Hypertensinogenic Potencies of Aldosterone and Deoxycorticosterone in the Rat

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SUMMARY Hypertensinogenic potency and other effects of acetate salts of aldosterone (ALA) and deoxycorticosterone (DOCA) were evaluated in 50-day-old mononephrectomized and saline-drinking Sprague-Dawley CD male rats. The steroids were administered by continuous subcutaneous infusion in a dose of 100 μg/24 hrs by means of Alzet osmotic minipumps implanted subcutaneously. Within 3 weeks of steroid treatment, systolic blood pressure, measured in the tail of conscious animals by a photoelectric cell method at 27°C environmental temperature, increased significantly in ALA rats as compared to that in DOCA rats, which was not different from controls. ALA rats exhibited marked polydipsia, decreased body weight, hypernatremia, hypokalemia, cardiomegaly, and kidney enlargement, whereas DOCA rats exhibited only cardiomegaly when compared with controls. The degree of cardiomegaly in ALA and DOCA rats was statistically much greater than the differences in their respective blood pressure levels when compared to controls. Under the conditions of this study, it is concluded that: 1) the hypertensinogenic potency of ALA is greater than that of DOCA; 2) ALA and DOCA may induce cardiomegaly, independent of their effects on blood pressure; 3) Alzet osmotic minipumps are effective tools for the administration of steroids by continuous infusion.

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KEY WORDS • hypertension • mineralocorticoids • electrolytes • cardiomegaly • osmotic minipumps • polydipsia

VOLUMINOUS work on mineralocorticoid-induced hypertension has concerned primarily two steroids with firmly-established hypertensinogenic properties. The acetate salt of 11-deoxycorticosterone (DOCA) was repeatedly shown to produce hypertension in several animal models, following initial studies on dogs1 and rats.2, 3

Prolonged administration of small doses of aldosterone into rats rendered them hypertensive within 12 weeks.4 These observations were confirmed in another study,5 where the effects of aldosterone on body weight, fluid intake, hypertension, and production of lesions in various organs in the rat was less pronounced than those of DOCA-treated rats. When comparing sodium-retaining potency of the two steroids, DOCA was judged to be more potent than aldosterone. Subsequently,6, 7 aldosterone was shown to produce only moderate hypertension and minimal, if any, vascular lesions in the rat. However, more frequent administration of the steroid resulted in a significant polydipsia, hypertension, cardionephromegaly, and development of cardiorenal and vascular lesions, as compared to control rats.8 Direct comparison of the two steroids in the rat9 showed them to have a similar effect on blood pressure and fluid intake; however, aldosterone produced more severe vascular damage than DOCA. Other investigators10 established the dose-effect relationship of aldosterone-induced hypertension in the rat, when administering small amounts of the hormone.

In view of the notion that the mechanism of mineralocorticoid-induced hypertension is thought to be mediated via sodium retention, the observation that mineralocorticoid potency of aldosterone in 30- to 70-fold that of deoxycorticosterone11 seemed to be at variance with conclusions from the above studies.6, 9 For these reasons, it was suggested that factors other than mineralocorticoid properties of steroids were responsible for development of hypertension.6, 12

In all previous studies, the steroids were administered by once- or twice-daily subcutaneous bolus injections in an oily solution. This form of treatment did not completely reflect the real pathophysiological state, and there were many other factors that could have affected the response, as was extensively discussed previously.6

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To minimize these factors, in this study we administered acetate salts of aldosterone and deoxycorticosterone by continuous subcutaneous infusion with minimal amount of carrier solution, by means of Alzet osmotic mini-pumps. By infusing equal amounts of both steroids under identical experimental conditions, we were able to compare directly their hypertensogenic potency and other effects. For purposes of this study, we disregarded any possible differences in the steroids' binding to proteins in blood as well as in their metabolism.

