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Failure of Verapamil and Diltiazem to Attenuate the Pressor Response to Hypothalamic Stimulation: A Possible Mechanism

ADEGO E. EFERAKEYA* AND UCHE A. OSUNKWO

Department of Pharmacology and Toxicology, University of Benin, Benin City, Nigeria

Abstract—The effects of verapamil or diltiazem on pressor responses to posterior hypothalamic stimulation, injected noradrenaline or tyramine were studied in urethane-anaesthetized normotensive, deoxycorticosterone acetate (DOCA), renal and spontaneously hypertensive rats at the early and established phases of hypertension. Pressor responses to the pressor stimuli were significantly enhanced in the early and established phases of hypertension when compared with the normotensives. While the magnitude of pressor responses in the established phase of renal or spontaneously hypertensive rats was significantly higher ($P < 0.05$) than the corresponding value in the early phase, in contrast, the pressor response in the early phase of DOCA hypertension was significantly higher than that of the established phase. Verapamil or diltiazem significantly ($P < 0.005$) inhibited pressor responses to injected noradrenaline or tyramine in all groups of rats but not that to hypothalamic stimulation, irrespective of the stage of hypertension. When the probable mechanism of the hypothalamic pressor response's resistance to the calcium antagonists was studied in-vitro, ATP significantly ($P < 0.005$) inhibited the relaxant effect of the calcium antagonists in the rat aortic strips. Our data indicate that verapamil or diltiazem is ineffective in inhibiting the pressor response to posterior hypothalamic stimulation. The probable mechanism of the resistance and the clinical implication of the findings are discussed.

Calcium antagonists (slow calcium channel blockers) are now widely used in the treatment of cardiovascular disorders including hypertension (Fleckenstein-Grun et al 1984). Like many other antihypertensive drugs their hypotensive effects are more pronounced in hypertensive states (Thievant et al 1982). Hypertensive animals or patients also respond more strongly to vasoconstrictor agents (Finch 1971; Folkow et al 1973; Finch & Haeusler 1974) and the hyperresponsiveness has been shown to occur in both early and late phases of hypertension (Hallback et al 1971; Wells et al 1985). We had earlier reported that pressor response to posterior hypothalamic stimulation is resistant to the inhibitory action of gallopamil or hydralazine while pressor responses to injected noradrenaline or angiotensin were susceptible (Eferakeya 1989; Eferakeya & Osunkwo 1990). The resistance of hypothalamic pressor responses to these antihypertensive drugs has some clinical implications since the blood pressure elevation induced by posterior hypothalamic stimulation is similar to that elicited by stress (Eliasson et al 1951; Folkow & Rubinstein 1966; Bunag & Eferakeya 1973).

The present investigation aimed to answer the following questions. Does the resistance of the hypothalamic pressor response to the inhibitory action of calcium antagonists occur in the early phase of hypertension? If it does occur, how does the magnitude compare with that observed in the established phase of hypertension? Does the phenomenon in the early phase vary in the different models of hypertension (deoxycorticosterone acetate, renal and spontaneously hypertensive rats) of hypertension and how do these possible variations compare with that of the normotensive rat? Lastly, what is the possible mechanism of the resistance? The calcium antagonists investigated were verapamil and diltiazem and pressor responses were induced by hypothalamic stimulation, injection of noradrenaline or tyramine. Tyramine injection and hypothalamic stimulation provide different methods of releasing noradrenaline from endogenous stores (Eferakeya & Bunag 1974).

* Present address and correspondence: A. E. Eferakeya, Department of Pharmacology, College of Medicine, King Faisal University, PO Box 2114, Dammam 31451, Saudi Arabia.

Materials and Methods

Whole-animal experiments

Four groups of female Wistar-Kyoto normotensive (NORM), deoxycorticosterone acetate hypertensive (DOCA), renal hypertensive (RHR) and spontaneously hypertensive rats (SHRs (Okamoto & Aoki 1963)) obtained from Ahmadu Bello University, Zaria, Nigeria, were used. Each group had four subgroups and each subgroup had 8 rats. Rats were operated on at 10 weeks of age under amylobarbitone anaesthesia (100 mg kg^{-1}, i.p). DOCA hypertension was produced by unilateral nephrectomy and subcutaneous implantation of DOCA (15 mg in silicon rubber implants (Ormsbee & Ryan 1973)) while renal hypertension was induced by unilateral nephrectomy and compression of the contralateral kidney with a figure-of-eight ligature (Grollman 1944). In both groups 0·9% NaCl (saline) solution was substituted for drinking water. Control normotensive rats were sham-operated. Normotensive and spontaneously hypertensive rats received tap water for drinking. All rats had free access to rat chow (Pfizer Nigeria Ltd) and were age-matched.

