นิพนธ์ต้นฉบับ

Effects of 3 α - dihydrocadambine isolated from anthocephalus chinensis leaves on blood pressure and cardiac activity in rats.

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Aroonsang P. Effects of 3 α - dihydrocadambine isolated from anthocephalus chinensis leaves on blood pressure and cardiac activity in rats. Chula Med J 1990 Jul; 33 (7) : 531-542

The blood pressure effects of 3 α - dihydrocadambine (ALKALOID) isolated from Anthocephalus Chinensis leaves have been investigated in anaesthetized rats and isolated right and left atria. An intravenous infusion of ALKALOID 0.4, 0.8, 1.6 and 3.2 mg/kg body weight (b.w.) caused dose-dependent sustained hypotensive effect on both systolic and diastolic blood pressures accompanied by bi-phasic initial reduction but followed by a small increase in heart rate. Prior administration of propranolol 1 and 2 mg/kg or mepyramine 10 mg/kg plus cimetidine 20 mg/kg b.w. did not inhibit hypotensive effect of the ALKALOID. Only atropine 0.3 mg/kg b.w. showed a partial reduction of the hypotensive effect at all doses significant. The ALKALOID produced dose-dependent reduction on isolated atrial rates and exhibited a slight depression of left atrial isometric tension.

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Received for publication. January 29, 1990.

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ผ่องพรรณ อรุณแสง, บังอร ชมเดช, ประสาน ธรรมอุปกรณ์, ราตรี สุดทรวง. ผลของ 3 แอลฟา-ไดไฮโดรคาดามบีน ที่แยกออกจากใบกระทุ่มใหญ่ ต่อความดันเลือด และการทำงานของหัวใจในหนูแรท จุฬาลงกรณ์เวชสาร 2533 กรกฎาคม ; 34 (7) : 531-542

ได้ทำการศึกษาฤทธิ์ต่อความดันเลือดของ 3 แอลฟา - ไดไฮโดรคาดามบีนซึ่งเป็นอัลคาลอยด์ที่แยกจากใบกระทุ่มใหญ่ในหนูแรทที่ให้ยาสลบและต่อหัวใจห้องบนทั้งสองที่แยกออกมา พบว่าการให้อัลคาลอยด์ขนาด 0.4, 0.8, 1.6 และ 3.2 มก/กก. ของน้ำหนักตัว หนูแรทสามารถทำให้ความดันเลือดทั้งซีสโตลิกและไดแอสโตลิกลดลงอย่างเป็นปฏิภาคโดยตรงกับความเข้มข้นที่สูงขึ้น อัตราการเต้นของหัวใจในช่วงแรกลดลง แล้วต่อมาเพิ่มขึ้น การให้ propranolol 1 และ 2 มก/กก. หรือ mepyramine 10 มก/กก. ร่วมกับ cimetidine 20 กม/กก. ของน้ำหนักตัวก่อนให้อัลคาลอยด์ไม่สามารถยับยั้งฤทธิ์การลดความดันเลือดของอัลคาลอยด์ได้ แต่การให้ atropine 0.3 มก/กก. สามารถยับยั้งฤทธิ์ของอัลคาลอยด์ทุกขนาดได้อย่างมีนัยสำคัญทางสถิติ สำหรับผลของอัลคาลอยด์ต่อหัวใจห้องบนทั้งสองที่แยกออกมา พบว่าอัลคาลอยด์สามารถลดอัตราการเต้นของหัวใจห้องบนขวาอย่างเป็นปฏิภาคโดยตรงกับความเข้มข้นที่เพิ่มขึ้น แต่ลดแรงบีบตัวของหัวใจห้องบนซ้ายน้อยมาก

Anthocephalus Chinensis A. Rich is known in thai as "Kra-thum,"[1] in English as "wild cinchona" and in Hindi as "Kadamb".[2] Its leaves have been used as a gargle in case of aphthae and stomatitis. The use of this plant in folk medicine stimulated an investigation of active principles present in the leaves. By means of alumina column chromatography, an indole glycosidic alkaloid was isolated from the leaves. The physical and chemical properties and spectroscopic evidences have shown that it is 3 α - dihydrocadambine which has a pentacyclic heteroyohimbinoid glycoside skeleton comprising of a carboline unit joined to an iridoid glucoside through C-3, N-4 bridges. The glucose unit is linked by an oxygen bond as an O-glycoside and is cleaved by the enzyme ß-D-glucosidase (Fig 1).[3]

