THE COMPARATIVE HYPERTENSIVE ACTIVITIES OF THE ACETATES OF D-ALDOSTERONE AND DEOXYCORTICOSTEROONE

By

C. E. Hall and O. Hall

ABSTRACT

The hypertensive effect of the acetates of d-aldosterone and deoxycorticosterone was compared by injecting 0.125 mg of each twice daily in oil subcutaneously into unilaterally nephrectomized rats given 1% saline solution to drink. The two hormones had entirely comparable activity in respect to the enhancement of saline consumption, the development of hypertension and the magnitude of cardiac enlargement induced. Aldosterone treatment caused a much greater degree of renal hypertrophy and far more severe vascular lesions in the heart and kidney. It is suggested that both of these effects may reflect the superior ability of aldosterone to cause potassium excretion and therefore a more severe hypokaliaemia. Only aldosterone caused thymic involution, believed to be an indirect response, and impairment of body growth.

Under the circumstances of this experiment the hypertensive potency of aldosterone was at least as great as that of deoxycorticosterone, and the ability to bring about vascular damage far greater. Although it is recognized that this relationship might not obtain at all dose levels of the two steroids, it is suggested that the lesser activity usually ascribed to aldosterone as compared with DCA when the two are given in dosages calculated to cause an equivalent degree of sodium retention, may reflect the operation of variables such as absorption rate, enzymatic inactivation rate and hence the respective quantities in the circulation at any given time, rather than differences in the inherent potency.

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Luxus consumption of sodium chloride causes hypertensive cardiovascular disease in rats (Sapirstein et al. 1950; Meneely et al. 1953; Koletsky 1958; Hall & Hall 1964) and has been implicated in the genesis of the condition in man (McDonough & Wilhelmj 1954; Dahl 1958). The adverse effect of salt in rats is aggravated by a reduction in effective renal mass (Koletsky 1959) or by treatment with deoxycorticosterone (Selye & Pentz 1943; Hall et al. 1952; Salgado 1955).

It is probable that deoxycorticosterone-induced hypertension merely represents an accelerated and aggravated form of salt hypertension since it fails to develop if the salt intake is severely curtailed (Selye et al. 1949; Tobian & Redleaf 1957). Therefore, it might reasonably be inferred that aldosterone, a stronger mineralocorticoid (Gross 1956), would even more effectively cause hypertension under experimental conditions than would deoxycorticosterone acetate (DCA). This is alleged not to be the case (Gross et al. 1955; Gross & Lichtlen 1958), and in fact it is commonly averred that under experimental conditions DCA has a much greater effect upon blood pressure than has aldosterone (Fregly & Arean 1959; Gross 1960; Nadasdi 1965). Hypertension induced by aldosterone is also said to be less severe than it is when DCA is employed, and not to be accompanied by the marked and extensive vascular lesions so commonly encountered in animals treated with the latter steroid (Gross et al. 1957; Gross & Lichtlen 1958; Masson et al. 1962). This is at variance with what would be expected in consequence of the disparity in sodium-retaining potencies, although in experiments where the two have been compared some attempt has usually been made to adjust the dosage of each so as to compensate for the inequality. The principal drawback to this maneuver is that aldosterone is 100 times as potent as DCA in its effect on the urinary Na+/K+ ratio, 25 times as potent in causing sodium retention, but only 5 times as active in promoting K+ excretion (Gross 1956), thus making it impossible to balance the activities of the two by any single proportional adjustment of dosage.

Recently we have reported that 0.125 mg of d-aldosterone acetate given subcutaneously twice daily in oil causes severe hypertensive vascular disease within three weeks in unilaterally nephrectomized immature rats drinking saline solution (Hall & Hall 1965). In that experiment DCA was not employed, precluding a comparison between the two mineralocorticoids, but the vascular lesions observed were quite as severe as those usually found in rats given a high dosage of DCA, in contrast to what has been reported (Gross & Lichtlen 1958; Masson et al. 1962). The present experiment was therefore designed to compare the two steroids under comparable circumstances and at the same dosage, rather than with dosages of each adjusted to compensate for the lesser potency of DCA.
MATERIALS AND METHODS

Thirty 60–80 g female rats of the Houston-Cheek strain, Sprague-Dawley derived, were unilaterally nephrectomized under ether anaesthesia and divided into three equal groups. Group 1 received 0.125 mg d-aldosterone acetate in sesame oil subcutaneously twice daily. Group 2 received 0.125 mg of deoxycorticosterone acetate in oil twice daily, and group 3 received similar injections of oil vehicle only. All animals were individually caged in temperature-controlled quarters and given 1% NaCl solution to drink. Purina laboratory chow ad libitum constituted the sole diet.

