CHAPTER 4
DISCUSSION AND CONCLUSION

In this chapter, the discussion is on the purification of the bioactive substance extracted from Khi-lek, the effect of anhydrobarakol hydrochloride on locomotor activity in rats and the main purpose is to discuss the effect of anhydrobarakol hydrochloride on alteration of dopamine or serotonin content in brain.

1. Preparation of anhydrobarakol hydrochloride

Purified bioactive substance from fresh young leaves and flowers of *Cassia siamea* Lamk. was obtained as a greenish yellow needle-shaped crystal. This substance was unstable, thus, it converted to stable form of anhydronium salt as a lemon yellow compound. The physical and spectrophotometric characteristics of the substance were shown; UV λ_{max} (EtOH) nm (log ε): 241(4.8) and 398(4.54) (Figure 10); IR ν_{max} (KBr) 3445, 1670, and 1271 cm^{-1} (Figure 11); ^{1}H-NMR (D_2O): δ 6.94 (1H), 6.90 (1H), 6.78 (1H), 6.71 (1H) and 2.61, 2.38 (6H, 2s, 2Me) (Figure 12). Mass spectra; m/z (rel.): M+ 216 (3), 214 (100), 186 (158), 115 (11), 89 (11), 63 (9) and 51 (9). These data were similar to those of anhydrobarakol hydrochloride in previous reports (Bycroft, 1970 and Kaokeaw, 1993). Thus, this confirmed that the lemon-yellow compound prepared in this experiment was anhydrobarakol hydrochloride.

2. Effect of anhydrobarakol hydrochloride on rat locomotor activity

In previous reports, they found anhydrobarakol hydrochloride decrease locomotor activity in mice related to the dosage (Jantarayota, 1987 and Kaokeaw, 1993), but no reports were done on rats. In this study it was found that the anhydrobarakol hydrochloride significantly induce a sedative effect in all rats in each dosage used. This result was consistent with those previously reported in mice. The anhydrobarakol hydrochloride induces the effect within 5 minutes after injection. This induction time is rather short which indicates the rapid distribution of the substances to the brain.

The action of anhydrobarakol hydrochloride on the rat is also related to the dosage. The increase of the effect is linear from dose 10-100 mg/kg body weight. The rat did not show any abnormal sign. This suggests that all dosages used in this study seemed to be quite safe.

3. Determination of monoamines in the brain nuclei by high performance liquid chromatography with electrochemical detection

Anhydrobarakol hydrochloride produces no effect on serotonin and its metabolite because the concentration of serotonin and its metabolite in all area of the rat brain is not changed when compared with the control group. This suggests that anhydrobarakol hydrochloride does not act on serotoninergic system.

On the other hand, in dopaminergic system, the concentration of dopamine in anhydrobarakol hydrochloride treatment group is changed in caudate head 2 area of the rat brain. The dopamine content in this area is decreased when compared with the control group. In the other area of the rat brain, the concentration of dopamine was not changed when compared with control group. Therefore, the action of anhydrobarakol hydrochloride may be on the dopamine content in the head of caudate in rat brain.

Caudate is a part of striatum (caudate and putamen) in basal ganglia. Striatum is a major input of structure of basal ganglia circuit. The input of striatum is from glutamate neuron from cortex and dopaminergic neuron located in substantia nigra pars compacta (SNpc) (Blandini et al., 2000 and Delong, 2000).

Although the striatum contains several distinct cell types, 90-95% of them are GABA (gamma aminobutylic acid) –ergic medium spiny neuron (Blandini et al., 2000). On the basis of the particular peptide neurotransmitters they contain and the type of dopamine receptor expresses, medium spiny neurons can be divided into two populations. One population contain GABA and substance-P and primarily express dopamine D_1

receptors. These neurons send axon directly to globus pallidus internal segment (GPi) and to substantia nigra pars reticulata (SNpr). The second populations contain enkephalin and GABA and primarily express dopamine D_2 receptors. These neurons project to globus pallidus external segment (GPe) and thus their influence on Gpi and SNpr is only expressed indirectly (Mink, 1999).

Dopamine in basal ganglia is from SNpc. Cell body of dopaminergic neurons are in SNpc and send axon to caudate and putamen. Dopamine release inhibits the indirect pathway by stimulating dopamine D_2 receptors, and excites the direct pathway by stimulating the express dopamine D_1 receptors (Figure 20) (Vermeulen, 1994).

The dopaminergic inputs to the two pathways lead to the same effect, reducing inhibition of the thalocortical neurons and thus facilating movements initiated in the cortex. Without the dopaminergic action in striatum activity in the output nuclei increase. This increased output in turn increased inhibition of the thalamocortical neurons that otherwise facilitate initiation of movement (Delong, 2000).