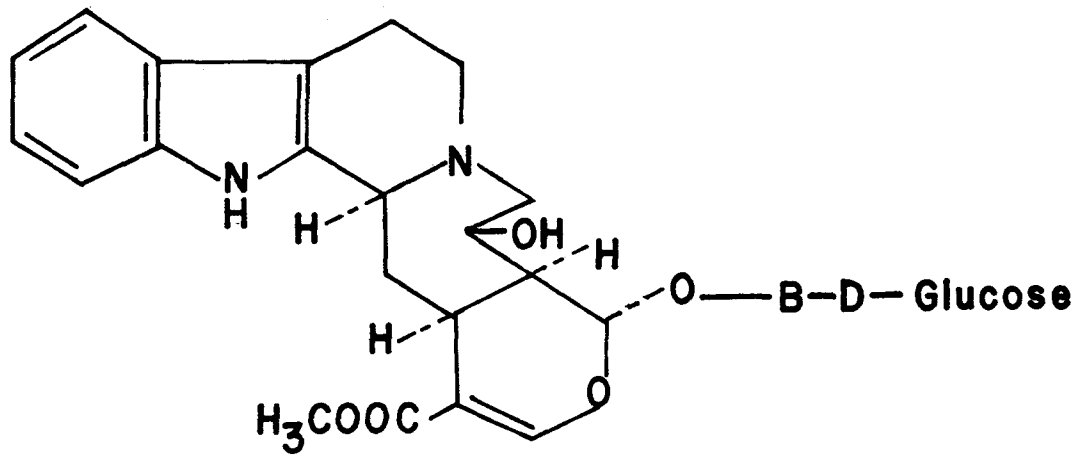

Figure 1. The structure of 3 α-dihydrocadambine, indole glycosidic alkaloid isolated from the leaves of Anthocephalus Chinensis A. Rich.

It is known that many indole alkaloids are physiologically active and some are of medicinal importance. For example the oxytocic ergoline, the antihypertensive and tranquilizer reserpine, vincaleucoblastine used in the treatment of leukemia, the stimulant strychnine and the cholinergic physostigmine. Most of which are either indole alkaloid complexes or their simple derivatives.

In the present study, the actions of 3 α - dihydrocadambine on blood pressure and cardiac activity were investigated in experimental animals. The results obtained from this study may be worthwhile in supporting the advanced studies of pharmacological actions of this alkaloid, and in turning a natural resource into a medicinal one.

Materials and Methods

1. Animals

Seventy-five albino rats of Wistar strain of both sexes with body weights varying between 200-300 gm were used. The animals were given ad libitum access to water and food for 1-2 week prior to experiments.

2. In Vivo Preparation

The animals were anaesthetized with sodium pentobarbital 40 mg/kg b.w. intraperitoneally. Supplementary doses of the same drug were given as necessary to maintain anaesthesia. The trachea was cannulated for spontaneous ventilation with room air and to facilitate respiration. Drugs were administered intravenously through a catheter in a jugular vein in which the tip of the catheter was advanced towards the heart. Arterial blood pressure and heart rate were measured from left carotid artery with pressure transducer connected to Bekaman Dynograph recorder (type RM).

3. In Vitro Preparation (Isolated Atrial Preparation)

The rats were killed by a blow on the head. The heart was quickly excised and placed in a petri-dish

containing oxygenated Locke solution. The left and right atria were then separated. the right atrium was used for chronotropic response by recording the rate and contractile force with an isometric force transducer connected to the Beckman Dynograph recorder. The atrium was allowed to equilibrate until the rate and amplitude of spontaneous contraction were stable before experiments began. The isolated left atria were used for inotropic response by electrical stimulation with square wave pulses of 6 msec duration at a frequency of 45 Hz and supramaximal voltage of 8 volts. The tissue was applied a tension of 1 gm, and allowed to equilibrate until the force of contractions were stable before they were exposed to the drugs.

