering into the formation of ionotropic (P2X) receptors for ATP. ATP reversibly increases the amplitude of electrically evoked GABAergic IPSCs and reduces paired-pulse inhibition or facilitation without affecting the IPSC kinetics. This effect is preferentially, but not exclusively, observed in neurons co-releasing ATP and GABA (Hugel and Schlichter 2000). Spinal endogenous ATP may play a role in formalin- and capsaicin-induced neurogenic pain *via* the P2X receptors (Tsuda et al. 1999).

ATP is rapidly metabolized to adenosine which in turn may activate a variety of adenosine (mainly A1 and A2) receptors in the dorsal horn. Adenosine and ATP exert multiple influences on pain transmission at peripheral and spinal sites (Sawynok 1998). At peripheral nerve terminals in rodents, adenosine A1 receptor activation produces antinociception by decreasing, while adenosine A1 receptor activation produces pronociceptive or pain-enhancing properties by increasing, cAMP levels in the sensory nerve terminal. Adenosine A3 receptor activation produces pain behaviors due to the release of histamine and 5-HT from mast cells and subsequent actions on the sensory nerve terminal (Gu and MacDermott 1997). It is most probable that adenosine is most effective as an inhibitor of dorsal horn neuronal activation mediated *via* non-NMDA EAA or SP receptors (Keil and Delander 1995).

Nitric oxide (NO)

Nitric oxide (NO) has been characterized as a neuronal messenger involved in a variety of neurotransmitter functions (Bredt and Snyder 1992; Snyder 1992; Meller and Gebhart 1993; Zorumski and Izumi 1993). The production of NO from the amino acid L-arginine (Arg) is catalyzed by a Ca^{2+} and calmodulin-dependent enzyme, NO synthase, which can be selectively inhibited by structural analogs of Arg, *e.g.*, N^{ω}-nitro-L-arginine methyl ester (L-NAME). The enzyme NO synthase has been localized within the spinal cord (Valtschanoff et al. 1992a, 1992b; Lee et al. 1993; Saito et al. 1994). NO is a small, reactive, lipophilic molecule with a half-life of milliseconds to several seconds. It may act in the neuron where it is produced, or diffuse through cell membranes to act on adjacent cells as an intercellular messenger (Garthwaite et al. 1988; Schuman and Madison 1994). The properties of NO may allow it to act as a "retrograde transmitter", *i.e.* when produced in a postsynaptic neuron, NO might diffuse to presynaptic sites, thereby altering the presynaptic function (Baringa 1991). This concept is particularly favored in studies of LTP, where it may account for the use-dependent changes in the efficacy of synaptic transmission. The increase in intracellular level of Ca^{2+} triggers a cascade of events that include stimulation of phospholipases to produce DAG and ecoisanoids, stimulation of the production of IP_3, activation of PKC and activation of the constitutive form of NO synthase. Allowing Ca^{2+} to enter the neuron, NMDA receptor activation results in an increase in production of NO, which diffuses to its site of action. Here, it activates soluble guanylate cyclase, which in turn increases the intracellular content of cGMP (Garthwaite et al. 1988).

It has been shown that the amount of intracellular Ca^{2+} produced by NMDA receptor activation is sufficient to prevent the activation of soluble guanylate cyclase by NO in the neuron where it is synthesized (Knowles et al. 1990; Vincent and Hope 1992). It has therefore been proposed that NO must diffuse to adjacent, cells where soluble guanylate cyclase is activated to increase the intracellular content of cGMP. Evidence that hemoglobin, which binds extracellular NO, prevents LTP supports this hypothesis (O'Dell et al. 1991; Schuman and Madison 1991).

