CHAPTER V

DISCUSSION AND CONCLUSION

The derivable ALK from the extraction and purification procedures was examined about the spectroscopic evidences consisting with Ultraviolet Absorption Spectrum, Infrared Absorption Spectrum and Nuclear Magnetic Resonance Spectrum. All of these showed that it is 3α - dihydrocadambine.

Low solubility of this ALK observing in the present study disagreed as to the previous study (Pongpan Aroonsang,1984) although the same ALK were used. While, from the physical properties of general alkaloids show that the alkaloids as free-base form are insoluble or Low solubility, but are good dissolved in organic solvents such as chloroform and ether (Fessenden and Fessenden,1977; Solomons,1980) ,which consisted this experiment.

The low solubility of 3α - dihydrocadambine require the usage of relatively large amounts of the solvent. Unfortunately, because of the limited volume of the solution, the usual solvent cannot be administered by IV or VENT in large quantities (Ganten, Unger and Lang , 1987). So the aqueous solutions of ALK using the cosolvents, polyethylene glycol 400, have been prepared.

The polyethylene glycols(PEGs) are polymeric compounds represented by a large number of ether linkages and terminal hydroxyl groups. There were the different consistencies, from liquids to solid materials, with molecular weights from 150 to 10,000 daltons. At room temperatures, substances with molecular weights up to 600 daltons are

liquids, whereas those with molecular weights above 1,000 daltons are solids (Maynard,1947; Benzon et al.,1987).

PEGs are heat stable , excellent coexisting water solubility, chemically inert and a minimal tendency to denature biomacromolecules (Atha and Ingham, 1981; Zeng, Suzuki and Alpert, 1990). Their low systemic toxicity, wide range of drug compatibility and solubilization characteristics make them useful as cosolvents or vehicles for drugs in biomedical industries (Atha and Ingham,1981; Benzon et al., 1987; Riad and Sawchuk,1991).

PEG 400; the number refers to the mean molecular weight, is a water soluble mixture of at least nine different polymers, ranging in molecular weights from 242 to 594 daltons (Benzon et al.,1987; Krugliak et al.,1990).

Based on the finding of Friedman(1944), the effects of the polyglycol on blood pressure was studied in nine dogs under pentobarbital anaesthesia and on six unanaesthetized rabbits. No effect was observed, when administered intravenously in 50 percent solution in distilled water. About the toxicity of the polyethylene glycol when given by intravenously, the Carbowax 1500 (the registered trade mark) was studied. Mongrel dogs were given each by vein 0.5 g/kg of the polyglycol and no disturbance was noted in respiration, pulse or temperature during the period of four hours following. When given 0.75 g/kg Carbowax per day for three days and blood studies were carried out for period of three weeks. The red and white count, hemoglobin and hematocrit remained normal and without any pathologic changes was found when the dogs were sacrificed one, four and six months later.

These evidents may prove that PEGs are suitable vehicles for the IV administration at least in the experimental work (Friedman, 1944).

The volume of ALK solution used in this study were selected from a series of pilot experiments, 0.3 ml for IV and 10 µl for VENT, which gave no noticeable systemic effects. Similarly to the rate of administration, the slowly bolus injection of ALK solution, 1.0 ml/min, were made to avoid the initial pressor responses that may occur after injection.

Different routes of administration, IV and VENT, were used to test possible mechanism of action of the ALK. The reason of these procedures is that the chemical substances may be introduced directly into the cerebral ventricular system, thereby diffusing the blood-brain barrier; thus the central action of the substances can be examined (Myers et al., 1971). Furthermore, Bolme et al., (1975) suggested that the drugs when given intraventricularly at doses that have no effect systemically, evokes very similar systemic changes as when given IV supports that it has a predominant central action. Therefore, when comparing between the two routes of administration, the central action of any chemical substances could be observed rapidly and more potent when administered directly into the cerebroventricular system.

