Glucocorticoid inhibition of mineralocorticoid action in the rat

C. J. KENYON*, N. A. SACCOCIO AND D. J. MORRIS

Department of Laboratory Medicine, The Miriam Hospital, Brown University, Providence, Rhode Island, U.S.A.

(Received 10 October 1983/7 March 1984; accepted 15 March 1984)

Summary

1. The mineralocorticoid activity of corticosterone based on acute changes in urinary Na+/K+ ratios in adrenalectomized rats was 1000 times less than that of aldosterone. However, corticosterone had only kaliuretic actions whereas aldosterone had both antinatriuretic and kaliuretic properties. Corticosterone inhibited the antinatriuretic actions of aldosterone.

2. Adrenalectomized rats infused continuously with a physiological dose of corticosterone (1 mg/day) were 5 times less sensitive to the antinatriuretic and 25 times less sensitive to the kaliuretic actions of aldosterone when administered acutely than were control adrenalectomized rats.

3. The long term effects of infusions of physiological doses of aldosterone and corticosterone were assessed in adrenalectomized rats maintained in metabolic cages. Aldosterone lowered plasma renin activity and reduced fluid (0.3% NaCl) intake; these effects were diminished when aldosterone and corticosterone were infused simultaneously. Plasma renin activity and fluid intake were correlated in long term infusion experiments. Both hormones had hypokalaemic effects but these were not additive. Corticosterone, but not aldosterone, increased systolic blood pressure and plasma sodium levels.

4. We conclude that glucocorticoid effects on water and electrolyte metabolism are different from those of mineralocorticoids, that glucocorticoids may antagonize mineralocorticoid effects and that interactions between mineralocorticoids and glucocorticoids may be important in long term blood pressure regulation.

Key words: aldosterone, corticosterone, plasma renin activity, urinary potassium/creatinine ratio, urinary sodium/creatinine ratio, urinary sodium/potassium ratio, water balance.

Introduction

Adrenal steroids have been described [1] as either mineralocorticoids (i.e. primarily affecting electrolyte metabolism) or glucocorticoids (i.e. regulators of intermediary metabolism). The distinction is not always clear. For example, plasma concentrations of corticosterone, the major glucocorticoid in rat, are 100-1000 times those of the principal mineralocorticoid, aldosterone [2]; accordingly, despite its relatively low affinity for renal mineralocorticoid receptors, corticosterone may compete for these receptors [3, 4]. It is not known whether such competition antagonizes the activity of mineralocorticoids and/or whether the very weak agonist effect resulting in the antinatriuretic and kaliuretic properties which are attributed to glucocorticoids [5] can assume physiological significance.

Previous studies have noted that glucocorticoids, but not mineralocorticoids, increase glomerular filtration rate (GFR) [6], promote the excretion of a water load in adrenal insufficiency [7], have immediate life-maintaining properties in otherwise moribund adrenalectomized dogs [8] and specifically increase Na,K-ATPase in medullary collecting tubules [9], as opposed to cortical collecting tubules [10]. These properties will interfere in any test for mineralocorticoid activity. For these reasons this study has compared both short and long term effects of aldosterone and corticosterone.

*Present address and address for correspondence: Dr C. J. Kenyon, MRC Blood Pressure Unit, Western Infirmary, Glasgow G11 6NT, U.K.
treatment on water and electrolyte balance in the rat.

Materials and methods

Acute effects of aldosterone and corticosterone

Male Sprague-Dawley rats weighing 170-190 g (Charles River, Wilmington, Massachusetts, U.S.A.) were bilaterally adrenalectomized and then maintained for a period of 5-10 days with free access to food and to 0.9% NaCl solution. Food was withdrawn 16 h before the test. All rats were injected with 3 ml of 0.9% NaCl subcutaneously to maintain urine flow and with 0.2 ml of a solution (0.9% NaCl/ethanol; 4:1, v/v) containing steroids. Rats were treated in one of four ways: (i) control (vehicle alone); (ii) with aldosterone (0.5 μg/kg body weight); (iii) with corticosterone (50 μg/kg body weight); (iv) with aldosterone + corticosterone (0.5 μg + 50 μg/kg body weight). Rats were induced to urinate immediately after injection and again at 1 and 3 h post-injection. During the test, rats were housed in wire-bar cages suspended inside large crystallizing dishes. Urine produced in the interval 1-3 h post-injection was collected and analysed for sodium, potassium and creatinine.