4. Drugs

To study the mechanisms of action of 3 α-dihydrocadambine, indole glycosidic alkaloid from **anthocephalus chinensis** A. Rich. leaf (ALKALOID), various drugs of well known mechanisms were used, such as acetylcholine chloride, atropine sulfate, isoproterenol hydrochloride, mepyramine maleate, cimetidine, hexamethonium bromide and tyramine hydrochloride. All these drugs were prepared as solutions in normal saline. ALKALOID was also dissolved in normal saline, except for the in vitro preparation ALKALOID was dissolved in Locke solution and doses were expressed as the salt.

5. Analysis of Data

Experimental data were expressed as means $\pm$ S.E.M. Statistical significance was tested according to Student's T-test for paired or unpaired variates.

Results

Anaesthetized Rats

1. The effects of ALKALOID on systemic blood pressure and heart rate.

A slow bolus intravenous infusion of ALKALOID caused dose dependent decrease in both systolic and diastolic blood pressures significantly ($p<0.001$). The higher the doses, the longer was the depression observed. As shown in Fig. 2, at the doses of 0.4, 0.8 and 1.6 mg/kg b.w., both systolic and diastolic blood pressures were similarly depressed. At the highest dose of 3.2 mg/kg b.w., most of the results showed more depression in diastolic than systolic blood pressure (Table 1)

Table 1. Effect of ALKALOID on blood pressure (B.P.)*

Dose of ALKALOID (mg/kg)	No.of Rats	Systolic B.P. (mmHg)		Diastolic B.P. (mmHg)		Mean B.P. (mmHg)	
		Before Infusion	After infusion	Before infusion	After infusion	Before infusion	After infusion
0.4	11	150.9 $\pm$4.21	129.82$\pm$3.52**	102.73$\pm$3.93	83.46$\pm$4.01**	118.79$\pm$3.77	98.01$\pm$3.61**
0.8	14	146.86$\pm$3.29	121.29$\pm$3.29**	106.71$\pm$2.54	82.71$\pm$3.72**	120.57$\pm$2.76	95.57$\pm$3.36**
1.6	12	145.50$\pm$3.18	118.33$\pm$2.67**	105.33$\pm$1.48	76.17$\pm$1.93**	118.83$\pm$1.78	90.22$\pm$1.71**
3.2	14	153.57$\pm$2.87	116.00$\pm$2.49**	105.43$\pm$3.61	67.29$\pm$3.80**	121.48$\pm$3.20	83.52$\pm$3.02**

* Values are expressed as mean $\pm$ S.E.M.

** $p < 0.001$

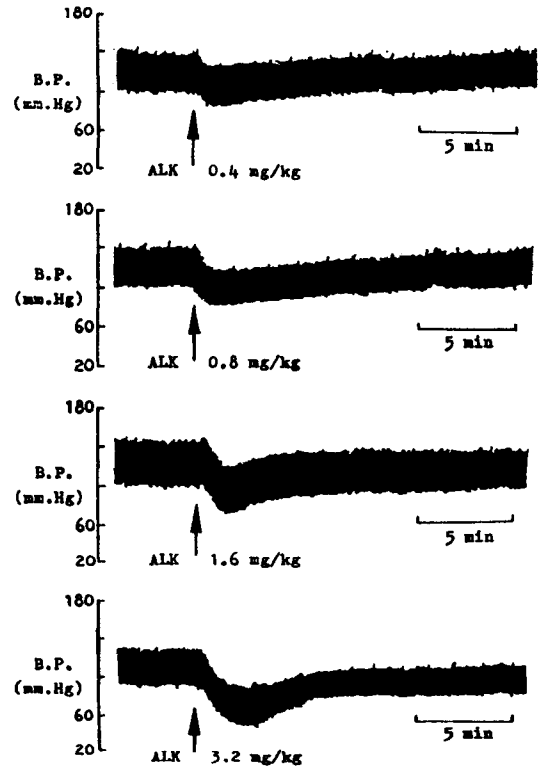

Figure 2. Records showing the effects of ALKALOID (ALK) in various doses on blood pressure in anaesthetized rats.

An intravenous infusion of ALKALOID elicited biphasic responses on the heart rate. The responses showed an initial reduction followed by an increase in heart rate. These changes were significantly seen in higher doses (Table 2)

Table 2. The effects of ALKALOID on heart rate in anaesthetized rats (mean ± S.E.M.).