**Materials and Methods**

Aldosterone acetate (ALA),* 02002, Batch 1349, and deoxycorticosterone acetate (DOCA), 03461, Batch 1768, were purchased from Steraloids, Inc., Wilton, New Hampshire. Propylene glycol, P-354, Lot 736261, and sodium chloride, S-721, Lot 755760, were obtained from Fisher Scientific Company, Fair Lawn, New Jersey; Punctilious ethyl alcohol — USP, anhydrous 200 proof, reagent quality, from U.S. Industrial Chemicals Company, Newark, New Jersey; and heparin, ammonium salt from Sigma Chemical Company, St. Louis, Missouri. Alzet osmotic minipumps, Model 1701, were purchased from Alza Corporation, Palo Alto, California. The osmotic minipump is a small capsule, 6.5 mm in diameter and 25 mm long, containing a collapsible reservoir surrounded by a sealed layer of salt solution, the osmotic driving agent, which is enclosed by a rate-controlling semipermeable membrane. The reservoir is prefilled with the steroid-containing solution at room temperature by means of a blunt tip needle and a syringe.

Sprague-Dawley CD male rats were obtained from Charles River Breeding Laboratories. Right mononephrectomized and 1% saline-drinking rats (50 days old) were divided into three comparable groups, on the basis of body weight and systolic blood pressure. The control group (CR), 13 rats, received vehicle only; the second group (DR), 10 rats, received DOCA; and the third group (AR), 10 rats, had ALA-containing mini-pumps implanted subcutaneously. The rats were housed in environmentally-controlled quarters, one per cage, and put on an ad libitum Purina Rat Chow diet and 1% saline. Saline intake was measured for 4 days preceding implantation of minipumps and continuously throughout the study by weighing all saline-containing bottles at intervals and, for calculation purposes, assuming that 1 ml of saline equals 1 g.

Systolic blood pressure (SBP) was measured at intervals in tail of conscious acclimated rats at constant room temperature of 27°C by photoelectric cell method, using Physiograph Life Science Instrumentation Desk Model DMP-4B (Narco Biosystems, Inc., Houston, Texas) and pulse amplifier, Model 59, cuff pump, Model PR-05, and manual scanner, Model 65 (International Imp. & Techn. Consultants, Landing, New Jersey). An average of six SBP measurements in quiescent state was used as representative blood pressure for each rat. At the end of the 7th and 14th days, when the previously-implanted minipump was irreversibly spent, an additional minipump was implanted under ether anesthesia, adjacent to the previous one, identically in all the groups. There was no mortality nor apparent morbidity in the rats in this study. The rats were sacrificed on the 22nd day by suffocation with carbon dioxide, and blood was withdrawn with an heparinized syringe from the inferior vena cava above adrenal glands. The organs were removed, blotted, weighed, examined, and stored in neutral 10% formalin. Upon sacrifice, all three osmotic minipumps were removed from each rat, for subsequent analysis of the amount of the steroid remaining in the minipumps. Tritium-labeled corresponding steroid was added to account for losses during the procedure. Fluid intake was calculated as per day and body weight. Plasma sodium and potassium were measured on Flame Photometer 143 with Dilutor 144 (Instrumentation Laboratory, Scientific Products, Bedford, Massachusetts). The data were statistically evaluated by analysis of variance and Newman-Keuls tests by comparing the three groups, and were reported as mean ± SEM.

**Results**

**Effect on Body Weight**

Before administration of the steroids, rat body weights were similar in all three groups: 181 ± 6 g in CR, 181 ± 9 g in DR, and 178 ± 5 g in AR. Therewith, CR and DR progressively gained weight, and their growth curves were almost identical, as seen in figure 1. In fact, DR, after the 10th day of treatment, slightly surpassed CR. On the other hand, AR lagged behind the other two groups from the beginning of the treatment, and the growth curve virtually leveled off beyond the 11th day, when the AR became hypertensive.