Systolic blood pressures were measured in the awake state by the tail-cuff method (Bunag 1973) just before operation and weekly post-operatively. Spontaneously hypertensive rats also had their systolic blood pressure measured. Two

weeks after operation (12 weeks old) all hypertensive rats (including SHRs) with systolic pressure levels of 135–145 mm Hg were considered to be in the early phase of hypertension, whereas at 18 weeks of age, rats with systolic pressure $\geqq 170$ mm Hg were considered to have established hypertension.

Rats were anaesthetized with urethane (1·25 g kg^{-1}, i.p.). Depth of anaesthesia was assessed and maintained as described previously (Bunag & Eferakeya 1973). A jugular vein and a common iliac artery were cannulated separately through cervical and abdominal incisions. The incisions were closed and the outer ends of both catheters were passed under the skin to emerge at the nape of the neck so that the rat could be attached to the stereotoxic instrument (David Kopf Instruments, Tujanga, CA, USA) in the prone position. To prevent clotting the arterial catheter was filled with dilute heparin solution (200 units mL^{-1}).

Phasic arterial pressure was recorded continuously using a polygraph (Grass Medical Instruments, MA, USA, Model 7D) which was connected to a pressure transducer (Statham, P23 1D) and the transducer was in turn connected to the arterial catheter. For simultaneous counting of heart rate, the phasic pressure signal from the transducer was fed into a biotachometer in one of the polygraph channels. The phasic arterial pressure was also integrated into mean arterial presure in the third channel of the polygraph. The polygraph speed was 10 mm min^{-1}.

For posterior hypothalamic stimulation a coaxial electrode with a diameter of 0·5 mm (Rhodes Medical Instruments, Woodland Hills, CA, USA) was placed in the posterior hypothalamus following de Groot's coordinates: anteroposterior 4·6, mediolateral 1·0 and dorsoventral −2·5 (Pellegrino et al 1979). An electrode was assumed to be correctly placed when stimulation with a 0·25 mA current (90 Hz, pulse width 1 ms) for 10 s produced a pressor response equal to or greater than 20 mm Hg (Bunag & Eferakeya 1973). Additionally, the brain was removed at the end of the experiment and the position of the electrode in the posterior hypothalamus was verified histologically (Bunag & Eferakeya 1976).

The rat's body temperature was kept constant by means of a heated blanket and was monitored with a thermistor probe in the rectum. The blanket and the probe were connected to a temperature controller (Model CFP 8185, Bioscience, UK) set at 37°C.

All drugs were injected through the jugular catheter with doses expressed as the weight of respective salt (kg body weight)$^{-1}$. Drugs injected were: noradrenaline bitartrate (Koch-Light Laboratory, UK) 2 μg, tyramine HCl (Sigma Chemical Co.) 1·2 μg, verapamil HCl (Knoll AG, Ludwigshafe, Germany) 1 μg and diltiazem chloride (LERS Synthelabo, France) 2 μg. Effective doses of calcium antagonists were determined in preliminary experiments as described previously (Eferakeya & Bunag 1974). Drug dose was injected in 0·05 to 0·1 mL solution followed by 0·1 mL saline.

After implantation of the hypothalamic electrode, the rat was allowed 30 min to stabilize before recording basal blood pressure levels and heart rates. Hypothalamic stimulation was followed by injection of noradrenaline, then tyramine, with 5 min intervals between the procedures. A calcium antagonist was then injected (bolus dose) and allowed 10 min (optimum time) to take effect. The sequence of hypothalamic stimulation and injection of agonists was repeated. Each rat was exposed to only one calcium antagonist.

In-vitro experiments

In-vitro experiments were carried out in normotensive rats only as hypothalamic pressor responses were qualitatively the same in the normotensive and hypertensive rats. Female normotensive rats (Wistar-Kyoto (Okamoto & Aoki 1963)), 18 weeks old, were killed by a blow on the head and the aorta removed. The aorta was transferred to physiological salt solution (PSS (Ebeigbe & Aloamaka 1985)). Under a dissecting microscope the aorta was dissected free of adhering connective tissue and then cut into helical strips. Each strip was superfused in a 10 mL organ bath with PSS of pH 7·4 and aerated with 95% O_2–5% CO_2 at 37°C.

The strip was fixed vertically to a metal base with the upper end (using fine thread) connected to an isometric transducer (Grass model FT.03, Quincey, MA, USA). The contraction signals from the isometric transducer were fed into a Grass polygraph (Model 7D) which recorded the aortic strip responses. The strip was given a resting tension of 600 mg and allowed to equilibrate for 90 min.

