Hypertensive Potency of 18-Oxocortisol in the Rat

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SUMMARY The hypertensogenic effect of 18-oxocortisol, an aldosterone analogue possessing both mineralocorticoid and glucocorticoid properties, was studied at the same dosage but under different experimental conditions in two experiments. Under experimental conditions conducive to the development of mineralocorticoid hypertension (i.e., rats with a single kidney on a high NaCl intake), there was an extremely rapid onset of saline polydipsia and hypertension accompanied by cardiac and renal enlargement, marked thymic involution without adrenal atrophy, cardiovascular lesions, and hypokalemia. With the exception of the thymic changes, the same changes occurred in rats given the biologically equivalent dose of deoxycorticosterone acetate. Under circumstances favoring the development of glucocorticoid hypertension (i.e., intact rats on a normal sodium intake), the same dose had only a transient blood pressure-elevating effect, attaining prehypertensive levels at most, and caused neither chronic hypertension nor hypokalemia. The biologically equivalent glucocorticoid dosage of cortisol was similarly ineffective. Under these circumstances, both steroids caused thymus involution but only 18-oxocortisol caused kidney enlargement. (Hypertension 8: 317-322, 1986)

KEY WORDS • mineralocorticoid • adrenal cortex • glucocorticoid • cortisol • NaCl • deoxycorticosterone • hypokalemia • cardiac hypertrophy

THE suspicion that low renin essential hypertension may be due to hypersecretion of an unusual adrenocortical steroid with appreciable mineralocorticoid activity has prompted an intensive search for a plausible candidate. The adrenal cortex can metabolize cortisol by means of an angular methyl oxidase to 18-hydroxycortisol and 18-oxocortisol. The same enzyme converts corticosterone to aldosterone and 18-hydroxycorticosterone. The hypertensive and metabolic syndrome accompanying primary hyperaldosteronism due to an adrenal adenoma is sometimes characterized by a disproportionality between its severity and the magnitude of the concurrent degree of hyperaldosteronism. Patients in whom this occurs may display excessive production of both 18-hydroxycortisol and 18-oxocortisol.

18-Oxocortisol has been synthesized, and its mineralocorticoid (MC) and glucocorticoid (GC) activities have been estimated. The affinity of 18-oxocortisol for the cytosol renal MC receptor has been given as 1.7% of that of aldosterone, whereas its relative biological potency has been reported as 1.2% and 0.6%. The GC biological activity was found to be 31% or 4% of that of cortisol, using two different bioassay procedures.

Because of the possible hyperproduction of 18-oxocortisol in human hypertension, and because the steroid has both MC and GC properties, this study was undertaken to examine the hypertensogenic activity of this steroid in rats under experimental conditions conducive to the induction of either MC or GC hypertension.

Materials and Methods

18-Oxocortisol was synthesized by the method of Akhtar et al. and was characterized as described elsewhere. Female Sprague-Dawley rats (Timco Breeding Laboratories, Houston, TX, USA) weighing 105 to 115 g were used in two experiments. In the first experiment, rats underwent right nephrectomy under ether anesthesia and were divided into three groups. Those in the first group (n = 8) were each given 1.5 mg of 18-oxocortisol in a single subcutaneous daily injection in a Ciba-Geigy CMC suspension vehicle (0.2 ml of a 7.5 mg/ml suspension). The second group (n = 8) was given a deoxycorticosterone acetate (DOCA; Sigma Chemical Company, St. Louis, MO, USA) suspen-
sion, 375 μg/day, in the same medium (1.875 mg/ml). The controls (n = 6) received only vehicle. All rats were individually caged in an environmentally controlled, windowless, room lighted 0800 to 2000 and received Purina Laboratory Chow (St. Louis, MO, USA) and 1% NaCl drinking fluid ad libitum. Fluid consumption was measured on the first 3 days of each week, and the average was taken to be representative for the week.

Systolic blood pressures were taken on warmed and restrained conscious rats by a tail method between 0900 and 1200 once weekly, on the day following the last of the fluid intake measurements, using a Mark IV physiograph integrated with a PE 300 programmed electrophysmomanometer and a DD 350 digital display unit (Narco Biosystems, Houston, TX, USA). The latter registered heart rates from paired needle electrodes inserted into the lateral thoracic skin on opposite sides of the chest. Systolic pressures were taken repetitively until three consecutive values agreed to within 10 mm Hg; these were averaged to obtain the value recorded. Values above 150 mm Hg were taken to be hypertensive. On the fourteenth day, the animals were anesthetized with ether and blood was withdrawn by cardiac puncture for measurement of Na⁺ and K⁺ by flame photometry (Model 343, Instrumentation Laboratories, Lexington, MA, USA). The rats were then allowed to die under anesthesia.

