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นิพนธ์ต้นฉบับ

Effect of 3α-dihydrocadambine isolated from wild cinchona leaves on blood pressure and heart rate in tree shrew.

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Sudsuang R, Vattanajun A, Kunluan P, Singhaniyom V, Boonsinsukh P. Effect of 3α-dihydrocadambine isolated from wild cinchona leaves on blood pressure and heart rate in tree shrew. Chula Med J 1995 Apr;39(4): 271-282

The effects of 3α-dihydrocadambine, indole glycosidic alkaloid (ALK), isolated from ***Anthocephalus chinensis*** *leaves on blood pressure (BP) and heart rate (HR) in tree shrews was investigated. The ALK was dissolved in 10% polyethylene glycol (PEG) in normal saline in preparation for intravenous injection (IV). It was found that 6.4 mg/kg B.W. was the optimal effective dose. In the case of intraventricular injection (VENT), 0.4 to 3.2 mg/kg B.W. were dissolved in 20% PEG in artificial cerebrospinal fluid (aCSF). It was found that after ALK injection, the BP and HR were significantly decreased when compared to a control group using both IV and VENT routes of administration. However, there was a difference in the pattern of hemodynamic change between the two routes of administration. In the IV injection group, the BP decreased immediately (about 20 sec after injection) while by VENT, there was a 20 min delay before the decreasing effect occurred. It was also found that placebo VENT injection of 20% PEG in aCSF evoked a significant BP change.*

We also observed a hypotensive effect of this agent during fastigial pressor response which was induced by fastigial nucleus stimulation. The hemodynamic changes in these groups were similar to the basal BP groups, when the same doses were compared.

From this study it may be postulated that this agent, 3α-dihydrocadambine, has an hypotensive effect which predominants peripheral action.

Key words: *Anthocephalus chinensis, Indole Glycoside, Blood Pressure, Heart rate, Tupaia glis.*

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ราตรี สุดทรวง, อนุสรา วัฒนจันทร์, พงษ์ศักดิ์ กรรณล้วน, วีระชัย สิงหะนิยม, ประโยชน์ บุญสินสุข. ผลของ 3 แอลฟา-ไดไฮโดรคาดามบีนที่แยกจากใบกระทุ่มใหญ่ต่อความดันเลือดและอัตราการเต้นของหัวใจในกระแต. จุฬาลงกรณ์เวชสาร 2538 เมษายน;39(4): 271-282

ได้ศึกษาผลของสาร 3 แอลฟา-ไดไฮโดรคาดามบีน ซึ่งเป็นอินโดลกลัยโคซิดิค อัลคาลอยด์ (ALK) ที่แยกจากใบของต้นกระทุ่มใหญ่ ต่อความดันเลือดและอัตราการเต้นของหัวใจในกระแต โดยการให้ ALK ที่ละลายใน 10% polyethylene glycol (PEG) ในน้ำเกลือในขนาดต่าง ๆ กัน ฉีดเข้าหลอดเลือดดำ ซึ่งพบว่าขนาดที่เหมาะสมมากที่สุดคือ 6.4 มิลลิกรัม/กิโลกรัม ของน้ำหนักตัว และให้ทาง ventricle ของสมอง (VENT) ขนาด 0.4 ถึง 3.2 มิลลิกรัม/กิโลกรัม ของน้ำหนักตัว ละลายใน 20 % PEG ในน้ำไขสันหลังเทียม (aCSF) พบว่าการให้ทั้งสองทางมีผลในการลดความดันเลือด และอัตราการเต้นของหัวใจ อย่างมีนัยสำคัญทางสถิติ ($p<0.05$) แต่อย่างไรก็ตาม ยังคงพบความแตกต่างของรูปแบบในการลดลงของความดันเลือดระหว่างสองทางที่ให้ โดยความดันเลือดจะลดลงทันที และลดลงมากที่สุดประมาณ 40 วินาทีหลังจากให้ ALK ทาง IV ในขณะที่ทาง VENT ความดันเลือดจะลดลงมากที่สุดหลังจากให้ ALK ประมาณ 20 นาที นอกจากนี้ยังคงพบว่าการให้ตัวทำละลาย 20% PEG ใน aCSF เพียงอย่างเดียวทาง VENT สามารถลดความดันเลือดได้อย่างมีนัยสำคัญทางสถิติ และเมื่อนำไปเปรียบเทียบกับกลุ่มที่ให้ตัวทำละลายร่วมกับ ALK ขนาดต่าง ๆ ทาง VENT ไม่พบว่ามีความแตกต่างอย่างมีนัยสำคัญทางสถิติ