Fluid intake of each rat was measured on three days in the middle of each week and the group average so obtained was considered to be representative of the intake for that week. Systolic arterial pressure was measured plethysmographically in unanaesthetized animals. On the 17th day the animals were killed by anaesthesia and various tissues and organs taken for weight and/or histologic examination placed in neutral 10% formalin. Organs to be weighed were removed from fixative, blotted, trimmed, and weighed on an analytical balance.

RESULTS

Ten days of treatment led to systolic hypertension in several members of the two steroid-treated groups. At this time there was no appreciable difference in response between them, either in terms of incidence or the magnitude of hypertension. A week later the average pressure of aldosterone-treated rats had reached 183 ± 7 mm Hg, whereas it stood at 168 ± 8 mm Hg in those getting DCA and 121 ± 1 mm Hg in controls. The difference between the two steroid treated groups was not statistically significant (P > .05), although each differed from controls (P < .001). However, all of the aldosterone-treated rats were hypertensive, whereas 20% of the DCA-treated animals were not. The data are given in Table 1.

Saline polydipsia was evident in steroid-treated rats in the first week of treatment, when fluid intake of both groups exceeded that of controls (P < .01 or better). It became even more conspicuous with longer treatment, so that in the final week of the experiment fluid consumption averaged 107 ± 8 ml/day in rats receiving aldosterone and 94 ± 5 ml/day in those given DCA, as compared to 51 ± 2 ml/day in controls. The response is compared in Table 1.

Animals treated with aldosterone did not grow as well as did controls or those getting DCA, the terminal weight of surviving animals given aldosterone 103 ± 5 g, being less than that of either of the two other groups (P < .05) which weighed substantially the same, 120 ± 5 g and 121 ± 5 g. Furthermore, there were three deaths in the aldosterone-treated group during the experiment, and none in either of the others. Two died in the first week of treatment, one from pneumonitis and one from atrophy of the remaining kidney,
Table 1.
Principal Findings in Aldosterone-treated, Deoxycorticosterone-treated and Control Unilaterally Nephrectomized Rats Drinking Saline.

<table>
<thead>
<tr>
<th>Data</th>
<th>Aldosterone</th>
<th>Deoxycorticosterone</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body Weight g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>66 ± 2*</td>
<td>66 ± 2</td>
<td>67 ± 2</td>
</tr>
<tr>
<td>Final</td>
<td>103 ± 5</td>
<td>120 ± 5</td>
<td>121 ± 5</td>
</tr>
<tr>
<td>Fluid Intake ml/rat/day</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 1</td>
<td>43 ± 4+++</td>
<td>37 ± 2+++</td>
<td>28 ± 2</td>
</tr>
<tr>
<td>Week 2</td>
<td>64 ± 5+++++</td>
<td>59 ± 3+++++</td>
<td>32 ± 3</td>
</tr>
<tr>
<td>Week 3</td>
<td>107 ± 8+++++</td>
<td>94 ± 5+++++</td>
<td>51 ± 2</td>
</tr>
<tr>
<td>Blood Pressure mm Hg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 2</td>
<td>151 ± 9+</td>
<td>148 ± 5++</td>
<td>121 ± 7</td>
</tr>
<tr>
<td>Week 3</td>
<td>183 ± 7+++++</td>
<td>168 ± 8+++++</td>
<td>121 ± 1</td>
</tr>
<tr>
<td>Organ Weight mg/100 g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body Weight</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kidneys</td>
<td>1392 ± 64++++</td>
<td>1086 ± 26++++++</td>
<td>829 ± 31</td>
</tr>
<tr>
<td>Heart</td>
<td>480 ± 11++++</td>
<td>449 ± 9++++++</td>
<td>396 ± 9</td>
</tr>
<tr>
<td>Thymus</td>
<td>179 ± 17++++</td>
<td>251 ± 20</td>
<td>270 ± 18</td>
</tr>
<tr>
<td>Adrenals</td>
<td>35.9 ± 2.3</td>
<td>33.9 ± 0.7</td>
<td>35.5 ± 0.8</td>
</tr>
</tbody>
</table>

* Mean ± S. E. M. ++ P < .01 ++++ P < .001
+ P < .05 +++ P < .005

which probably should not be charged to the hormone, but one died in the third week with severe hypertension and hypertensive cardiomegaly.

