CHAPTER IV

DISCUSSION

The present work was designed to explore the role of 5-HT_{1A} and 5-HT_{2A} receptors in modulating nociceptive transmission in rat spinal cord by utilizing selective agonists for these receptors. The present data indicated the nociceptive attenuating effect of 8-OH-DPAT and nociceptive facillitating of DOI. Nociceptive attenuating effect of 5-HT_{1A} agonist was demonstrated by an increase time of tail flick latency. On the contrary, 5-HT_{2A} agonist reduced the tail flick latency, which indicated its nociceptive facilitating effect. In addition, administration of 8-OH-DPAT was found to decrease formalin-induced nociceptive behavior. However, no significant change in formalin-induced nociceptive behaviors was evident. Our immunohistochemistry study also showed that administration of 5-HT agonists can alter that pattern on noxious stimulation-induced FLI in the spinal dorsal horn neuron.

Effects of 5-HT_{1A} and 5-HT_{2A} agonists on thermal nociception

The efforts to correlate the antinociceptive effects of 5-HT in the spinal cord with subtypes of putative 5-HT receptors have produced contradictory results. The present result was in accord with the previous studies using thermal nociceptive model, i.e. tail-flick and hot plate tests (Ali et al., 1994; Archer et al., 1987; Berge, 1982; Eide et al., 1988; Eide and Hole; 1989, 1991a; Sawynok and Reid A, 1994; Xu et al., 1994; Zemlan et al., 1980) as well as niception induced by electrical stimulation (Gjerstad et al., 1996). Nociceptive aattenuating effect of 5-HT_{1A} receptor was also evident in the study using co-administration of 5-HT and a variety of 5-HT receptor antagonists (Schmauss et al., 1983). In contrast, other results suggested that 5-HT_1 receptors may

mediate facilitation of spinal nociceptive reflexes (Alhaider and wilcox, 1993; Crisp et al., 1991; Murphy and Zemlan; 1987; Zemlan et al., 1983), or do not modify nociception (Mjellem et al., 1992; Millan, 1994; Solomon and Gebhart, 1988). Activation of 5-HT_2 receptors may increase (Alhaider, 1991; Banks et al., 1988; Barber et al., 1989; Eide and Hole, 1989; Eide and Hole, 1991a; Iverfeldt et al., 1986; Wilcox and Alhaider, 1990), inhibit (Solomon and Gebhart, 1988) or do not alter (Sawynok and Reid, 1994; Sawynok and Reid, 1996) the nociceptive transmission. The marked inconsistency between the results reported by different laboratories may reflect the complexity of the problem as well as the technical problems associated with the techniques.

Tail flick test has been used widely as an index of nociception. Being as spinal reflex, tail-flick reflex is constantly under the modulation of suprasegmental control. Conventional nociceptive procedures such as the tail flick and hot plate tests cannot differentiate between presynaptic (on primary afferent fibers) and postsynaptic (on intrinsic neurons in the spinal cord) actions of a drug. Furthermore, results from thermal tests may be confounded by drug-induced changes in skin temperature at the site of exposure (Eide et al., 1988). Therefore, the conflicting results may be related to variation in experimental model, e.g. differences in skin temperatures in hot plate and tail flick test (Tjølsen et al., 1989), different administration routes of drug and species differences, etc.

Dorsal horn is of significance in controlling nociceptive induced spinal reflexes, including tail flick response. Therefore, the demonstration of 5-HT agonist-induce drugs in tail flick latency may reflect its role in modulation of the spinal sensory control. This hypothesis was supported by anatomical localization of these receptors in the spinal dorsal horn. High density of 5-HT_{1A} receptors was demonstrated in the dorsal horn, especially in the superficial lamina I/II involving in the nociceptive impulses processing (Daval

