SUBSTANCE P: TRANSMITTER OF NOCICEPTION (MINIREVIEW)

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Substance P plays the role of a neurotransmitter and neuromodulator in the central and peripheral nervous system. The presence of substance P in demyelinated sensory fibres, as well as in the small and medium-sized neurons of spinal dorsal horn *substantia gelatinosa* gives a structural basis for the hypothesis that SP plays an important role of a mediator in the processing of nociceptive information. In this paper recent advances on the effect of SP on conduction and modulation of nociceptive impulsation are reviewed. The studies concerning the role of SP in the prevertebral ganglia and spinal dorsal horns has been presented, including the distribution of SP in the spinal cord, as well as its pre- and postsynaptic actions on excitatory and inhibitory postsynaptic potentials (EPSP and IPSP) and the effect of opiates on tachykinin transmission.

1. Distribution of substance P in the spinal cord

Substance P (SP) is synthesised in the spinal ganglia, from where it is transported centrally to the *substantia gelatinosa* of spinal dorsal horn and peripherally to the nerve endings in many tissues of the organism. SP is released at the level of the first synapse from so-called primary neurons whose perikarya are localized in the spinal ganglia in the *substantia gelatinosa* of spinal dorsal horns as well as in the Gasserian ganglion and the trigeminal nucleus (Hoekfelt et al. 1975; Takahashi and Otsuka 1975).

Electron microscopy revealed that SP is highly concentrated in the superficial layers (lamina I-III) of the dorsal horn , where most primary afferent fibers terminate (Hoekfelt et al.1975). In other areas of the spinal cord a medium- or high-density of SP-immunoreactive fibers has also been detected in lamina V of the dorsal horn (Ljungdahl et al. 1978), lamina X surrounding the central canal (Lamotte and Shapiro 1991), the *nucleus dorsalis*, interomediolateral cell column, and the ventral horn (Pioro et al.1984). Electric stimulation of the dorsal root, or peripheral nerve endings, causes a substantial release of SP within the *substantia gelatinosa* of spinal dorsal horns (Otsuka and Kanishi 1976).

In the dorsal regions of the spinal dorsal horn, besides SP also large quantities of gamma-aminobutyric acid (GABA) are present, but reduction of GABA levels has no effect on the SP content, and similarly, reduction of SP levels does not cause any decrease of GABA concentration in this region of the spinal cord (Takahashi and Otsuka 1975). The above suggests that SP and GABA are localized in different spinal neurons and confirms the location of SP in primary afferents, while GABA is located ininhibitory interneurons (Miyata and Otsuka 1975).

2. Distribution of tachykinin receptors in the spinal cord

Tachykinins exert their effect by activation of specific receptors located on target cells. The distribution of tachykinin receptors in the rat spinal cord has been examined by autoradiographic studies, which suggest that tachykinin binding sites are located on postsynaptic membrane of spinal neurons (Helke et al. 1986). Numerous diversities between the distribution of tachykinin receptors and localisation of SP immunoreactivity in the CNS have been demonstrated. In some cerebral regions there is a close correlation between the density of tachykinin containing

Table 1
Distribution of tachykinins and their receptors in the spinal cord of the rat

Regions	Tachykinins pmol/g			Receptors		
	SP	NKA	NKB	NK-1	NK-2	NK-3
Substantia nigra	1154,2	115,0	2,8	–	–	–
Medulla oblongata	226,4	7,1	7,2	++	–	–
Spinal cord:						
Dorsal horn	503,7	65,9	9,1	++	–	++++
Ventral horn	117,4	16,1	2,0	++	–	–

fibres and density of receptors, whereas in others, e.g. in the *substantia nigra* and ventral segmental region the correlation between these two values is not significant (Mantyh et al.1989).

The distribution of NK-3 binding sites is restricted to the superficial dorsal horn while NK-1 binding sites are more widely distributed in the rat spinal cord (Ninkovic et al.1984). NK-2 binding sites were detected in the dorsal horn (Mantyh et al.1989) (Table 1), whereas NK-2 receptor mRNA was undetectable in the rat spinal cord (Tsuchida et al.1990), but it was detected in the sensory ganglia or in peripheral inflammed tissues reached by IR-SP. It was also demonstrated that the level of NK-1 receptor mRNA was elevated in the tissues affected by inflammation, which indicates that the flexibility of expression of NK-1 receptor gene may regulate the sensitivity to SP in a way similar to that observed in the spinal dorsal horn (Mc Carson 1999). No significant correlation between the distribution of tachykinins and their receptors in the CNS was found. Moreover, it has been suggested that the binding sites of labelled tachykinins in autoradiography reflect the receptor binding sites of high affinity, whereas those with low affinity remain undetected, although they play an important physiological role (Stroes and Bast 1986).

