A SPECIFIC INCREASE IN CARDIOVASCULAR REACTIVITY RELATED TO SODIUM RETENTION IN DOCA-SALT-TREATED RATS

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SUMMARY

1. Exchangeable body sodium was measured in deoxycorticosterone acetate (DOCA)–salt-treated rats by whole body γ-counting after equilibration with a 22Na isotope.

2. The blood pressure of DOCA–salt-treated rats is positively correlated with their exchangeable body sodium.

3. After pithing, the heart rate is significantly lower in DOCA–salt-treated than in control rats: in conscious DOCA–salt-treated rats the heart rate is also lower than in control rats but the difference was not statistically significant.

4. Cardiac and vascular responses to sympathetic stimulation are positively correlated with exchangeable body sodium in pithed rats, but there is no correlation between either cardiac or vascular responses to noradrenaline injections and exchangeable sodium.

5. Pressor responses to sympathetic stimulation in pithed DOCA–salt-treated rats are potentiated less by cocaine than are responses of untreated control rats.

6. It is suggested that sodium retention in mildly hypertensive rats specifically enhances responses to sympathetic stimulation by increasing the availability of the sympathetic transmitter.

Key words: cardiovascular reactivity, deoxycorticosterone acetate–salt hypertension, sodium retention, sympathetic stimulation.

INTRODUCTION

Experimental hypertension in most strains of rats may be successfully produced by a combination of unilateral nephrectomy, deoxycorticosterone acetate (DOCA) administration, either as injections or by subcutaneous implant, and replacement of drinking water by a 1% solution of sodium chloride (Grollman, Harrison & Williams, 1940; Selye, Hall & Rowley, 1943). There
is also evidence that salt intake may be an important factor influencing the severity of essential hypertension in man (Ambard & Beaujard, 1904; Meneely & Dahl, 1961; Knudsen & Dahl, 1966). There is general agreement that sodium retention results in an increase in peripheral resistance, but the exact mechanism of this change is disputed.

Structural changes in the walls of small vessels may lead to narrowing of the lumen (Folkow, Grimby & Thulesius, 1958), but many of the changes such as vascular smooth muscle hypertrophy and fibrinoid necrosis in arteries from hypertensive animals appear to be secondary to the raised arterial pressure, being either adaptive or a pathological consequence of the hypertension (Folkow et al., 1958; Hinke, 1965; Hollander, Kramsch, Farmelant & Madoff, 1968; Pickering, 1968).

The alternative mechanism through which peripheral resistance could be raised is by increased vascular tone, which may be a result of either increased stimulation by nervous impulses or chemical stimuli or both, or of greater reactivity of the resistance vessels to vasoconstrictor stimuli. There is some evidence that DOCA–salt-treated hypertensive rats may have increased pressor activity in the plasma (Hinke, 1965; Dahl, Knudsen & Iwai, 1969), but there are a number of reports that blood vessels from these hypertensive animals are hyperreactive to a variety of pressor stimuli (Sturtevant, 1956; Hinke, 1965; Beilin, Wade, Honour & Cole, 1970; Finch, 1971a). There are also many reports of hyperreactivity in other types of experimental hypertension, including renal hypertension (Phelan, 1966; McGregor & Smirk, 1968; Davey & Reinert, 1968) and genetic hypertension (Laverty, 1961; Haeusler & Haefely, 1970), and in essential hypertension in man (Doyle & Fraser, 1961; Kaneko, Takeda, Nakajima & Ueda, 1966; Sivertsson & Olander, 1968).

In the present studies, the relationships were explored between exchangeable body sodium, blood pressure and cardiovascular reactivity in DOCA–salt-treated rats, since it has been suggested that the sodium load is a determinant of hypertension (Guyton & Coleman, 1969). The hypertension induced was mild compared with that found by other workers, so that secondary changes in cardiac and vascular structure that occur in severe or prolonged hypertension are likely to be less important. Cardiovascular reactivity was studied after pithing, when the resting blood pressures are similar for hypertensive and normotensive rats, so a better comparison can be made than is possible with either anaesthetized or conscious animals in which the resting blood pressures are different.