จากการศึกษาในครั้งนี้ยังพบว่า การให้ ALK ทาง IV สามารถลด fastigial pressor response ที่เกิดจากการกระตุ้น fastigial nucleus ได้ แต่อย่างไรก็ตาม เมื่อนำมาเปรียบเทียบกับผลของ ALK ขนาดเท่ากันในระดับความดันปกติแล้ว ไม่พบว่ามีความแตกต่างกัน

จากการศึกษานี้สรุปได้ว่า สาร 3 แอลฟา-ไดไฮโดรคาดามบีน มีฤทธิ์ในการลดความดันเลือด โดยออกฤทธิ์เด่นในส่วนของระบบประสาทส่วนปลายมากกว่าระบบประสาทส่วนกลาง

Anthocephalus chinensis Achille Richard is known in Thai as "Kra-thum"(1) in English as "wild cinchona" and in Hindi as "Kadamb".(2) A decoction of the leaves has been used as a gargle in cases of aphthae and stomatitis. The compound fruit is used as an astringent in cases of diarrhoea. By means of alumina column chromatography, an indole glycosidic alkaloid was isolated from the leaves.(3) The physical and chemical properties and spectroscopic evidence have shown that it is 3α-dihydrocadambine (Figure 1).

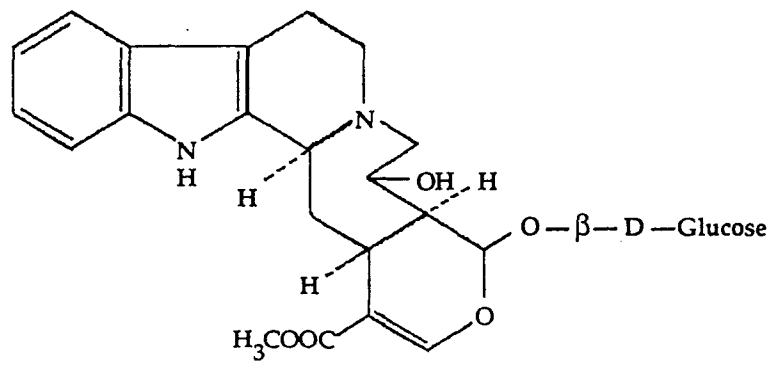

Figure 1. The structure of 3α-dihydrocadambine ($C_{27}H_{34}N_2O_{10}$).

Endo et al.(4) was the first to show that this agent, extracted from the Uncaria hooks, elicited strong and long-lasting hypotension after injection into rats. Later, Aroonsang(5) investigated the pharmacological actions of this alkaloid (ALK) in rats and reported that the ALK caused hypotensive effects which were accompanied by biphasic initial reductions followed by a small increase in the heart rate. However, the mechanism of ALK effects on the cardiovascular system was not clear.

The aim of our study was to reconfirm the effects of 3α-dihydrocadambine on blood pressure and heart rate in tree shrew *(Tupaia glis)*, and to clarify the site of action of ALK on the central and peripheral nervous systems. Furthermore, we also wished to determine whether the powerful hypertensive responses from stimulation of fastigial nucleus (FN) and the hypotensive effects of ALK also mediated from the same mechanism.

Materials and Methods

1. Extraction and purification of 3α-dihydrocadambine (ALK)(3)

The dried coarsely powdered leaves of *A. chinensis* were macerated with 95% ethyl alcohol for 10-15 days and filtered. The filtrate was concentrated under reduced pressure to a syrupy mass and then evaporated, mixed with glacial acetic acid and then filtrated. The filtered acid extract was made to alkaline and extracted with chloroform. The chloroform extract was dried and concentrated to yield a syrupy crude based. The crude base was purified by aluminium oxide column chromatography. Identification of the ALK compound was confirmed by UV, IR and NMR in comparison with the standard sample.

