CHAPTER V

DISCUSSION

This study was conducted to investigate the role of the 5-HT_{2A} receptor in the chronic pain model as well as the development of the chronic pain state. The second objective of this study was to investigate the effect of 5-HT depletion on the changes of pain sensation including the role 5-HT_{2A} receptor as well.

The benefits of the nociceptive behavioral study are the expressions of overall animal behaviors in painful conditions. The monitored reactions are almost always motor responses ranging from spinal reflexes to complex behaviors. The nociceptive behavioral models also have the limit that they may be associated with, or modulated by, other physiological functions. The limitations are highlighted, including 1) that in most tests responses are monitored around a nociceptive threshold, whereas clinical pain is almost always more severe; 2) differences in the fashion whereby responses are evoked from healthy and inflamed tissues; and 3) problems in assessing threshold responses to stimuli, which continue to increase in intensity (Le Bars, 2001). However, the expressed behaviors show the body's homeostatic mechanisms.

Moreover, the sensitization of the somatosensory cortex was detected by Fos protein immunohistochemical study, in both conditions mentioned above.

1. The role of the 5-HT_{2A} receptor in chronic pain model and the development of the chronic pain state

From the experiment, CFA was used as a chronic pain model. This model was applied from the formalin test (Le Bars et al., 2001). The formalin test produced the specific pattern of behaviors that could classify to first and second phases of nociceptive behaviors. However, the nociceptive pattern was not found in the CFA model. CFA produced a non-specific pattern of nociceptive behaviors. The nociceptive behavior was transiently expressed during the observation time.

The results of the first experiment showed that CFA (1) induced the changes of the rats' behaviors (2) shorten of paw withdrawal latency of thermal noxious stimuli (3) increase Fos protein expression in the somatosensory cortex in both hemispheres. CFA injection not only resulted in a decrease in non-nociceptive behaviors but also an increase in nociceptive as well as still but alert behaviors. Moreover, the rats expressed their behaviors in rest or sleep category in the CFA induced-peripheral inflammation around 220 to 300 seconds during the experiment, while in the control group rarely expressed theses behaviors along the experiment. The data indicated that rats had a trend to reduce their movement to avoid the nociception.

On the other hand, thermal induced noxious response of the CFA group became indifferent in the Day 7 group. Similar evidence was found in Okamoto and colleagues' experiment in 2002. They found that the difference of paw withdrawal latency in CFA-group was reduced 3 days after introduction of CFA, and the different latency decreased to the

significant level in 7 days compared with the first day. They also found that the paw withdrawal latency of inflamed hind paw returned to normal in day 11 after CFA injection. The lengthening of the withdrawal latency may indicate the recovery process or the adaptation of thermo-nociceptors in the inflammatory area.

The relationship between thermal hyperalgesia and *c-fos* was reported by Sugiyo and colleagues in the year 2001. They found that intrathecally administration of *c-fos* anti-sense lengthened the paw withdrawal latency in CFA induced inflammation. They suggested that the expression of CFA-induced *c-fos* in the dorsal horn might facilitate thermal nociception.

This study revealed that chronic inflammation evoked Fos expression in all areas of somatosensory cortex. The evoked For-IR neurones were not confined in functionally representative cortical area as expected by its topographic organisation. On the contrary, Fos-IR neurones were distributed evenly throughout both hemispheres. This pattern reflected that, in chronic nociceptive condition, somatosensory cortical neurones are diffusely sensitised. The wide spread sensitisation of somatosensory cortex demonstrated in this study may explain the generalised decrease in pain tolerance observed in some chronic painful syndrome such as fibromyalgia. This finding also advocates the role of sensitisation of somatosensory cortex in the development of chronic pain.

In this study, the treatment of ketanserin in CFA-group resulted in the decrease of nociceptive behaviors, the lengthening of the paw withdrawal latency as well as suppressing Fos protein expression.

The role of 5-HT_{2A} receptor in nociception is quite controversy. Previous studies aimed at investigating this issue revealed conflicting result.

Some studies show that 5-HT_{2A} receptor may have role in attenuating nociception (Bardin et al., 2000). Recent studies showed that intrathecally administration of 5-HT receptor's agonists suppressed neuropathic pain, which were reversed by ketanserin (Obata et al., 2001; Sasaki et al., 2001). These reports suggest that activation of spinal 5-HT_{2A} receptors mediate analgesia for chronic pain. On the other hand, several studies showed facilitating effect of this receptor in the process of nociception (Eide and Hole, 1993; Saika et al., 1997; Tokunaga et al., 1998). The present findings of the effect of 5-HT_{2A} antagonist in reducing hyperalgesia secondary to chronic inflammation despite no efficacy in normal physiological nociception indicated that this class of receptor may play minor role in physiological nociception but may be of importance in the chronic pain state. This conclusion agreed with the previous anatomical studies which demonstrated an up-regulation of this receptor in painful condition (Xei et al., 2002). Recent study has shown that activation of 5-HT_{2A} receptor leads to an enhancement of nitric oxide synthase expression (Srikiatkhachorn et al., 2002). It is known that nitric oxide is strongly implicated in the process of sensitisation of central neurones. Therefore, up-regulation of this receptor may increase the sensitivity of central somatosensory neurons and contributes the development of central sensitisation.

2. The role of 5-HT_{2A} receptor in 5-HT depleted state on the changes of pain sensation

Many clinical conditions such as depression, fibromyalgia, migraine were found that low serotonin level in blood and brain as well. It was known that perception of painful stimuli in those patients was higher than normal.

The results of this study showed that PCPA-induced 5-HT depletion in the dose of 100 mg/kg body weight seemed not altering the behaviors of the rats. It was suggested that single dose of PCPA-administered could not impair the overall physiological functions of 5-HT or it may have the compensatory system that maintain the homeostasis.

The paw withdrawal latency of in left side Day 0-PCPA-administered group was higher than control group significantly. In Day 7 group, it was found that the latency of PCPA group, both sides was lower than in control group. In addition, the latencies in 5-HT depletion group seemed un-stable during the experiment. It might be implicate that the impairment of 5-HT affect the sensitivity of the peripheral thermo-nociceptor.

There was not found the changes of Fos expression during the experiments. It indicated that the impairment of 5-HT level without painful stimulation does not affect the cortical activity.

The application of 5-HT_{2A} receptor antagonist, ketanserin caused the incretion of rest or sleep behaviors in 5-HT depletion animals. The behaviors of the rats in low serotonin condition strongly supported previous studies that 5-HT_{2A} receptor has roles in alteration of motor function, alertness and sleep (Minami et al., 1997; Huibobro-Toro and Harris 1996; Swainston and Perry 2004).

On the other hand, ketanserin did not alter the withdrawal latency neither in the normal nor low level of serotonin. In addition, the data showed that ketanserin did not affect the changes of the number of Fos protein expression in those groups. It may implicated that 5-HT_{2A} receptor does not modulates the sensitivity of thermo-nociceptor.

In summary, overall findings indicated the some roles of 5-HT_{2A} receptor in pain pathways. In the development of the chronic pain state, 5-HT_{2A} receptor play a minor role in facilitating the pain perception. On the contrary, this receptor does not have a role in nociception in low 5-HT condition.