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Cardiovascular reactivity in the experimental hypertensive rat

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Summary

1. In pithed preparations, deoxycorticosterone (DOCA)/NaCl-induced hypertensive rats showed increased cardiovascular reactivity to injected noradrenaline, DMPP, tyramine, angiotensin and to sympathetic nerve stimulation.

2. Pithed, chronic renal hypertensive rats showed similar hyperreactivity to noradrenaline, DMPP and tyramine but responses to sympathetic nerve stimulation were within normal limits.

3. In the isolated, Krebs perfused mesentery preparation, vessels obtained from both DOCA/NaCl and renal hypertensive rats showed hyperreactivity to injected noradrenaline. Responses to periarterial nerve stimulation were also markedly increased in preparations from DOCA/NaCl hypertensive rats, while preparations from renal hypertensive rats showed this effect only at higher rates of stimulation (12 and 25 Hz).

Introduction

The renin-angiotensin system is thought to be an important factor in experimental renal hypertension (Page & McCubbin, 1968), but other workers consider increased cardiovascular reactivity to be one of the principal factors (Laverty, McGregor & McQueen, 1968). Vascular hyperreactivity to a variety of pressor stimuli has been reported in renal (Phelan, 1966 ; McGregor & Smirk, 1968), deoxycorticosterone (DOCA)/sodium chloride (Sturtevant, 1956 ; Beilin & Wade, 1970) and in genetically hypertensive rats (Laverty, 1961 ; Haeusler & Haefely, 1970). However, several reports indicate that aortic smooth muscle from genetically and renal hypertensive rats do not show supersensitivity to noradrenaline (Redleaf & Tobian, 1958 ; Mallov, 1959 ; Clineschmidt, Geller, Govier & Sjoerdsma, 1970).

In this study, attempts have been made to investigate hyperreactivity to a variety of pressor agents in the intact experimental hypertensive rat. This was thought to be of interest since recent studies have shown that hypertension can develop and be maintained in the absence of the sympatho-adrenal nervous system (Finch & Leach, 1970a, b). Studies were also carried out using the isolated perfused mesentery preparation (McGregor, 1965), as this contains both large and small blood vessels and constitutes one of the principal sites of peripheral resistance.

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Methods

All experiments were carried out using male C.S.E. rats (Scientific Products), of 370–420 g body weight. Renal or DOCA/NaCl hypertension was induced by the methods described previously (Finch & Leach, 1970a). Chronic hypertension was considered to be present when systolic blood pressure in excess of 160 mmHg (1 mmHg≡1·333 mbar) was maintained for a period of 8 weeks, and measured using the tail cuff method (Finch & Leach, 1970a).

Anaesthetized and pithed preparations were set up as described previously (Finch & Leach, 1969). When investigating responsiveness to the pressor agents only one drug was used in each preparation to avoid errors due to interactions between the drugs. Additionally, normotensive and hypertensive preparations were set up in pairs, simultaneously side by side, and the responses monitored on a Devices M4 recorder. Pressor agents were injected every 20 min and the mean of two responses calculated. In some experiments, pithed preparations were used for stimulation of the entire sympathetic outflow (Gillespie & Muir, 1967); submaximal stimulation from a Multitone stimulator at strengths of 15–30 V, 0·03 ms duration and frequencies of 3 Hz and 6 Hz were applied for periods of 18 seconds. Stimulation was repeated at not less than 10 min intervals. Atropine (0·5 mg/kg) and tubocurarine (1 mg/kg) were given before commencement of stimulation.

Isolated mesentery preparations were set up as described by McGregor (1965). Preparations were perfused with oxygenated Krebs solution (37° C) at a constant flow rate of 3·5–4 ml/minute. For stimulation of the periarterial nerves a subminiature bipolar electrode was placed in contact with the nerve plexus. Stimuli of 1 ms duration at various frequencies and supramaximal voltage were applied every 3 minutes. Dose-response curves for noradrenaline were obtained by injecting the drug in various doses into the perfusion fluid close to the cannula attached to the superior mesenteric artery. Individual dose-response curves were constructed from the means of only two responses for each dose as the sensitivity increased with longer experimental periods.

For statistical evaluation of mean differences, Student's *t* test was used ; differences of $P<0·05$ were considered significant. Throughout the paper, mean values are given together with standard error of the mean (S.E.) ; *n* is the number of experiments.