et al., 1987; Marlier et al., 1991). The electrophysiological studies in the dorsal raphe and locus coeruleus indicate that activation of 5-HT_{1A} receptor hyperpolarizes neurons by opening potassium ion channels (Aghajanian et al., 1988; Bobker and Williams, 1990). Such a direct action would inhibit rather than excite neurons. A mechanism of action that possibly underlies the effect of 8-OH-DPAT in modulating tail flick responses in rats would be a decreased efficacy of coupling to intracellular transduction mechanism. Irrespective of the precise mechanisms underlying the attenuation of tail flick responses by direct 5-HT_{1A} agonists, these data suggest that tail flick responses was elicited by the inhibition of 5-HT_{1A} receptor postsynaptic dorsal horn neurons. Inhibitory 5-HT_{1A} autoreceptors in dorso raphe nuclei (DRN) was coupled via the G-protein to K^+-channels and their activation leads to hyperpolarization and inhibition of neuronal firing (Blier et al., 1989; Luam and Piercey, 1988; Sprouse and Aghjanian, 1989).

The 5-HT_2 receptor has an uneven distribution in the CNS, with cerebral cortex having higher levels than the cord as revealed by radioligand experiments and dot blot hybridization studies (Hoyer et al., 1986; Molineaux et al., 1989). In the rat spinal cord, the 5-HT_2 receptor is present mostly in the sympathetic area and in the ventral horn, while the dorsal horn exhibits few 5-HT_2 receptors (Marlier et al., 1991). Concerning the possible role played by the 5-HT_2 receptor in spinal pain modulation, literature data present a picture of great divergence. Activation of the 5-HT_2 receptor has been reported to facilitate (Wilcox and Alhaider, 1990; Eide et al., 1991a) or to inhibit (Solomon and Gebhart, 1988).

In the spinal cord, local application of 5-HT to the cell of secondary nociceptive afferents in the dorsal horn can selectively inhibit the depolarizing action of substance P. Local administration of 5-HT can also inhibit the firing rate of the primary afferent induced by noxious stimulation upon their receptive

field. The 5-HT receptor subtype involved in mediating this action has not been definitively characterized, but is probably a 5-HT_1 receptor. Since a descending inhibitory serotonergic pathway carried in the dorsolateral funiculus is believed to synapse onto such secondary nociceptive dorsal horn neurons and inhibit the forward transmission of nociceptive information by the spinothalamic tract at the segmental level, this inhibitory 5-HT receptor may well have physiological relevance for pain gating. In agreement with this is the observation that intrathecal serotonin increases the pain threshold in several species. The 5-HT receptor involved in mediating this effect has also not been definitively characterized, although both 5-HT_1 and more recently 5-HT_2 receptors antagonist ketanserin blocks the nociceptive action of 5-HT. Further studies using more selective agonists and antagonists are required before a definitive statement regarding the subtype(s) of 5-HT receptors involved can be made.

In the brain stem the local application of 5-HT to cells of the NRM, which is believed to be the origin of the serotonergic pathway, causes excitation through a 5-HT_2 receptor mechanism (Pycock et al., 1981). Previous study demonstrated that NRM receives serotonergic input from the ventromedial and ventrolateral parts of the periaqueductal gray. Electrical stimulation of these regions leaded to activation of NRM via 5-HT_2 receptor-mediated mechanism (Llewelyn et al., 1984). Previous studies also showed that activation of the descending serotonergic system at any level (i.e. by electrical stimulation of PAG or NRM, microinjection of 5-HT into NRM, iontophoretic application of 5-HT to the dorsal horn etc.) lead to excitation of the forward transmission of nociceptive input. Based on the excitatory effect of 5-HT_{2A} receptors, it seems possible that the spinal serotonergic pathway can be activated via the same mechanism. Accepting this assumption, stimulation of 5-HT_{2A} receptors may enhance the nociceptive impulse transmission in the spinal level.