To record control responses, noradrenaline was introduced into the organ bath and while the strip was at the plateau of its contractile response to noradrenaline, the calcium antagonist was introduced to produce aortic strip relaxation. After obtaining maximum relaxation, the drugs were washed out of the organ bath and the strip allowed to return to control resting tension. ATP was introduced, then one min later the same control dose of noradrenaline was introduced. At the maximum response to noradrenaline the calcium antagonist was injected into the organ bath and responses recorded as before. A strip was only exposed to one calcium antagonist. Results of strips from each animal were pooled together, 'n' represents the number of rats studied. The concentrations of drugs used were: noradrenaline 10^{-7} M, ATP 10^{-4} M, verapamil 10^{-6} M and diltiazem 10^{-6} M.

The data for whole animal experiments were analysed using analysis of variance and Tukey's test for multiple comparisons when n is the same for all groups and the Spearman rank correlation coefficient test (Daniel 1978). The in-vitro data were analysed using Student's *t*-test for paired samples (Daniel 1978).

Results

Systolic blood pressure levels in conscious rats

Fig. 1 shows the curves of average systolic pressure for the normotensive and hypertensive groups. Whether in the early phase or in the established phase (Fig. 1) of hypertension, systolic pressure levels were significantly higher ($P < 0·005$) in the hypertensive groups than in the normotensive rats (Tables 1, 2). Although the SHRs continued to have higher systolic pressure levels than either DOCA or RHRs the differences were not significant ($P > 0·05$). As expected there were no significant differences in the blood pressures of 12 and 18 week old normotensive rats (Fig. 1, Tables 1, 2).

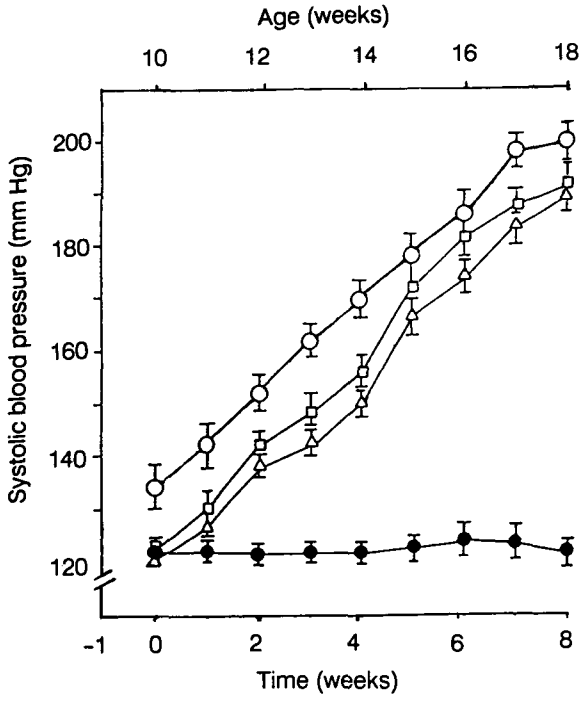

FIG. 1. Temporal course of the development of hypertension in deoxycorticosterone acetate (DOCA, △), renal (RHRs, □) and spontaneous hypertensive rats (SHRs, ○). Mean ± s.d. of systolic pressure mm Hg (n = 16 for each group). The systolic pressures of sham-operated normotensive rats (●) are also shown. $P < 0.005$ when hypertensive groups are separately compared with the normotensives at each point from two weeks onwards.

Basal mean arterial pressures (MAP) and heart rates (HR)

Fig. 2 shows a typical tracing of phasic and mean arterial pressures and heart rate. When rats were anaesthetized, mean arterial pressure levels in the early phase of hypertension were nearly the same ($P > 0.05$) in normotensive and in hypertensive groups (Tables 1, 2). On the other hand, rats in the established phase of hypertension still had MAP values that were significantly higher ($P < 0.01$) than those in the normotensive group (Tables 1, 2) in spite of anaesthesia. Basal heart rates of rats in the early phase of hypertension were not significantly different ($P > 0.05$) from those of normotensive controls. In the established phase of hypertension, heart rates in DOCA or RHRs were significantly lower ($P < 0.05$) than those of normotensive or SHRs (Tables 1, 2).