Drugs

The doses of the following drugs were calculated as salt: atropine sulphate (Northern Pharmaceuticals) ; dimethylphenylpiperazinium iodide (DMPP ; Aldrich) ; tubocurarine chloride (Burroughs Wellcome) ; and tyramine hydrochloride (B.D.H.). Noradrenaline acid tartrate (Hoechst), was stored in 0·01 N HCl and diluted in 0·9% W/V NaCl solution before use ; doses were calculated as base. Val-5-angiotensin II amide (Ciba) was dissolved in saline immediately before use.

Results

Cardiovascular reactivity in the experimental hypertensive rat after pithing

In experimental renal hypertensive preparations, after pithing, the sensitivity to exogenous noradrenaline was increased by a factor of approximately 2 when com-

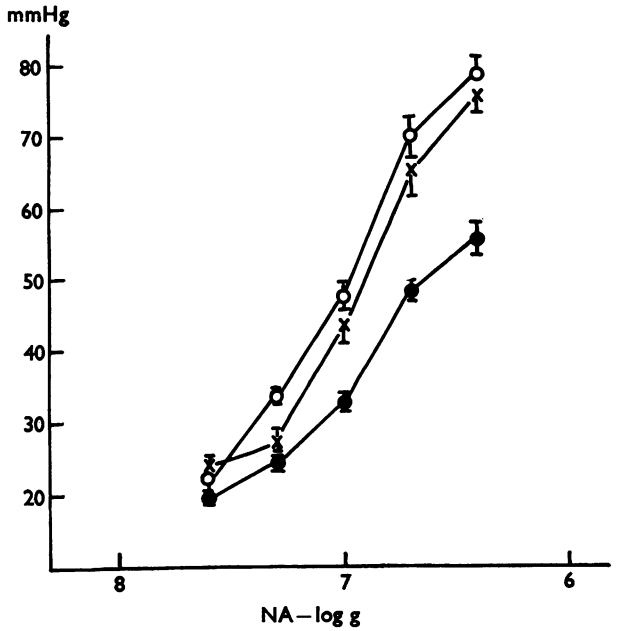

FIG. 1. Pithed rat preparations. Mean rise in blood pressure to various doses of noradrenaline (NA). (●——●), Normotensive controls ($n=20$); (○——○), rats with experimental renal hypertension ($n=10$); (×——×), rats with DOCA/NaCl hypertension ($n=10$). Vertical bars, S.E. of mean.

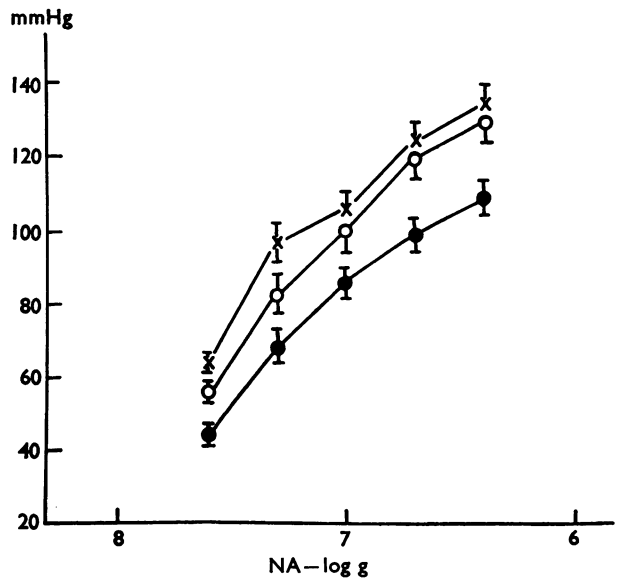

FIG. 2. Pithed rat preparation. Mean rise in blood pressure to various doses of noradrenaline (NA) after administration of desmethylimipramine, 0·5 mg/kg intravenously. (●——●), Normotensive controls ($n=20$); (○——○), rats with experimental renal hypertension ($n=10$); (×——×), rats with DOCA/NaCl hypertension ($n=10$). Vertical bars, S.E. of mean.

pared with normotensive controls (Fig. 1). A similar increase in sensitivity using DOCA/NaCl hypertensive preparations was also observed.

After administration of desmethylimipramine (0·5 mg/kg) intravenously, the pressor responses to exogenously administered noradrenaline were markedly potentiated in both magnitude and duration using both normotensive and hypertensive preparations. However, both renal and DOCA/NaCl hypertensive preparations still showed an increased sensitivity to noradrenaline, by a factor of approximately 2 when compared with the similarly treated normotensive group (Fig. 2).

