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EVIDENCE FOR A ROLE OF INHIBITION OF SYMPATHETIC NEUROTRANSMISSION IN THE CARDIOVASCULAR EFFECTS OF INTRAVENOUSLY ADMINISTERED VERAPAMIL

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ABSTRACT

The effects of intravenous administration of verapamil, nifedipine and diltiazem on sympathetic stimulation-induced increase in heart rate (HR) and blood pressure (BP) have been investigated in chloralose-anaesthetized and artificially - ventilated cats. Verapamil (300 µg kg^{-1}i.v.) produced a significant inhibition of sympathetically-induced tachycardia and pressor responses. The same dose of verapamil did not significantly alter adrenaline (2 µg kg^{-1}i.v.) induced increase in HR and BP. In contrast, neither the sympathetically-induced nor the adrenaline-induced pressor and tachycardiac responses were significantly affected by nifedipine or diltiazem. These results demonstrate that peripherally administered verapamil but not nifedipine and diltiazemcan inhibit cardiovascular sympathetic neurotransmission and this can possibly contribute to its effects on HR and BP.

Keywords : verapamil, diltiazem, nifedipine.calcium channel blockers, heart rate, blood pressure, sympathetic neurotransmission.

I. INTRODUCTION

Calcium channel blockers (CCBs) have been found to be of benefit in several clinical conditions such as migraine [1], convulsive disorders [2,3], behavioral states and experimental behavioral syndromes [4-6]. The targets of CCB actions are thus not restricted to cardiac and vascular smooth muscles but also include neural structures. To effectively alter neural and behavioral states it is necessary that the end result of CCB actions should in some way affect the availability of neurotransmitters or the efficiency of their actions on the respective receptors. Among the number of evidences which suggest that CCBs may affect neuronal functions are the presence of both L and N type channels on neurons [7], presence of CCB binding sites in CNS |3. 8, 9|, their interaction with the autonomic nervous system [10-12] and experimental evidences for CCB induced alterations of neuronal release of neurotransmitters in vitro[4. 13-17]. We have already shown that verapamil produces a functional inhibition of noradrenergic neurotransmission in vitro in the cat nictitating membrane [18]]. Since sympathetic tone is an important determinant of cardiovascular functions any alteration in this can lead to changes in heart rate and blood pressure which could possibly contribute to the cardiovascular effects of CCBs. The present investigation was undertaken to test whether CCBs also affect the sympathetic influences on cardiovascular functions in intact animals.

II. MATERIALS AND METHODS

The study was conducted in healthy young adult cats of either sex weighing between 2.5 and 4.0 kg, anaesthetized with α-chloralose (80 mg kg^{-1}i.v.) and maintained on artificial respiration throughout the experiment. A femoral vein and artery were cannulated with polyethylene catheters. The venous catheter was used for administration of drugs and the arterial catheter was connected through a pressure transducer (p23dB) to one channel of a polygraph (Medicare. India) for recording the blood pressure (BP). Heart rate (HR) was calculated from the pressure pulse tracings. Bilateral mid cervical vagotomy was performed in all experiments to eliminate reflex vagal activation during sympathetic stimulation.

A stainless steel stimulating electrode was positioned along the left side of the C4 to T3 vertebrae for stimulating the sympathetic chain on that side. The ground electrode was placed under the skin on the left side of the chest. The stimulation parameters were 5 V, 50 Hz, 1 msx10 s. Stimulation of the sympathetic chain in this manner was found to elicit reproducible and consistent elevations of BP and HR for up to 3 hr (maximum observation period). Drug

treatment was commenced in all the experiments only after obtaining at least three consistent pressor and tachycardiac responses of comparable magnitude at 5 min intervals. A minimum of 1 h was allowed for stabilization of HR and BP after completion of surgical procedures and placement of electrodes, before commencing drug treatment.

Not more than two doses of any agent were administered per animal and an interval of at least 60 min was allowed between complete recovery from the effect of the first and administration of the second dose.

