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AN INCREASED REACTIVITY IN HYPERTENSIVE RATS UNAFFECTED BY PROLONGED ANTIHYPERTENSIVE THERAPY

L. FINCH

School of Studies in Pharmacology, University of Bradford, Bradford 7, Yorkshire

1 Isolated perfused mesenteric arteries obtained from chronic experimental hypertensive rats (deoxycorticosterone/NaCl) exhibited an increased reactivity to noradrenaline, 5-hydroxytryptamine and adenosine 5'-triphosphate (ATP) when compared with similar preparations from age-matched normotensive animals.

2 The dose-response curves to all three vasoconstrictor agents obtained from hypertensive animals exhibited a steeper slope, and higher maximum without any significant change in the threshold dose suggesting that adaptive/structural changes in the blood vessels had taken place.

3 Ten week treatments with antihypertensive combinations of hydrallazine, hydrochlorothiazide and reserpine or hydrallazine and mecamylamine lowered the systolic blood pressures of the hypertensive rats to those of normotensive animals and also reversed secondary changes such as periarteritis nodosa of the mesentery and cardiac hypertrophy.

4 The reactivity of these blood vessels to all these vasoconstrictor agents from the hypertensive rats with a normalized blood pressure was similar to those obtained with untreated hypertensive animals.

5 The persistent increased reactivity in the hypertensive rats after long-term anti-hypertensive treatment suggests that the hyperresponsiveness is secondary to the elevated blood pressures and that the adaptive/structural changes of the blood vessels in chronic hypertensive rats cannot be reversed by prolonged antihypertensive therapy.

Introduction

In several models of experimental hypertension a hyperreactivity has been reported for various vasoconstrictor stimuli (McGregor & Smirk, 1970; Finch, 1971, 1974a; Nicholas, 1971). Since this increased reactivity can be demonstrated in several isolated preparations it would seem to be independent of both circulating humoral agents and neurogenic control (Haeusler & Haefely, 1970; Haeusler & Finch, 1972; Beilin & Ziakas, 1972).

The underlying mechanism for this hyperresponsiveness and its role in hypertension is not clear. However, one possible mechanism is that due to the elevated blood pressure, adaptive structural changes take place in the blood vessels resulting in an increased wall/lumen ratio (Folkow, 1971; Weiss, 1974a; Lundgren, 1974a). As a consequence of the adaptive/structural changes, theoretical and experimental data show that the slope of dose-response curves to various vasoconstrictor stimuli become steeper and a higher maximum is obtained. Also, in most cases there is no change in the threshold dose for the various agonists (Folkow, Hallbäck, Lundgren & Weiss, 1970; Haeusler & Finch, 1972; Finch, 1974a). Furthermore, these adaptive structural changes seem to be reversible in those animals with hypertension of a short duration (Weiss, Lundgren & Folkow, 1974; Lundgren, 1974b).

Some observations are more compatible with the view that individual smooth muscle cells of the hypertensive animals display a true supersensitivity and would therefore lead to a permanently increased vascular tone and presumably be the causal factor of the elevated blood pressure (Bandick & Sparks, 1970; Bohr & Sitrin, 1970; Holloway & Bohr, 1973).

The present studies were carried out on rats with a hypertension of long-standing duration and the reactivity was measured in the mesenteric bed. Even after prolonged lowering of the blood pressure by means of antihypertensive therapy, an increased reactivity was observed in

vascular preparations from hypertensive animals. Preliminary results have been reported to the British Pharmacological Society (Finch, 1974b).

Methods

Male normotensive rats from a closed randomized colony (Bradford C.F.E.) were used for normotensive controls and for inducing deoxycorticosterone (DOCA)/NaCl hypertension.

Induction of experimental hypertension

Surgical procedures were carried out under ether anaesthesia in 6-7 week old animals. DOCA/NaCl hypertension was induced by subcutaneous implantation of DOCA acetate pellets (2 x 25 mg), unilateral nephrectomy and replacement of the drinking water with 0.9% w/v NaCl solution for a period of 4 weeks. Blood pressures of conscious animals were measured by an indirect tail cuff method. Rats with systolic blood pressures of at least 185 mmHg were considered hypertensive and those with a blood pressure between 110 and 140 mmHg were considered normotensive.