Enhancement of pressure responses during the development of hypertension

Fig. 2A shows control pressor responses to posterior hypothalamic stimulation, exogenous noradrenaline and tyramine. The magnitude of the pressor response in the early phase of hypertension, irrespective of the pressor stimuli, was generally higher in the hypertensive groups than in the normotensive controls (Tables 3, 4). The differences were significant ($P < 0.05$) for DOCA and SHRs. In the established phase of DOCA hypertension, the average of all pressor responses to stimuli was 47 ± 5 mm Hg; this value is significantly lower ($P < 0.05$) than that for the early phase (57 ± 4 mm Hg). On the other hand the average of all pressor responses to stimuli in the established phase of RHRs (82 ± 10 mmHg) or SHRs (69 ± 6 mm Hg) was significantly higher ($P < 0.005$) than the respective pressure responses in the early phase 43 ± 3 mm Hg for RHRs and 46 ± 5 mm Hg for SHRs. Thus quantitative alteration in pressor responsiveness exists during the development of DOCA, renal or spontaneous hypertension.

Cardiovascular effects of verapamil or diltiazem during development of hypertension

Verapamil or diltiazem significantly ($P < 0.005$) depressed MAP in both early and established phases of hypertension in all groups of rats. The rats in the established phase of hypertension were more susceptible to the calcium antagonist effect than those in the early phase of hypertension but the differences were not significant. The basal heart rates were also depressed by calcium antagonists but in contrast

Table 1. Effects of verapamil (1 μg kg^{-1}) on basal blood pressure levels (mm Hg) and heart rates (beats min^{-1}) in the early and established phases of hypertension (mean ± s.d.)***.

	NORM	DOCA	RHR	SHR	F ratio
Early phase	n = 8	n = 8	n = 8	n = 8	
SBP (conscious)	120 ± 6	138 ± 7	142 ± 4	145 ± 5	3·39*
MAP (anae.)[a]	84 ± 4[c]	88 ± 3[c]	89 ± 2[c]	94 ± 2[c]	2·78
MAP (anae.)[b]	52 ± 3	54 ± 2	56 ± 2	55 ± 2	2·65
% depression	38 ± 2	39 ± 3	38 ± 2	41 ± 3	2·88
Heart rate (anae.)[a]	400 ± 18[c]	373 ± 16[c]	365 ± 20[c]	394 ± 19[c]	2·89
Heart rate (anae.)[b]	378 ± 14	298 ± 15	314 ± 14	328 ± 16	3·41*
% depression	5 ± 1	20 ± 4	14 ± 2	17 ± 3	2·97*
Established phase	n = 8	n = 8	n = 8	n = 8	
SBP (conscious)	122 ± 5	189 ± 12	187 ± 10	199 ± 12	4·61*
MAP (anae.)[a]	84 ± 2[c]	120 ± 5 [c]	125 ± 8[c]	121 ± 4[c]	4·78*
MAP (anae.)[b]	65 ± 2	64 ± 5	67 ± 4	69 ± 3	2·86
% depression	24 ± 2	48 ± 3	46 ± 3	49 ± 3	3·80*
Heart rate (anae.)[a]	410 ± 20	338 ± 20[c]	376 ± 19[c]	404 ± 21[c]	3·75*
Heart rate (anae.)[b]	389 ± 16	281 ± 14	336 ± 15	353 ± 12	4·90**
% depression	6 ± 3	17 ± 2	11 ± 2	13 ± 1	2·78

*** One-way analysis of variance. NORM = normotensive rats, DOCA = DOCA hypertensive rats; RHR = renal hypertensive rats, SHR = spontaneously hypertensive rats, SBP = systolic blood pressure, MAP = mean arterial blood pressure, anae. = anaesthetized. [a]Before verapamil, [b]after verapamil, % depression = (a − b)/a × 100. Degrees of freedom = 3,28. *$P < 0.05$, **$P < 0.01$, [c]$P < 0.005$ when 'before' value is compared with the corresponding 'after' value.

Table 2. Effects of diltiazem (2 $\mu g\ kg^{-1}$) on basal arterial blood pressure levels (mm Hg) and heart rates (beats min^{-1}) in the early and established phases of hypertension (means ± s.d.)***.