3. Effects of stimulation of primary afferents on substance P release

Effect of SP on nociception depends on its concentration. Intraventricular or intraperitoneal administration of low concentrations of SP exerts analgesic effect (Stewart et al. 1976), whereas in high concentrations it may induce hyperalgesia which has been explained by its pre- or postsynaptic action (Frederickson et al. 1978). Substance P at low concentrations probably stimulates the secretion of endogenous opioid peptides, and at high concentrations it stimulates neuronal transmission in the nociceptive pathways (Oehme et al. 1980). Application of SP at low concentration was shown to induce a dose-dependent depolarization of motoneurons in the isolated spinal cord of the newborn rat, as recorded extracellularly from the ventral root or intracellularly from motoneurons (Konishi and Otsuka 1974). This SP-induced depolarization is predominantly due to a transsynaptic action through interneurons, since it is largely blocked by tetradoxin (Otsuka and Yanagisawa 1980; Yanagisawa and Otsuka 1990).

Ionophoretic application of SP produces a prolonged depolarization of dorsal horn neurons in the spinal cord *in vivo* (Henry 1976) and in cultured spinal neurons (Nowak et al. 1982). Studies with spinal cord slices of young rats by Urban and Randic (1984) showed that both the application of SP and electrical stimulation of a dorsal root induced a slow depolarization of dorsal horn cells as recorded intracellulary. Both the SP-induced and the electrical stimulus-induced depolarizations were blocked by tachykinin antagonists or SP antibodies.

4. Role of substance P in nociceptive transmission

It is believed that SP together with other tachykinins is responsible for nociceptive transmission from the peripheral to the central nervous system (Iversen 1982). The structural basis for such hypothesis is the fact that SP occurs in small and medium-sized

neurons of *substantia gelatinosa* of the spinal dorsal horn, as well as in peripheral and central endings of primary afferent fibres. Among primary afferent fibers, unmyelinated C-fibers are known to convey delayed pain to second-order neurons in the superficial dorsal horn of spinal cord and *medulla oblongata*. In rats C-fibers constitute of about 70 % of nerve fibers and 83 % of saphenous nerve fibers (Nagy and Van der Kooy 1983). In rats, 80 % of C-fibers are polymodal nociceptors (Lynn and Hunt 1984). It seems likely, therefore, that most SP-immunoreactive C-fibers belong to polymodal nociceptors. Intrathecal injection of SP in mice elicits the behavior suggesting the pain sensation (Hylden and Wilcox 1981), whereas tachykinin antagonists (Lembeck et al. 1981; Zubrzycka et al. 1997) or SP antibody (Kuraishi and Satah 1990) administered by the same route produce an analgesic effect. Intrathecal injection of SP in rats also facilitates a spinal nociceptive reflex (Yashpal and Henry 1983). Wiesenfeld-Hallin (1986) found that intrathecally injected SP as well as C-afferent stimulation increased the magnitude of the spinal flexion reflex elicited by noxious mechanical or thermal stimuli in the rat and thus sugested that SP may be released from polymodal nociceptors. Treatment with capsaicin resulted in a decrease in the SP content in the dorsal horn and concomitant elevation of pain threshold (Nagy and Van der Kooy 1983).

5. Substance P-mediated excitatory postsynaptic potentials

In central processing of nociceptive information in all chemical synapses of the nociceptive system the electric signals are transformed many times into chemical ones and vice versa. The impulses induce the release of neurotransmitters on the nerve endings. These substances bind to their appropriate receptors causing the alterations in cell membrane excitability, taking part in induction of excitatory postsynaptic potentials (EPSP) or inhibitory postsynaptic potentials (IPSP). EPSP generates further impulses, whereas IPSP reduces the excitability in the postsynaptic neuron. All these bioelectric and biochemical processes lead at the end of the information chain to the subjective phenomenon – sensation of pain.