Long-term antihypertensive therapy

Eight weeks after the surgical procedures hypertensive rats and unoperated, age-matched normotensive rats were selected into groups for antihypertensive therapy. The animals were then given the drug combinations in the drinking water for a period of 10 weeks (Solution A: hydrallazine 0.1 g/litre + hydrochlorothiazide 0.25 g/litre + reserpine 5 mg/litre; Solution B: mecamylamine 0.05 g/litre + hydrallazine 0.05 g/litre). Blood pressures of treated and untreated groups were measured every 2 weeks and also measured within 48 h of setting up the perfused mesentery preparation.

Isolated perfused mesenteric artery preparations

Vascular reactivity to noradrenaline (base), 5-hydroxytryptamine (base) and adenosine 5′-triphosphate (salt) were determined in the isolated Krebs perfused mesenteric artery preparations by the method described previously (Haeusler & Finch, 1972). The perfusion experiments in treated animals were all carried out during the eleventh week after the antihypertensive therapy using double organ baths for paired experiments.

Statistical analysis

For evaluation of data, Student's t test was used. Throughout the paper, mean values are given together with the standard error of mean (s.e.); n is the number of estimations.

Drugs

The following drugs were used: adenosine 5′-triphosphate (ATP, Koch-Light), deoxycorticosterone acetate (Organon Laboratories); hydrallazine hydrochloride (Ciba Laboratories); hydrochlorothiazide (Merck, Sharpe & Dohme); 5-hydroxytryptamine creatinine phosphate (5-HT, Koch-Light); mecamylamine hydrochloride (Merck, Sharpe & Dohme); noradrenaline acid tartrate (Hoechst) and reserpine phosphate (Ciba Laboratories).

Table 1 Effect of long-term anti-hypertensive therapy on the resting systolic blood pressures of normotensive and chronic DOCA/NaCl hypertensive rats

	Before	*After*	*Treatment*
Normotensive	129 ± 6 (12)	122 ± 7 (12)*	Normal
Normotensive	124 ± 5 (10)	102 ± 7 (10)†	Solution A
Normotensive	127 ± 8 (10)	117 ± 8 (10)*	Solution B
Hypertensive	192 ± 9 (12)	188 ± 9 (9)*	Normal
Hypertensive	195 ± 11 (12)	130 ± 8 (8)†	Solution A
Hypertensive	203 ± 8 (12)	133 ± 9 (8)†	Solution B

Values indicate systolic blood pressures (mmHg ± s.e. mean) measured by indirect tail/cuff method. Antihypertensive therapy for 10 weeks. Drinking water of solution A contained hydrochlorothiazide 0.25 g/litre, reserpine 5 mg/litre and hydrallazine 0.1 g/litre. Solution B contained mecamylamine 0.05 g/litre and hydrallazine 0.05 g/litre.

* No significant differences. † $P < 0.05$.

Results

Effect of antihypertensive therapy on the resting blood pressures of normotensive and experimental hypertensive rats

The mean values of the resting blood pressures of normotensive and DOCA/NaCl hypertensive rats did not alter significantly during the 10 week period of the experiment when given normal drinking water (Table 1). In the normotensive rats treated with antihypertensive combination A, the blood pressures fell significantly (22 mmHg) whilst solution B produced only a moderate fall (10 mmHg) in normotensive animals. Both antihypertensive combinations (A and B) produced a marked fall in blood pressures in hypertensive animals and these were maintained within normotensive levels throughout the 10 week period of drug therapy (Table 1).