The changes in HR and BP induced by sympathetic .stimulation were recorded before and at 2. 5. 15. 30 and 60 min after administration of the drugs. The means and standard errors were calculated for the observations at different time intervals. Statistical significance of the differences between the means of different groups were determined by comparing the post-treatment data at different time intervals with their respective pre-treatment (control) values by employing Student's t-test for paired data.

In one set of experiments pressor and tachycardiac responses to intravenous administration of adrenaline (2 µg kg^{-1}) before and after treatment with calcium channel blockers was recorded. The effects of CCBs on adrenaline were compared with those obtained with sympathetic stimulation.

The following drugs were used verapamil hydrochloride (German Remedies. India) nifedipine (Merck.India), diltiazem hydrochloride (Torrent laboratories.India), adrenaline bitartrate (Loba. India). Verapamil and diltiazem were dissolved in normal (0.9% w/v) saline. Nifedipine was first dissolved in 95% alcohol and then diluted with normal saline to the required strength. Doses of the drugs mentioned in the text refer to their salts. All drug solutions were prepared fresh for each experiment and nifedipine was stored in absolute darkness, till just prior to injection. The doses selected were those which have been previously found by us to be optimal for eliciting cardiovascular effects in cats [12].

III. RESULTS

Following vagotomy the HR and BP of the cats stabilized between 150 and 180 beats per minutes (bpm) and 140 to 170mmHg respectively. The initial responses obtained with sympathetic stimulation were variable but these became consistent and reproducible within 30-60 min after surgical procedures. Only when a minimum of three responses of comparable magnitude 5 min apart, were obtained, was the experiment continued further. The doses of CCBs employed produced alterations in resting HR and BP which were steep immediately after administration but recovered substantially within 5 min and with none of the three CCBs used did the resting BP settle at more than 20 mmHg below the control levels. Likewise, the resting HR did not show a decrease of more than 12 to 18 bpm below the control levels.

The sympathetic – stimulation – induced changes in HR and BP before and after administration of CCBs are given in table 1A and B.

Table 1 Effect of IV CCBs on sympathetic – stimulation – induced tachycardia and increase in BP
(A)

Drug	Dose	Mean increase in HR (bpm±se)					
		Control	5 min	15 min	30 min	60 min	n
Verapamil	300 µg kg^{-1}	25.7±2.1 (n=7)	-6.5*** ±5.1	3.0** ±3.2	16.5* ±4.5	16.5 ±7.7	4
Diltiazem	300 µg kg^{-1}	23.3±1.9(n=10)	17.2 ±4.4	19.6 ±4.0	24.4 ±3.1	28.0 ±5.0	5
Nifedipine	10 µg kg^{-1}	23.3±1.3(n=10)	18.4 ±2.0	19.6 ±2.1	19.6 ±3.1	22.0 ±3.5	5

(B)

Drug	Dose	Mean increase in BP (mmHg±se)					
		Control	5 min	15 min	30 min	60 min	n
Verapamil	300 µg kg^{-1}	21.3±2.3(n=7)	12.3*±3.3	8.5*±3.9	7.3*±5.6	5.7***± 0.6	4
Diltiazem	300 µg kg^{-1}	20.0±3.9(n=10)	27.3±5.6	32.3±6.7	23.3±8.6	28.3±7.4	5
Nifedipine	10 µg kg^{-1}	22.8±3.1(n=10)	12.4*±2.3	14.2±2.3	14.2±3.4	15.4±2.6	-5

Verapamil (300 µg kg^{-1} i. v.) produced a marked suppression of stimulation induced tachycardia and pressor responses within 5 min. In this group, the mean increases in HR and BP following sympathetic stimulation was 25.7±2.1 bpm and 21.3±2.3 mmHg respectively abolished and instead a bradycardia was observed in two cases, following sympathetic stimulation. Significant suppression of the tachycardiac response persisted for 30 min and recovery was seen at 60 min. The inhibitory effect of verapamil on the sympathetic – stimulation – induced pressor response was more prolonged and a significant suppression persisted throughout the 60 min post – injection observation period.