At necropsy, white epicardial scars were visible in five of seven rats getting aldosterone and in only one of ten DCA-treated animals. Two of the former had marked renal glomerular haemorrhages, a change not seen in either of the other two groups. Macroscopic polyarteritis, a lesion common in rats with arterial hypertension, was not visible in any animal.

Organ Weights
The kidneys were significantly enlarged in both steroid-treated groups as compared to controls (P < .001 or better), but were also larger in aldosterone-treated rats than in those which had received DCA (P < .001), on either a relative (68% versus 31% enlargement) or absolute (39% versus 30% enlargement) basis.

Cardiac hypertrophy was also evident in both of the hormone-treated groups (P < .001 or better). Although the effect was somewhat greater in rats
given aldosterone, the difference between these and DCA-treated rats was not statistically significant.

DCA did not cause thymus atrophy, but the glands of rats receiving aldosterone were significantly smaller than in either DCA-treated \((P < .01)\) or control \((P < .001)\) animals.

There was no discernible effect of hormone treatment upon the weight of adrenal glands. Organ weights are given in Table 1.

Vascular Lesions

Cardiac lesions were found only in rats that had received hormone. They were present in all except one of the aldosterone-treated animals, and absent from all but one of those given DCA. The mildest changes from aldosterone treatment consisted of focal areas of varying size in which the striations of myocardial fibers were entirely absent (Fig. 1). In the more severely affected rats there was extensive necrosis of arterial walls and perivascular infiltration of inflammatory cells (Figs. 2 and 3). Both types of lesion were sometimes present in the same heart.

The one rat given DCA that had detectable cardiac changes (Fig. 4) was also noted to have a few hyalinized renal glomeruli and occasional hyaline tubular casts. The remaining animals of that group had only slight glomerular enlargement and a few casts as indices of the treatment they had received. Generally, the changes were less pronounced in rats given DCA (Fig. 5) than in those given aldosterone (Fig. 6). The incidence of glomerular hyalinization was eightfold higher among aldosterone-treated rats, and the changes were far more severe than in the DCA-treated animals (Figs. 7–10). Controls were free of lesions.

Polyarteritis of the pancreatic and mesenteric arteries was seen only in rats of the aldosterone group, more than half of which were affected although the changes were not particularly severe (Fig. 11).

The lesions were arbitrarily graded in severity on a 0 to 3+ scale and the incidence in any group expressed as a percentage of the theoretical maximum. Comparative figures are given in Table 2.

**DISCUSSION**

Reports on the hypertensenogenic properties of aldosterone are at variance. Using very small doses several investigators have noted a detectable increase in the blood pressure of rats in either acute \((Friedman et al. 1958)\) or long-term \((Kumar et al. 1955, 1957; Gornall et al. 1960)\) experiments. On the other hand, even chronic experiments have frequently given negative results \((Gross et al. 1955; Fregly & Arean 1959; Gaunt et al. 1957)\). With larger doses such


3. An area of patchy necrosis (at lower left) and perivascular inflammation coexisting in the heart of a rat given aldosterone. Haematoxylin and Phloxine × 128.

4. The only cardiac lesion found in a DCA-treated rat. showing replacement fibrosis. Haematoxylin and Phloxine × 160.

5. Low power view of most seriously damaged kidney in DCA-treated series. The architecture is not grossly distorted. Haematoxylin and Phloxine × 25.

6. Low power view of kidney from aldosterone-treated rat. Tubules show dilation and luminal casts, and areas of atrophy are present. Haematoxylin and Phloxine × 25.
Figs. 7-11.

7. Higher power of Fig. 5, showing one glomerulus undergoing hyaline degeneration alongside a more normal glomerulus. There are scanty tubular casts. Haematoxylin and Phloxine × 160.

8. Higher power of kidney in Fig. 6. All three glomeruli show hyaline degeneration, atrophy, and adhesions between tuft and Bowman’s capsule. Tubules are dilated and distorted and hyaline and granular casts are present. Haematoxylin and Phloxine × 140.

9. Typical glomerulus from DCA-treated rat. Some capillary thickening is present, but parenchyma appears normal. Haematoxylin and Phloxine × 250.

10. Glomerulus from aldosterone-treated rat showing capsular fibrosis, fibrinoid necrosis of glomerular capillaries and obliteration of capsular space. Haematoxylin and Phloxine × 320.

11. Polyarteritis nodosa in pancreatic artery of aldosterone-treated rat. This lesion was common in the group, but not seen in those on DCA. Haematoxylin and Phloxine × 160.
Table 2.
Blood Pressure and Vascular Changes Due to Hormone Treatment.