DOSE OF ALKALOID (mg/kg)	NO. OF RAT	HEART RATE (beats/min)		
		BEFORE ALKALOID INFUSION	AFTER ALKALOID INFUSION	
			✪ INITIAL	●MAXIMUM
0.4	10	339.00±12.43	337.00±12.31*	340.00±12.57★
0.8	11	340.91±12.17	336.36±11.99*	349.00±11.48★
1.6	10	341.00± 9.94	336.00± 9.69*	347.00±10.33★
3.2	13	347.69± 9.55	340.77±10.33*	353.08± 9.09★

✪ INITIAL = at initial hypotensive effect of ALKALOID.
● MAXIMUM = at maximum hypotensive effect of ALKALOID.
* Significant decrease from control, $P < 0.05$, paired t-test.
★ Significant increase from control, $P < 0.05$, paired t-test.

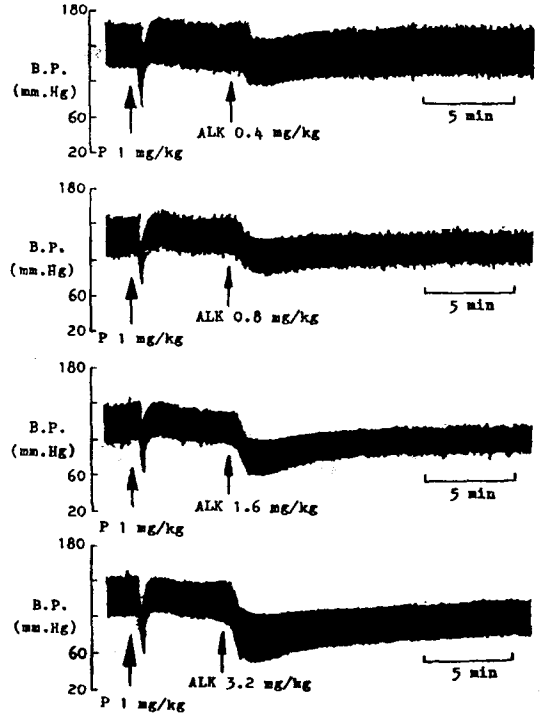

Figure 3. Records showing the effects of ALKALOID (ALK) in various doses on blood pressure after propranolol (P) 1 mg/kg blockade for 5 min in anaesthetized rats.

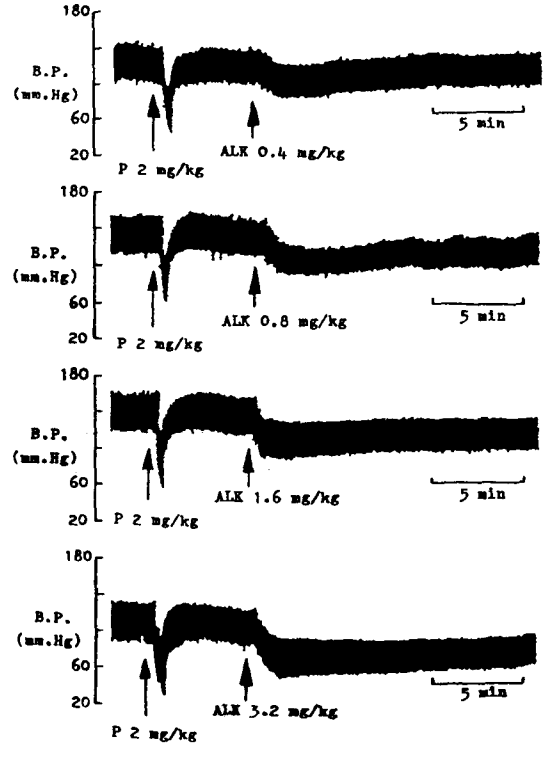

Figure 4. Records showing the effects of ALKALOID (ALK) in various doses on blood pressure after propranolol (P) 2 mg/kg blockade for 5 min in anaesthetized rats.