Effects of 5-HT_{1A} and 5-HT_{2A} agonists on formalin-induced nociceptive behavior responses

An intraplantar injection of formalin induced biphasic nociceptive responses in mice and rats. The first and second phases were considered to represent tissue injury phase and inflammatory phase, respectively (Dubuisson and Dennis, 1977; Shibata et al., 1989). Substance P may be related to the manifestation of the first phase response. Bradykinin inhibitor also inhibited the first phase response, and the second phase as well, and so bradykinin may be released in the formalin injury reaction. Some data reported previously that a combination of bradykinin with substance P exerted a synergistic effect on pain response in mouse paws (Shibata et al., 1986). It may be that formalin stimulation causes substance P release mediated by axon reflex, and substance P may play a role through cooperation with bradykinin in the first phase response. However, no action by 10 mg/kg of bromelain, which causes a specific depletion of high molecular weight kininogens (Katori et al., 1978), was detected at the first phase response, suggesting that participation of bradykinin may not be very important for the manifestation of the first phase response. Histamine and 5-HT were related to the second phase response as well as bradykinin. Prostaglandin synthetic inhibitors such as indomethacin, aspirin and dexamethasone only inhibited the second phase, and bromelin potentiated the indomethacin-induced analgesia in the second phase. This indicates that both prostaglandin and bradykinin cooperated to produce the second phase response. Therefore, it was concluded that the first phase response is evoked by the direct formalin stimulation of the nerve endings followed by substance P release, and the second phase may be mainly due to subsequent inflammation.

While there has been ample evidences suggesting an inhibitory role of 5-HT receptors in thermal and chemical nociceptive transmission, there have been

only a few studies on their roles in formalin-induced nociceptive transmission (Fasmer et al., 1986; Giordano, 1991; Giordano and Rogers, 1989; Giordano and Dyche, 1989). It has been hypothetized that the serotoninergic pathway involving 5-HT_1 and 5-HT_2 receptors plays an inhibitory role, not only in the tissue injury phase but also the inflammatory phase of the formalin test. The former part of the speculation in line with previous observations dealing with acute pain models (Alhaider, 1991; Alhaider and Wilcox, 1990; Crisp et al., 1991; Eide et al., 1990; Eide and Hole, 1991a; Eide and Tjølsen, 1988; El-Yassir and Fleetwood-Walker, 1990; Murphy and Zemlan, 1990; Xu et al., 1994; Zemlan et al., 1983). For instance, Fasmer et al. (1986) showed that 8-OH-DPAT 1 mg/kg subcutaneous elicited hypoalgesia in phase I of the formalin test.

The role of 5-HT_2 receptors in chemical nociception has been investigated, and conflicting results have been presented whether they inhibit (Solomon and Gebhart, 1988) or enhance nociceptive transmission (Eide and Hole, 1993; Kjørsvik et al., 1997; Wilcox and Alhaider, 1990). It has been suggested that different methods probably give conflicting results. Activation of spinal 5-HT_2 receptors produced nociceptive biting and scratching behavior (Solomon and Gebhart, 1988) and produced a dose-dependent behavioral syndrome consisting of biting and licking directed toward the caudal part of the body and reciprocal hind limb scratching (Eide et al., 1991). It has also been shown that the 5-HT_{2A} receptors agonist DOI enhance the nociceptive behavior elicited by intrathecal administration of the glutamate receptor agonists in mice (Mjellem et al., 1992). The nociceptive behavioral response to i.t. 5-HT and DOI was blocked by SP receptor blocker, supporting the hypothesis that the 5-HT_2-induced hyperalgesia is mediated via the release SP (Eide and Hole, 1991a,b). On the other hand, anti-nociceptive effects of 5-HT_{2A} receptors was also observed in some studies (Solomon and Gebhart, 1988; Yaksh and Wilson, 1979).

In this experiment, although the nociceptive facilitating effect of 5-HT_2 agonist was observed in tail flick test, we could not demonstrate the same response in formalin test. However, it was observed that administration of DOI produced obvious motor depression effects during the last period of the second phase response. This motor depression effect was profound and could mask the nociceptive behavior responses. Interestingly, some studies have shown that different 5-HT receptors have different laminar and rostro-caudal distributions within the spinal cord. The presence of 5-HT_2 receptor in the ventral horn also implies its involvement in motor modification (Malier et al., 1991). Indeed, 5-HT_2 receptor-induced altered motor behavior has already been shown in the previous study by Fone et al (1991).