**Blood Pressure Response to Treatment**

SBP was comparable in all three groups before steroid treatment began (fig. 2). Thereafter, within 6

* Nomenclature and abbreviations used: aldosterone acetate, (AA), 4-pregnen-11β, 21-diol-3, 18, 20-trione-21-acetate; deoxycorticosterone acetate, (DOCA), 4-pregnen-21-ol-3,20-dione acetate, propylene glycol, 1,2-propanediol, saline, 1% sodium chloride solution in distilled water (w/v).
days of treatment, SBP increased by 10 and 11 mm Hg in AR and DR, respectively, and subsequently by the 11th day, it was 32 and 10 mm Hg above that of CR. From this time on, SBP in AR was significantly above that of DR and CR, whereas in the latter two groups it was virtually the same (fig. 2).

Effect on Saline Intake

Baseline saline intake, expressed per rat body weight at the particular time, was not significantly different among the three groups, although in DR it was slightly higher than in the other two groups. Steroid treatment resulted in no change in saline intake in DR as compared to CR; however, AR exhibited a significant polydipsia soon after administration of the hormone, which thereafter almost tripled as compared to the other two groups (fig. 3). Cumulative saline intake for duration of the study was significantly higher in AR as compared to the other two groups (table 1); this difference is even larger when related to the lower body weight in AR (fig. 1).

Effect on the Organs

Steroid treatment did not affect hematocrit values, as seen in table 1. The adrenal glands in AR were insignificantly larger and the thyroid and thymus smaller as compared to the other two groups. On the other hand, the heart weights of each group were significantly different from each other, the highest being in AR and the lowest in CR. Also, the kidneys in AR were markedly enlarged as compared to the other two groups, which were similar (table 1).

Effect on Electrolytes

ALA-treated rats exhibited profound changes in plasma electrolyte levels (table 1), with marked hypernatremia and hypokalemia as compared to the other two groups. Likewise, the sodium/potassium ratio was much higher in AR than in DR and CR. Conversely, electrolyte values in DR were identical to those of CR, thus further confirming negligible mineralocorticoid activity of DOCA in this study.
**TABLE 1. Effect of Steroid Treatment on Followed Parameters in the Rat**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Deoxycorticosterone acetate</th>
<th>Aldosterone acetate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cumulative fluid intake* (ml/rat/21 days)</td>
<td>1,493 ± 71</td>
<td>1,467 ± 137</td>
<td>4,007 ± 233**††</td>
</tr>
<tr>
<td>Final blood pressure† (mm Hg)</td>
<td>137 ± 4</td>
<td>135 ± 9</td>
<td>162 ± 4**§</td>
</tr>
<tr>
<td>Sacrifice body weight (g)</td>
<td>316 ± 9</td>
<td>320 ± 15</td>
<td>266 ± 9††</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>56.2 ± 1.6</td>
<td>54.6 ± 1.2</td>
<td>56.3 ± 2.0</td>
</tr>
<tr>
<td>Plasma sodium (mEq/liter)</td>
<td>147.3 ± 0.6</td>
<td>146.9 ± 0.6</td>
<td>151.3 ± 0.7††${\ddagger}$</td>
</tr>
<tr>
<td>Potassium (mEq/liter)</td>
<td>6.38 ± 0.02</td>
<td>6.40 ± 0.38</td>
<td>5.06 ± 0.29††</td>
</tr>
<tr>
<td>Sodium/potassium ratio</td>
<td>23.31 ± 0.66</td>
<td>23.63 ± 1.28</td>
<td>30.77 ± 1.66††</td>
</tr>
<tr>
<td>Organ weight (µg/g of body weight)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adrenal glands</td>
<td>217 ± 11</td>
<td>201 ± 7</td>
<td>225 ± 13</td>
</tr>
<tr>
<td>Thymus</td>
<td>1,915 ± 94</td>
<td>1,929 ± 110</td>
<td>1,730 ± 126</td>
</tr>
<tr>
<td>Thyroid</td>
<td>2,345 ± 59</td>
<td>2,348 ± 70</td>
<td>2,240 ± 54</td>
</tr>
<tr>
<td>Heart</td>
<td>3,374 ± 44</td>
<td>3,594 ± 48††</td>
<td>4,284 ± 58††</td>
</tr>
<tr>
<td>Kidney</td>
<td>8,839 ± 208</td>
<td>8,861 ± 198</td>
<td>13,616 ± 589††</td>
</tr>
</tbody>
</table>

Values are the mean ± SEM.