MATERIALS AND METHODS

Methods

DOCA–salt treatment. Female hooded Wistar rats weighing 100–120 g underwent unilateral nephrectomy under ether anaesthetic and were given a subcutaneous implant of 50 mg of DOCA. Drinking water was replaced by sodium chloride solution (161 mmol/l) and the rats were fed ad libitum on a cube diet over a 15 week period, by which time they weighed 150–200 g.

Rats of the same strain, sex and age and of the same weight range were used for control experiments.

Exchangeable body sodium. This was measured after replacing the cube diet by a sodium-free diet (unhusked rice, casein and peanut oil in the proportion 15 : 15 : 1, by weight, and a vitamin supplement). A drinking solution containing $^{22}$Na (1.25 μCi/litre) in sodium chloride
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solution (161 mmol/l) was supplied. The $^{22}$Na content of a rat was measured by a whole body counting in an Armac whole body counter attached to a Packard autogamma spectrometer Model 3002 and a weighed volume of the $^{22}$Na-labelled sodium chloride solution was also counted. Exchangeable body sodium in mmol/kg was calculated from the expression:

$\frac{(\text{counts/kg of rat} \times 161)}{(\text{counts/kg of drinking solution})}$

A group of rats placed on the regime were subjected to daily whole body counting to determine the number of days required for equilibration. Exchangeable body sodium in these rats increased, until, after 6 days, the rats were fully equilibrated with the isotope, as indicated by the constant values obtained for exchangeable body sodium, which varied over the next 14 days by only 3% of the mean (Fig. 1).

All other rats were placed as a routine on the regime 7 days before whole body counting to estimate total exchangeable body sodium. In a group of twenty normal rats the mean exchangeable body sodium was $45.2 \pm 0.6$ mmol/kg.

![Equilibration of twenty normotensive rats with $^{22}$Na during administration of a salt-free diet and a drinking solution containing 161 mmol/l of $^{22}$Na-labelled NaCl.](image)

**Plasma sodium concentration.** This was determined on a flame photometer, blood samples (100 $\mu$l) being taken from the orbital sinus under ether anaesthesia on the same day as exchangeable body sodium was determined.

**Blood pressure and heart rate (conscious).** Arterial systolic pressure and heart rate were measured in the conscious rat, after warming for 30 min at 38°C, by tail-cuff sphygmomanometry. Tail artery pulses were detected with a strain-gauge sensor and displayed on an oscilloscope screen for measurement of systolic pressure, and were also recorded on a galvanometric chart recorder for heart rate determinations. Blood pressure and heart rate values quoted are the means of at least three readings for each rat taken on separate days over an interval of less than 1 week.

**Observations on pithed rats.** Rats were pithed and the thoracolumbar sympathetic outflow was stimulated by following the method of Gillespie & Muir (1967), but neither tubocurarine
nor atropine was given. The rats were artificially ventilated by using a Palmer small animal respiration pump at a frequency of 100 strokes/min with a stroke volume of 1 ml/100 g body weight. Arterial pressure was monitored from one carotid artery with a Stratham 23 Db transducer and beat to beat arterial pressure, mean arterial pressure and heart rate triggered from the pulse record were recorded on a Gilson 5 MP polygraph recorder. Square wave pulses from an AEL laboratory stimulator were delivered at 15 min intervals by using a voltage of 150 V, which was near maximal, and a pulse width of 1 ms for 10 s at frequencies indicated in the text. Drugs used were diluted in 150 mmol/l of sodium chloride. Injections in a volume of 0.1–0.2 ml were given between periods of spinal stimulation through a cannula in a femoral vein.

Drugs

The following drugs were used, and the doses in the text refer to these salts: cocaine hydrochloride (Drug Houses of Australia); (-)-noradrenaline bitartrate (Winthrop).