The pressor responses to tyramine (25 μg and 50 μg intravenously) were significantly increased ($P<0{\cdot}001$) in magnitude in both renal and DOCA/NaCl hypertensive preparations when compared with the responses obtained using normotensive rats. Renal and DOCA/NaCl hypertensive preparations showed an even greater increase in sensitivity to DMPP, a nicotinic ganglion stimulating agent, when compared with responses obtained with normotensive controls (Fig. 3). In DOCA/NaCl hypertensive preparations there was also an increased sensitivity to angiotensin ($P<0{\cdot}002$) at all doses tested when compared with responses obtained in the normotensive preparations. However, renal hypertensive preparations did not show a significant increase in sensitivity to angiotensin throughout the whole dose range used (Fig. 4).

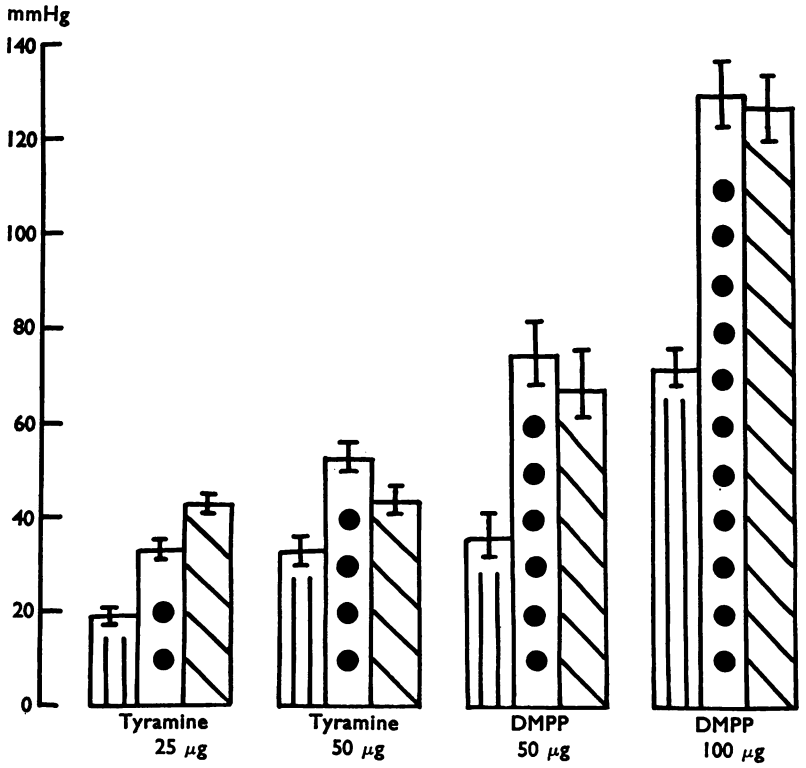

FIG. 3. Pithed rat preparations. Histogram representing the mean rise in blood pressure after intravenous doses of tyramine and DMPP. ▯, Normotensive control ($n=18$); ●, rats with experimental renal hypertension ($n=8$); ▩, rats with DOCA/NaCl hypertension ($n=8$). Vertical bars, S.E. of mean.

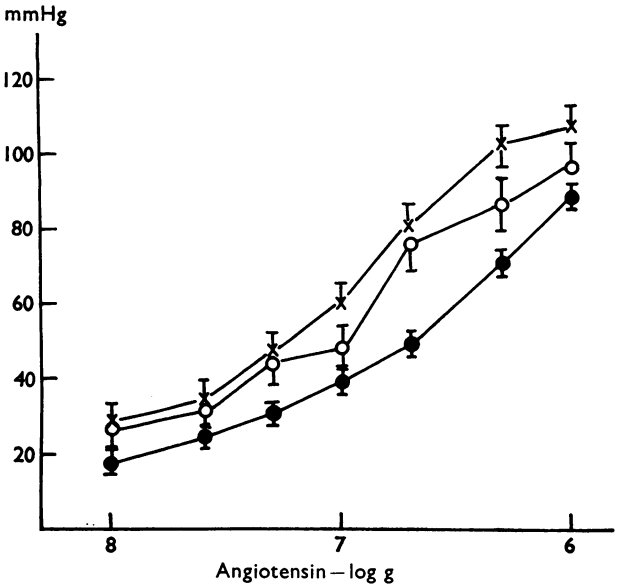

FIG. 4. Pithed rat preparations. Mean rise in blood pressure to various doses of Val.5 angiotensin 11 amide. (●——●), Normotensive controls ($n=15$); (○——○), rats with experimental renal hypertension ($n=8$); (×——×), rats with DOCA/NaCl hypertension ($n=8$). Vertical bars, S.E. of mean.