<table>
<thead>
<tr>
<th>Item</th>
<th>Aldosterone</th>
<th>DCA</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Group Hypertensive Week 2</td>
<td>62.5</td>
<td>60</td>
<td>0</td>
</tr>
<tr>
<td>Lowest Pressure</td>
<td>108</td>
<td>124</td>
<td>102</td>
</tr>
<tr>
<td>Highest Pressure mm Hg</td>
<td>186</td>
<td>170</td>
<td>144</td>
</tr>
<tr>
<td>Average Pressure</td>
<td>151 ± 9</td>
<td>148 ± 5</td>
<td>121 ± 7</td>
</tr>
<tr>
<td>% Group Hypertensive Week 3</td>
<td>100</td>
<td>80</td>
<td>0</td>
</tr>
<tr>
<td>Lowest Pressure</td>
<td>152</td>
<td>118</td>
<td>114</td>
</tr>
<tr>
<td>Highest Pressure mm Hg</td>
<td>204</td>
<td>196</td>
<td>124</td>
</tr>
<tr>
<td>Mean Pressure</td>
<td>183 ± 7</td>
<td>168 ± 8</td>
<td>121 ± 1</td>
</tr>
<tr>
<td>Cardiac lesions</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Incidence</td>
<td>85.8</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Average Severity</td>
<td>57.6</td>
<td>9.6</td>
<td>0</td>
</tr>
<tr>
<td>Nephrosclerosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Incidence</td>
<td>85.8</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Average Severity</td>
<td>48.0</td>
<td>9.6</td>
<td>0</td>
</tr>
<tr>
<td>Polyaeritis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Incidence</td>
<td>57.2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Average Severity</td>
<td>24.0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

as used in this experiment hypertension has been induced, although not necessarily in all animals, and without significant vascular lesions (Gross et al. 1957; Gross & Lichtlen 1958; Masson et al. 1962). Since in our hands, both previously (Hall & Hall 1965) and presently, aldosterone causes greater hypertension than has been reported by others, induces severe vascular lesions reputed to be absent, causes a larger effect upon saline intake than has hitherto been observed, and appears to be more rather than less effective than DCA as has been alleged, comment as to the possible reasons for the discrepant findings is required.

The biological half-life of aldosterone in the circulation is shortest of any of the naturally occurring corticoids, about 30 minutes (Tait et al. 1961). It is to be anticipated, therefore, that injections given with greater frequency than has usually been the case (once daily or even less often) (Gross et al. 1955, 1957; Kumar et al. 1955, 1957; Gaunt et al. 1957; Fregly & Arean 1959; Gornall et al. 1960; Masson et al. 1962) would exert a more pronounced effect. Furthermore, many studies have been conducted with dl-aldosterone (Gross et al. 1957, 1958; Friedman et al. 1958; Fregly & Arean 1959), whereas in others it was not stated whether or not the racemate was employed (Kumar et al. 1955; Gornall et al. 1960), and since only the d form is biologically
active, the effective quantity administered has usually been even less than the rather small microgram quantities would suggest. There is also the possibility that l-aldosterone might competitively interfere with the activity of d-aldosterone, as several other steroids with weak mineralocorticoid potency are known to do.

A related problem exists with respect to evaluation of reports on the relative salt-retaining and hypertensigenic potency of the two steroids. Almost without exception the free alcohol of d1-aldosterone has been compared with the acetate of DCA (Gross et al. 1955; Kumar et al. 1957; Fregly & Arean 1959; Gornall et al. 1960). Differences in the rate of absorption and inactivation, and of biological survival and hence duration of action, undoubtedly contribute appreciably to the greater effect of DCA on blood pressure under such circumstances, particularly in view of the fact that far larger amounts of the latter have usually been administered to compensate for its weaker sodium-retaining effect. Even comparisons between the acetates of DCA and d1-aldosterone are difficult to interpret, because apart from the dubious role of l-aldosterone there is the added question of precisely how much of the greater activity ascribed to DCA is to be credited to the fact that five (Gross et al. 1957) to twenty-five times (Gross et al. 1955; Fregly & Arean 1959) as much of it was used, an important consideration where relative rates of absorption, inactivation, and duration of action are involved. Although hypertensive activity undoubtedly depends upon sodium retention, the reliability of the latter as a criterion of the former is uncertain. Gross & Schmidt (1958) contend that despite the fact that aldosterone has a greater Na-retaining activity than DCA, it must be given at about the same dosage in order to produce similar alterations in the distribution of tissue electrolytes, the nature and extent of which cannot be predicted from the accompanying changes in the pattern of serum and urinary electrolytes. There is good reason to believe that the vascular reactivity upon which hypertension depends would more closely conform to aberrations of intracellular electrolyte concentrations than to those which occur in blood and urine, although it is the latter upon which potency estimates of mineralocorticoid hormones are commonly based.

