The Differential Effect of Aldosterone and Dexamethasone on Pressor Responses in Adrenalectomized Rats

Yoram Yagil and Lawrence R. Krakoff

SUMMARY The effect of selective glucocorticoid or mineralocorticoid replacement on pressor responses to angiotensin I and II and norepinephrine was studied in adrenalectomized rats given high salt intake. Four groups were prepared by 1) adrenalectomy only (n = 5); 2) adrenalectomy plus aldosterone, 6 μg/24 hr i.p. (n = 5); or 3) adrenalectomy plus dexamethasone, 10 μg/24 hr i.p. (n = 5), using miniosmotic pumps; and 4) sham adrenalectomy (controls; n = 5). Plasma corticosterone was undetectable in all three adrenalectomized groups. Plasma aldosterone concentration was similar in aldosterone-replaced and sham-operated controls. Pressor responses to various doses of angiotensin I, angiotensin II, and norepinephrine were determined in unanesthetized, undisturbed rats. Compared with both control and dexamethasone-replaced rats, pressor responses to all three agonists were significantly reduced in both nonreplaced adrenalectomized and aldosterone-replaced groups. Comparing the ratios of the pressor responses to angiotensin I and angiotensin II in the four groups over the entire dose range suggests that a greater fraction of injected angiotensin I was converted to angiotensin II in nonreplaced adrenalectomized rats than in the other three groups. We conclude that glucocorticoid action markedly contributes to the systemic pressor effect of angiotensin and norepinephrine. However, glucocorticoid deficiency does not impair in vivo conversion of angiotensin I to angiotensin II. (Hypertension 11: 174-178, 1988)

Key Words • norepinephrine • adrenal insufficiency • cardiovascular reactivity • angiotensin • mineralocorticoid • glucocorticoid
angiotensin I to angiotensin II. Sham-operated rats and nonreplaced adrenalectomized rats served as controls.

Materials and Methods

Animals

Weight-matched male Sprague-Dawley rats (age, 9–10 weeks) were obtained from Perfection Breeder (Douglasville, PA, USA). The animals were housed in groups, and standard Purina laboratory chow (certified rodent chow 5002, Lab Chows, St. Louis, MO, USA) and tap water ad libitum were provided until the time of the studies.

Study Groups

Four groups were prepared: 1) sham-adrenalectomized (CONT; n = 5); 2) adrenalectomized (ADX; n = 5); 3) adrenalectomized, replaced with aldosterone (ALDO; n = 5); 4) adrenalectomized, replaced with dexamethasone (DEX; n = 5).

Study Protocol

The rats were adrenalectomized or underwent a sham procedure (exposure of adrenals) through flank incisions during ketamine anesthesia (100 mg/kg i.p.). Alzet miniosmotic pumps (Models 1701 and 2001, Alza, Palo Alto, CA, USA) containing aldosterone (Sigma Chemical, St. Louis, MO, USA) diluted with polyethylene glycol at a dose calculated to deliver 6 μg/24 hr, dexamethasone (Elkins-Sinn, Cherry Hill, NJ, USA) in normal saline delivering 10 μg/24 hr, or the vehicle polyethylene glycol were used for hormone replacement in the ALDO, DEX, CONT and ADX groups, respectively. The pumps were introduced into the peritoneal cavity through flank incisions. The animals were subsequently given 1% NaCl and 2.5% glucose to drink. Adequate aldosterone delivery was confirmed by measurements of plasma aldosterone levels on completion of the study. The dose of dexamethasone given is similar to that used by others in similar experiments and is estimated to equal the daily endogenous glucocorticoid secretion in intact rats.1 11

Approximately 24 hours after adrenalectomy or sham operation, the animals were reanesthetized with ketamine. A PE-50 cannula was placed in the femoral artery for arterial pressure measurements, and three PE-10 catheters were placed in the femoral vein for drug injection. Catheters were exteriorized at the back of the neck. The arterial line was connected to a Physiograph DMP-4A recorder (Narco Bio-Systems, Houston, TX, USA) through a Statham P23Db transducer (Gould, Hato Rey, Puerto Rico), and the animals were allowed to recover in their native cages.

While in their cages, baseline pulsatile arterial pressure and heart rate were recorded in the conscious, undisturbed rats 4 to 6 hours after operation. Mean arterial pressure was obtained by electronic damping. After blood pressure stabilized, angiotensin I (Sigma) was injected in doses increasing from 25 to 200 ng/kg, followed by injection of angiotensin II (Sigma) at equal doses and norepinephrine (levarterenol bitartrate, Winthrop Laboratories, New York, NY, USA) at doses of 300 to 2400 ng/kg. The injections were administered at intervals that allowed arterial pressure to return to preinjection levels and in duplicates with a microinjector (Gilmont Instruments, Great Neck, NY, USA) in aliquots ranging from 2 to 40 μl. The peak pressor responses at each dose were recorded and averaged.