In the present study, it was found that 3α - dihydrocadambine 0.8 to 6.4 mg/kg B.W. administered intravenously resulted in dose-dependent decreases in both systolic and diastolic blood pressure significantly ($p<0.05$) (Table 1,2). The most effective dose was observed at ALK 6.4 mg/kg B.W.. And at the higher doses of 16.0 and 24.0 mg/kg the decreasing effect was also similarly to the dose 6.4 mg/kg. Then, in figure 7

which shows the concentration response curves of ALK, a plateau decreasing effect had been observed at the doses ranging 6.4 to 24.0 mg/kg. In case of the heart rate, it was also reduced at initially hypotensive effect of ALK and significant reduction was observed at doses 3.2 and 6.4 mg/kg (Table 5.). After that, when the peak of effect was reached the heart rate was increasing. This suggests that part of the initial bradycardia was caused by direct depression on the heart, as has been reported by previous investigator (Pongpan Aroonsang,1984). In addition, the sustained hypotensive effect of ALK may induce the baroreceptor reflex and cause secondary to increase in heart rate (Guyton,1981; Ganong,1991). However, at higher dose 16.0 and 24.0 mg/kg the heart rate was elevated at initial hypotensive effect of ALK. It may be due to the baroreceptor mechanism. These findings suggest that at doses 16.0 and 24.0 mg/kg the more powerful hypotensive effect was also observed, at the same time the discharge rate of the buffering nerves (IX,X) in baroreceptor circuit was declined. No impulses generated in an inhibitory pathways from nucleus of the tractus solitarius (NTS) to vasomotor area (C1 area). Then the more active of vasoconstrictor nerves was observed during the vagal innervation of the heart was also inhibited. These resulted in producing tachycardia and also following increase in AP. This response is the compensatory mechanism for maintenance the AP(Figure 15). The significantly increased in heart rate was observed only in dose 16.0 mg/kg but not in 24.0 mg/kg ,it may be due to the greater standard diviation.

The placebo injection of 10%PEG in NSS intravenously evoked no significant systemic change when was compared to neither base line control before injection nor control NSS itself (Table 4, 5 and