	NORM	DOCA	RHR	SHR	F ratio
Early phase	n = 8	n = 8	n = 8	n = 8	
SBP (conscious)	118 ± 5	140 ± 8	139 ± 4	146 ± 5	3·38*
MAP (anae.)[a]	86 ± 6[c]	83 ± 3[c]	88 ± 3[c]	84 ± 1[c]	2·61
MAP (anae.)[b]	54 ± 2	54 ± 1	52 ± 1	51 ± 3	2·58
% depression	37 ± 2	35 ± 3	41 ± 1	39 ± 1	2·69
Heart rate (anae.)[a]	400 ± 17[c]	390 ± 18[c]	386 ± 14[c]	426 ± 20[c]	2·93
Heart rate (anae.)[b]	368 ± 16	328 − 12	288 ± 13	362 ± 10	3·51*
% depression	8 ± 2	16 ± 3	25 ± 4	15 ± 2	3·12*
Established phase	n = 8	n = 8	n = 8	n = 8	
SBP (conscious)	120 ± 4	185 ± 13	190 ± 11	195 ± 10	4·60*
MAP (anae.)[a]	86 ± 2	119 ± 6	121 ± 5	120 ± 4	4·68*
MAP (anae.)[b]	66 ± 2	62 ± 4	61 ± 2	68 ± 4	2·85
% depression	23 ± 2	54 ± 3	50 ± 3	44 ± 2	3·51*
Heart rate (anae.)[a]	411 ± 14[c]	329 ± 15[c]	392 ± 14[c]	420 ± 18[c]	3·64*
Heart rate (anae.)[b]	379 ± 19	244 ± 12	323 ± 20	351 ± 10	3·72**
% depression	8 ± 2	26 ± 2	17 ± 5	9 ± 1	3·13

*** One-way analysis of variance. NORM = normotensive rats, DOCA = DOCA hypertensive rats, RHR = renal hypertensive rats, SHR = spontaneously hypertensive rats, SBP = systolic blood pressure, MAP = mean arterial blood pressure, anae. = anaesthetized. [a]Before verapamil, [b]after verapamil. % depression = (a − b)/a × 100. Degrees of freedom = 3,28, $*P < 0.05$, $**P < 0.01$; [c]$P < 0.005$ when 'before' value is compared with the corresponding 'after' value.

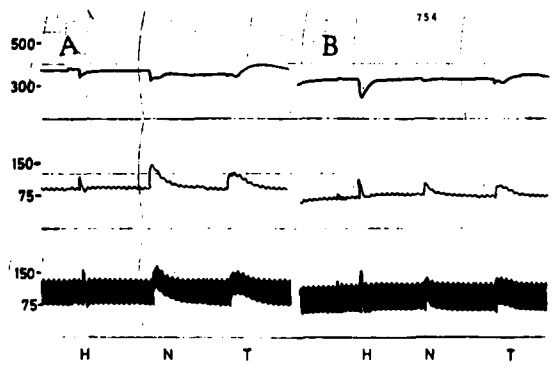

FIG. 2. Non-inhibition of posterior hypothalamic pressor response by verapamil in a normotensive rat. Panel A recorded before and panel B after injection of verapamil. Bottom and middle tracings are phasic and mean blood pressures in mm Hg, respectively; the top tracing is heart rate in beats min^{-1}. H = posterior hypothalamic stimulation at 0·25 mA for 10 s, N = noradrenaline 2·0 $\mu g\ kg^{-1}$, T = tyramine 1·2 $\mu g\ kg^{-1}$. Pressor response to noradrenaline or tyramine is inhibited by verapamil. Recorder speed 10 mm min^{-1}. Rats were anaesthetized with urethane.

the degree of depression was the same in early and established phases of hypertension (Tables 1, 2). However, the hypertensives were more susceptible to the negative chronotropic effects of the calcium antagonists than the normotensive rats. For example, the % depression of heart rate by verapamil in normotensive rats was 5 ± 1, and in the early phase of hypertension, 20 ± 2 (DOCA), 14 ± 2 (RHR) and 17 ± 3 (SHR). In the established phase of hypertension the corresponding values were; 17 ± 2 (DOCA); 11 ± 2 (RHR); and 13 ± 1 (SHR), whereas that for normotensive rats was 6 ± 3. For each group, heart rates after administration of calcium antagonists were significantly decreased. Verapamil was more potent ($P < 0.01$) in depressing the heart rates than diltiazem ($P < 0.05$) when absolute values of depression are compared (Tables 1, 2).

Panels A and B of Fig. 2 (bottom and middle tracings) show the pressor responses to the three stimuli before and after calcium antagonist, respectively. Pressor responses to posterior hypothalamic stimulation were resistant to the inhibitory action of verapamil (Fig. 2B) or diltiazem irres-

Table 3. Variable effects of verapamil (1 $\mu g\ kg^{-1}$) on pressor responses (mm Hg, mean ± s.d.) in the different phases of the development of hypertension in the rat***.