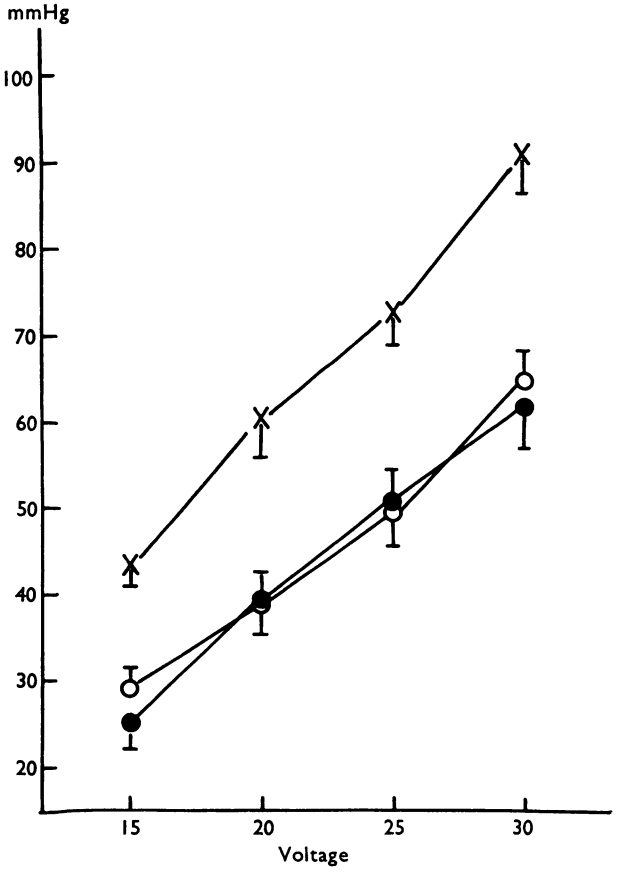

FIG. 5. Mean rise in blood pressure obtained using the Gillespie & Muir preparation (1967). Sympathetic outflow stimulated for a period of 18 s, pulse duration 0·03 ms, frequency 3 Hz and repeated every 10 min at varying voltage. (●——●), Normotensive controls ($n=18$); (○——○), rats with experimental renal hypertension ($n=6$); (×——×), rats with DOCA/NaCl hypertension ($n=9$). Vertical bars, S.E. of mean.

Using pithed preparations for stimulation of the entire sympathetic outflow (Gillespie & Muir, 1967), it was found that with low frequencies of stimulation (3 and 6 Hz) it was possible to obtain graded pressor responses with alterations in the voltage. In this manner DOCA/NaCl hypertensive preparations showed a marked increase in the responsiveness to sympathetic nerve stimulation (Fig. 5), while renal hypertensive preparations showed no statistically significant difference when compared with the responses of the normotensive controls.

Vascular reactivity in the perfused mesentery preparation obtained from normotensive and experimental hypertensive rats

In preparations from both DOCA/NaCl and renal hypertensive rats the perfusion pressures were the same as those from normotensive controls. The DOCA/NaCl group showed twice the sensitivity to exogenously administered noradrenaline when compared with the normotensive group. In preparations from the renal hypertensive rats there was also an increased sensitivity to noradrenaline but this was not as marked as the DOCA/NaCl group (Fig. 6).

Responses to nerve stimulation of the mesentery preparation varied considerably with the placing of the electrode. However, in preparations from DOCA/NaCl hypertensive rats the responses to periarterial nerve stimulation were consistently elevated using frequencies of 6, 12 and 24 Hz (Fig. 7). Preparations from renal hypertensive rats showed hyperresponsiveness only at 12 and 24 Hz while at the lower frequency of 6 Hz there was no difference when compared with the responses obtained using preparations from the normotensive rats.