Until the 2nd week of the experiment there was little difference in response to the two steroids. Thereafter saline polydipsia was slightly greater, the incidence of hypertension higher and the growth impaired in aldosterone-treated rats. Greater responsiveness to that steroid was also indicated by thymus involution, increased renal hypertrophy and the much higher frequency of cardiac lesions observed among them.

It seems doubtful that the greater effect of aldosterone upon the heart and kidney can be attributed to the slightly higher blood pressure achieved by rats treated therewith. Potassium depletion is known to cause kidney enlargement because of tubular hyperplasia, hypertrophy and dilation, and specifically to
cause loss of striations and necrosis of myocardial fibers (Follis et al. 1942; Kornberg & Endicott 1946; Spargo 1954; Welt et al. 1960). These changes are said to be indistinguishable from those caused by large doses of DCA in the rat, and the increased kidney size and myocardial injury provoked by that steroid have been ascribed to hypokaliaemia (Darrow & Miller 1942; Durlacher et al. 1942). The response to aldosterone differed from that to DCA principally in the greater degree of renal enlargement and tubular dilation and in the more severe myocardial lesions produced by the former. In the present experiment the quantity of DCA injected and duration of its administration were insufficient, with but a single exception, to cause severe cardiorenal changes. Since aldosterone has five times the activity of DCA on K+ excretion, it seems reasonable to implicate potassium deficiency in the genesis of lesions known to be of the type caused by such a deficiency. Hypokaliaemia, however, causes neither glomerular hyalinization nor necrosis of cardiac arteries. These alterations are known to accompany hypertension, although it may well be that the damage is greater when potassium deficiency co-exists. In the case of the heart it certainly does not strain credulity to suppose that the simultaneous imposition of two influences, hypokaliaemia and relative ischaemia, either of which alone is capable of causing parenchymal injury, might have cumulative effects.

A number of studies have shown aldosterone to lack any direct effect upon thymus tissue. The thymus involution which did occur in aldosterone-treated rats in this experiment was therefore most likely due to the stress of treatment, which was indicated also by impaired body growth. Potassium deficiency alone was noted by Kornberg & Endicott (1946) to impede growth, although Spargo (1954) did not observe such an effect. Whatever the basis may have been, the co-existence of severe cardiorenal lesions, impaired growth and thymus involution would seem adequate to substantiate belief that a state of stress was, in fact, induced.

If the hypertensive response to mineralocorticoid excess is, in fact, determined principally by the degree of sodium retention brought about, then the minimal effects upon blood pressure should be detectable with lower dosages of aldosterone than of deoxycorticosterone. Furthermore, once a dose is reached which affords maximal sodium retention there should be no additional response to further quantities. This, the maximally effective dose, should also be quantitatively less for aldosterone than for deoxycorticosterone. There must, however, be a maximal rate at which the cardiovascular response to sodium excess can occur, and this would be expected to limit the rate at which hypertension develops. Additional complications are introduced by the relatively short half-life of aldosterone, by the fact that it is far more active than DCA in respect to causing sodium retention than it is in facilitating potassium excretion, and by the fact that the absorption rate of the two differ, particularly when far more of one is given than of the other. Under these circumstances it is possible that
the dose-response curves of the two hormones might depend upon factors other than intrinsic potency. In the present experimental circumstances the two steroids appeared to exhibit the same order of activity as regards increasing saline consumption and causing hypertension, but aldosterone had a greater effect upon kidney enlargement and was more effective in causing cardiorenal vascular lesions. At other dose levels these relationships might not obtain.

This study does not support the contention that deoxycorticosterone is far more active than aldosterone in causing hypertension, although a case could be made for a potency disproportionate to its sodium-retaining activity. This, and the previous experiment with aldosterone, leave no doubt that this hormone is eminently capable of causing the same proliferative inflammatory lesions that deoxycorticosterone causes, about which some doubt has been expressed (Gross et al. 1957; Gross & Lichtlen 1958).

REFERENCES

Hall C. E., Hall O. & McCleshey O.: Acta endocr. (Kbh.) 9 (1952) 199.

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