Chemical stimulation evoked Fos expression in dorsal horn neuron

The present results show that a tonic noxious chemical stimulus, produced by subcutaneous formalin injection can evoke an expression of Fos protein-like immunoreactivity (FLI) in spinal cord neurons of awake rats. This finding supports the use of Fos immunocytochemistry to map functionally related neural circuits in the CNS (Hunt et al., 1987; Sagar et al., 1988; Menetrey et al., 1989). The principle advantage of this technique in the study of nociception is that it reveals large populations of presumed nociceptive neurons in individual animals, with resolution at the single-cell level. Although it is possible to quantitate noxious stimulus-evoked neural activity with the 2-deoxy-glucose method (Abram and Kostreva, 1986), this procedure does not have cellular resolution and is much more difficult to perform. The cytochrome oxidase method (Wong-Riley and Kageyama, 1986) having cellular resolution and cutting high baseline levels of cytochrome oxidase acitivity in the spinal cord would make it difficult to detect noxious stimulus evoked changes in enzyme activity. A particularly important advantage of monitoring c-*fos*

expression for spinal cord studies is that FLI is very low in the spinal cord of unstimulated rats and thus induction of FLI by noxious stimuli is readily detected. In fact, the absence of substantial basal FLI distinguishes the spinal cord form certain brain stem and forebrain areas, including the solitary nucleus, cerebral cortex, hippocampus, striatum, and cerebellum (Morgan et al., 1987; Sagar et al., 1988).

It has been previously demonstrated that the appearance of Fos protein within the spinal cord was dependent upon the activation of small diameter high-threshold primary afferents, and that the pattern of labeling within the first few hours following stimulation closely followed the pattern of termination of particular sensory afferents within the dorsal horn (Hunt et al., 1987). At 2 hours the pattern of positive neurons from either mode of stimulation was topographically consistent with induction by synaptic activation by primary afferent nerve fibers, possibly in a monosynaptic fashion (Swett and Woolf, 1985). The events from cell surface stimulation leading to IEG expression in the nucleus are complex and involve multiple second messenger pathway. The details of these pathways have been described else where (Armstrong and Montminy, 1993; Ginty et al., 1992) but in general those neurotransmitters associated with the processing of nociceptive information, such as glutamate and substance P, increase the concentration of Ca^{2+} in the postsynaptic neurons leading to c-*fos* activation.

Some data have demonstrated that population of both nociceptive specific and wide-dynamic-range nociceptive neurons are located predominantly in the superficial dorsal horn (lamina I and II) and largely restricted to the neck of the dorsal horn (lamina V) (reviewed by Besson and Chaoch, 1987). Lamina I, II and V receive input from the Aδ and C-fibers which respond to noxious stimulation. To achieve Fos expression in these superficial laminae of the spinal cord it is essential to use noxious stimulation.

Since the majority of small-diameter thinly-myelinated and unmylinated afferents terminate in the superficial dorsal horn (Light and Perl, 1979; Sugiura et al., 1987), it is reasonable to hypothesize that most of the neurons in the superficial dorsal horn which express the c-*fos* gene are driven monosynaptically by small-diameter, presumed nociceptive primary afferents from the injured paw.

The appearance of c-*fos* messenger RNA (mRNA) from previous work with cell lines has been regarded as a transient even and translating into protein with a substantially longer half-life (Greenberg et al., 1985). Using *in situ* hybridization , mRNA for c-*fos* was found to be heavily expressed in ipsilateral neurons of lamina I and II from 5 to 120 min after cutaneous heat stimulation, before decaying over the next 120 min (Wisden and Hunt, 1989).