The hearts, kidneys, thymus, and adrenal glands were then removed and placed in neutral 10% formalin for subsequent weight and histology. After fixation, they were removed, trimmed, blotted, and weighed on an analytical balance.

The second experiment was conducted similarly. In this instance, however, 24 female rats were divided into three equal groups and were not subjected to kidney removal. The first group (n = 8) received 1.5 mg/day of 18-oxocortisol subcutaneously in the CMC vehicle (7.5 mg/ml), and the second group (n = 8) received 46.5 μg/day of cortisol (Sigma) in the same volume of vehicle. The eight controls received only vehicle. The rats were maintained and housed as in the preceding experiment, except that tap water was given to drink and fluid intake was not measured. Blood pressures were taken weekly as in the first experiment, and the same criteria applied. On the 29th day the rats were anesthetized with ether and blood was withdrawn by cardiac puncture for flame photometry, after which they were allowed to die. Organs were removed and treated as in the first experiment.

For statistical comparisons in both studies Student's t test was used to compare values in steroid-treated and control rats. A p value below 0.05 was regarded as significant.

Results

In the first experiment, prominent saline polydipsia of substantially equivalent severity developed in both groups of steroid-treated rats (Figure 1). Those treated with 18-oxocortisol failed to gain weight normally in

![Figure 1](http://hyper.ahajournals.org/)

**Figure 1.** Average daily volume consumption of 1% saline solution by steroid-treated and control rats. Asterisks indicate significant differences from control values (p < 0.05). DOCA = deoxycorticosterone acetate

![Figure 2](http://hyper.ahajournals.org/)

**Figure 2.** Weight gain during the 2-week experiment. Deoxycorticosterone acetate (DOCA) had no significant effect, but 18-oxocortisol inhibited growth in the second week (p < 0.001)
HYPERTENSIVE ACTION OF 18-OXOCORTISOL

There was no significant difference in heart rate between the three groups in any of the periods. Both steroid-treated groups exhibited marked hypokalemia, which was particularly severe in those given 18-oxocortisol (Table 1). The serum sodium concentrations of 18-oxocortisol-treated rats were slightly but not significantly below those of controls, whereas DOCA treatment produced levels slightly but not significantly above levels in control rats. The result was that DOCA-treated rats had Na⁺ concentrations significantly higher than those seen in rats treated with 18-oxocortisol (p < 0.05; see Table 1).

Organ weights revealed that both steroids caused a slight but not statistically significant adrenal atrophy and that 18-oxocortisol, but not DOCA, caused thymus involution (p < 0.005). Both DOCA-treated and 18-oxocortisol-treated rats exhibited cardiac hypertrophy (p < 0.005, p < 0.0001, respectively) and nephromegaly (p < 0.01, p < 0.005, respectively), as shown in Table 1.

Microscopic examination of the hearts and kidneys revealed that all steroid-treated rats, whether normotensive or hypertensive, had the renal tubular lesions characteristic of hypokalemia, chiefly swelling and vacuolization of proximal tubular cells. The renovascular lesions of hypertension (i.e., hyalinization and/or fibrinoid necrosis or rupture of glomerular capillary tufts, with an abundance of tubular hyaline casts and the hyalinization and/or fibrinoid necrosis of arteriolar walls) were confined to hypertensive rats. Thus, their incidence was 75% (6 of 8 rats) in Group 1 and 88% (7 of 8 rats) in Group 2.

Cardiac lesions (i.e., periarterial inflammatory foci, fibrinoid necrosis of arterial walls and degeneration of myocardial fibers, and their replacement by fibrinoid material and connective tissue) were present in 50% of Group 1 (4 of 8 rats) and in 75% of Group 2 (6 of 8 rats) but absent in control rats.

Cardiac and renovascular lesions were graded on an arbitrary 0 to 4 scale, as described elsewhere. Hypertension of substantially equivalent duration and severity affected both steroid-treated groups; thus, vascular lesions were equally widespread and advanced in the groups, and no statistical difference in severity or incidence occurred between the groups (see Table 1).