2. Animal preparation

Sixty tree shrews (Tupaia glis) of either sex weighing between 100-150 g each were used. The animals were anaesthetized with sodium pentobarbital 25 mg/kg given intraperitoneally. Supplementary doses of the same drug were given whenever necessary to maintain the anaesthesia. Drugs were administered intravenously through a catheter inserted into a femoral vein of which the tip of the catheter was advanced toward the heart. Arterial blood pressure (BP) and heart rate (HR) were monitored from a femoral artery with a pressure transducer connected to a Harvard Universal oscillograph. The animals were placed in a stereotaxic apparatus for rats (Narishige SR-G) with the bite-bar set 5.0 mm below the ear bars. The brain was exposed through a burr hole in the occipital bone and with the underlying dura removed. The animals were divided into 3 groups as the following

Group 1. To study the effects of intravenous admistration of ALK, six dosages of ALK were used in 30 tree shrews.

Group 2. Effects of intracerebroventricular ALK on BP and HR were studied in 20 tree shrews. In this group, 4 doses of ALK were used.

Group 3. To study the effects of intravenous injection of ALK during FN stimulation, 2 dosages of ALK were used in 10 tree shrews.

3. ALK administration

Due to the slight solubility of the ALK, it was dissolved in 10% polyethylene glycol (PEG) in normal saline solution (NSS) for intravenous administration (IV), and in 20% PEG in artificial cerebrospinalfluid (aCSF) for intraventricular administration (VENT). For IV, ALK at 0.8, 1.6, 3.2, 6.4, 16.0 and 24.0 mg/kg BW doses in 10% PEG in NSS in 0.3 ml volumes were injected. For VENT, ALK at 0.4, 0.8, 1.6 and 3.2 mg/kg BW doses in 20% PEG aCSF in 10 µl volumes were slowly injected into the lateral cerebral ventricle through a microsyringe. At autopsy, the placement of the microsyringe and the ALK distribution were verified by slow injection of alcian blue dye through the needle to outline the 3rd and 4th ventricles as well as portions of the lateral ventricle and the spinal canal.

4. Electrical stimulation of the fastigial nucleus (FN)

The FN was stimulated cathodally through a monopolar microelectrode. The microelectrode was mounted on a stereotaxic micromanipulator and lowered through the cerebellum into the FN to localize the most sensitive sites from which stimulation could elicit a maximal elevation of BP and tachycardia. The electrode was then left in place at that most active site. FN stimulaton was continued for 30-40 sec. During this phase, the BP was allowed to stabilize. At the end of this phase, while stimulation continued, the injection of ALK was begun. At the end of the experiment the site of FN stimulation was confirmed by histological techniques.

5. Analysis of Data

The data were analyzed for statistical significance with either student's paired t-test for comparing between control and treatment in each group or student's unpaired t-test when comparing between groups difference. In multiple comparisons, the data were evaluated by analysis of varience (ANOVA). Values are expressed as mean ± S.E.M. A p value < 0.05 was considered to indicate statistical significance.

Results

1. Effect of intravenous ALK on BP and HR

An IV injection of ALK caused significant dose dependent decreases in both systolic and diastolic blood pressure. The higher the doses, the longer the depression was observed (figure 2). In terms of time-action, the BP began to fall immediatly after injection and the peaks were always reached by 40 sec after injection. These effects were still evident after about 30 minutes and then they returned to baseline. As shown in table 1, heart rate was also decreased initially by IV ALK injection and then followed by a HR increase when the maximum hypotensive effect was observed. However, a statistically significant decrease was found in 3.2 and 6.4 mg/kg BW dose at initial hypotensive effect. Where as at the higher, 16.0 mg/kg BW dose the heart rate increased significantly from that of the control.