The formalin stimulus also evoked Fos immunoreactivity in neurons of lamina III and IV regions that predominantly contain cells which only responsive to non-noxious stimulation. Hunt et al. (1987) demonstrated that continuous non-noxious stimulation would evoke Fos staining in lamina III and IV. Fos induction in these lamina were possibly derived from the vigorous licking and scratching of the injured paw. It was suggested that Fos protein was induced by neurotransmitters, possibly peptides, known to be present in small-diameter afferents. The low-threshold inputs could induce Fos protein through peptidergic interneurons appears likely which respond only to non-noxious inputs are readily excited by ionophoretic application of neuropeptides (Pini and Ryall, 1986).

Our observations clearly demonstrated that, in the dorsal horn, primary afferent input generates rapid trans-synaptic expression of FLI. The lamina distribution of these evoked FLI are related to the nature of the sensory stimulation.

Effects of 5-HT_{1A} and 5-HT_{2A} agonists on noxious stimulation-evoked Fos expression

In this experiment, it was observed that administration of 5-HT agonists could change the pattern of FLI in spinal dorsal horn neurons. A greater density of FLI was evident in the 8-OH-DPAT group than those observed in the DOI group. However, degree of changes in FLI did not reach the level of statistical significance when the results were compared to the controls.

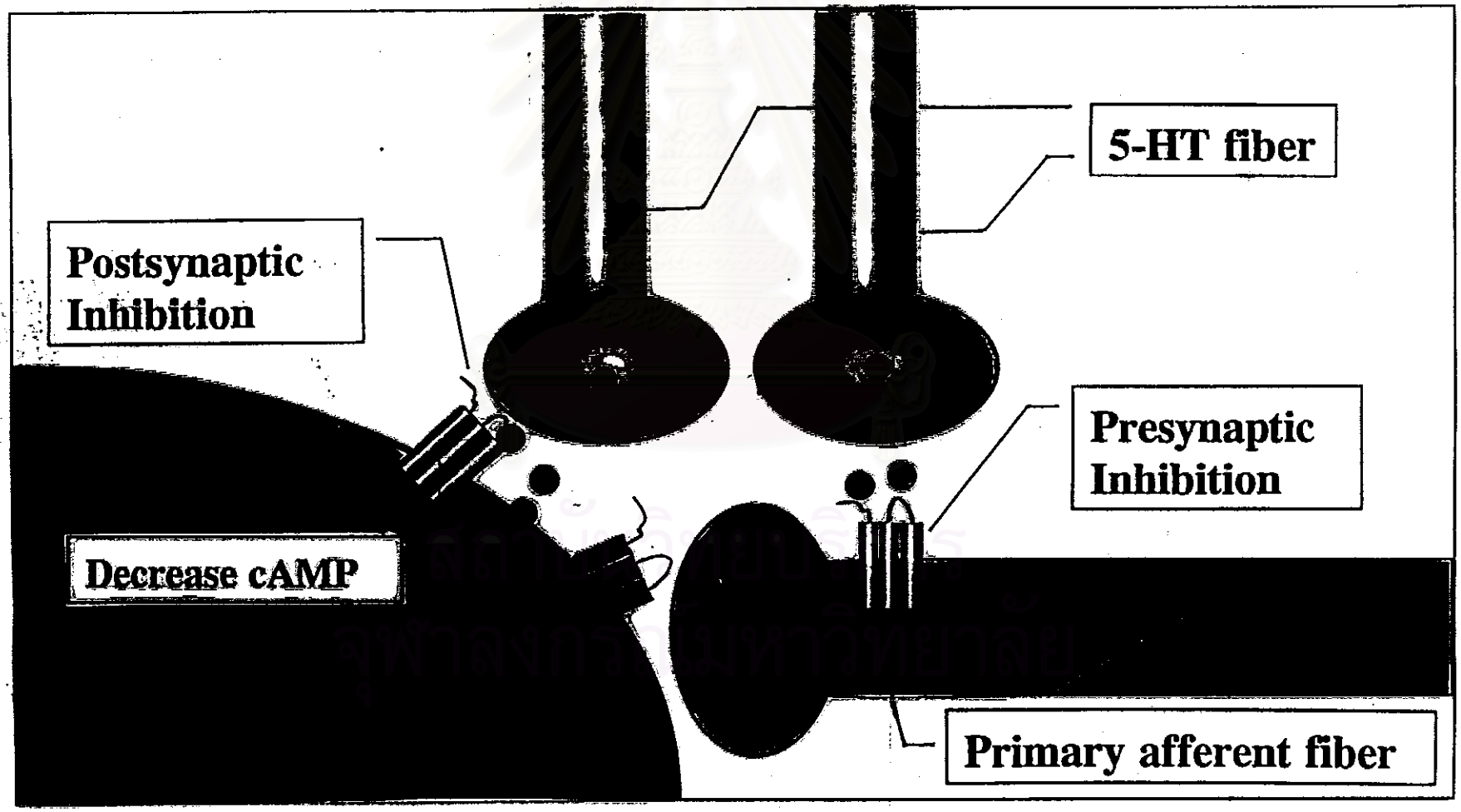
Regarding the effect of 5-HT_{1A} receptor on noxious stimulation-evoked FLI, this result showed that administration of 8-OH-DPAT produced a slight decrease in number of FLI in lamina V. As previously noted, lamina V neurons are considered to be nociceptive-specific and wide-dynamic-range neurons which respond primarily to noxious stimulation. A decrease in FLI density in this lamina therefore implied an antinociceptive effect of 5-HT_{1A} receptor in nociceptive transmission. However, the difference of FLI in lamina V was not statistically significant. This statistically negative result may be due to the limited number of sample.