Considerable evidence suggests that NO plays a role in spinal somatosensory processing. The NMDA receptor subtype, activated by endogenous agonists such as L-glutamate, L-aspartate or L-homocysteate, appears to be involved in multisynaptic nociceptive transmission and plasticity in the spinal cord (for reviews see Wilcox 1991; Meller and Gebhart 1993; Wilcox 1993; Wilcox and Seybold 1997). Intrathecal administration of NMDA elicits a transient hyperalgesia, which is inhibited by prior treatment with L-NAME, methylene blue or hemoglobin (Kitto et al. 1992). Intrathecal injection of NO-donating compounds such as L-arginine, sodium nitroprusside or hydroxylamine also results in hyperalgesia (Kitto et al. 1992; Meller et al. 1992a, 1992b; Meller and Gebhart 1993). In the formalin pain model, inhibition of NO synthase by L-NAME produces long-lasting antinociception (Moore et al. 1991). Topical application of L-NAME onto the spinal cord attenuates both the first and second peaks of the neuronal responses to formalin (Haley et al. 1992). L-Arginine, which surprisingly is antinociceptive itself, reverses the antinociceptive effects of L-NAME in the formalin-induced paw licking test (Moore et al. 1991), but not the decrease in neuronal firing rate caused by L-NAME (Haley et al. 1992). L-Arginine, a constituent amino acid of kyotorphin (L-tyrosyl-L-arginine), an endogenous Met-enkephalin releaser in the brain and spinal cord, is considered to be an effective kyotorphin precursor. It has been proposed that L-arginine plays a dual role in nociceptive processing, being antinociceptive *via* the kyotorphin-Met-enkephalin pathway and nociceptive *via* the L-arginine-NO pathway (Kawabata et al. 1993).

In *in vivo* electrophysiological studies, inhibition of NO synthase by L-NAME has been reported to reduce the responses of single dorsal horn neurons to the electrical or chemical nociceptive stimulation of peripheral nerves (Haley et al. 1992) and it selectively inhibits the responses of these cells to iontophoretically applied NMDA (Radhakrishnan and Henry 1993). Iontophoretically applied L-NAME causes reciprocal changes in EAA responses: the NMDA-evoked responses are significantly decreased, whereas the responses to the iontophoretically applied non-NMDA agonists (AMPA and KA) are increased (Budai et al. 1995). Intravenous application of L-NAME suppresses ongoing activity recorded from dorsal rootlets that are responsive to electrical stimulation of the axotomized sciatic nerve. This effect of L-NAME is reversed by L-arginine (Wiesenfeld-Hallin et al. 1993). *In vivo* electrochemical studies have shown that NO is released from substantia gelatinosa cells upon electrical stimulation. NO release in the dorsal horn is significantly decreased in animals treated with capsaicin for C-fiber denervation (Kimura et al. 1999). Activation of Glu (NMDA) receptors may account for the release of NO (Rivot et al. 1999). Neuronal NO synthase mRNA is upregulated in rat sensory neurons after spinal nerve ligation (Luo et al. 1999). Experimental data also suggest that NO contributes to the development and maintenance of central sensitization of spinothalamic tract cells and to the resultant mechanical hyperalgesia and allodynia after peripheral tissue damage or inflammation (Lin et al. 1999). Involvement of NO in the development of central sensitization may affect nociceptive processing by increasing *Fos* expression. Since many other substances which are related to pain mechanisms can be induced by *Fos*, it is suggested that nitric oxide may regulate production of these substances through activation of *Fos* (Wu et al. 2000).

Capsaicin and vanilloid receptors

Capsaicin (8-methyl-N-vanillyl-6-noneamide), the main pungent ingredient in "hot" peppers, evokes a sensation of burning pain by selectively activating C-polymodal nociceptors (but not mechanoreceptors or cold receptors) that convey information about noxious stimuli to the central nervous system (Jancsó et al. 1977; Bevan and Szolcsányi 1990). At low concentrations, capsaicin selectively desensitizes nerve fibers to subsequent stimuli in adult neurons. Experimentally, spinal dorsal horn cells show two types of excitatory responses to intradermal injection of capsaicin. The first excitatory response shown by the majority of wide dynamic range and nociceptive specific cells is consistent with their sensitization by capsaicin. The cells produce an acute and prolonged increase in ongoing activity with capsaicin injection. Responses to mechanical stimuli are substantially increased after capsaicin and an expansion of receptive field is often observed. The responses of the same cells to EAA agonists applied locally by iontophoresis are also increased (Dougherty et al. 1999). At high concentrations in newborn animals, capsaicin selectively degenerates primary afferent C-fibers and a subset of small primary sensory neurons within the DRG. The neurotoxic effect may result from the rise in the concentrations of intracellular Ca^{2+} (Jancsó et al. 1977, 1984) as a consequence of the activation of nonspecific cation channels by capsaicin. Systemic application of capsaicin as an analgesic is unattractive because of its extensive activation of C-polymodal nociceptors, yet there may be therapeutic value in desensitization of nociceptors by local application of capsaicin-related compounds (the ultrapotent resiniferatoxin; Szállási and Blumberg 1996).