Stimulation		Early phase					Established phase				
		NORM	DOCA	RHR	SHR	F ratio	NORM	DOCA	RHR	SHR	F ratio
		n = 8	n = 8	n = 8	n = 8		n = 8	n = 8	n = 8	n = 8	
Hypothalamic	a	36 ± 3	60 ± 7	34 ± 1	40 ± 3	3·98*	34 ± 3	40 ± 2	90 ± 8	68 ± 12	7·42**
(0·25 mA)	b	36 ± 2	42 ± 1	34 ± 2	38 ± 4	2·75	32 ± 1	37 ± 2	76 ± 8	59 ± 5	6·83**
Noradrenaline	a	34 ± 3[c]	63 ± 8[c]	43 ± 3[c]	45 ± 2[c]	3·61*	36 ± 4[c]	46 ± 2[c]	93 ± 11[c]	78 ± 10[c]	7·65**
(2 $\mu g\ kg^{-1}$)	b	16 ± 3	35 ± 3	25 ± 1	30 ± 1	3·38*	19 ± 3	23 ± 2	56 ± 8	42 ± 4	7·72**
Tyramine	a	34 ± 4[c]	56 ± 6[c]	36 ± 2[c]	47 ± 2[c]	3·32*	33 ± 4[c]	53 ± 4[c]	79 ± 5[c]	67 ± 7[c]	7·54**
(1·2 $\mu g\ kg^{-1}$)	b	17 ± 2	26 ± 3	46 ± 3	38 ± 2	6·48**	18 ± 1	29 ± 1	21 ± 1	24 ± 1	3·36*

*** Two-way analysis of variance. NORM = normotensive rats, DOCA = DOCA hypertensive rats, RHR = renal hypertensive rats, SHR = spontaneously hypertensive rats. a = before verapamil, b = after verapamil; $*P < 0.05$, $**P < 0.01$; [c]$P < 0.005$ when 'before' value is compared with the corresponding 'after' value.

Table 4. Variable effects of diltiazem (2 $\mu g\ kg^{-1}$) on pressor responses (mm Hg, mean ± s.d.) at the different phases of the development of hypertension in the rat***.

Stimulation		Early phase					Established phase				
		NORM	DOCA	RHR	SHR	F ratio	NORM	DOCA	RHR	SHR	F ratio
		n=8	n=8	n=8	n=8		n=8	n=8	n=8	n=8	
Hypothalamic	a	33±5	53±7	41±2	45±5	3·38*	30±3	43±3	71±6	66±5	6·74**
(0·25 mA)	b	30±5	46±4	37±1	38±3	3·32*	29±3	37±2	65±5	61±5	6·44**
Noradrenaline	a	35±6[c]	56±5[c]	45±4[c]	45±2[c]	3·34*	33±3[c]	45±2[c]	83±10[c]	60±5[c]	7·35**
(2 $\mu g\ kg^{-1}$)	b	18±2	33±2	23±2	31±2	3·39*	18±1	20±1	40±4	30±3	4·26*
Tyramine	a	33±2[c]	55±6[c]	45±4[c]	51±5[c]	3·32*	32±4[c]	54±3[c]	78±8[c]	77±8[c]	6·78**
(1·2 $\mu g\ kg^{-1}$)	b	16±3	26±2	24±1	32±2	3·36*	16±2	21±1	36±4	40±3	4·09*

***Two-way analysis of variance. NORM = normotensive rats, DOCA = DOCA hypertensive rats, RHR = renal hypertensive rats, SHR = spontaneously hypertensive rats. a = before diltiazem, b = after diltiazem; $*P<0·05$, $**P<0·01$; $^cP<0·005$ when 'before' value is compared with the corresponding 'after' value.

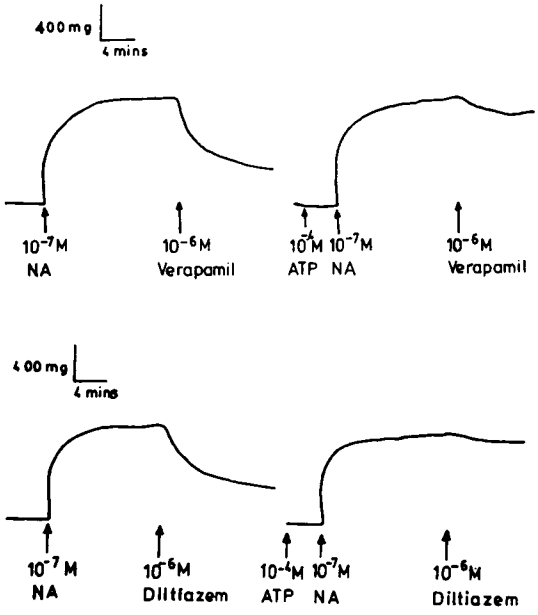

FIG. 3. Inhibition of verapamil- or diltiazem-induced relaxation of the rat aortic strip by ATP. Left top and bottom tracings show control relaxation by verapamil or diltiazem of aortic strip contractile response to noradrenaline (NA). Right top and bottom tracings show the inhibition of the relaxation by ATP.

pective of the phase of hypertension (Tables 3, 4); % pressor depression ranged from 0 to 16±2. On the other hand, the pressor responses to injected noradrenaline or tyramine were significantly ($P<0·005$) inhibited by the two calcium antagonists; % pressor depression ranged from 39±2 to 61±3 (Tables 3, 4; Fig. 2B).