RESULTS

Conscious blood pressure, heart rate and exchangeable body sodium

Fifteen weeks after unilateral nephrectomy, DOCA implantation and provision of sodium
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chloride (161 mmol/l) in the drinking water, there was a significant increase in mean arterial pressure of 22 mmHg compared with untreated rats of similar age and weight ($t = 9.97, P < 0.001$). The mean heart rate of the untreated group was higher than that of DOCA–salt-treated rats, but the difference was not significant ($t = 1.80; 0.05 < P < 0.1$). The mean exchangeable

![Graph showing correlation between exchangeable body sodium ($E_{Na}$) and mean systolic blood pressure. The correlation coefficient of 0.637 is highly significant ($P < 0.001$). The line shows the calculated regression of blood pressure on exchangeable body sodium; its equation is shown.](image)

**FIG. 3.** Correlation between exchangeable body sodium ($E_{Na}$) and mean systolic blood pressure. The correlation coefficient of 0.637 is highly significant ($P < 0.001$). The line shows the calculated regression of blood pressure on exchangeable body sodium; its equation is shown.

**TABLE 1. Blood pressure and heart rate after pithing**

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of rats</th>
<th>Mean blood pressure (mmHg)</th>
<th>Heart rate (beats/min)</th>
<th>Exchangeable body sodium (mmol/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOCA–salt treated</td>
<td>11</td>
<td>47.3 ± 1.7</td>
<td>257 ± 6</td>
<td>61.9 ± 2.0*</td>
</tr>
<tr>
<td>Normotensive controls</td>
<td>10</td>
<td>51.9 ± 1.4</td>
<td>295 ± 6</td>
<td>$45.2 ± 0.6^\dagger$ (20)</td>
</tr>
</tbody>
</table>

Values shown are means ± SEM.

* Determined 1–3 days before pithing.

† Determined in a separate control group with number of animals used in parentheses.

Body sodium was significantly greater in DOCA–salt-treated than in control rats ($t = 7.65, P < 0.001$).

Individual and mean values of conscious blood pressure, heart rate and exchangeable body sodium measurements for DOCA–salt-treated and control rats are shown in Fig. 2.
There was a significant positive correlation \( r = 0.637, P < 0.001 \) between blood pressure and exchangeable body sodium of thirty-five DOCA-salt-treated rats (Fig. 3).

Plasma sodium concentrations in a group of eighteen DOCA-salt-treated rats also showed a significant positive correlation \( r = 0.583, P < 0.001 \) with exchangeable body sodium.

### Blood pressure and heart rate of pithed rats

Mean blood pressures and heart rates of DOCA-salt-treated and control rats after pithing are shown in Table 1. DOCA-salt-treated rats did not have a higher mean arterial pressure than normotensive controls after pithing: in fact, the mean arterial pressure of the DOCA-salt-treated rats was 4.6 mmHg (9\%) lower and this difference was just significant \( t = 2.10, P < 0.05 \). The heart rate of the DOCA-salt-treated rats after pithing was 38 beats/min (13\%) less than in the normotensive controls and this difference was highly significant \( t = 4.63, P < 0.001 \).

Fig. 4. Records of pulsatile blood pressure (BP, upper channel), mean blood pressure (mean BP, centre channel) and heart rate (HR, lower channel) of a pithed, DOCA-salt-treated rat. Responses are shown to spinal stimulation and to noradrenaline before and after a dose of cocaine. Stimulation was with frequencies of 5 (●), 2 (□) and 0.5 (○). Hz for 10 s periods, and noradrenaline was injected in doses of 0.5 (●), 0.2 (△) and 0.1 (△) µg/kg. The time between panels was 10 min.

### Responses to stimulation of thoracolumbar sympathetic nerves

A typical record from which mean blood pressure and heart-rate responses were obtained is shown in Fig. 4.

Mean blood pressure and heart-rate responses to stimulation at a frequency of 5 Hz were on the linear part of the frequency–response curve. Mean blood pressure and heart-rate responses of DOCA-salt-treated rats to electrical stimulation of the sympathetic outflow were compared with the exchangeable body sodium by correlation analysis (Fig. 5). For eleven rats, significant positive correlations were obtained with mean arterial pressure \( r = 0.699, P < 0.02 \) and heart rate \( r = 0.705, P < 0.02 \).

### Responses to noradrenaline in pithed rats

Mean arterial pressure and heart-rate responses to 0.5 µg/kg of noradrenaline were found to
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occur on the linear portion of the dose–response curves. In the eleven rats used for spinal stimulation referred to above, the mean arterial pressure and heart-rate responses to noradrenaline (0.5 µg/kg) were not significantly correlated with exchangeable body sodium ($r = 0.135$ and $r = 0.112$, respectively).