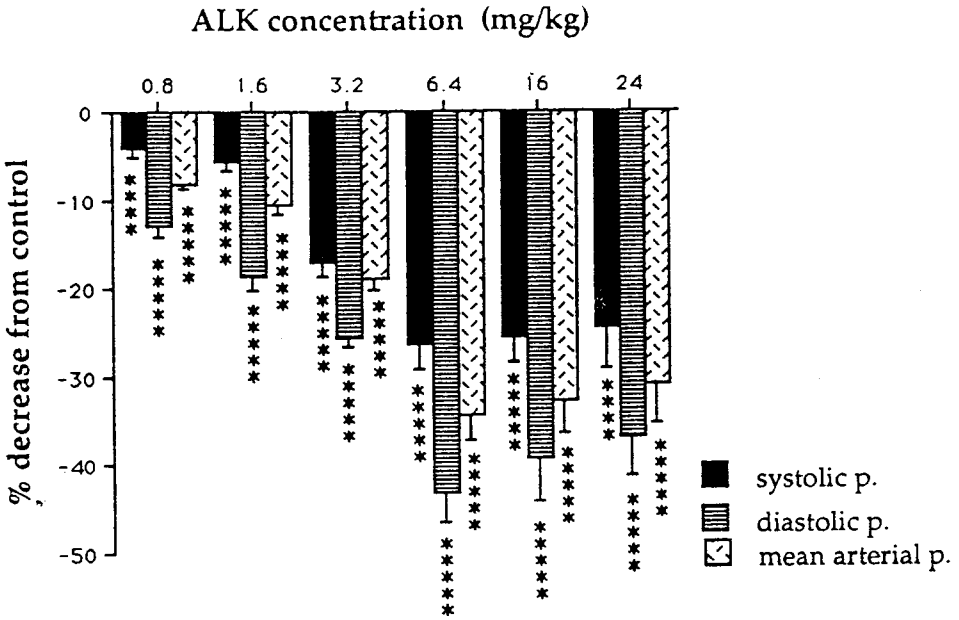

Figure 2. Dose-dependent decreases in systolic, diastolic and mean arterial pressure produced by intravenous administration of 3α-dihydrocadambine (ALK) in anaesthetized tree shrews (n=5). Bar graphs represent mean ± SEM.
**** $p<0.005$, ***** $p<0.0005$.

Table 1. The effect of intravenous ALK in various doses on heart rate in anaesthetized tree shrews (n=5). Values are expressed as mean ± SEM. * p<0.05.

	Dose of Heart rate (Beats/min)				
ALK(mg/kg)	Control	Initial	% Change	Maximum	% Change
20 % PEG	315.00 ± 10.25	312.00 ± 14.69	-1.08 ± 1.81	329.50 ± 20.65	5.05 ± 3.28
0.8	370.00 ± 50.00	358.50 ± 43.50	-2.95 ± 1.35	372.00 ± 42.00	0.87 ± 2.27
1.6	301.50 ± 13.50	293.50 ± 11.50	-2.65 ± 0.55	299.00 ± 11.00	-0.79 ± 0.79
3.2	292.86 ± 24.46	284.57 ± 24.56	-2.97 ± 0.68*	297.43 ± 24.73	2.27 ± 0.97*
6.4	370.00 ± 28.57	361.20 ± 27.52	-2.33 ± 0.96*	362.40 ± 22.01	-1.15 ± 2.57
16.0	402.00 ± 36.49	412.00 ± 36.72	2.53 ± 0.57*	422.00 ± 34.87	5.14 ± 0.96*
24.0	388.00 ± 26.46	398.00 ± 26.00	2.62 ± 1.08	413.00 ± 21.17	6.46 ± 2.82

INITIAL = at initial hypotensive effect of ALK,
MAXIMUM = maximum hypotensive effect of ALK.

2. Effect of intracerebroventricular ALK on BP and HR

As shown in figure 3, intraventricular injection of ALK significantly decreased both the systolic and diastolic blood pressures. The peak effect of ALK was observed about 20 min after VENT administration. However, the placebo VENT injection of 20% PEG aCSF itself also evoked significant reductions in systolic and diastolic blood pressure, while the peak effect was observed about 10 minutes after injection. The heart rates were decreased (table 2) and the peak was reached about 20 min after VENT ALK injection.