Pressor responses were accompanied by reflex bradycardia. For example in early phase of DOCA hypertension, the pressor responses and the accompanying bradycardia were as follows: hypothalamic stimulation 60±7 mm Hg, −60±12 beats min^{-1}, noradrenaline 63±8 mm Hg, −80±15 beats min^{-1}; tyramine 56±6 mm Hg, −70±12 beats min^{-1}. Similar values were obtained for other groups of rats irrespective of the phase of hypertension. There was poor positive correlation (0·2–0·5) between magnitude of pressor response and the accompanying bradycardia. After calcium antagonist treatment, the bradycardia was only

Table 5. Inhibition of verapamil- or diltiazem-induced aortic smooth muscle relaxation (means ± s.d.) by adenosine 5'triphosphate (ATP, 10^{-6} M).

	n	Percent relaxation* Before ATP	Percent relaxation* After ATP**	% Inhibition by ATP
Verapamil	5	48±10	15±6	68±5
Diltiazem	5	39±6	8±6	79±7

** $P<0·01$ when before value is compared with the corresponding after value. * Percent relaxation = relaxation/control contraction to noradrenaline × 100. n = number of animals.

reduced in the instances where the magnitude pressor response was depressed. For example in the established phase of hypertension in SHRs, where there was no significant difference in hypothalamic response, the pressor responses and the accompanying bradycardia before and after verapamil were as follows: before, 68±12 mm Hg and −134±23 beats min^{-1}; after, 59±5 mm Hg and −130±12 beats min^{-1}. Similar results were obtained for diltiazem. The reflex bradycardia was blocked by atropine and not by calcium antagonists.

Inhibition of verapamil- or diltiazem-induced aortic strip relaxation by ATP

The contractile response of rat aortic strips in-vitro to noradrenaline (10^{-7} M) was significantly relaxed (39–48%) by either verapamil or diltiazem (Fig. 3; Table 5). When ATP (10^{-4} M) was added to the organ bath before verapamil or diltiazem was added the relaxing effect of either calcium antagonist was significantly ($P<0·01$) antagonized by ATP (Fig. 3; Table 5). ATP did not affect the magnitude of the aortic smooth muscle contractile response to noradrenaline as the magnitude of the response in the absence or presence of ATP were nearly the same (Fig. 3).

Discussion

In the early phase of hypertension systolic blood pressures in the conscious state were significantly ($P<0·05$) higher in the hypertensive groups than in the normotensive rats but after anaesthesia, although mean arterial blood pressures (MAP) were still higher in the hypertensive groups, the differences

were no longer significant ($P > 0.05$). This finding suggests the hypertensive groups were more sensitive to the depressant effect of anaesthesia which supports previous reports in established hypertension (Bunag et al 1975; Eferakeya & Osunkwo 1990). After treatment with verapamil or diltiazem, MAP values were nearly the same in all groups of rats and differences became even less apparent when depression of MAP after calcium antagonists were expressed as percentage of respective baseline MAP levels (Tables 1, 2). However, in the established phase of hypertension although after treatment with either verapamil or diltiazem, baseline MAP values were no longer significantly different, percentage depressions were significantly higher ($P < 0.05$) in the hypertensive groups. This indicates that in the established phase of hypertension, hypertensive rats were more susceptible than normotensive rats to the hypotensive action of the calcium antagonists. Similar findings have been reported for calcium antagonists and other anti-hypertensive agents (Thievant et al 1982) but these reports contrast with the present finding of equi-hypotensive responsiveness in the early phase of hypertension.

In all rat groups, baseline heart rates were not significantly different ($P > 0.05$) in early phases of hypertension but in the established phase, the hypertensives had lower heart rates than the normotensive groups. This finding in the established phase could be due to either hypersensitivity to anaesthesia or reflex lowering of heart rate via baroreceptor mechanisms in an attempt to normalize arterial blood pressure. However, the latter possibility is unlikely since baroreceptors are usually reset upwards in hypertension (McCubbin et al 1956; Jones & Floras 1980; Bunag & Miyajima 1984). Verapamil or diltiazem significantly depressed baseline heart rates in all rat groups (normotensive and hypertensive) but the degree of depression was higher in the hypertensive groups ($P < 0.05$, irrespective of the phase of hypertension) than in the normotensives (Tables 1, 2), thus suggesting that hypertensive rats were more susceptible to the negative chronotropic action of the calcium antagonists.