2. The effects of ß -adrenergic blocking drug (propranolol) on the hypotensive effect of ALKALOID.

As shown in Fig. 3 and 4 administration of propranolol 1.0 mg/kg b.w. (Fig. 3) and 2.0 mg/kg b.w. (Fig.4) 5 min before ALKALOID could not significantly reduce the hypotensive effect of ALKALOID. By observation, some of the initial reduction in blood pressure at the doses of 1.6 and 3.2 mg/kg b.w. showed a shorter duration of the peak hypotension than the effect of ALKALOID alone.

3. The effects of selective antihistaminic drug (H_1 and H_2 receptor antagonists) on the hypotensive effect of ALKALOID.

Administration of mepyramine 10 mg/kg b.w. plus cimetidine 20 mg/kg b.w. 2 min before ALKALOID showed no significant reduction in hypotensive effect of ALKALOID (Fig 5).

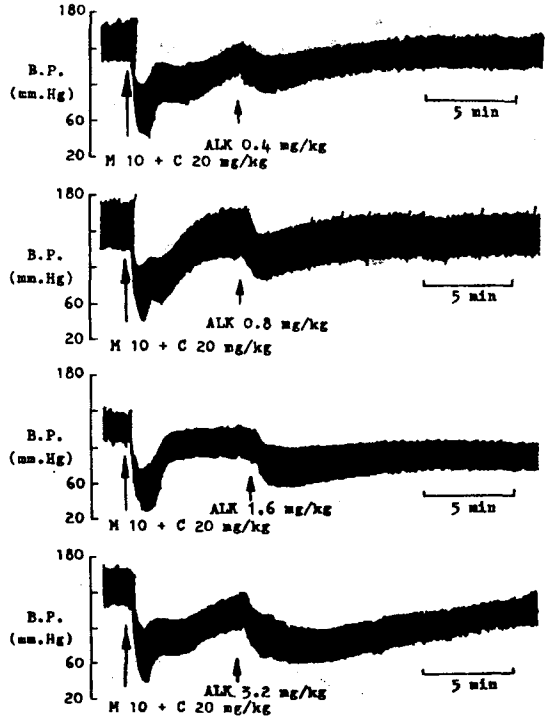

Figure 5. Records showing the effects of ALKALOID (ALK) in various doses on blood pressure after mepyramine (M) 10 mg/kg plus cimetidine (C) 20 mg/kg blockade for 5 min in anaesthetized rats.

4. The effects of cholinergic blocking drug (atropine) on the hypotensive effect of ALKALOID.

As shown in Fig. 6, prior administration of atropine 0.3 mg/kg b.w. 5 min before ALKALOID could partially reduce the hypotensive effect of ALKALOID at all doses significantly ($p<0.05$ in dose 0.4, 0.8 and 0.6 mg/kg b.w., $p<0.001$ in dose 3.2 mg/kg). The reduction in blood pressure showed a slight decrease which was sustained.

5. The effects of ganglionic blocking drug (hexamethonium) on the hypotensive effect of ALKALOID.

An intravenous infusion 3.5 mg/kg b.w. of hexamethonium caused a dramatic reduction in systemic blood pressure. This reduction gradually recovered, toward the control level within 15-20 min after administration. With a 3 min prior administration of hexamethonium 3.5 mg/kg b.w., the hypotensive effect of ALKALOID was variably reduced (fig. 7). However, the percentage changes of these reductions were relatively depended on the blood pressure at that period. The effects of ALKALOID 1.6 mg/kg b.w., at period of 3, 5 and 10 min after hexamethonium showed that hexamethonium could reduce the hypotensive effect of ALKALOID (Fig. 7). Again the percentage changes of these reductions were dependent on the pressure level at that period.