*Data were adjusted for before-treatment intergroup difference in fluid intake.

†Data are means of the last two blood pressure measurements (Days 18 and 21).

‡Blood was withdrawn into heparinized syringe from the interior vena cava, above the adrenal glands, at the time of sacrifice, and centrifuged in graduated vessels at 3000 g for 30 minutes. Hematocrit was calculated from the ratio of red blood cell to whole blood volume.

**p < 0.05, and ††p < 0.01 or better, as compared to controls.

§p < 0.05, and ‡p < 0.01 or better, as compared to DOCA group.

Examination of the Minipumps

At necropsy, the minipump implantation sites showed no significant degree of fibrosis, nor necrosis or tissue reaction in the area of their openings. The amount of steroids remaining was determined in six rats from each group. Minipumps in AR contained 202 ± 40 µg of ALA, and in DR, 190 ± 32 µg of DOCA. Since all three minipumps initially contained 2210 µg of ALA or DOCA, the uninfused amount of the hormones thus represents 9.1% for ALA and 8.6% for DOCA. Therefore, the amount of steroids actually infused was lowered from 110 to 100 µg/24 hrs.

Discussion

In this study, using improved means of steroid administration, by continuous subcutaneous infusion and with minimal amount of carrier solution, we confirmed the hypertensinogenic potency of aldosterone.** Unlike in previous studies, in this investigation we were able to produce hypertension (HTN) with daily doses of 100 µg/rat. Assuming that the secretion rate of aldosterone in stressed rats is 1.4 µg/24 hrs, we found that the infused dose of ALA was 70-fold higher, and plasma levels of aldosterone thus produced were 10-fold higher than in CR (our unpublished data). Since the secretion rate of deoxycorticosterone is 20 µg/24 hrs, the failure of 100 µg/24 hrs in our study to produce HTN over such a short period of time is not surprising.

Decreased growth in AR as compared to other groups in this study resulted from the effect of ALA and possibly also of HTN. Profound polydipsia in AR that occurred shortly after administration of ALA and preceded the development of HTN (figs. 2 and 3) is considered to be a direct effect of ALA. It was shown previously that ALA enhances saline intake by stimulating sodium ion appetite, which appears to be centrally mediated, since it can be completely eliminated by damaging the dorsolateral hypothalamus. The cardionephromegaly in AR (table 1) occurred as expected in mineralocorticoid-induced HTN.* However, when comparing the level of SBP and heart and kidney weight in AR with those in CR, as shown in table 1, the degree of cardiomegaly and nephromegaly in AR markedly surpassed that of HTN. This observation strongly suggests that ALA independently stimulates cardiac and renal enlargement. If it is assumed that this phenomenon is common to mineralocorticoids in general, the heart appears to be more sensitive to their action than the kidney, since DR, being normotensive, were found to have cardiomegaly and no nephromegaly (table 1). DOCA-induced dissociation between the heart and kidney size was not reported previously. ALA-induced
hypernatremia, hypokalemia, and increased sodium/potassium ratio in plasma had occurred as expected (table 1). However, equal doses of DOCA, under similar conditions, proved to be ineffective in producing any such changes. Hypertensinogenic doses of DOCA were shown repeatedly to produce hypernatremia, hypokalemia, and altered sodium/potassium ratio.\textsuperscript{16, 17} Therefore, the lack of these findings confirms the observation of no effect of DOCA on blood pressure.