**Effect of cocaine on responses to thoracolumbar sympathetic stimulation and noradrenaline**

Cocaine (10 mg/kg) increased the blood pressure responses of the pithed rat to sympathetic

![Graphs showing correlation between responses to sympathetic nerve stimulation at a frequency of 5 Hz and exchangeable body sodium ($E_{Na}$). The left-hand diagram represents mean blood pressure responses and the right-hand diagram represents heart-rate responses. The correlation coefficients ($r$) are significant ($P<0.02$). The lines are calculated regression lines: their equations are shown.](image)

**TABLE 2. Potentiation of pressor responses by cocaine**

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of rats</th>
<th>Stimula-</th>
<th>Noradrena-</th>
<th>Exchangeable body sodium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>tion (5 Hz)</td>
<td>line (0.5 µg/kg)</td>
<td>(mmol/kg)</td>
</tr>
<tr>
<td>DOCA-salt treated</td>
<td>6</td>
<td>243±15</td>
<td>172±12</td>
<td>57.0±1.6</td>
</tr>
<tr>
<td>Normotensive controls</td>
<td>10</td>
<td>483±82</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8</td>
<td></td>
<td>256±51</td>
<td>45.2±0.6† (20)</td>
</tr>
</tbody>
</table>

*Potentiation of responses are expressed as percentages of responses before cocaine. Values shown are means±SEM.
† Determined in a separate control group with the number of animals used in parentheses.
nerve stimulation (5 Hz) and to noradrenaline (0.5 µg/kg) by extending the duration of the response more than by increasing the maximal height of the response (Fig. 4, right-hand panel). Quantitation of the cocaine effect was obtained by measuring the area of the responses before and after cocaine administration and expressing the effect of cocaine as a percentage increase in the response.

Cocaine increased responses to sympathetic nerve stimulation more in control than in DOCA–salt-treated rats (Table 2). This difference was just significant ($t = 2.23, P<0.05$). Similarly, noradrenaline responses were increased more in the control than in the DOCA–salt-treated rats, but the difference was not significant ($t = 1.40, 0.1 < P<0.2$). Responses to sympathetic nerve stimulation were more potentiated by cocaine than were responses to noradrenaline in both control and DOCA–salt-treated rats, confirming the findings of Glover & McCulloch (1970).

**DISCUSSION**

In this study cardiovascular reactivity was studied in DOCA–salt-treated rats by using a pithed preparation. This preparation has the advantage that hypertensive and normotensive rats have similar arterial pressures, overcoming the problem caused by the different initial arterial pressures present in the conscious rat (Nicholas, 1971). The pithed rat has certain advantages over isolated preparations (Beilin et al., 1970) since the cardiovascular system is intact and the effects of pressor stimuli on the whole cardiovascular system can be observed in the absence of counteracting reflexes.

Rats made hypertensive by DOCA–salt administration had a lower basal mean blood pressure when pithed than had normotensive rats; this finding conflicts with the observations of Finch (1971b). The difference in the results might be related to the lower degree of hypertension induced by DOCA–salt-treatment in our experiments than in Finch’s; the higher level of pressure in his rats may have caused vascular hypertrophy so the rats have a higher peripheral resistance even in the absence of neurogenic tone.

The lower basal blood pressure after pithing in DOCA–salt–treated rats is probably a direct result of the lower heart rate. Assuming stroke volumes to be similar in DOCA–salt–treated and control rats, DOCA–salt treatment decreased cardiac output by 13%, but blood pressure was only decreased by 9%, showing that peripheral resistance is probably slightly greater in the DOCA–salt–treated rats.

The lower basal heart rate in the DOCA–salt–treated than in control rats may be caused by the raised plasma sodium decreasing the rate of depolarization of pacemaker action potentials. Also, in pithed rats in the absence of neurogenic drive, the heart rate might be elevated slightly by a slow leakage of noradrenaline from neuronal stores (Burn & Rand, 1958). It is well established that storage of noradrenaline is greatly decreased in DOCA–salt–treated hypertensive rats (DeChamplain, Krakoff & Axelrod, 1966, 1967, 1968), and this may result in a decreased rate of noradrenaline leakage.