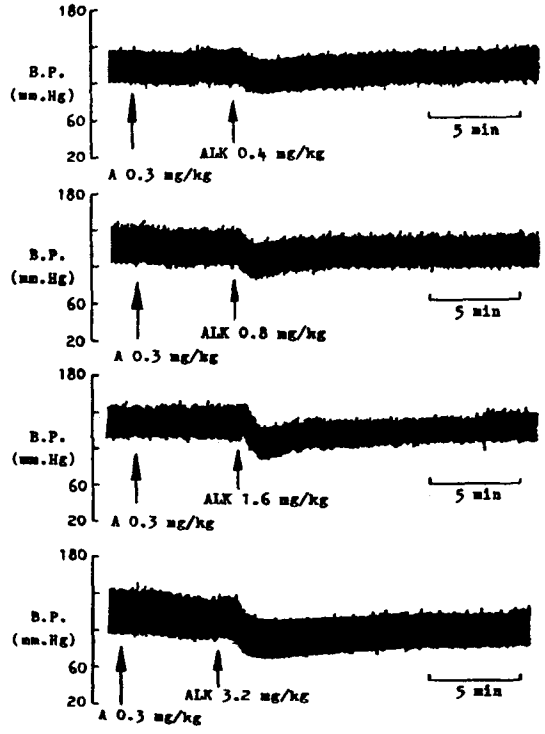

Figure 6. Records showing the effects of ALKALOID (ALK) in various doses on blood pressure after atropine (A) 0.3 mg/kg blockade for 5 min in anaesthetized rats.

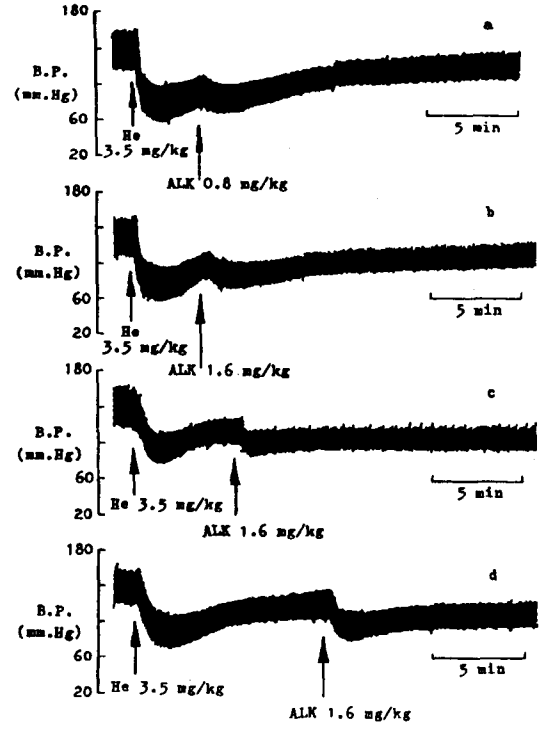

Figure 7. Records showing the effects of ALKALOID (ALK) on blood pressure after hexamethonium (He) 3.5 mg/kg blockade for 3 min (a and b), 5 min (c), 10 min (d) in anaesthetized rats.

6. The effects of ALKALOID on norepinephrine releasing sympathomimetic amine (tyammine).

Intravenous infusion of tyramine 0.3 mg/kg b.w. elicited an increase in the mean blood pressure. Administration of ALKALOID 3.2 mg/kg b.w. caused a dramatic reduction in systemicblood pressure which could be reversed immediately after by an infusion of tyramine (fig. 8). comparison of the effects of tyramine on mean blood pressure before and after ALKALOID blookade were not statistically significant.

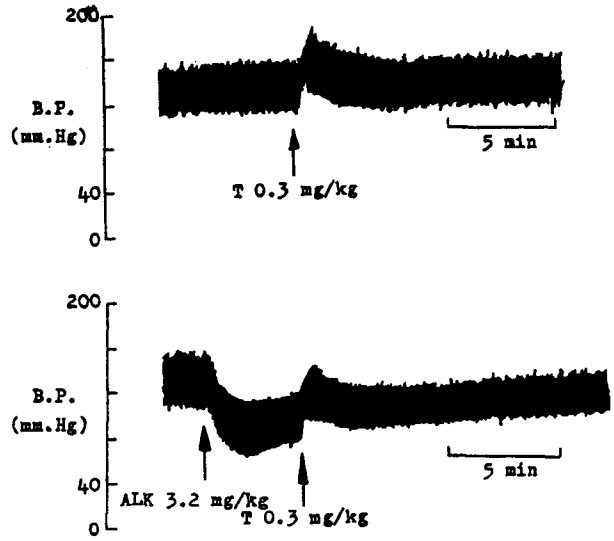

Figure 8. Records showing the effects of tyramine (T) 0.3 mg/kg on blood pressure before and after ALKALOID (ALK) 3.2 mg/kg blockade for 3 min in anaesthetized rats.