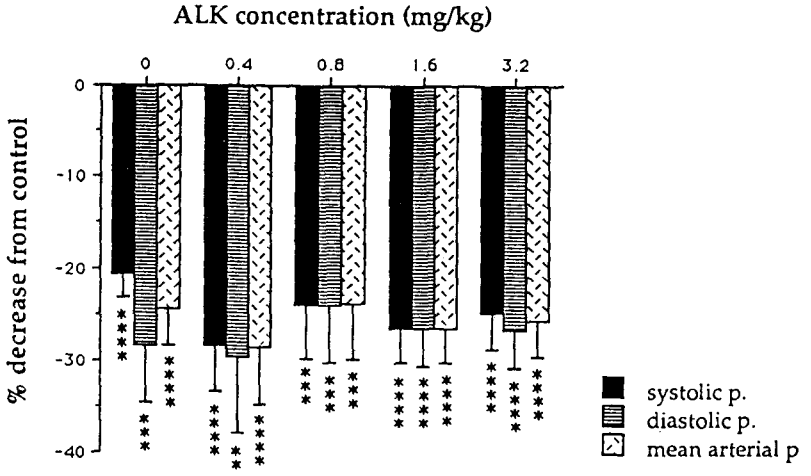

Figure 3. The mean of percent decreases in systolic, diastolic and mean arterial pressure produced by intraventricular administration of 3α-dihydrocadambine (ALK) in anaesthetized tree shrews (n=5). Bar graphs represent mean ± SEM.
** p<0.025, *** p<0.01, **** p<0.005.

Table 2. The absolute values and the percent changes of heart rate before and after intraventricular administration in various doses of ALK. Values are expressed as mean ± SEM. * $p<0.05$.

Dose of ALK(mg/kg)	Heart rate (Beats/min)						
	Control	5 min	% Change	15 min	% Change	25 min	% Change
20 % PEG	326.66±17.64	318.00± 9.17	-2.29±3.96	332.00± 1.73	2.33 ±6.49	344.00±4.00	10.47±2.83*
0.4	368.00±26.23	336.00±30.19	-8.88±2.49*	324.00±30.19	-12.22±1.83*	328.67±28.39	-10.87±1.50*
0.8	392.00±32.00	350.67±18.52	-10.06±3.64	324.00± 6.93	-16.54±4.98*	327.33±16.34	-15.16±9.14
1.6	365.00± 5.00	329.00±19.00	-9.76±6.46	297.00±27.00	-18.52±8.52	302.00±22.00	-17.16±7.16
3.2	380.00±10.00	320.00±10.00	-15.80±0.42*	285.00±15.00	-25.06±1.98*	305.00±25.00	-19.85±4.47*

3. The hypotensive effect of IV ALK during FN stimulation

FN stimulation consisted of 0.1 ms duration pulses at a frequency of 50 Hz and a stimulus current of 0.15 mA. This resulted in significant increases in both systolic and diastolic blood pressure (figure 4) which began to elevate within 2-3 seconds of the onset of stimulus, and then rose rapidly to a peak. During the continued stimulation, the BP was sustained, but it rapidly declined to the control level after the stimulation was terminated. The IV injection of ALK when the BP was sustained during FN stimulation showed statistically significant decreases after 3.2 and 6.4 mg/kg doses (figure 4, table 3). Furthermore, when comparing the hypotensive effect of the ALK during basal BP to that during fastigial pressor response at the same dose, there were no significant systemic change between them except in systolic blood pressure of the dose 3.2 mg/kg (figure 5).

Discussion and Conclusion

The results of the present study demonstrated that 3-α dihydrocadambine (ALK) produced a dose-dependent reduction of arterial blood pressure in tree shrews by both routes of administration. However, the IV injection decreased the BP immediately (about 20 sec after injection) while by VENT, a 20 min delayed decreasing effect occurred. It was also found that the placebo VENT injection of 20% PEG in aCSF itself evoked a significant BP change. The low solubility property of ALK required the use of PEG which is heat stable, has excellent coexisting water solubility, is chemically inert and has a minimal tendency to denature biomacromolecules.[6] Based on the findings of Friedman,[7] PEG had no effect on blood pressure in dogs and rabbits when administered intravenously, and he concluded that PEG is a suitable vehicle for IV administration. The introduction of ALK directly into the cerebral ventricular system could be used to examine the central action of the substance.[8] Furthermore, Bolme et al.,[9] suggested that the drugs, when given intraventriculary at doses that have no effect systemically, evoked very similar systemic changes as when given via IV. This supported the belief that it has a predominant central action. Therefore, when comparing the two routes of administration, the central action of any chemical substance could be observed more