Diltiazem (300 µg kg^{-1} i. v.) and nifedipine (10 µg kg^{-1} i. v.) however, produced only a mild statistically insignificant inhibition of the stimulation induced tachycardia. The pressor response was not affected by diltiazem but was mildly inhibited by nifedipine being statistically significant only during the first 5 min and recovering to statistically insignificant levels thereafter.

IV. DISCUSSION

There is now sufficient evidence that CCBs affect CNS functions [1-6, 20]. Their ability to alter behavioural function, interaction with autonomic nervous system [10-12] effectiveness in certain behavioural and convulsive states [2, 3] all point towards an action of CCBs on neuronal tissues. Coupled with this is the evidence from in vitro studies that CCBs alter release of neurotransmitters from neurons [4. 13 – 17]. We have earlier provided evidence for an inhibition of sympathetic neurotransmission by intravenously administered CCBs in the eat nictitating membrane [18]. The results of the present study demonstrate that a similar inhibition of sympathetic influences on cardiovascular functions also occurs after i. v. administration of verapamil.

Table – 2 Effect of IV administration of CCBs on adrenaline (2 µg kg^{-1} i. v.) induced tachycardia and increase in BP

(A)

Drug	Dose	Mean increase in HR (bpm±se)					
		Control	5 min	15 min	30 min	60 min	n
Verapamil	300 µg kg^{-1}	30.0±4.1 (n=10)	25.0±5.0	23.0±2.9	21.0±3.0	21.0±3.7	6
Diltiazem	300 µg kg^{-1}	21.0±2.2 (n=9)	17.3±5.3	20.3±5.7	18.8±4.3	23.3±3.9	4
Nifedipine	10 µg kg^{-1}	25.2±2.9 (n=9)	25.2±4.4	26.4±3.6	27.6±5.6	27.6±6.2	5

(B)

Drug	Dose	Mean increase in BP (mmHg±se)					
		Control	5 min	15 min	30 min	60 min	n
Verapamil	300 µg kg^{-1}	67.6±7.8 (n=10)	70.7±15.8	64.5±11.6	56.8±15.2	59.8±14.4	6
Diltiazem	300 µg kg^{-1}	75.7±9.2 (n=9)	69.5±11.7	78.8±7.5	81.3±12.2	76.5±11.6	4
Nifedipine	10 µg kg^{-1}	63.5±7.5 (n=10)	64.8±8.6	68.4±11.9	68.0±12.8	70.6±14.0	5

The model chosen in the present study was similar to that described by Szaboet. Al. [19].One representative each of the three major classes of CC'B.s VIA phenyl alkyl amine (verapamil) dihydropyrtdsne (nifedipine) and benzothiazepine (diltiazem), was chosen. The magnitude of decrease in BP and HR produced by these agents in the present study compared favourably with-our earlier observations. Sympathetic-stimulation-induced tachycardia andpressor responses were significantly inhibited by i.v. verapamil (300 µg kg^{-1}) for up to 30 and 60 min post injection respectively (Table 1A and B). The same doses of verapamil, however, did not affect adrenaline (2 µg kg^{-1} i. v.) induced responses thus ruling out any role of the direct inhibitors effects of verapamil on the effector site.

In view of the failure of verapamil to inhibit adrenaline responses, it appears reasonable to conclude that the likely mechanism for verapamil--induced inhibition of sympathetic-stimulation-induced tachycardia and pressor responses is an interference with catecholamine transmission. An action of verapamil at ganglionic sites cannot be ruled out from the present study. However, in our earlier study [18] on the nictitating membrane we observed a similar inhibition of response following direct stimulation of the ganglion. Thus the most likely mechanism appears to be an inhibition of release of neurotransmitter from the terminals. Nifedipine and diltiazem do not seem to share this-property of verapamil since they did not inhibit either sympathetic-stimulation- or adrenaline-induced responses to any appreciable extent. A similar difference was also observed in our earlier study [18] wherein verapamil but not nifedipine inhibited cervical sympathetic – stimulation induced contraction of nictitating membrane [16]. We therefore conclude that peripherally administered verapamil, in doses which alter resting HR and BP. can possibly interfere with catecholamine neurotransmission which may contribute to the cardiovascular effects of this drug.

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