In contrast with the result of FLI observed in the nociceptive-related lamina, the present result showed a significantly greater number of FLI neurons in lamina III and IV. Normally, these two laminae do not directly relate to the nociceptive processing. By using electrophysiological method, Dickenson and Sullivan (1987a,b) showed that formalin injection did not activate neurons which only respond to innocuous stimulation found in lamina III and IV. Therefore, it should be concluded that the increase in Fos immunoreactivity in neurons of lamina III and IV resulted from a combination of a formalin excitation of these neurons and the peripheral input that is secondary to the behavior produced by the nociceptive stimulus.

Pretreatment with DOI prior to intraplantar formalin injection of forepaw facilitated the formalin-induced increase in FLI in the ipsilateral spinal cord. Concerning to the 5-HT_{2A} receptor agonist, DOI, it stimulate FLI in lamina III, IV, however, was not statiscally significant when compared to the control. The excitation of the Aδ and C-fiber responses could be mediated through presynaptic n the dorsal horn but 5-HT_{2A} receptors located no primary afferent Aδ and C-fibers or by a postsynaptic mode of action on spinal neurons.

In summary, the present study showed that 5-HT can exert different roles on nociceptive process. Activation of 5-HT_{1A} receptor tends to inhibit the nocieption as evident in the tail flick and formalin tests. However, the precise mchanism underlying the attenuation of both heat and chemical nociception by 5-HT_{1A} receptor cannot be elucidated in this study. Based on its transduction mechanism, stimulation of this receptor may attenuate the nociception by inhibiting Ca^{2+} entry into the primary afferent fibers, which consequently decrease the releasing of substance P or glutamate. The direct inhibition to postsynaptic of thalamic projection neurons is another alternative mechanism (Figure 20). In contrast, nociceptive facilitating effect was evident in the animals receiving 5-HT_{2A} receptor agonist. The mechanism underlying this phenomenon may involve the effect of this receptor in potentiating the release of algogenic peptides from primary afferents or direct stimulation of thalamic projection neuron in the spinal dorsal horn (Figure 21).

Proposed Mechanism of 5-HT_{1A}-Related Nociceptive suppression (Figure 20)



Proposed Mechanism of 5-HT_{2A} Related Nociceptive Facilitation (Figure 21)