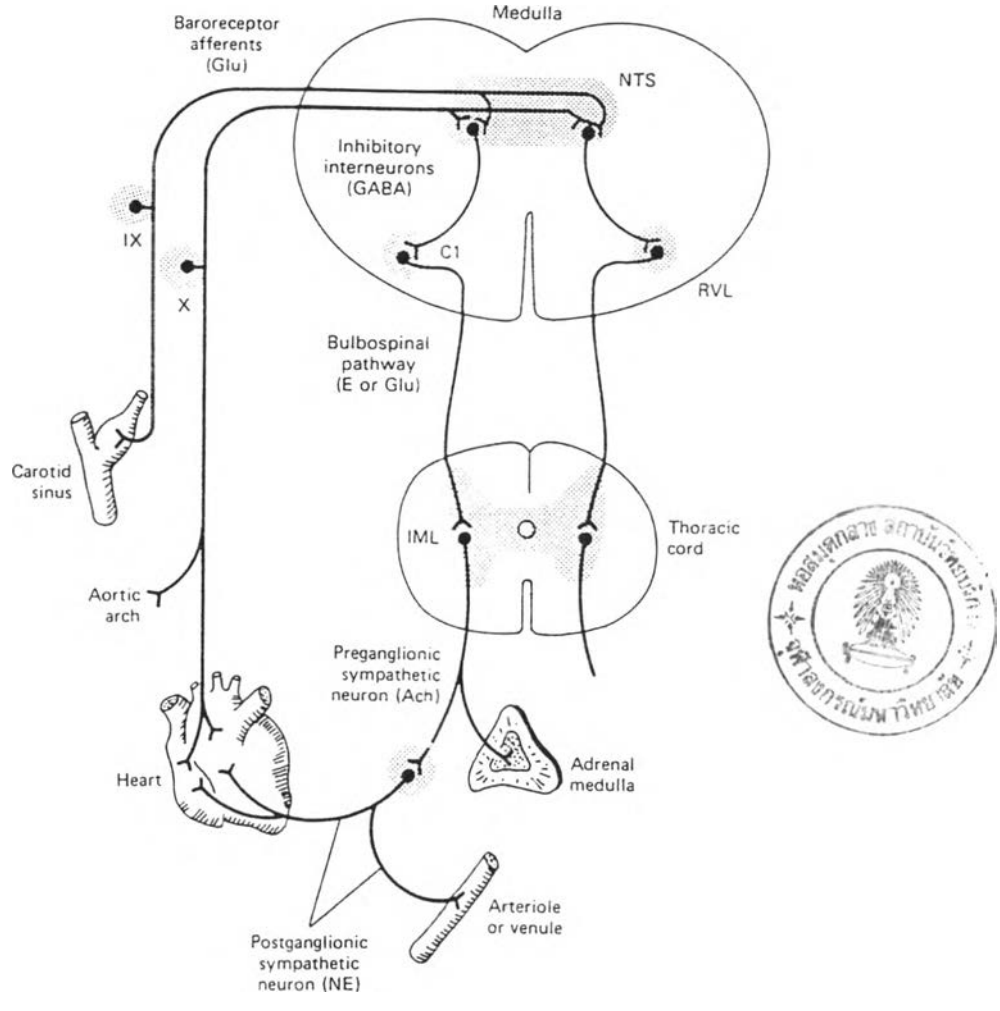

Figure 15. Basic pathways involved in the medullary control of blood pressure. The vagal efferent pathways that slow the heart are not shown. The putative neurotransmitters in the pathways are indicated in parentheses. Glu, glutamate; GABA, γ-aminobutyric acid; E, epinephrine; Ach, acetylcholine; NE, norepinephrine. IML, intermediolateral gray column; NTS, nucleus of the tractus solitarius; RVL, rostral ventrolateral reticular nucleus (Ganong, 1991).

Figure 8). By VENT a significant decrease in AP occurred after injection of 20%PEG in aCSF, whereas the normal aCSF itself evoked no significant change (Table 6, 7, 8 and Figure 10, 11). The ability of the more concentrated PEG solution to evoke significant systemic change may be related to their increased osmotic pressure. Since the osmotic pressure of a solution is direct proportion to the concentration of the solute, 20%PEG are more osmolality can be considered hyperosmotic or hypertonic solution when compared to the nerve axon and the myelin (Benzon et al., 1987).

At normal, axon are highly hydrated containing 75 to 90% water by weight (Shapiro,1966). In the presence of a hypertonic solution, water passes from nerve axon to the solution, leading to shrinkage of nerve fibers. On the other hand, the water content of myelin is about 40 to 50% (Finean,1957). The lyophilic molecules and ions in the hypertonic solution together with its bound water penetrate the myelin and increase its volume. These structural changes in nerve axon and myelin may reduce the ion permeability of the axonal membrane, threrby elevating the external K^+concentration and depolarizing the nerve membrane, rendering it refractory to nerve impulse conduction (Shapiro,1966). As pointed out by Benzon et al., (1987) which investigated the effect of a 1-hour exposure to different concentrations of PEG on rabbit nerve impulses. They reported that 20% PEG caused mild depression of the compound action potential amplitudes and marked slowing of the conduction velocities in 10% PEG. Furthermore, exposure of desheated nerves to PEG showed the same degrees of block as the sheathed nerves and showed independent of the pH of solution. So in this study, 20%PEG in aCSF was administered directly into the cerebral ventricular system which closely related with the areas controlling the cardiovascular of central nervous system. The

mechanism as previously stated may occur in those areas thus causes ultimate result in changeable of AP.

Comparing the results between the two groups, IV and VENT administration, it was found that 3α- dihydrocadambine could lower AP significantly ($p<0.05$) from control by both routes of administration (Table 1,2,3,6,7 and 8). However, there was the difference in pattern of hemodynamic changes, in the term of time-action, between them (Table 4,9 Figure 8,11). The IV injection decreased AP immediately while by VENT, delayed decreasing effect was occurred. This suggests that in IV group, systemic change was caused by direct peripheral action of the ALK then the peak were reached rapidly about 40 seconds after injection. In part of VENT group, the time-action curve of ALK 3.2 mg/kg in bottom panel of figure 11 shows the delayed decreasing effect of the ALK. It should be pointed out that this curve may derive from another two curves. Firstly, the curve shows decreasing effect of the solvent solution (20%PEG in aCSF) (top panel of figure 11.) which has time to peak about 10 minutes after injection. Secondly, shows the decreasing effect of the ALK itself which may diffuse from the cerebral ventricular system into peripheral part, thereby diffusing the blood-brain barrier. Therefore, after ALK in solvent solution was injected into lateral ventricle, the solvent solution shows decreasing effect and then were added with the following decreasing effect of ALK thus the curve in bottom panel of figure 11 was so observed.