The heart rates of conscious DOCA–salt–treated and control rats did not differ significantly, so that the difference in endogenous basal heart rate must be counteracted by centrally mediated neurogenic mechanisms.

Cardiovascular reactivity has been compared with exchangeable body sodium, which, it has been suggested, is an important determinant of arterial pressure (Guyton & Coleman, 1969),
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particularly in DOCA–salt hypertension. We found that both systolic blood pressure and plasma sodium concentration were correlated with exchangeable body sodium.

In pithed DOCA–salt-treated rats, the arterial pressure and heart-rate responses to stimulation of the sympathetic nerves were positively correlated with exchangeable body sodium. The increased pressor responses are probably largely due to increased vasoconstriction, since selective stimulation of the cardiac sympathetic nerves produces pronounced tachycardia but only a slight rise of blood pressure (Gillespie, Maclaren & Pollock, 1970); elimination of the cardiac component of the response with β-receptor antagonists does not significantly decrease the pressor response (Gillespie & Muir, 1967; Atkinson, Dusting & Rand, 1973). In contrast, the pressor and heart-rate responses to noradrenaline injections were not significantly correlated with exchangeable body sodium. The correlation between exchangeable body sodium and pressor and heart-rate responses to sympathetic nerve stimulation, but not to noradrenaline, suggests that sodium retention increases the effective concentration of transmitter noradrenaline.

Cocaine potentiated responses to noradrenaline and to sympathetic nerve stimulation by inhibiting the neuronal uptake (or re-uptake) of noradrenaline. It was found to be less effective in potentiating pressor responses to sympathetic nerve stimulation in DOCA–salt-treated rats than in control rats. This is consistent with the observations of Pomeroy & Rand (1971) and Harris & Palmer (unpublished work) that increasing the sodium ion concentration of the perfusing medium in isolated rabbit ear arteries decreased the potentiated responses to sympathetic nerve stimulation in the presence of cocaine. A raised extracellular sodium concentration increases the affinity of the neuronal uptake mechanism for noradrenaline (Bogdanski & Brodie, 1969). Cocaine has the opposite effect, and there may, therefore, be competition between sodium ions and cocaine. Since an increase in extracellular sodium ion concentration facilitates noradrenaline uptake, the process will be more effective in DOCA–salt-treated rats which have increased exchangeable body sodium, hence increased plasma sodium concentration, than in normal rats.

It follows that sodium retention must greatly enhance the amount of transmitter released on sympathetic nerve stimulation, since responses are greater despite the operation of a more efficient re-uptake process, which inactivates the transmitter. Responses to noradrenaline are not affected by the facilitated neuronal uptake process, since uptake is thought to remove only a small proportion of circulating (or injected) noradrenaline from peripheral vascular beds (Vane, 1969). Any small decrease in the amount of noradrenaline reaching the vascular receptors is probably not reflected by a decreased response since it might be expected that the vascular response to noradrenaline would be slightly greater than normal, being superimposed on a slightly greater basal peripheral resistance.

The findings suggest that in DOCA–salt-treated rats the increase in body sodium is associated with an increased response to sympathetic nerve stimulation which is due to a change in noradrenaline dynamics at the neuro-effector junction, since noradrenaline responses are not changed. The loss of potassium that occurs in DOCA–salt-treated rats would have the same effect on transmitter dynamics at adrenergic nerve terminals as does the retention of sodium, since decreasing the potassium concentration in the medium has the same effect as raising the sodium concentration in increasing noradrenaline release, and noradrenaline uptake (Bogdanski & Brodie, 1969). Although the present study does not allow us to determine which ion is responsible for the effects on adrenergic transmission in DOCA–salt-treated rats, it seems more likely that hypertension is related to hypernatraemia than to hypokalaemia.
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REFERENCES


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Sivertsson, R. & Olander, R. (1968) Aspects of the nature of the increased vascular resistance and increased 'reactivity' to noradrenaline in hypertensive subjects. Life Sciences, 7, 1291-1292.