In general, the finding of hyperresponsiveness to vasoconstrictor stimuli supports reports that hypertensive animals or patients have exaggerated pressor responses to pressor stimuli (Goldenberg et al 1948; Doyle & Black 1955; Kaplan & Silah 1964; Finch & Haeusler 1974; Bunag et al 1975; Wells et al 1985). This hyperresponsiveness has been attributed to functional (Yamori 1976; Noon et al 1978) and structural (Folkow et al 1973, 1977) vascular changes in hypertension. Structural changes are not prominent in the early phase of hypertension (Wells et al 1985), rather functional changes could probably explain the hypersensitivity (Bunag et al 1975; Katovich et al 1984; Wells et al 1985). In contrast to the findings in RHRs and SHRs, the pressor response in established DOCA hypertension, although higher than that of the normotensive controls, was significantly lower than that obtained in the early phase of DOCA hypertension. It seems that some of the initial hormonal phenomena in DOCA hypertension (Berecek et al 1980; Crofton et al 1980) tend to disappear with the progression of hypertension even though blood pressures of DOCA rats were elevated equally to those of SHRs and RHRs.

Pressor responses were accompanied by reflex bradycardia and after addition of the calcium antagonists correlation between the magnitude of the pressor response and the bradycardia were poor. Verapamil and diltiazem depressed heart rates to the same extent. Thus results of changes of heart rate accompanying pressor responses were not analysed because the bradycardia does not significantly influence the magnitude of the pressor response (Bunag & Eferakeya 1973).

Verapamil or diltiazem failed to inhibit the hypothalamic pressor responses in normotensives and in early and established phases of hypertension. This finding suggests that the phenomenon exists not only in established phases but also in the early phase of the development of hypertension irrespective of the method of induction of the hypertension. Worcel (1978) found that the innervated proximal artery of normotensive rats was unresponsive to the relaxant effect of hydralazine. This phenomenon has been attributed to the released ATP at the nerve endings since exogenous ATP inhibited the relaxant effect of hydralazine (Chevillard et al 1981) and ATP is released with noradrenaline during normal sympathetic nerve activity (Holton 1959). As the pressor response to posterior hypothalamic stimulation is due to increased sympathetic nerve activity (Eferakeya & Bunag 1974), the ATP released would tend to antagonize the verapamil or diltiazem smooth muscle relaxant effect. This possibility was investigated in-vitro and it was found that ATP significantly inhibited ($P < 0.005$; Table 5) the relaxant effect of verapamil or diltiazem on noradrenaline-induced contraction of aortic strips, thus suggesting that the probable reason why diltiazem or verapamil failed to inhibit hypothalamic pressor responses was due to antagonism by the ATP released from sympathetic nerve endings.

On the other hand, in our studies, verapamil or diltiazem significantly inhibited pressor responses to exogenous noradrenaline to nearly the same extent in all rat groups. This finding is in agreement with our previous reports using gallopamil or hydralazine (Eferakeya 1989; Eferakeya & Osunkwo 1990) and that of van Breemen et al (1982) that noradrenaline-induced contraction of, or calcium influx into, vascular smooth muscle was partially inhibited by calcium antagonists.

However, calcium antagonists significantly inhibited pressor responses to injected tyramine but not those to hypothalamic stimulation even though the pressor responses by both procedures are due to endogenously released noradrenaline from sympathetic nerve endings. While this discrepancy remains to be investigated, a plausible explanation is that hypothalamic stimulation produces nerve action potentials (Takeda & Bunag 1980) which cause the release of noradrenaline by exocytosis of all the contents (noradrenaline, ATP and dopamine β-hydroxylase) of the vesicles. On the other hand, tyramine causes release of noradrenaline at the nerve endings by displacing noradrenaline from the vesicles without the process of exocytosis (Weiner & Taylor 1985). Thus, tyramine probably does not cause the release of ATP which would inhibit the action of the calcium antagonist.

In conclusion, our data indicate that verapamil or diltiazem is ineffective in inhibiting pressor responses to posterior hypothalamic stimulation irrespective of the type and stage of hypertension. The probable mechanism involved is inhibition of calcium antagonist relaxation of vascular smooth muscle by ATP released from sympathetic nerve endings.

These findings may have clinical implications in the choice of antihypertensive drugs for patients who are repeatedly exposed to stress.

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