In this experiment, anhydrobarakol hydrochloride decreased dopamine concentration in the head of caudate in rat brain and from the fact discussed in the above paragraph, anhydrobarakol hydrochloride may act on the dopaminergic system and decrease activity in animals.

Caudate is separated into three parts - Head, Body (Middle) and Tail (Mink, 1999). From previous report, there is a high density of dopamine D_2 receptors in head of caudate but low densities in the other areas of caudate. On the other hand, there is a high density of dopamine D_1 receptors in body of caudate but low densities in the other areas of caudate (Beckstead, 1988). In this experiment, anhydrobarakol hydrochloride decreased dopamine concentration significantly in the head of caudate 2 but in the head of caudate 1 it was decreased but not significant. This result suggests that anhydrobarakol hydrochloride may affect dopamine D_2 receptors in the head of caudate.

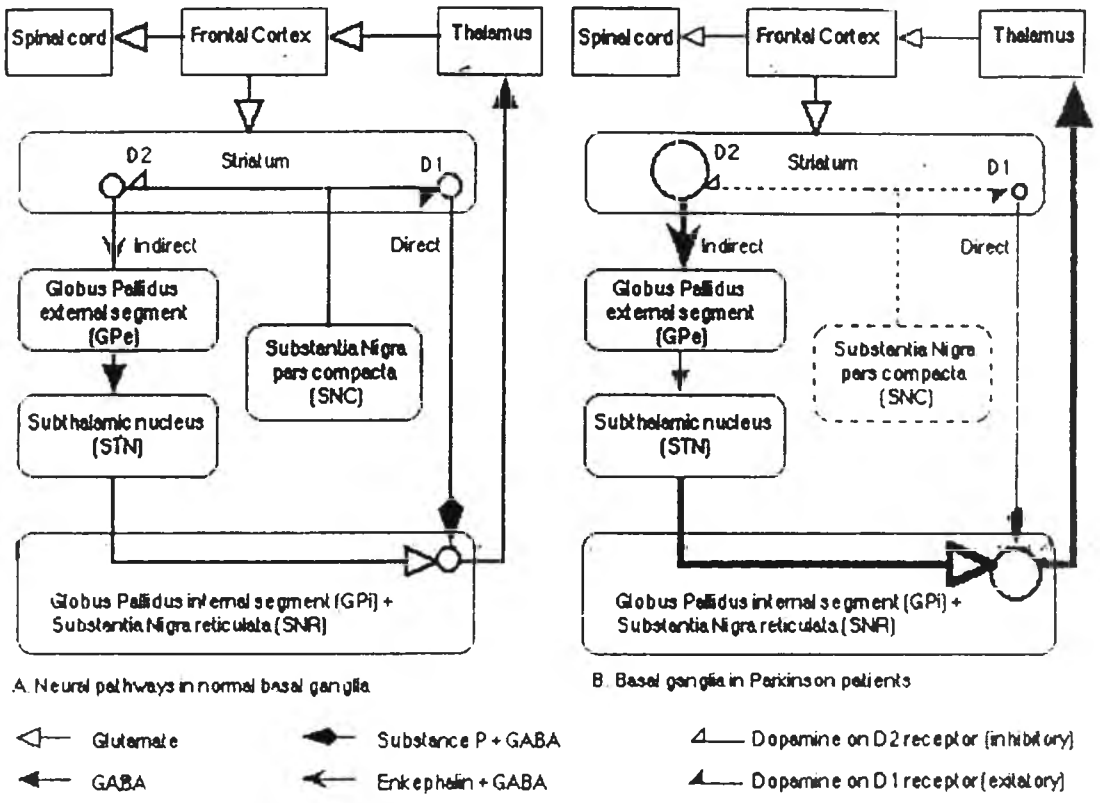

Figure 20 Schematic of basal ganglia circuits in normal and Parkinson's patient.

The level of dopamine metabolite (DOPAC) in that anhydrobarakol hydrochloride treatment group was not changed. This result suggests that the decrease of dopamine level dose not affect dopamine metabolism. It seems to be the direct effect of anhydrobarakol hydrochloride on dopamine content.

In this experiment, the dopamine level in other areas including the substantia nigra was not changed. This effect shows that anhydrobarakol hydrochloride may directly affect dopamine release but not dopamine synthesis.

In conclusion, this study suggests that anhydrobarakol hydrochloride has an effect on dopaminergic system to decrease dopamine level in the head of caudate and decrease activity of animals.