However, no significant difference in the multiple comparisons between the 20%PEG in aCSF group and various doses of ALK in 20%PEG groups by using analysis of variences ($p>0.05$). Therefore,

it may be postulated that most of the decreasing effects as were noted resulting from the solvent solution, 20%PEG in aCSF.

This part of the present study shows the peripheral action of the ALK contrastingly to the previous report of Pongpan Aroonsang (1984) which indicated that hypotensive effect of ALK may act on central nervous system. This controvercy may concern with the difference in the solvent system as earlier stated. And in the present study, when compare the results between the two routes of administration of ALK, IV and VENT, the hypotensive effect of IV ALK was observed immediately after injection. Whereas by VENT , delayed decreasing effect was occurred and then found that this decreasing effect resulting from the solvent solution. While the evidence from injecting of alcian blue dye shown that the ALK which was injected into VENT, distributed through the 4th ventricle. The areas near the 4th ventricle such as the nucleus of the tractus solitarius, the vasomotor area involved in the medullary control of blood pressure (Ganong, 1991). Thus , if the ALK has central action, the direct injection of ALK into VENT, the rapid and more potent effect could be observed. In addition, the data from previous report shown that hexamethonium could partially reduced (not significant) the effect of ALK . So these evidences confirmed the peripheral action of the ALK.

To test the hypothesis whether the mechanism of hypotensive effect of ALK and the powerful hypertensive response from stimulation of the FN be mediated from the same mechanism. The hypotensive effect of the ALK was elicited under the FN stimulation.

The cardiovascular responses to FN stimulation in anaesthetized tree shrews have been described by Chakkrit Luk-in (1992). Similarly to this

study, as shows in figures 12, 13 and table 11 during FN stimulation, significantly increased in systolic, diastolic and also mean arterial pressure ($p<0.05$). In this part of the experiment an active site of the FN stimulation was defined as one from which electrical stimulation elicited the highest elevation of FPR. And then from the electrolytic lesion and histological techniques shown that the active site which was selected was placed at the site in white matter anterior to the rostral pole of FN. This is not a new finding, as has been reported by Chakkrit Luk-in (1992) which indicated that electrical stimulation of this area gave the greatest response when compared to the area 640 µm, posterior to the rostral pole. Anatomical studies shown that reciprocal connections exist between FN and two medullary nuclei implicated in baroreflex activity, the parasolitary nucleus of tractus solitarius (NTS : the first relay nuclei of baroreceptor afferents) and nucleus paramedian reticularis. In addition to the evidences as has been reported by other investigators such as Miura and Reis, 1969; Achari and Downman, 1970; Lisander and Martner, 1971; Dormer and Stone, 1978; Bradley, Paton and Spyer,1986 concluded that these cardiovascular changes is the result of widespread activation of the sympathetic nervous system.

The results of ALK during FN stimulation was summarized in table 11. It was found that during FPR, IV ALK 3.2 mg/kg could reduced the blood pressure significantly ($p<0.005$) and more significance was observed at the dose 6.4 mg/kg ($p< 0.0005$). However, when comparing these data to the data from basal blood pressure groups at the same dose in percentaged decrease, there were no statistically significant differences between them except in systolic blood pressure parameter at 3.2 mg/kg B.W.(unpaired *t*-test $p<0.05$) (Table 12, 13 and 14). This means the fall in blood pressure

produced by ALK was not being interfered by the rise in blood pressure during FN stimulation. These results indicated that the hypotensive effect of ALK and the FPR may not be exert through the same mechanism.

In conclusion, from all above evidences in particularly about the different patterns of the ALK's effects, such as the time-action which was observed between the two routes of administration. It may be postulated that the primary action of ALK, 3α - dihydrocadambine, was not in the central nervous system but predominantly occurred in peripheral. In addition, in the part studying about the antagonistic effect, indicated that the action of ALK is not mediated with sympathetic nervous system. Although the clearly mechanism of action of the ALK remains undetermined at this time. At least, the results obtained from this present study are further evidences that supporting the peripheral action of this ALK. However, more studies about the detail mechanism of hypotensive effect of this ALK are also required.