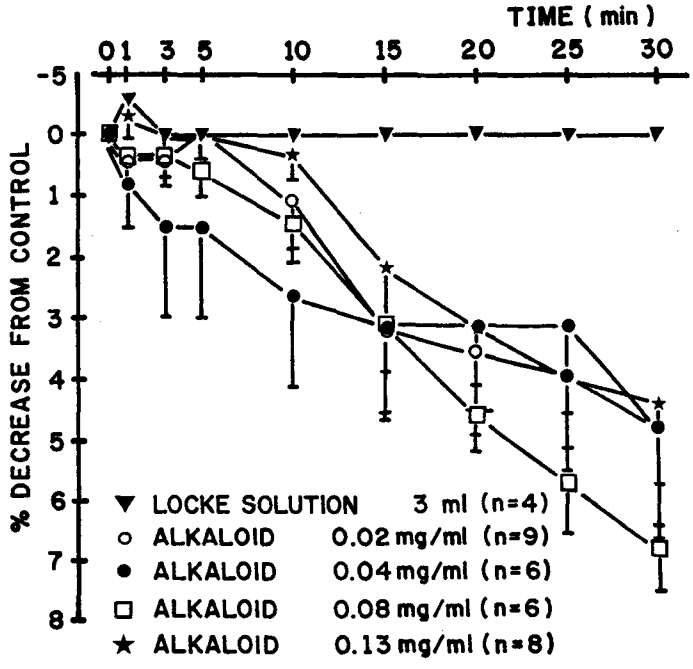

Figure 9. The concentration response curves for negative chronotropic effect of ALKALOID on isolated right atrial strips of the rats. Each point is the mean ± S.E.M. The ordinate scale is percent decrease in heart rate; the abscissa scale is the time in minute.

Isolated Rat Atrial Strips

1. The effects of ALKALOID on the isolated right atrial strips.

Contractile response of the right atria when exposed to various concentration of ALKALOID (0.02, 0.04, 0.08 and 0.13 mg/ml) were measured. As shown in Fig. 9, ALKALOID caused significant ($p<0.05$) negative chronotropic effect and the effect was dose-dependent. This negative chronotropic effect was not significantly inhibited by prior administration of atropine 0.3 ug/ml. ALKALOID 0.13 mg/ml induced cardiac arrhythmias in 3 out of 7 right atrial strips.

2. The effects of ALKALOID on the isolated left atrial strips.

ALKALOID (0.02, 0.04, 0.08 and 0.13 mg/ml) caused slight variable depression on myocardial contractile tension in electrically paced left atrial strips. At 30 min after administration, there was no difference between the maximum effects obtained from each dose of ALKALOID. (Fig 10).

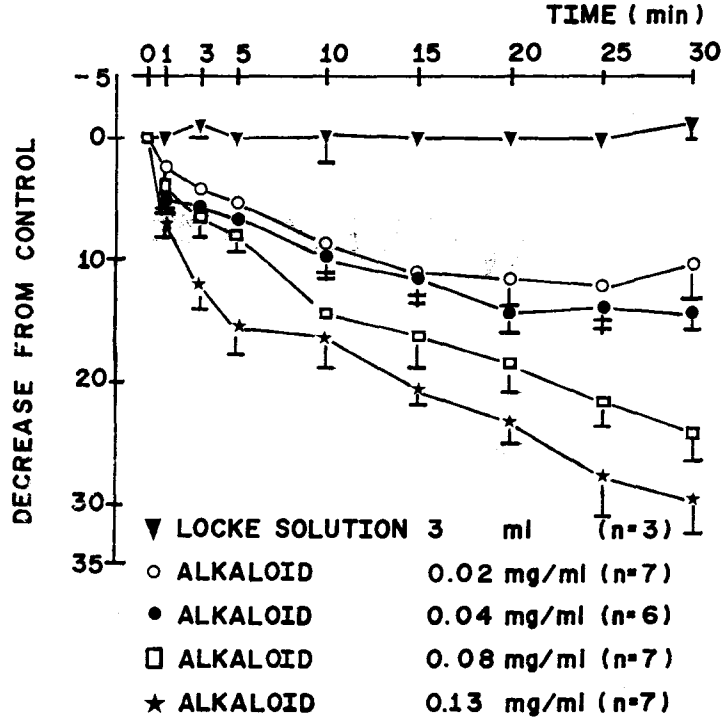

Figure 10. The effect of ALKALOID on inotropic response of the rats isolated left atrial strips. Each point is the mean ± S.E.M. The ordinate scale is percent decrease in contractile tension; the abscissa scale is the time in minute.

