Chapter IV

Discussion and conclusion

Different types of pain test in aminal can be used to classified the level that responses to the test. For example, tail flick response is a spinal reflex (Carroll and Lim,1960; Irwin et al., 1951). The sensory component of experimental pain is set at the spinal interneuron (Chan and Dallaire, 1989) so that tail flick test can be used in evaluating analgesic agent. The hot plate test is another pain test in which responses are thought to involved an integrated escape response that may be mediated by supra-spinal mechanisms (Eddy et al., 1950; Eddy and Leimbach, 1953; Woolfe and McDonald, 1944). In the formalin test, response is relatively complex and pain mechanism appears to be located in the forebrain level of the CNS (Amodei and Paxinos, 1980). In this study demonstrated that mitragynine, an alkaloid found in *Mitragyna speciosa* Korth., produced antinociceptive action on both chemical (acetic acid and formalin) and thermal (hot plate) stimuli suggesting that sites of antinociceptive action are at supraspinal and forebrain level in CNS. In addition, that antinociceptive action of mitragynine was not reversed by naloxone, whereas antinociceptive action of morphine was, suggested that antinociceptive action of mitragynine does not mediated by opiate receptors. In formalin test , time course of response during the first hour was described as an early response (a short-lasting pain caused by direct effect on nociceptors(first 10 min)) and a late response (a long lasting pain due to inflammation(after first 10 min to an hour)) (Hunskaar et al., 1986). Hunskaar and Hole (1987) tested several analgesics of different classes and demonstrated an analgesic effect of steriods and non-steroidal anti-inflammatory in the late phase. None of these drugs had any effect in the early phase. In

present study mitragynine was effective in both early phase and late phase suggesting that mechanism of action of mitragynine was not the inhibition of prostaglandin synthesis. At this point, mechanism of action of mitragynine should be somehow different from analgesics available at the present time.

The result of this study also showed that mitragynine increased locomotor activity in mice that reflexed to the stimulating effect of mitragynine. This result supported the report of Grewal (1932) that mitragynine was found to be a central nervous system stimulant rather than depressant. The increase in locomotor activity is well known related to dopamine since apomorphine, a dopamine agonist, increased locomotor activity (Matsumoto et al., 1990) and in this study haloperidol, a dopamine antagonist, decreased locomotor induced by mitragynine. This is the reason why some experimental models were employed to determine the dopaminergic effect of mitragynine. In the rotational behavior, the results showed that mitragynine did not act as dopamine agonist or dopamine antagonist because it had no effect to the 6 hydroxydopamine induced brain lesion. Mitragynine did not induce turning neither contralaterally nor ipsilaterally when given to the rat and did not potentiate or inhibit turning when given to lesioned rat prior to subcutaneous injection of apomorphine. The dopamine receptor binding assay confirmed the result from rotational behavior. Mitragynine, only the high concentration, bound to the dopamine 2 receptor whereas other agonists or antagonists bound to the receptor at lower concentration. At the concentration mitragynine bound to the receptor was too high to be selective binding (10^{-6} M). The concentration of mitragynine that began to bind with dopamine receptor was approximate 100 fold compared with yohimbine, bromocriptine and sulpiride. From these results suggested that mitragynine was not dopamine selective agonist or antagonist and mechanism of action of mitragynine was not via dopaminergic system

To determine the level of neurotransmitters in striatum, microdialysis was employed. The neurotransmitters and their metabolites that found in dialysate was HVA, DOPAC (metabolites of dopamine) and 5-HIAA (metabolite of serotonine). In this study, no significantly different in neurotransmitter level was found even though there was tendency to increase level of DOPAC in the dialysate when mitragynine 30 mg/kg was administered.

Mitragynine had no effect on number of head weaving induced by 5-MeODMT . Head weaving was found to be induced by variety of serotonin agonists such as 5-methoxy-N,N-dimethyltryptamine (5-MeODMT) or serotonin-releasing drug, fenfluramine (Grahame-Smith, 1971; Green and Kelly, 1976). Head weaving is also induced by 5-HT_{1A} agonist 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT)(Arvidsson et al., 1981). This suggests that head weaving is mediated by 5-HT_{1A} receptor. Mitragynine did not induce head weaving and did not block head weaving induced by 5-MeODMT. Therefore, mitragynine is not neither 5-HT_{1A} agonist nor antagonist.

Head twitch is another serotonin syndrome produced by 5-MeODMT (Green and Heal, 1985). In this study mitragynine was found to significantly decrease in number of head twitch induced by 5-MeODMT .Ritanserin (5-HT_2 antagonist) and α_2 antagonist can inhibit head twitch. This suggested that mitragynine acted as an antagonist of 5-HT_2 receptor or α_2 receptor. Using the tail flick and writhing tests, significant antinociceptive effects of 5-HT_2 antagonists, ritanserin and ketanserin, have been described in rat (Barber et al., 1989). The effects are not triggered at the spinal level, but more probably via the blockade of supraspinal 5-HT_2 receptor, leading indirectly to an activation of descending monoaminergic neurons (Barber et al.,1989). However ketanserin also exhibit α adrenergic antagonist properties which might well account for their effects on nociception.(Sanders-Bush,1988).

As one of the results, it was shown that mitragynine was also able to decrease core body temperature. Concerning only 5-HT_2 receptor, 5-HT_{1A} and 5-HT_2 receptors works in opposition to regulate body temperature (Gudelsky et al.,1986) in case that 5-HT_{1A} agonist caused hypothermia and 5-HT_2 agonist caused hyperthermia. If we propose that mitragynine is an 5-HT_2 antagonist, the effect of mitragynine induced hypothermia can also be explained.

In conclusion, the present study demonstrated an antinociceptive activity of mitragynine with the different mechanism of action from the available analgesics. It did not mediated neither via opiate receptor nor as a postaglandin synthesis inhibitor. Possibly serotonin 2 antagonist is the mechanism of action of mitragynine. However the other possible mechanism of action that should be a point of interest is α_2 adrenergic antagonist. To clarify the mechanism of action precisely further studies using appropiate experimental models, both in vivo and in vitro model, should be performed.

However. there is a limitation in this study. From the fact that kratom is an illegal plant in Thailand, the amount of mitragynine used in this study depended on how much kratom leaves were obtained. In this study, the highest dose of mitragynine was not more than 60 mg/kg in mice 30 mg/kg in rats. The peak time of analgesic action of a dose of mitragynine was not determined. Moreover, there was no scanning for the appropiate dose of analgesic activity. These were the results from the small amount of tested compound, mitragynine. In further study all stated above should be determined.