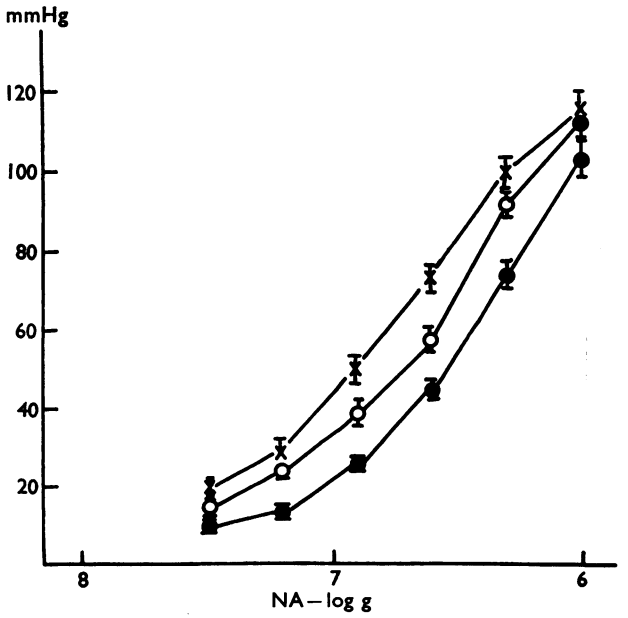

FIG. 6. Vasoconstrictor responses to noradrenaline of the isolated perfused mesentery preparation. (●——●), Normotensive controls ($n=18$); (○——○), rats with experimental renal hypertension ($n=6$); (×——×), with DOCA/NaCl hypertension ($n=9$). Vertical bars, S.E. of mean.

Discussion

The chief difficulty in comparing the pressor responses of various drugs in normotensive and hypertensive animals is that the initial levels of blood pressure are different and therefore influence the respective pressor responses. In order to overcome this problem all intact preparations were spinalized and after this treatment the blood pressures of normotensive and hypertensive rats were approximately the same (Taquini, 1963 ; Finch, 1970). The responsiveness to noradrenaline, in both DOCA/NaCl and renal hypertensive rats, was generally increased before and after the administration of desmethylimipramine, which suggests a ' postjunctional ' type supersensitivity. Increased sensitivity to noradrenaline has also been reported in anaesthetized DOCA/NaCl and renal hypertensive rats (Sturtevant, 1956 ; Phelan, 1966). In the case of angiotensin the increased cardiovascular reactivity was not so marked in either DOCA/NaCl or renal hypertensive rats ; however, it has been shown that angiotensin releases catecholamines in the pithed rat only at the higher doses (Finch & Leach, 1969).

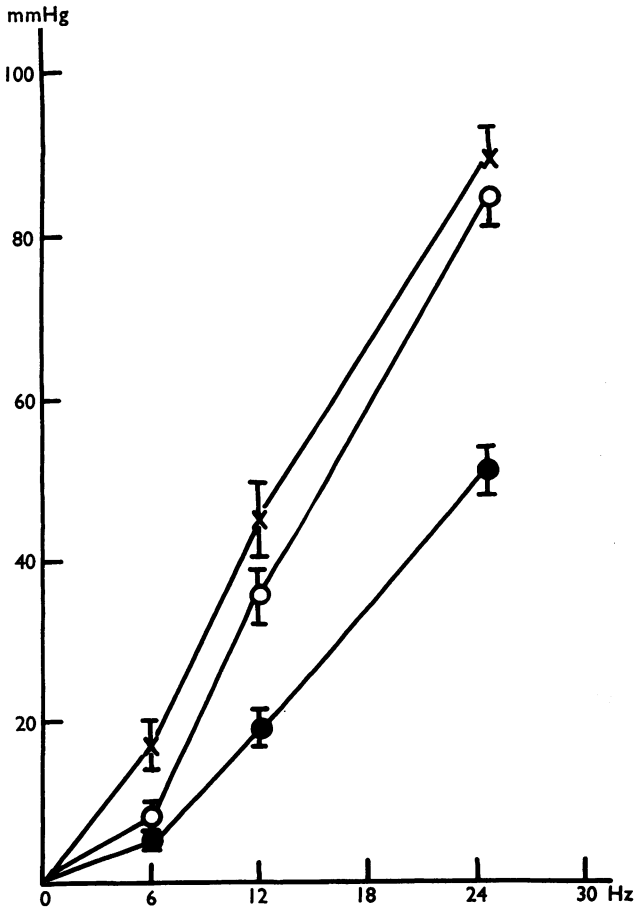

FIG. 7. Vasoconstrictor responses to periarterial nerve stimulation of the isolated, perfused mesentery preparation. Sympathetic nerves stimulated for a period of 15 s, 1 ms pulse duration, supramaximal voltage, every 3 min at varying frequency. (●——●), Normotensive controls ($n=12$) ; (○——○), rats with experimental renal hypertension ($n=6$) ; (×——×), rats with DOCA/NaCl hypertension ($n=6$). Vertical bars, S.E. of mean.