Vascular reactivity of the perfused mesenteric artery preparation from normotensive and hypertensive animals

Using the isolated perfused mesenteric artery preparation the vasoconstrictor responses to noradrenaline, 5-HT and ATP were determined in normotensive and hypertensive animals. Noradrenaline, 5-HT and ATP produced dose-dependent vasoconstrictor responses in preparations from both normotensive and hypertensive rats (Figures 1-3). In preparations obtained from hypertensive animals the dose-response curves produced by all three vasoconstrictor agents all exhibited a steeper slope and a higher maximum was obtained when compared with the curves from preparations of normotensive rats. However, the threshold doses of noradrenaline, 5-HT and ATP were not significantly different from those in normotensive controls (Figures 1-3). At the completion of each experiment higher doses of each vasoconstrictor agent were added to show that the responses were truly maximal.

In preparations obtained from normotensive rats treated with either antihypertensive solution A or B, the dose-response curves to noradrenaline, 5-HT and ATP were identical to those obtained in preparations from untreated normotensive rats (Figures 1-3). Using the preparations from 'hypertensive' rats in which the blood pressures had been normalized for a ten-week period by the use of antihypertensive therapy (solutions A and B), the dose-response curves for noradrenaline, 5-HT and ATP were almost identical to those obtained in preparations from age-matched hypertensive rats which had not received the antihypertensive therapies (Figures 1-3). The maximal responses to all three vasoconstrictor agents were also not significantly changed from those obtained with the untreated hypertensive rats.

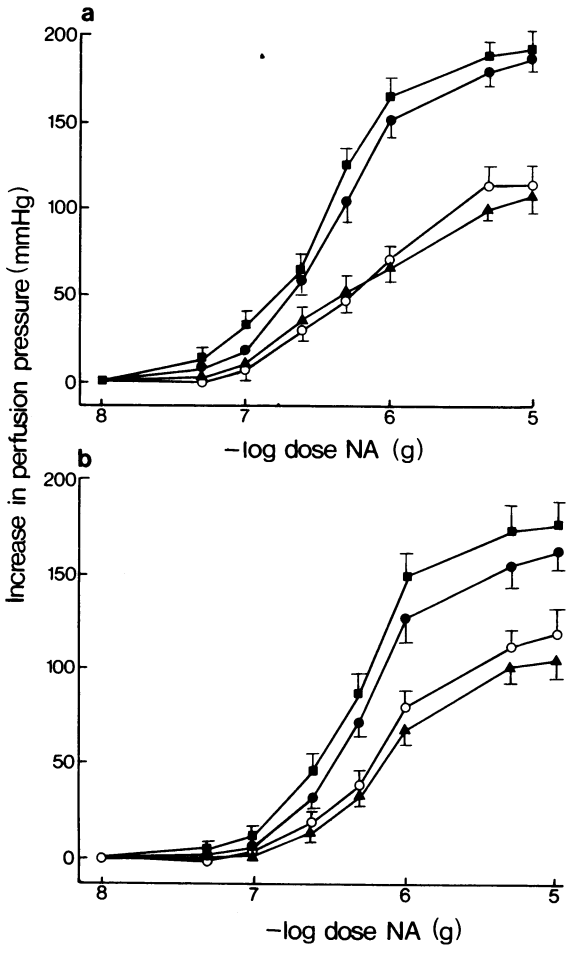

Figure 1 Dose-response curves for the vasoconstrictor effect of noradrenaline (NA) in isolated perfused mesenteric artery preparations: (a) from normotensive rats (○); normotensive rats after antihypertensive treatment A (▲); DOCA/NaCl hypertensive rats (●); DOCA/NaCl 'hypertensive' rats after antihypertensive treatment A (■); (b) from normotensive rats (○); normotensive rats after antihypertensive treatment B (▲); DOCA/NaCl hypertensive rats (●); DOCA/NaCl 'hypertensive' rats after antihypertensive treatment B (■). The mean values are shown; vertical bars indicate s.e. mean. Blood pressures before and after the 10-week treatment are shown in Table 1.