Two hours after completion of the dose-response studies, blood was obtained from the conscious animals through the arterial catheter for plasma electrolytes, aldosterone, and corticosterone concentrations.

Blood samples were obtained by decapitation for measurement of plasma renin activity from other groups of rats prepared similarly to those used for the pressor assays.

Hormone and Biochemical Assays

Plasma renin activity was measured by radioimmunoassay as previously described,12 except that phenylmethylsulfonyl fluoride was used in place of disopropylfluorophosphatase as an inhibitor of angiotensinas. Interassay variation was 10%.

Plasma aldosterone was measured by radioimmunoassay13 using the aldosterone liquid phase kit (Diagnostic Products, Los Angeles, CA, USA) after extraction with methylene dichloride. Interassay variation was 7%. Plasma corticosterone was determined by radioimmunoassay, using antibody provided by BioFlex Laboratories (So. Ozone Park, NY, USA). Electrolytes were determined by flame photometry.

Biometric Analyses

The results are presented as means ± SEM and were statistically analyzed by regression analysis, analysis of variance, and multisample variance analysis (Fisher’s least-significant-difference test), as applicable. Differences were considered significant at a p value below <0.05 level.

Results

Thirty hours after adrenalectomy or the sham procedure, body weight and baseline mean arterial pressure were not significantly different in the four groups, although the ADX and ALDO groups tended to have slightly lower arterial pressures than CONT or DEX (Table 1). Plasma potassium levels in the ALDO and CONT groups were similar and tended to be lower than in the ADX group. Plasma aldosterone was similar in the ALDO and CONT groups but was undetectable in the ADX group. In the DEX group, plasma potassium was elevated and similar to that for the ADX group. A small amount of aldosterone-like activity was measured by radioimmunoassay in the plasma of several DEX rats and probably was due to cross-reaction with dexamethasone or one of its metabolites (see Table 1). Corticosterone was undetectable in the plasma of all three adrenalectomized groups (i.e., ADX, ALDO, and DEX).

The arterial pressure responses to injections of angiotensin I, angiotensin II, and norepinephrine in the four groups of rats are displayed in Figures 1, 2, and 3.
TABLE 1. Body Weight, Baseline Mean Arterial Pressure, Plasma Electrolytes, Aldosterone, and Corticosterone in the Four Groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>ADX</th>
<th>ALDO</th>
<th>DEX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>353 ± 7</td>
<td>367 ± 13</td>
<td>339 ± 15</td>
<td>366 ± 16</td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>114 ± 3</td>
<td>108 ± 4</td>
<td>106 ± 3</td>
<td>118 ± 3</td>
</tr>
<tr>
<td>Plasma Sodium (mmol/L)</td>
<td>134 ± 3</td>
<td>136 ± 3</td>
<td>137 ± 2</td>
<td>136 ± 4</td>
</tr>
<tr>
<td>Potassium (mmol/L)</td>
<td>4.4 ± 0.4</td>
<td>5.0 ± 0.3</td>
<td>3.8 ± 0.4*</td>
<td>5.2 ± 0.3</td>
</tr>
<tr>
<td>Aldosterone (ng/dl)</td>
<td>27 ± 7</td>
<td>ND</td>
<td>21 ± 5</td>
<td>5 ± 2†</td>
</tr>
<tr>
<td>Corticosterone (μg/dl)</td>
<td>26 ± 4</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

Values are means ± SEM. ADX = adrenalectomy; ALDO = aldosterone treatment; DEX = dexamethasone treatment; ND = nondetectable.

* p<0.05, compared with ADX and DEX groups.
† Due to cross-reactivity with dexamethasone or its metabolites.

respectively. When the four groups were compared, similar patterns were evident in the responses to all three agonists. The ADX and ALDO groups consistently had the least elevations in arterial pressure. The DEX group displayed pressor responses that were always higher than those of the ADX and ALDO groups and that were in most instances not significantly lower than those of the CONT group.

Angiotensin I and II were injected at doses of equal weight per kilogram of body weight in this study. Since angiotensin II lacks the His-Leu dipeptide, its molecular weight is approximately 20% less than that of angiotensin I. Theoretically, if all of the injected angiotensin I is converted to angiotensin II, the ratio of pressor responses (angiotensin II/angiotensin I) at equal doses should be nearly 1.2. A defect in conversion of angiotensin I to angiotensin II or inactivation of the decapeptide before conversion to the octapeptide would result in a ratio higher than this theoretical limit of 1.2. In these experiments, the ratio of pressor responses (angiotensin II/angiotensin I) at each dose was calculated. These ratios were then plotted against the dose employed, and the regression relationship for each group of animals was determined as shown in Figure 4. It is evident that at the lower doses, a higher ratio of pressor responses (angiotensin II/angiotensin I) was observed in the DEX, CONT, and ALDO groups compared with the ADX group. At the highest dose, there was convergence of the pressor ratio toward the theoretical limit.