Discussion and Conclusion

The present study indicated that administration of 3α-dihydrocadambine (ALKALOID) resulted in a fall in systemic blood pressure. Low doses of ALKALOID (0.4, 0.8 and 1.6 mg/kg) reduced both systolic and diastolic blood pressures, whereas the highest dose (3.2 mg/kg) showed more initial reduction in diastolic than systolic blood pressure (Fig.2 Table 1). ALKALOID caused biphasic responses on the heart rate (Table 2). It produced a transient significant decrease in heart rate as well as the initial fall in blood pressure. These revealed the primary depression of ALKALOID on he heart. The effect of ALKALOID on isolated right atrial contraction rate of the rats showed dose-dependent depression (Fig. 9). It could be suggested that ALKALOID cause an initial direct depression on the heart. However, a sustained hypotensive effect may induce reflex mechanisms and cause secondary increase in heart rate.

Beta-adrenergic blocker such as propranolol could not inhibit the hypotensive effect of ALKALOID (Figs. 3,4). It could be suggested that the hypotensive action of ALKALOID does not mediate via beta-

adrenergic receptors. by the observation, it could be explained that propranolol may potentiate the hypotensive effect of ALKALOID. The hypotensive effect of ALKALOID may not be mediated via histaminic receptors, since mepyramine (H_1 -receptor antagonist) in combination with cimetidine (H_2 -receptor antagonist) can not block the hypotensive effect of ALKALOID (fig. 5). This combination of antagonists has been demonstrated to inhibit the hypotensive effect of histamine which mediated by H_1 and H_2 receptors completely.[4-7]

Cholinergic agonists or some other substances which act on muscarinic cholinergic receptors cause vasodilatation and decrease in cardiac rate and force of contraction.[9] The action of atropine is a competitive antagonism of acetylcholine at muscarinic cholinergic receptors.[9,10] From this study, the preadministration of atropin E (0.3 mg/kg) resulted in partial reduction of hypotensive effect of ALKALOID significantly (Fig. 6). It could be suggested that hypotensive effect of ALKALOID may partly mediate via muscarinic cholinergic receptors.

In order to determine the hypotensive effect of ALKALOID which may act through the hypothalamic or brain stem centers, ganglionic blocking drug was used to prevent the impulse from these centers. Hexamethonium which blocks the transmission of impulse from the preganglionic axon by occupying receptor sites at the postganglionic axon[11] was used. It was found that hexamethonium 3.5 mg/kg partially reduced the hypotensive effect of ALKALOID (Fig. 7). It would be suggested that ALKALOID may act somehow on central nervous system which resulted in hypotension.

ALKALOID may produce hypotensive effect by acting at sympathetic nerve terminal. In order to investigate this possible action, tyramine which releases norepinephrine from storage sites in sympathetic nerves was used. The response was therefore similar to those of norepinephrine[11,12]. Pretreatment with ALKALOID could not abolish the increase in blood pressure of tyramine (Fig. 8). This result indicated that the hypotensive effect of ALKALOID may not be due to the depletion of norepinephrine from storage sites in sympathetic nerve terminal.

In order to investigate the effects of ALKALOID on cardiac contractility, experiments were performed on isolated rat atria. It was found that ALKALOID exhibited dose-dependent negative chronotropic response (Fig. 9) and limited negative inotropic response (Fig.10). The negative chronotropic effect was not inhibited by prior administration of atropine. Therefore this effect of ALKALOID was independent of cholinergic mechanism.

In conclusion, it could be postulated that there are more than one mechanisms of ALKALOID which mediate a sustained reduction of blood pressure. The first is ALKALOID mediating via cholinergic receptors, while the second is ALKALOID acting somehow on central nervous system which finally resulted in reduction of blood pressure. The other, a direct action on vascular resistance may be suggested. However, more studies are required to elucidate the detail mechanism of ALKALOID on cardiovascular system.

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