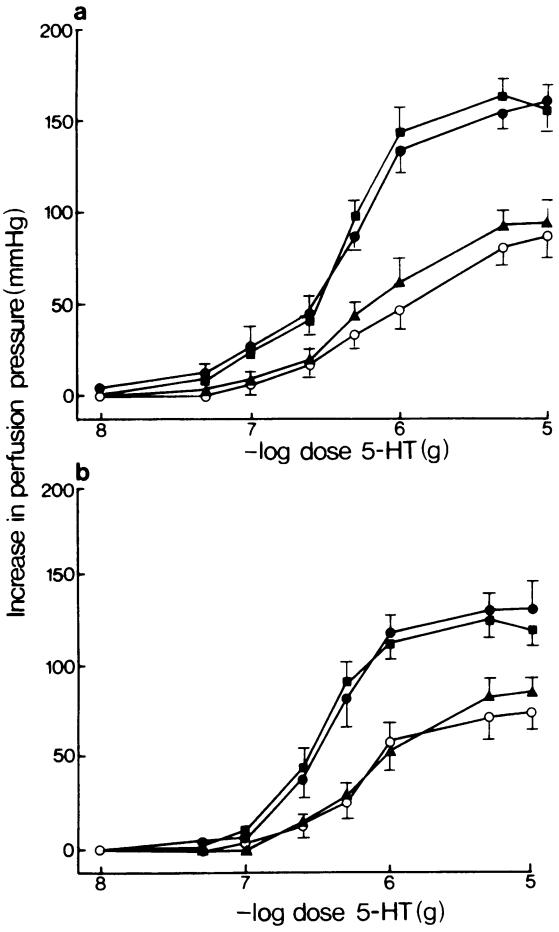

Figure 2 Dose-response curves for the vasoconstrictor effect of 5-hydroxytryptamine (5-HT) in isolated perfused mesenteric artery preparations: (a) from normotensive rats (○); normotensive rats after antihypertensive treatment A (▲); DOCA/NaCl hypertensive rats (●); DOCA/NaCl 'hypertensive' rats after antihypertensive treatment A (■). (b) from normotensive rats (○); normotensive rats after antihypertensive treatment B (▲); DOCA/NaCl hypertensive rats (●); DOCA/NaCl 'hypertensive' rats after antihypertensive treatment B (■). The mean values are shown; vertical bars indicate s.e. mean. Blood pressures before and after the 10-week treatment are shown in Table 1.