The same dose of 18-oxocortisol that had caused hypertensive vascular disease in mononephrectomized, salt-loaded rats caused only a slight and transient elevation of systolic pressure in rats with intact kidneys on a normal NaCl intake. The effect was

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**TABLE 1** Serum Electrolytes, Organ Weights, and Cardiovascular Lesions in Steroid-treated, Mononephrectomized Rats Drinking Saline Solution

<table>
<thead>
<tr>
<th>Variable</th>
<th>18-Oxocortisol (n = 8)</th>
<th>DOCA (n = 8)</th>
<th>Control (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Na⁺ (mEq/L)</td>
<td>137 ± 2</td>
<td>144 ± 2</td>
<td>141 ± 1</td>
</tr>
<tr>
<td>Serum K⁺ (mEq/L)</td>
<td>3.1 ± 0.2*</td>
<td>3.5 ± 0.2*</td>
<td>4.7 ± 0.1</td>
</tr>
<tr>
<td>Organ weight (mg/100 g body weight)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adrenal glands</td>
<td>24.3 ± 0.7</td>
<td>24.9 ± 0.8</td>
<td>27.0 ± 1.3</td>
</tr>
<tr>
<td>Thymus</td>
<td>176.6 ± 8.9†</td>
<td>217.4 ± 18.4</td>
<td>225.5 ± 8.6</td>
</tr>
<tr>
<td>Heart</td>
<td>487.4 ± 18.5†</td>
<td>453.0 ± 14.6*</td>
<td>386.0 ± 8.5</td>
</tr>
<tr>
<td>Kidney</td>
<td>1007.0 ± 43.3*</td>
<td>936.9 ± 51.2‡</td>
<td>724.2 ± 20.1</td>
</tr>
<tr>
<td>Renovascular lesions</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incidence (%)</td>
<td>75.0</td>
<td>87.5</td>
<td></td>
</tr>
<tr>
<td>Severity</td>
<td>1.0 ± 0.3</td>
<td>1.5 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>Cardiac lesions</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incidence (%)</td>
<td>50.0</td>
<td>75.0</td>
<td></td>
</tr>
<tr>
<td>Severity</td>
<td>0.6 ± 0.3</td>
<td>1.3 ± 0.4</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SEM. Severity of renovascular and cardiac lesions was graded on an arbitrary 0 to 4 scale.

* p < 0.005, † p < 0.0001, ‡ p < 0.01, compared with values in control rats.
closely paralleled in those receiving cortisol treatment (Figure 4). In both treated groups, blood pressure rose faster than in controls during the first 2 weeks of treatment, slower in the third week, and fell in the fourth week, while pressures in control rats rose continuously during the experiment. Although both steroid-treated groups had statistically elevated blood pressures in Weeks 2, 3, and 4, pressures in both groups fell in the fifth week. Blood pressures in the 18-oxocortisol–treated rats were higher than those in rats given cortisol (\( p < 0.05 \)), but neither value differed significantly from that seen in controls. The pressures of cortisol-treated rats were actually numerically below those of controls. Only two rats in Group 2 acquired pressures of 133 and 135 mm Hg, respectively, during the experiment, and both rats had lower levels at the end of treatment. Five 18-oxocortisol–treated rats also had pressures at these levels during the experiment. Levels in two (in the second and third weeks, respectively) exceeded 145 mm Hg, a level that we regard as prehypertensive; levels in both rats had declined to 122 mm Hg by the fifth week. Heart rates did not differ among the groups in any period.

Neither steroid affected body weight (Figure 5). The adrenals were slightly but not significantly smaller in both steroid-treated groups than in controls. Neither steroid affected heart weight. Both caused thymus involution, but only 18-oxocortisol caused kidney enlargement (Table 2).

**Discussion**

Administration of 18-oxocortisol, 1.5 mg/day, to mononephrectomized, salt-loaded rats had substantially the same effects, increasing saline consumption and blood pressure, depressing serum K\(^+\) concentrations, and causing heart and kidney enlargement and cardiovascular lesions, as did 2.5% of that amount of DOCA (375 \( \mu \)g/day), in agreement with their respective MC potencies. However, 18-oxocortisol also impaired body growth and caused thymus involution. We were initially inclined to ascribe both effects to the known slight GC properties of 18-oxocortisol. However, in the second experiment, the same quantity of steroid given for twice as long failed to impair growth, although it led to thymus involution. Evidently, impaired weight gain in the first experiment was not a reflection of GC overdosage per se.

In rats without either mononephrectomy or a high salt intake, neither 18-oxocortisol, 1.5 mg/day, given at an equivalent GC dosage (3.1% of that of cortisol), nor cortisol caused progressive, sustained hypertension. Although both groups displayed statistically elevated pressures from the second to the fourth week of treatment, we are not inclined to equate this finding with hypertension. It is quite possible for various treatments to cause statistically elevated pressures that still fall within the normotensive range. Such a response is not necessarily progressive or sustained, in which case it produces neither heart enlargement nor cardiovascular damage. Although many investigators equate statistical significance with biological significance (an unjustified conclusion) and allude to slight but significant elevations of blood pressure as hypertension, we do not. In the present study, this response typically was not sustained; at the final determination, the blood pressures of both steroid-treated groups were declining, and those of the cortisol-treated group were neither statistically different from nor numerically lower than those of controls.