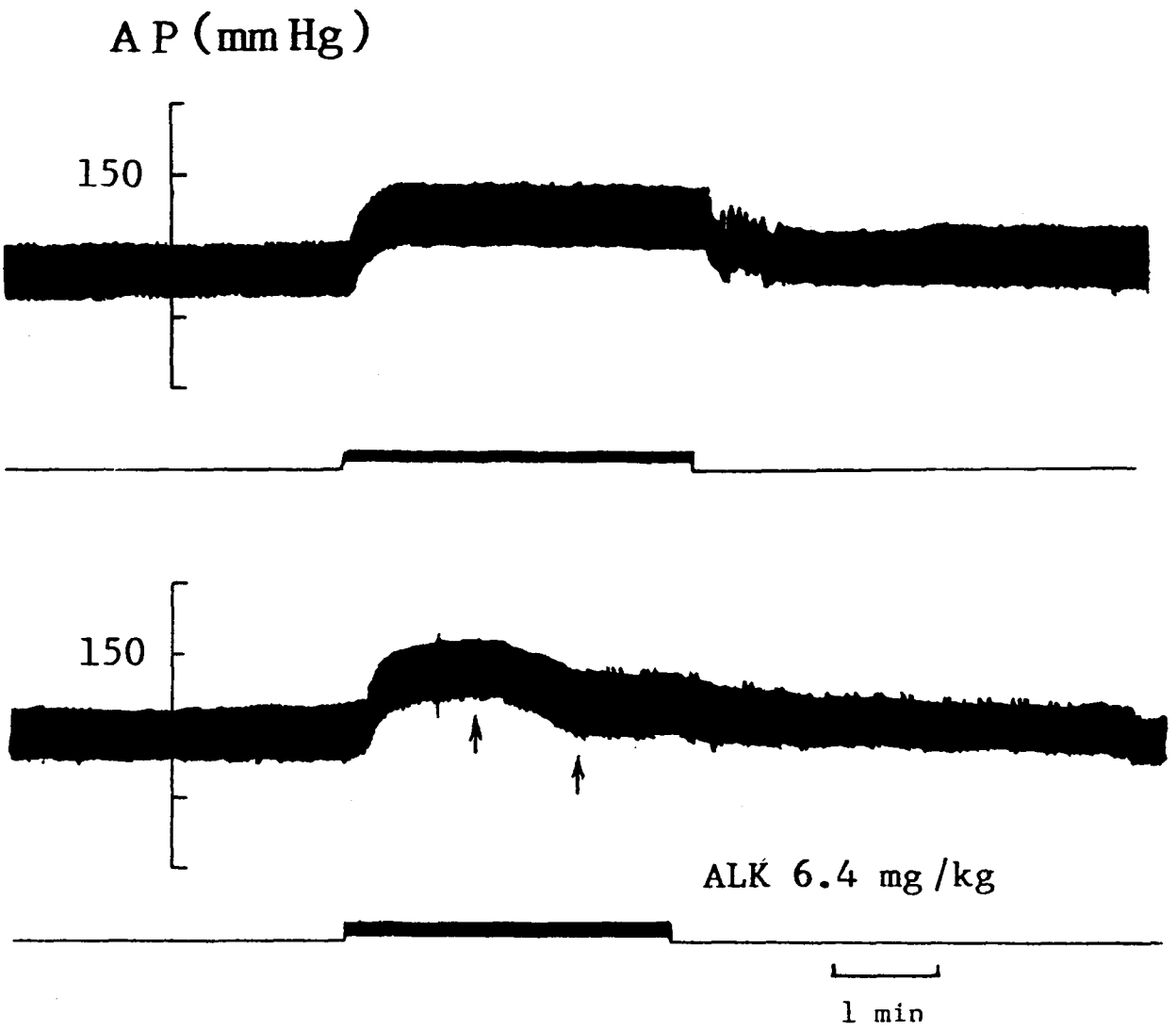

Figure 4. Records showing fastigial pressor response in anaesthetized tree shrews (top panel) and its attenuation by intravenous ALK 6.4 mg/kg (bottom panel).