The pressor responses to tyramine and DMPP in both pithed DOCA/NaCl and renal hypertensive preparations were significantly increased when compared with responses in control preparations, which is in partial agreement with earlier findings using chronic renal hypertensive dogs (Page, Kaneko & McCubbin, 1966 ; Davey & Reinert, 1968). However, the responses to DMPP were increased by a greater extent than those to tyramine, and this could be due to the fact that DMPP releases catecholamines from the adrenal medulla and by its nicotinic ganglion stimulant action, whilst tyramine releases noradrenaline by a different mechanism (Lindmar, Löffelholz & Muscholl, 1967). Even so, recent evidence has shown that the adrenal medulla does not play an important part in the pressor response to DMPP in the rat (Chinn & Weber, 1970 ; Drew & Finch, unpublished observations). In a further series of experiments it was found that the responses to sympathetic nerve stimulation in the Gillespie & Muir preparation (1967) were markedly increased in DOCA/NaCl hypertensive preparations, but there was no significant difference in the responses of renal hypertensive preparations when compared with control responses. This latter result with renal hypertensive rats seems to contradict the findings using DMPP, tyramine and injected noradrenaline. One possible explanation is that less noradrenaline may be released per impulse in the renal hypertensive rat. This assumption does not, however, explain why the responses to DMPP were elevated unless, in addition to its ganglion stimulating action, at the dose used it also releases catecholamines directly from sympathetic nerve endings (Lindmar, Löffelholz & Muscholl, 1968).

Using the perfused mesentery preparation it was found that tissues isolated from both DOCA/NaCl and renal hypertensive rats showed hyperresponsiveness to injected noradrenaline. The increased sensitivity was comparable to that observed in preparations isolated from normotensive rats which had previously been treated with 6-hydroxydopamine (Finch & Leach, 1970c). These results are in general agreement with those of Haeusler & Haefely (1970) who used Krebs perfused mesentery preparations from genetically hypertensive rats and from rats made hypertensive with a combination of DOCA/NaCl and kidney encapsulation. McGregor & Smirk (1968) also reported hyperresponsiveness to angiotensin and noradrenaline in blood perfused mesentery preparations isolated from both renal and genetically hypertensive rats. In this laboratory it was not possible to study the effect of angiotensin on the Krebs perfused mesentery preparation since rapid tachyphylaxis occurred, and this confirms the earlier findings of Zumani (1969). Also, using the isolated tail preparation it has been reported that DOCA/NaCl derived preparations show increased reactivity to noradrenaline but not to angiotensin (Beilin & Wade, 1970). However, complete dose-response curves to angiotensin were not possible because of prolonged tachyphylaxis. Using periarterial nerve stimulation it was found that mesentery preparations from DOCA/NaCl hypertensive rats were hyperresponsive at both low and high rates of stimulation while preparations from renal hypertensive rats only showed hyperresponsiveness at the higher rates (12 and 25 Hz).

One or several of the following factors may contribute to the increased cardiovascular reactivity observed in both DOCA/NaCl and renal hypertensive rats. (a) Ionic changes occurring in the plasma, blood vessels and tissues could alter cardiovascular reactivity and also might affect the release of catecholamines (Tobian & Redleaf, 1968 ; Phelan & Wong, 1968 ; Clarke, Smookler & Barry, 1970). (b) Structural changes in the media of the blood vessels may interfere with the release

and/or passage of noradrenaline (Wolinsky, 1970). (c) Structural changes which interfere with the wall/lumen ratio of blood vessels may affect cardiovascular reactivity (Folkow, Hallaeck, Lungren & Weiss, 1970). (d) Alterations in the storage and turnover rates of noradrenaline may affect the amount available for release by sympathetic nerve stimulation (Krakoff, DeChamplain & Axelrod, 1967 ; DeChamplain, Mueller & Axelrod, 1969 ; Henning, 1969 ; Louis, Spector, Tabei & Sjoerdsma, 1969). (e) Differences in the metabolism may explain the increased reactivity since metabolism rather than reuptake is an important factor in the termination of noradrenaline induced contractions in the rabbit aorta (Kalsner & Nickerson, 1969). Although several of the above defects occur in either DOCA/NaCl or renal hypertension it is rather hard to correlate them with the generalized increase in cardiovascular reactivity found in the present studies. Furthermore no real conclusions can be made as to whether this increased sensitivity is a contributing factor of the elevated blood pressure in the experimental hypertensive rat.

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