Since hypertension was induced by 18-oxocortisol under conditions conducive to MC hypertension and was matched in its development and manifestations by a comparable MC dosage of DOCA, it is clear that MC hypertension was being expressed. On the other hand, the same quantity given over a longer period failed to induce GC hypertension when given under conditions appropriate to the expression of that condition. Perhaps at a higher dosage it might have done so, for cortisol given at a biologically equivalent dosage was equally ineffective; however, the amount of 18-oxo-
cortisol available did not permit examination of the possibility.

Glucocorticoid hypertension is more capricious in development than is the MC form in rats. Several synthetic glucocorticoids have been reported to be efficacious; however, cortisone has been effective in some experiments, whereas in others the blood pressure, after rising for a period of days, returns to normal despite continued treatment. In our experience, the latter result has been the characteristic response to high dose corticosterone treatment in the rat at both normal and elevated levels of salt intake. Conversely, other investigators have found it to cause severe hypertension. Haack et al. contended that corticosterone treatment caused sodium extrusion from the intracellular to the extracellular compartment, which was therefore accountable for both the development of hypertension and the fact that additional dietary salt was not a requirement. However, treatment lasted only 4 days, during which blood pressure rose but frank hypertension did not develop. Furthermore, this laboratory had reported a year earlier that the same dosage given for 6 days to rats of the same size and sex had no effect on blood pressure.

We believe that the tendency for rising blood pressures to be reversed unpredictably during glucocorticoid treatment accounts for the blood pressure responses seen in the second experiment when 18-oxocortisol and cortisol were tested under conditions conducive to the development of GC hypertension. Blood pressures showed a slight, transient elevation that was succeeded by a decline. Both steroids caused thymus involution, but only 18-oxocortisol caused kidney enlargement, which indicates that this was an MC-induced effect.

Although in this study 18-oxocortisol treatment was great enough at the dosage employed to cause both MC hypertension under conditions favorable to its expression and hypokalemia, the same dosage failed to cause either GC hypertension or hypokalemia when circumstances appropriate to elicitation of GC hypertension were established. In any event, the inherent MC hypertensogenic activity of 18-oxocortisol is enough to endow it with the potential of causing hypertensive disease in humans when excessive quantities circulate more or less continuously, as, for example, in primary aldosteronism due to an adrenal adenoma or in GC-suppressible aldosteronism. Its possible role in other hypertensive disorders is unknown.

**Table 2. Serum Electrolytes, Body and Organ Weights in Steroid-treated Nonnephrectomized Rats Drinking Water**

<table>
<thead>
<tr>
<th>Variable</th>
<th>18-Oxocortisol (n = 8)</th>
<th>Cortisol (n = 8)</th>
<th>Control (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>109 ± 3</td>
<td>109 ± 2</td>
<td>109 ± 3</td>
</tr>
<tr>
<td>Final</td>
<td>196 ± 3</td>
<td>202 ± 4</td>
<td>204 ± 5</td>
</tr>
<tr>
<td>Serum Na⁺ (mEq/L)</td>
<td>129 ± 1 ± 6</td>
<td>130 ± 1 ± 8</td>
<td>132 ± 5 ± 2 ± 5</td>
</tr>
<tr>
<td>Serum K⁺ (mEq/L)</td>
<td>4.9 ± 0 ± 2</td>
<td>4.7 ± 0 ± 1</td>
<td>4.5 ± 0 ± 1</td>
</tr>
<tr>
<td>Organ weight (mg/100 g body weight)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adrenal glands</td>
<td>22.7 ± 1 ± 1</td>
<td>21.7 ± 0 ± 6</td>
<td>23.4 ± 0 ± 8</td>
</tr>
<tr>
<td>Thymus</td>
<td>142 ± 10 ± 8</td>
<td>141 ± 8 ± 8</td>
<td>176 ± 11</td>
</tr>
<tr>
<td>Heart</td>
<td>337 ± 10</td>
<td>320 ± 11</td>
<td>317 ± 5</td>
</tr>
<tr>
<td>Kidneys</td>
<td>816 ± 26 ± 6</td>
<td>745 ± 16</td>
<td>733 ± 24</td>
</tr>
</tbody>
</table>

Values are means ± SEM
*p < 0.05, compared with values in control rats

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