A suitable model to investigate slow SP-induced EPSP is the inferior mesenteric ganglion of the guinea pig. Brief pulse application of SP induces a slow depolarization lasting tens of seconds in cultured spinal neurons (Nowak and MacDonald 1982). Application of SP at low concentrations produced a depolarization of inferior mesenteric ganglion cells of the guinea pig (Konishi and Otsuka 1985). When the potential was recorded intracellulary from ganglion cells, stimulation with a single or repetitive shocks of the lumbar splanchnic nerves induced fast cholinergic EPSPs followed by a noncholinergic slow EPSPs (Tsunoo et al. 1982). Upon stimulation of dorsal roots, a slow EPSP was evoked in ganglion cells that was not accompanied by fast EPSPs (Konishi et al. 1980). Slow EPSP and the depolarization induced by short pulse application of SP have a similar time course of 30 s to a few minutes and are associated with a similar conductance change (Tsunoo et al. 1982). Both, the slow EPSP and the SP-induced depolarization are blocked by tachykinin antagonists and augmented by isoprenalin (Konishi and Otsuka 1985). During a prolonged application of SP, witch produces a sustained depolarization of ganglion cells, the slow EPSP is obliterated. The obliteration is not due to the depolarization per se, but probably to saturation of the receptor by SP, because the slow EPSP does not recover even if the membrane potential is brought back to the level of the resting potential by passing current. The slow EPSP is greatly depressed after *in vitro* treatment with capsaicin (Tsunoo et al. 1982), witch evokes a release and depletion of SP from the ganglion (Konishi et al. 1980). Neurotensin increases the release of SP-like immunoreactivity upon electrical stimulation, and at the same time augments the amplitude and duration of the slow EPSP (Stapelfeldt and Szurszewski 1989).

It is believed that SP released from primary C-afferent terminals produces a slow EPSP in second order neurons in the spinal cord and thus contributes to transmission of delayed pain signals (Otsuka and Yanagisawa 1987). The majority of SP-immunoreactive nerve endings in the rat dorsal horn can be stained with antisera for glutamate (Merighi et al. 1991). Therefore, it seems possible that the SP-mediated slow EPSP in the second order neurons is accompanied by a glutamate mediated fast EPSP. In

their recent study, Ren et al. (1999) observed that glutamates, through a metabotropic GluRs receptor inhibited slow EPSP and potentiated slow IPSP recorded intracellularly in the submucosal neurons of the intestinal ganglion in guinea pig.

6. Other sensory neuropeptides

Numerous neuromodulators and neurotransmitters are involved in transmission and processing of nociceptive impulsation (Furst 1999). These biologically active compounds, produced in the neural tissue, are released into the cerebrospinal fluid and their concentrations are altered as a result of excitation or inhibition of the cerebral structures involved in transmission and processing of nociceptive impulsation (Zubrzycka et al., 2000).

In the spinal horn the peripheral noxious stimulation causes a release not only of SP, but also of other peptides such as NKA, NKB, CGRP and somatostatin, but not of galanin (Morton et al. 1988; Morton and Hutchison 1989). NKA exerts an excitatory action similar to that of SP on spinal neurons (Yanagisawa et al. 1990). Duggan et al. (1990) reported that the release of NKA caused by noxious stimuli in the dorsal horn of the cat spinal cord is more widely spread and longer lasting than that of SP. This may be due to resistance of NKA to degrading enzymes in the tissue and suggests that, in the spinal cord, the mode of function of NKA may be different from that of SP. In contrast, somatostatin and galanin exert inhibitory actions on spinal neurons and release of SP (Yanagisawa et al. 1986), while CGRP enhaces the release of SP from primary afferents (Oku et al. 1987). Numerous neuropeptides have been shown to be present in primary afferent neurons in various combinations (Cameron et al. 1988). Therefore, some of these peptides released upon noxious peripheral stimulation may cause complicated and probably slow processes in the dorsal horn, which will contribute to the spinal nociceptive processing (Woolf and Wiesenfeld-Hallin 1986). Recent studies by Furst (1999) demonstrate that nitric oxide and prostanoids, which enhance the transmission of nociceptive impulsation, activate of the N-methyl-D-aspartate receptor (NMDA). Excitation of receptors causes also the penetration of calcium ions into the cells, which triggers the cellular neuroplastic mechanisms. NO synthase is thus activated and nitric oxide produced. Nitric oxide plays the role of a retrograde neurotransmitter. Long-term excitation of receptors activates the second messenger system involving the changes in G-proteins, which leads to alterations of neuron excitability (Trafton et al. 1999).