Aldosterone dose responses with and without corticosterone infusions

Male rats were used as above except that at the time of adrenalectomy they were given a subcutaneous implant of a mini-osmotic infusion pump (Alzet, Palo Alto, California, U.S.A.) which infused corticosterone dissolved in propylene glycol (1 mg/day) continuously for up to 7 days. The acute kaliuretic (urinary K+/creatinine) and antinatriuretic (urinary Na+/creatinine) effects of various doses of aldosterone (0-1.25 μg/rat) in these corticosterone-treated rats were compared with those in rats given a sham implant by the method outlined above.

Long term effects of aldosterone and corticosterone

Male rats were housed individually in metabolic cages with free access to food containing 0.21 mmol of Na+/kg and 0.28 mmol of K+/kg. Rats were given 0.3% NaCl as drinking water since this has been shown to maintain body weight gain after adrenalectomy rather than 0.9% NaCl which would obviate the need for mineralocorticoid replacement [10, 11]. After 10 days (to familiarize rats with daily handling procedures) rats were adrenalectomized or sham adrenalectomized and steroid treatments were started. Steroids were dissolved in propylene glycol and administered as a continuous infusion using mini-osmotic pumps. Groups of six to seven rats were used to compare the effects of five treatments: (i) adrenalectomy + vehicle; (ii) adrenalectomy + 2 μg of aldosterone/day; (iii) adrenalectomy + 1 mg of corticosterone/day; (iv) adrenalectomy + 2 μg of aldosterone and 1 mg of corticosterone/day; (v) sham adrenalectomy + vehicle.

Body weight, food and fluid intake and urine volume were monitored for 7 consecutive days. On day 7, systolic blood pressures were recorded from the tail of each rat with an electrophysgmomonometer (Narco Biosystems).

Analysis

Na+ and K+ content of food, plasma and urine were measured by flame photometry. Urine osmolality was determined using an Advanced Osmometer (Advanced Instruments, Massachusetts, U.S.A.). Plasma renin activity was estimated by radioimmunoassay of generated angiotensin I [12]. Unpaired t-tests were used to test the significance of differences between means. Linear regression analysis was performed on plasma renin activities and fluid intakes.

Results

The acute effects of corticosterone and aldosterone

Urine collected 1-3 h after injection with aldosterone or corticosterone had lower ratios of Na+/K+ (Table 1) than urine collected from rats injected with vehicle alone (P < 0.05); the urinary Na+/K+ ratios of rats injected with aldosterone and corticosterone in combination were similar to those of rats given either steroid alone. Closer analysis of individual electrolyte effects revealed that aldosterone had both antinatriuretic (P < 0.05) and kaliuretic properties (P < 0.02), whereas corticosterone increased only the urinary K+/creatinine ratio (P < 0.01).

Aldosterone dose-response curve in rats infused with corticosterone (Fig. 1)

Aldosterone injections had no acute effect on creatinine excretion in either corticosterone-infused or sham-treated control rats. The corticosterone-treated group excreted more creatinine than controls (0.21 ± 0.01 mg/h per rat, n = 28, compared with 0.16 ± 0.01 mg/h per rat, n = 31, P < 0.001). Nevertheless values of Na+/creatinine and K+/creatinine for rats injected with 0.2 ml of
TABLE 1. The acute effects of aldosterone and corticosterone on urinary sodium and potassium in male adrenalectomized rats

Values shown are the means ± SE of six to ten observations in urine collected between 1 and 3 h post-injection. * Indicates significant \((P < 0.05)\) difference from controls.

<table>
<thead>
<tr>
<th></th>
<th>Urinary (\text{Na}^+)/K(^+) (mmol/g)</th>
<th>(\text{Na}^+)/creatinine (mmol/g)</th>
<th>K(^+)/creatinine (mmol/g)</th>
<th>Creatinine excretion ((\mu)g/h per rat)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle-injected controls</td>
<td>5.76 ± 1.26</td>
<td>604 ± 44</td>
<td>143 ± 24</td>
<td>146 ± 9</td>
</tr>
<tr>
<td>Aldosterone (0.5 (\mu)g/kg body weight)</td>
<td>*1.73 ± 0.25</td>
<td>*414 ± 76</td>
<td>*240 ± 22</td>
<td>144 ± 8</td>
</tr>
<tr>
<td>Corticosterone (50 (\mu)g/kg body weight)</td>
<td>*2.49 ± 0.31</td>
<td>637 ± 69</td>
<td>*264 ± 26</td>
<td>155 ± 5</td>
</tr>
<tr>
<td>Aldosterone + corticosterone (0.5 (\mu)g + 50 (\mu)g/kg body weight)</td>
<td>*2.02 ± 0.17</td>
<td>602 ± 95</td>
<td>*288 ± 31</td>
<td>155 ± 5</td>
</tr>
</tbody>
</table>