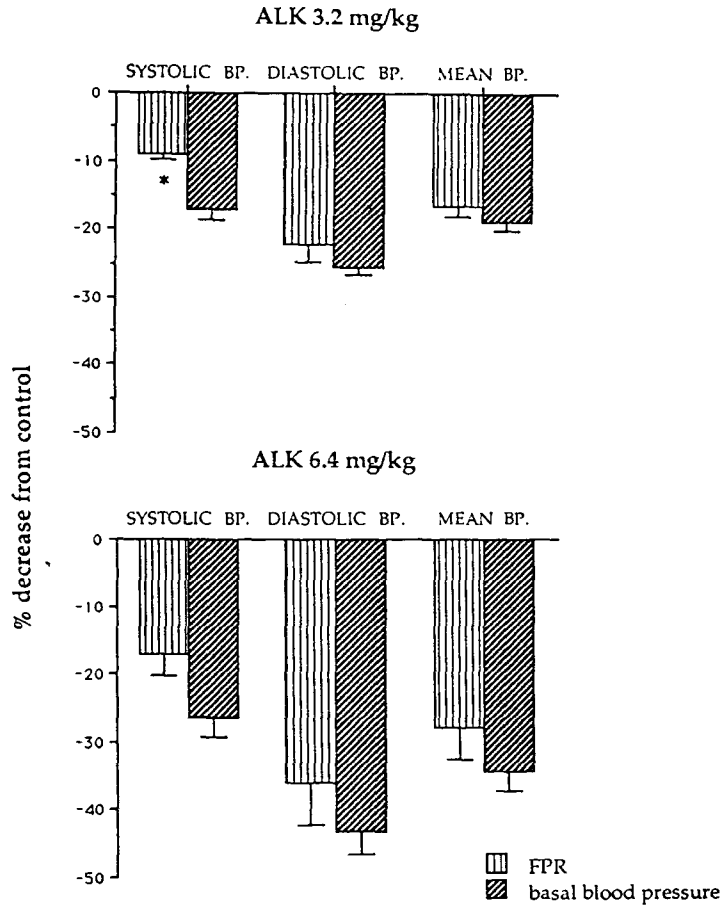

Figure 5. Comparison of the effect of intravenous ALK on basal blood pressure and during fastigial pressure response (FPR) at doses 3.2 mg/kg (top panel) and 6.4 mg/kg (bottom panel) in anaesthetized tree shrews (n=5). Bar graphs represent mean ± SEM. * $p<0.05$.

Table 3. Comparison of the effect of fastigial nucleus stimulation on arterial pressure and the effect of intravenous alkaloid 3.2 and 6.4 mg/kg during fastigial pressor responses. Values are expressed as mean ± SEM.

Dose of	Number of	Arterial pressure (mmHg)						
ALK(mg/kg)	animal	Control	Stimulate	Increase	% Increase	After ALK	Decrease	% Decrease
Systolic P.								
3.2	5	113.60±14.55	142.00±17.65	28.40±8.19	27.68± 9.98*	128.80±15.00	13.20±2.71	8.98±0.75****
6.4	5	124.00± 8.67	156.60± 4.28	32.60±6.12	27.96± 6.68**	130.20± 7.78	24.60±4.63	17.06±3.27***
Diastolic P.								
3.2	5	70.60±10.13	93.00± 6.59	22.40±4.15	42.40±19.21*	72.60± 6.73	20.40±2.01	22.34±2.48****
6.4	5	77.40± 2.42	108.40± 5.66	31.00±7.61	41.02±10.38**	70.20± 9.55	38.20±5.67	36.08±6.08***
MAP								
3.2	5	84.94±11.35	109.32± 9.79	24.38±4.91	35.18±14.05*	91.34± 9.14	17.98±1.45	16.70±1.34****
6.4	5	92.96± 4.35	124.46± 3.75	31.50±6.78	35.32± 8.84**	90.20± 8.16	34.26±5.30	27.94±4.82***

* $p<0.5$, ** $p<0.01$, significant increase from normal control,*** $p<0.005$,

**** $p<0.0005$, significant decrease from stimulate control.

rapidly and more strongly when it was administered directly into the cerebroventricular system.