Measurements of plasma renin activity are also displayed in Figure 4. An inverse relationship between the pressor ratio and the plasma renin activity level was suggested at the lower dose of injected angiotensin I and II. Thus, at the lower doses, the most efficient conversion of injected angiotensin I (lowest pressor ratio) occurred in the groups with the higher plasma renin activity (ADX, DEX), whereas the higher pressor ratio was observed in the groups with the lower plasma renin activity (ALDO, CONT).
Discussion

The results of this study in conscious rats confirm previous observations from anesthetized rats that adrenalectomy results in a reduced systemic pressor responsiveness to pressor substances.2-6,14 We found this effect with both the adrenergic agonist (norepinephrine) and the two angiotensins (angiotensin I and II). Effective replacement with the mineralocorticoid aldosterone did not significantly enhance the pressor responses to norepinephrine or the angiotensins. In contrast, replacement of glucocorticoid activity with dexamethasone increased the pressor responses to all three agonists when compared with responses in either nonreplaced adrenalectomized rats or those given aldosterone. These findings lend further support to the view that impaired cardiovascular responsiveness may be a contributing factor to the abnormal regulation of arterial pressure in the absence of endogenous glucocorticoid secretion by the adrenal glands.1

Previous studies have shown that glucocorticoids increase pressor responses to sympathetic nerve stimulation in anesthetized rats2,3 and dogs.9 Glucocorticoids have also been shown to increase vascular responses to catecholamines or adrenergic agonists in the isolated hindlimb preparation of the rat,6 pithed rats,15 and in isolated rabbit aortic strips.16

Replacement with dexamethasone may not be identical to replacement with corticosterone, the primary endogenous glucocorticoid in the rat. Our study, however, was designed to address the specific effect of glucocorticoid replacement, and the choice of dexamethasone resulted from its more specific glucocorticoid effect compared with corticosterone. The latter may have a major mineralocorticoid action at its physiological plasma levels in some tissues.17

Several mechanisms have been proposed to account for reduced pressor or vasoconstrictor responses to catecholamines after adrenalectomy. It has been suggested that steroid action inhibits catecholamines metabolizing enzymes (catechol O-methyl transferase or monoamine oxidase) or alters the effectiveness of the catecholamine uptake systems of neuronal or extraneuronal tissue.7,14,16,18 In addition, adrenergic receptors may be regulated by a glucocorticoid effect.15,19 However, our results demonstrate that glucocorticoid replacement alone increased the pressor responses to norepinephrine and angiotensin in adrenalectomized rats. It is possible that the major effect of glucocorticoid action in maintaining normal vascular responsiveness occurs at a common site shared by both catecholamine and peptide pressor agonists. However, separate
actions of glucocorticoids on both the sympathetic system and the renin-angiotensin system still might account for a parallel increase in pressor responses to norepinephrine and the angiotensins.

The report by Mendelsohn et al., 10 that glucocorticoid-deficient rats have reduced in vitro lung converting enzyme activity, led us to compare the relationships between the pressor responses to angiotensin I and II within the four groups studied. If glucocorticoid deficiency resulted in a functionally notable impairment of in vivo conversion of angiotensin I to II, then the difference in the responses to angiotensin I and angiotensin II, or the ratio of responses (angiotensin II/angiotensin I), would be highest in the two groups without glucocorticoid replacement (ADX and ALDO). Furthermore, this ratio would be expected to increase with higher doses of the injected peptides, as the rate of conversion in these two glucocorticoid-deficient groups became more limiting. Instead, we found that the lowest ratio was observed in the ADX group and the highest in the ALDO group, neither group being replaced with glucocorticoids. These findings suggest that short-term glucocorticoid deficiency does not diminish in in vivo conversion of angiotensin I to II.

In summary, unanesthetized, adrenalectomized rats have reduced pressor responsiveness to norepinephrine, angiotensin I, and angiotensin II. Selective replacement with dexamethasone, but not aldosterone, significantly increases the pressor response to all three agonists. In this short-term model of adrenal insufficiency, the relationship between the pressor effects of angiotensin I and angiotensin II, or the ratio of responses (angiotensin II/angiotensin I), would be highest in the two groups without glucocorticoid replacement (ADX and ALDO). Furthermore, this ratio would be expected to increase with higher doses of the injected peptides, as the rate of conversion in these two glucocorticoid-deficient groups became more limiting. Instead, we found that the lowest ratio was observed in the ADX group and the highest in the ALDO group, neither group being replaced with glucocorticoids. These findings suggest that short-term glucocorticoid deficiency does not diminish in in vivo conversion of angiotensin I to II.

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