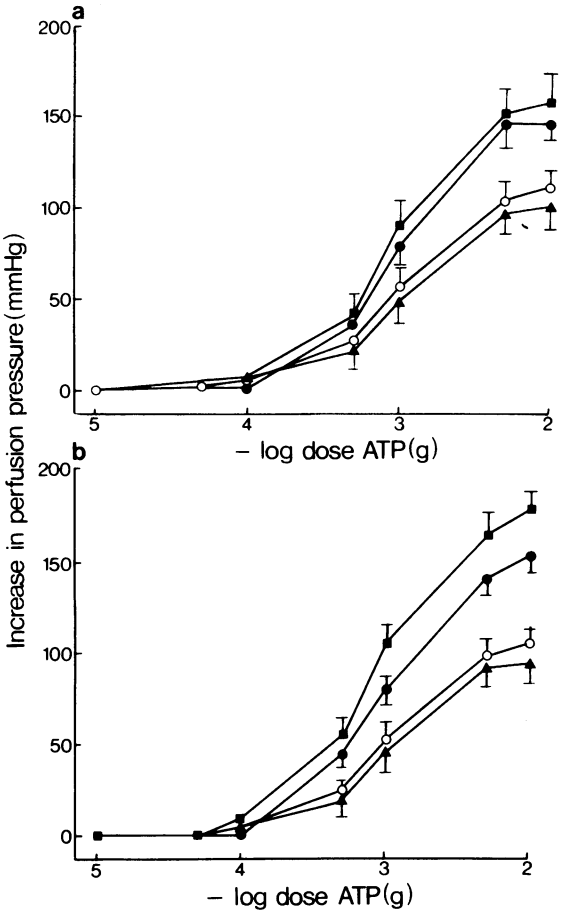

Figure 3 Dose-response curves for the vasoconstrictor effect of adenosine 5'-triphosphate (ATP) in the isolated perfused mesenteric artery preparations: (a) from normotensive rats (○); normotensive rats after antihypertensive treatment A (▲); DOCA/NaCl hypertensive rats (●); DOCA/NaCl 'hypertensive' rats after antihypertensive treatment A (■); (b) from normotensive rats (○); normotensive rats after antihypertensive treatment B (▲); DOCA/NaCl hypertensive rats (●); DOCA/NaCl 'hypertensive' rats after antihypertensive treatment B (■). The mean values are shown; vertical bars indicate s.e. mean. Blood pressures before and after the 10-week treatment are shown in Table 1.

Effect of antihypertensive therapy on the incidence of hypertensive lesions and heart weight in DOCA/NaCl hypertensive and normotensive rats

In order to discover whether the long-term antihypertensive treatment produced any reversal of the pathological states involved in DOCA/NaCl hypertension, heart weight (ventricles) and incidence of perarteritis nodosa of the mesentery was determined in all animals used for vascular reactivity studies. In normotensive rats the heart weights of a group of ten rats was found to lie

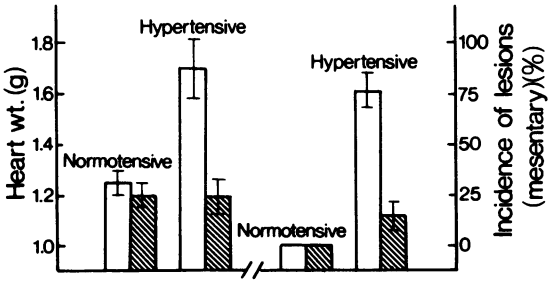

Figure 4 Effect of long term antihypertensive therapy on the incidence of arterial lesions and change in heart-weight in normotensive and DOCA/NaCl hypertensive rats. Each column represents the mean value obtained from 10 animals. Hatched columns: effect of 10 weeks antihypertensive therapy (hydrochlorothiazide 0.25 g/litre, reserpine 5 mg/litre, hydrallazine 0.1 g/litre).

within a very small weight range (Figure 4). The age-matched DOCA/NaCl hypertensive rats (18 weeks post DOCA treatment/nephrectomy) exhibited a massive cardiac hypertrophy, the heart weight being increased by 50% compared with normotensive animals (Figure 4). This cardiac hypertrophy was completely reversed by 10 weeks of antihypertensive treatment with a combination of hydrochlorothiazide, reserpine and hydrallazine. In normotensive animals this antihypertensive therapy did not significantly change the heart weight compared with those from untreated normotensive animals.

In the mesentery of DOCA/NaCl hypertensive rats, with a hypertension of approximately 18 weeks duration, the incidence of lesions (perarteritis nodosa) was 75% whilst no lesions were seen in the age-matched normotensive animals (Figure 4). The long-term antihypertensive treatment almost completely reversed these lesions in the hypertensive rats with a normalized blood pressure (Table 1. Figure 4).

Discussion

An increased vascular reactivity of the perfused mesenteric artery preparation to various vasoconstrictor agents was demonstrated using 7-month old DOCA/NaCl hypertensive rats. These findings confirm earlier results with spontaneously hypertensive rats (Folkow *et al.*, 1970; Finch, 1974a), in DOCA/NaCl hypertensive rats (Beilin & Ziakas, 1972; Finch & Haeusler, 1974) and in renal hypertensive rats (Lundgren, 1974a, b). As in previous studies with spontaneously hypertensive rats the maximal responses obtained to ATP, or noradrenaline were higher than those to 5-HT (Finch, 1974a). The absence of any significant increased threshold response to any of the vasoconstrictor agents confirms previous work in many regions of the cardiovascular system (Haeusler & Finch, 1972; Finch, 1974a; Lundgren, 1974a, b; Weiss, 1974a, b), and is strong evidence for adaptive/structural changes, an encroachment of the lumen, and not any true hyperresponsiveness of the smooth muscle cells of hypertensive animals (Folkow *et al.*, 1970; Folkow, 1972). Even so, there are some experimental results in support of a true hyperresponsiveness of the excitation contraction coupling mechanism since in completely depolarized mesentery preparations there is no increased reactivity to calcium in preparations from hypertensive animals (Finch & Haeusler, 1974).