The hypotensive effect of IV injection of ALK in this study showed that the most effective dose was 6.4 mg/kg BW while the higher doses of 16 and 24 mg/kg caused a decreasing effect that was similar to the 6.4 mg/kg dose (figure 2). Regarding the heart rate, it was also reduced at the initial hypotensive effect of ALK and significant reductions were observed at 3.2 and 6.4 mg/kg BW doses (table 1). After that, when the peak of the effect was reached the heart rate was increasing. This suggests that part of the initial bradycardia was caused by direct depression on the heart,[5] thus the sustained hypotensive effect of ALK may induce the baroreceptor reflex and cause secondary increases in heart rate.[10] However, at the higher 16 and 24 mg/kg doses, the heart rate was elevated at the initial hypotensive effect of ALK and this may be due to the baroreceptor mechanism. The significant increase in heart rate was observed only in 16 mg/kg doses but not in 24 mg/kg doses, and it may be due to the greater standard deviation.

The placebo injection of 10% PEG in NSS intravenously evoked no significant systemic change when compared to the base line control before injection or control NSS itself. By VENT a significant decrease in BP occurred after injection of 20% PEG in aCSF, whereas the normal aCSF itself evoked no significant change. The ability of the more concentrated PEG solution to evoke significant systemic changes may be related to increased osmotic pressure. 20% PEG can be considered to be a hyperosmotic or hypertonic solution when compared to the nerve axon and the myelin.[11] Benson et al.[11] reported that 20% PEG caused mild depression of the compound action potential in rabbit nerves. In this study, 20% PEG was administered directly to the cerebral ventricular system which was closely related to the areas controlling the cardiovascular central nervous system. This may lead to shrinkage of nerve axons and myelin, and reduce the ion permeability, thereby causing the ultimate result of changeable of the BP.

Both IV and VENT administration of ALK significantly lowered BP (figure 2, 3), but there was a difference in time-action. The IV injection decreased the BP immediately by direct peripheral action. The VENT group showed the delayed decreasing effect of the ALK which may derive from the decreasing effect of the solvent solution plus the diffusion of ALK from the cerebral ventricular system into the peripheral part. However, there was no significant difference in multiple comparisons between the 20% PEG in aCSF group and various doses of ALK in 20% PEG groups. This used analysis of variences methods. Therefore, it maybe postulated that most of the decreasing effects of VENT group resulting from the solvent solution, 20% PEG in aCSF. In addition, the evidence from injection of alcian blue dye showed that the ALK was distributed through the 4^{th} ventricle which the areas nearby situated the vasomotor center control of BP.[10] Thus, if the ALK has central action, the direct injection of ALK into VENT caused, the most rapid and strong effect to be observed.

To test the hypothesis that the hypotensive effect of ALK and the powerful hypertensive response from stimulation of FN mediated from

the same mechanism, the hypotensive effect of the ALK was elicited under the FN stimulation, as shown in figure 4 and table 3. Anatomical studies showed that reciprocal connections exist between FN and two medullary nuclei implicated in baroreflex activity, the parasolitary nucleus of tractus solitarius and the nucleus paramedian reticularis which results in widespread activation of the sympathetic nervous system.[12,13] In this study, during fastigial pressor responses IV ALK significantly reduced the BP (table 3). However, when comparing these data to the data from basal blood pressure groups at the same doses, there were no statistically significant differences, except in the systolic BP parameter at 3.2 mg/kg doses (figure 5). This means that the fall in BP produced by the ALK was not being interfered with by the rise in BP during FN stimulation. These results indicated that the hypotensive effect of ALK and the fastigial pressor responses may not be exerted through the same mechanism.

In conclusion, the present investigation demonstrated that ALK, 3-α dihydrocadambine caused a dose-dependent reduction in BP in tree shrews. This action was not in the central nervous system but predominantly occurred in the peripheral. In addition, the action of ALK may not be mediated via the 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 ALK. However, more detailed studies about the hypotensive effect mechanism of ALK are required.

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