[^{3}H]Resiniferatoxin autoradiography reveals high densities of capsaicin binding sites in rat DRG as well as in the superficial dorsal horn of the spinal cord, known to contain the cell bodies and central terminals, respectively, of capsaicin-sensitive, sensory neurons (Szállási et al. 1994a, 1994b). Capsaicin binds to a specific binding site that opens a nonselective cation channel that is voltage-independent and structurally related to members of the TRP family of ion channels. The cloned capsaicin receptor, also termed as the vanilloid receptor subtype 1 (VR_1) (Szállási and Blumberg 1999), is also activated by increases in temperature in the noxious range and by protons, suggesting that it functions as a transducer of painful thermal or chemical stimuli *in vivo* (Caterina et al. 1997). As revealed by mRNA distribution studies, the VR_1 vanilloid receptor subtype is expressed not only in primary sensory neurons but also in several brain nuclei (Mezey et al. 2000). There are indications that cannabinoids may be the endogenous ligands for the VR_1 capsaicin receptor (Szolcsányi 2000).

Conclusions

Somatosensory processing in the dorsal horn of the spinal cord implicates the role of the same transmitters and receptors sub-serving neurotransmission in other areas of the central and peripheral nervous systems. Transmitters released from primary afferent fibers interact with receptors located on secondary neurons in the dorsal horn of the spinal cord and/or with receptors located on terminals of descending axons and primary afferent fibers including the ones from which they were released (autoreceptors). Many observations support the concept that the afferent terminal may release more than one transmitters when depolarized. A number of transmitters involved (some 18 reviewed here) including simple molecules such as EAAs whose rapid actions are in the millisecond time range as well as complex molecules such as peptides, which have effects lasting for seconds to minutes or longer. The spinal dorsal horn is a primary receiving area for somatosensory input and contains high concentration of various receptors. These receptors show a great deal of variety and mediate a number of cellular actions and, thus, determine the neural response to the synaptic input. The complex combination of neurotransmitters involved and the modes of interactions among them make the dorsal horn an obvious choice to produce analgesia.

Many neurons within the dorsal horn respond to noxious stimuli. EAAs, especially AMPA and KA receptors, are associated with the fast excitatory neurotransmission between nociceptors and spinal neurons. The AMPA component of the Glu action requires less than a millisecond. This is probably followed by the activation of NMDA receptors whose activation is contingent on prior depolarization and whose actions last for several tens of milliseconds. The perception of pain requires consideration of events taking place on an even longer time scale such as seconds, minutes and hours. Activation of metabotropic receptors that act *via* intracellular second messenger systems, *e.g.* neurokinin or metabotropic Glu receptors, has been implicated in longer term changes in the responsiveness of dorsal horn neurons. It is hypothesized that an appropriate combination of several afferent excitatory actions with an appropriate temporal distribution could explain progressive development of abnormalities in various pain states (reviewed by Wilcox 1991). It is noteworthy that the number of inhibitory neurotransmitters and receptors greatly outnumbers the excitatory ones in the dorsal horn. It seems probable that transmission of the somatosensory information from the primary afferent fibers to the secondary dorsal horn neurons depends on the complex interaction between the excitation by EAAs and the inhibitory actions of several other transmitter systems.

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