Since aldosterone was shown to produce HTN,\textsuperscript{4, 8} several investigators\textsuperscript{4, 8, 9} compared its hypertensinogenic potency to that of deoxycorticosterone. Although aldosterone is the most potent mineralocorticoid,\textsuperscript{11} its hypertensinogenic potency was considered though aldosterone is the most potent mineralocorticoid. Previous studies\textsuperscript{4, 8} were done with d’l-aldosterone, and in all,\textsuperscript{4-10} the steroid in oil was administered as bolus injections and employing different treatment schedules.\textsuperscript{4} Therefore, a direct comparison of results from the different studies is not appropriate, for in addition to the aforementioned reasons, different strains of rats were used, of different sex and age, and there were dissimilar conditions in animal quarters and mode of blood pressure measurement employed, all of which have some effect on the outcome of the study. In this study, we administered equal amounts of ALA and DOCA to the rats by continuous subcutaneous infusion, thus exposing target tissues to constant amounts of the hormone. Under these conditions, hypertensinogenic potency of ALA proved to be superior to that of DOCA, which was further confirmed by polydipsia, cardioneprhymegaly, hypokalemia, and decreased body weight in AR.

Although mineralocorticoids are known to cause sodium retention,\textsuperscript{1, 18-20} their mode of hypertensinogenic activity is not universally agreed upon.\textsuperscript{4, 13} Mineralocorticoid potency of steroids has been tested in toad bladder,\textsuperscript{21, 22} adrenalectomized rats,\textsuperscript{11, 19-20} and most recently, by binding to kidney receptors.\textsuperscript{25-28} By bioassay in rats, aldosterone is a 43-fold more potent mineralocorticoid than deoxycorticosterone.\textsuperscript{11} Biological actions of steroids appear to be mediated via the receptor mechanism.\textsuperscript{29} The affinity of deoxycorticosterone for renal mineralocorticoid Type I receptors is 85% of that of aldosterone,\textsuperscript{30} equivalent to aldosterone for dexamethasone Type II,\textsuperscript{29} and 24-fold higher for corticosterone Type III receptors.\textsuperscript{30} It was shown previously that there was an excellent correlation between the degree of binding of agonist steroids to kidney mineralocorticoid Type I receptors and their sodium-retaining activity.\textsuperscript{30, 31} It has also been observed that only free steroid determines receptor occupancy\textsuperscript{44} and binding of steroids to receptors is inversely related to their binding to plasma proteins.\textsuperscript{34, 35} These findings, however, apply to physiological amounts of hormones only. With pharmacological doses, at least some of the effects may be mediated by separate, currently undefined pathways; for, upon saturation of the stereospecific, high-affinity and low-capacity receptors, the steroid in question binds to the alternate receptors of low affinity and high capacity.\textsuperscript{27}

The presence of receptors may not necessarily indicate the ability of the tissue to respond to hormones.\textsuperscript{36}

Determination of mineralocorticoid potency of steroids by bioassay in the rat is widely used but it may not be an ideal model, for these rats are adrenalectomized with no adrenal steroids present, and therefore steroid-blood and tissue-binding dynamics do not reflect the physiological state.\textsuperscript{36} Furthermore, definition of mineralocorticoid activity of the hormones is not fully elucidated, for aldosterone is 100 times as potent as deoxycorticosterone in its effect on the urinary sodium/potassium ratio, 25 times as potent in causing sodium retention, but only five times as active in producing potassium excretion.\textsuperscript{18} Similar observations were reported with 18-hydroxydeoxycorticosterone.\textsuperscript{17} Thus, different hormones have dissimilar effects on sodium and potassium metabolism.

In addition, selection of the rat model plays a major role in the outcome of the study, since administration of aldosterone to adrenalectomized rat produces both sodium retention and potassium excretion, while in intact rats, there is only a kaliuretic effect.\textsuperscript{32} Accordingly, the antinatriuretic and the kaliuretic effects of aldosterone may be separable. The picture may be even more complex in the uninephrectomized rat, the commonly-used hypertensive model, with the adrenals intact.

Whereas the relationship among the steroids' mineralocorticoid activity, binding to its specific receptors and hypertensinogenic potency is a complex one, they appear to correlate. To what extent sodium retention and other factors, like increased cardiac output and peripheral resistance, affect the development of mineralocorticoid-induced HTN and whether the mechanisms whereby aldosterone produces HTN differ from those in deoxycorticosterone HTN\textsuperscript{39} remains to be elucidated. Regardless of the mechanisms involved, as shown in this study, ALA is a more potent hypertensinogenic agent than DOCA.

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