The phenomenon of antinociception is closely associated with endogenous opioid peptides (EPO). It is believed that elevating the pain threshold is influenced by serotonin (5-HT) and EPO, including, in particular, ß-endorphin (Selley and Bidlack 1992).

7. Interactions of opioids with tachykininergic transmission

Enkephalins are present in cell bodies, fibres and nerve endings in many structures of the CNS. Immunocytochemical studies indicate that many enkephalinergic neurons are present in the PAG, pontine, medullary and spinal raphe nuclei. The *substantia gelatinosa* of the dorsal horn is rich in both enkephalin (Pollard et al. 1989) and opioid receptors (Atweh et al. 1977). The opioid receptors on neuronal cell membranes are associated with endogenous opioids produced in the CNS: enkephalins, ß-endorphins and dynorphins, which exert their analgesic effect via these receptors. Besides three main types of opioid receptors (mu, delta and kappa) the recent studies detected the presence in the brain of an epsilon receptor coupled with G-protein (Narita and Tseng 1998). It has been determined that endogenous Met-enkephalin (Met-Enk) released in the spinal cord due to activation of supraspinal opioid epsilon receptors activates spinal delta-2 receptors to exert analgesic effects.

Dorsal rhizotomy results in a marked decrease in opioid receptors in the *substantia gelatinosa*, suggesting that a significant portion of the receptors is located on presynaptic terminals of primary afferent neurons (Jessell et al. 1979). Disruption of the continuity of primary afferents, similarly as mechanical or chemical dissection of the dorsal root, by neurotoxin or capsaicin, abolishes almost completely the endomorphine-2-like immunoreactivity in the dorsal horn. These results indicate that endomorphine-2 is present in primary afferents where it plays the role of an endogenous ligand of nociception for pre- and postsynaptic mu receptors (Martin-Schild et al. 1998).

SP and enkephalins exert opposite effects on the neurons of the structures associated with nociception. The inhibition of the SP release from primary afferents by opioids has been confirmed by many investigators. Ultrastructural immunocytochemical studies, however, have revealed that enkephalin containing terminals within the superficial layers of the dorsal horn form mostly axodendritic synapses (Newton and Hamill 1989). Ma et al. (1997) using ultrastructural immunocytochemical methods characterising the contents of SP-IR and enkephalins in specific nociceptive and non-nociceptive neurons of cat spinal dorsal horn. Most of the nociceptive neurons contained SP-IR, whereas no enkephalins were detected in any non-nociceptive neurons. Boutons containing Enk-IR and SP-IR coincident with Enk-IR were never presynaptic to boutons with SP-IR. The authors put forward a hypothesis that modulation of ascending nociceptive impulsation by enkephalinergic neurons in the dorsal horn takes place via a postsynaptic mechanism and suggest that the enkephalinergic neurons of the dorsal horn constitute a part of a local inhibitory feedback loop on a pathway separate from the previously postulated, opioid-mediated reduction of SP release from primary afferent terminals. Also the results obtained by Trafton et al. (1999) suggest that first of all postsynaptic inhibitory mechanisms and presynaptic control of primary nociceptive afferents not containing SP are involved in opioid analgesia.

To conclude, it seems likely that endogenous opioid peptides released from interneuron terminals spread diffusely to exert both presynaptic inhibition on nociceptive afferent terminals and postsynaptic inhibition on dorsal horn neurons. A recently discovered endogenous selective mu agonist, endomorphine-2, an important modulator of nociception, coincides with SP in primary sensory afferents, in the superficial laminae of the spinal cord and in the spinal trigeminal nucleus as an endogenous ligand for the pre- and postsynaptic mu receptors. It is suggested that the analgesic effect of enkephalin involves co-operation with the serotoninergic system. The relative importance of the pre- and postsynaptic mechanisms in the action of endogenous opioid peptides, however, remains to be clarified.

Reviewing the advances in the field of nociception it can be stated that a significant progress has been made in our understanding of the role of the neurotransmitters and their blockers involved in transmission and modulation of pain. At present the research in this field is mainly concerned with the efficacy of non-peptide SP receptor antagonists in the processes of inhibition of pain transmission. These compounds yield promising results in the search for potent analgesics which could be used in rational pharmacotherapy of pain.

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