In DOCA/NaCl hypertensive animals in which the blood pressure had been elevated for approximately 8 weeks, it was found that with antihypertensive combinations of reserpine, hydrochlorothiazide and hydrallazine or hydrallazine and mecamylamine the blood pressures could be normalized for a 10-week period. These drug combinations have been shown to be effective in lowering the blood pressures in spontaneously hypertensive rats (Freis, Ragan, Pillsburg & Mathews, 1972).

The 10-week treatment with both antihypertensive combinations did not affect the vascular reactivity to ATP, 5-HT or noradrenaline in the mesentery preparations from normotensive animals which suggests that no impairment of receptors sites and/or uptake mechanism were involved. Furthermore, no pathological changes were observed in spontaneously hypertensive rats given a similar treatment for 6 months (Freis *et al.*, 1972). However, it was surprising to find that in the 'hypertensive' animals which had normalized blood pressures after the 10-week antihypertensive treatments, the vascular reactivity to 5-HT, ATP and noradrenaline was still increased and identical to that from untreated hypertensive animals. Similar results were obtained in a previous study in which antihypertensive treatment was carried out for only a 4-week period (Finch, 1974a).

In studies with both renal and spontaneously hypertensive rats adaptive/structural changes have been shown to take place rapidly in response to the elevated blood pressure (Folkow *et al.*, 1970; Lundgren, 1974a; Weiss & Hallbäck, 1974). Also these groups of workers have shown that a rapid regression takes place in younger hypertensive animals but found in the older established hypertensive rats that the blood pressures could

not be normalized by some antihypertensive agents and therefore full regression of vascular design was not obtained (Weiss & Hallbäck, 1974; Weiss *et al.*, 1974).

Factors which may alter vascular reactivity during the prolonged hypertensive phase may include lesions of the vasculature. The incidence of these lesions in the mesentery and the marked cardiac hypertensive seen in these chronic DOCA/NaCl hypertensive animals was reversed by the antihypertensive treatment. Similar results have also been observed in spontaneously and renal hypertensive rats using antihypertensive agents such as α-methyl dopa and propranolol (Lundgren, 1974b; Sen, Tarazi, Khairallah & Bumpus, 1974; Weiss *et al.*, 1974). All these observations suggest that at least some of the secondary changes involved in experimental hypertension can be reversed by prolonged antihypertensive treatment.

The exact role of the increased reactivity to vasoconstrictor stimuli in experimental hypertension must remain in doubt. Although the preparations used in this study contained only arteries and arterioles, with no precapillary resistance vessels, and therefore cannot be compared directly with results using intact resistance vessels (Weiss, 1974a; Lundgren, 1974a, b), the preparations from 'hypertensive' animals still exhibited an increased reactivity even after a normalized blood pressure for a 10-week period. It is possible that the adaptive/structural changes, with an increased amount of supporting tissues containing elastin-collagen within the blood vessels, account for the higher maximal responses to vasoconstrictor agents and that no regression of this supporting tissue takes place. This hypothesis is strengthened by pathological studies in which the elastin-collagen deposition persists in the aortae of chronic hypertensive rats (Wolinsky, 1971, 1972). The regression of structural changes observed in 'true' resistance vessels may reflect a hypertrophy of the vessles, but this still depends on the extent of loading since it has been reported that chronic hypertensive animals become resistant to antihypertensive agents (Lundgren, 1974a).

In conclusion, the experimental results have shown that an increased reactivity to various vasoconstrictor stimuli occurs in blood vessels of the mesentery from chronic DOCA/NaCl hypertensive rats. Furthermore, this increased reactivity persists after the blood pressures had been normalized by antihypertensive treatment, suggesting that the hyperresponsiveness is only secondary to the hypertension.

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