Fig. 1. Effects of corticosterone infusions on the mineralocorticoid activities of aldosterone. The antinatriuretic (\(\text{Na}^+\)/creatinine decreases) (a) and kaliuretic (K\(^+\)/creatinine increases) (b) activities of various doses of aldosterone (0-1.25 \(\mu\)g/rat) were measured in the urine of male adrenalectomized rats collected between 1 and 3 h post-injection (continuous line) and compared with the activities of aldosterone in rats infused with 1 mg of corticosterone/day (dotted line). Each point is the mean ± SE of five to nine observations. * Denotes significant difference from control rats \((P < 0.05)\).

Vehicle alone were similar for corticosterone-treated and control animals (Fig. 1). Differences in \(\text{Na}^+\)/creatinine and K\(^+\)/creatinine between control and corticosterone-infused rats when injected with aldosterone are therefore assumed to reflect differences in mineralocorticoid responsiveness. For example, K\(^+\)/creatinine values with corticosterone treatment were significantly lower \((P < 0.05)\) than control values for every dose of aldosterone, indicating a 25-fold difference in sensitivity (Fig. 1). Approximately 5 times more aldosterone was needed by corticosterone-treated rats compared with controls to elicit the same antinatriuretic effect although the difference in \(\text{Na}^+\)/creatinine values was only statistically significant \((P < 0.05)\) at a dose of 0.25 \(\mu\)g of aldosterone/rat.

Long term effects of aldosterone and corticosterone (Figs. 2 and 3)

Adrenalectomy and maintenance on 0.3% NaCl solution was followed by an increase in fluid intake \((P < 0.01)\), plasma renin activity \((P < 0.05)\),...
FIG. 2. Effects of aldosterone and corticosterone infusions on fluid and electrolyte metabolism. Groups of six to seven male adrenalectomized or sham adrenalectomized rats were infused via mini-osmotic pumps with either vehicle, aldosterone (2 μg/day), corticosterone (1 mg/day) or aldosterone + corticosterone (2 μg and 1 mg/day). Values are from samples taken from 7 days of continuous subcutaneous infusion. ○, Adrenalectomized rats given vehicle alone; □, sham adrenalectomized rats given vehicle alone; ■, adrenalectomized rats given aldosterone; ▲, adrenalectomized rats given corticosterone; ◼, adrenalectomized rats given aldosterone + corticosterone. * Significantly different (P < 0.05) from adrenalectomized rats. † Significantly different (P < 0.05) from sham adrenalectomized rats. ‡ Significantly different (P < 0.05) from rats infused with aldosterone + corticosterone. Abbreviation: ANG I, angiotensin I.

and plasma potassium concentration (P < 0.001), and lowered urine osmolarity (P < 0.001) and blood pressure (P < 0.05); plasma sodium levels were not significantly affected. Aldosterone treatment suppressed renin activity (P < 0.01) and fluid intake (P < 0.01) to values which were even lower than in sham adrenalectomized rats (P < 0.05 and P < 0.01 respectively). Plasma potassium levels with aldosterone treatment were less than adrenalectomized rats (P < 0.001) but not significantly different from those in sham adrenalectomized rats. Blood pressure and plasma sodium levels were not affected by aldosterone treatment.

Corticosterone did not significantly affect renin activity, fluid intake or urine osmolarity but did have a slight hypokalaemic effect (P < 0.01), and elevated blood pressure (P < 0.001) and plasma sodium levels (P < 0.05) to values greater than in
Glucocorticoid-mineralocorticoid interactions

FIG. 3. Relationship between fluid intake and plasma renin activity. Values shown are means ± SE of observations made at the end of 6–7 days treatment in groups of six to seven male rats. From left to right points are from: (a) adrenalectomized rats treated with 2 μg of aldosterone/day; (b) sham adrenalectomized rats; (c) adrenalectomized rats treated with 1 mg of corticosterone + 2 μg of aldosterone/day; (d) adrenalectomized rats; (e) adrenalectomized rats treated with 1 mg of corticosterone/day. \( r = 0.84; P < 0.001 \).

sham adrenalectomized rats (\( P < 0.02 \) and \( P < 0.01 \) respectively).

Renin activity, plasma potassium levels, fluid intake and urine osmolarity in rats treated with aldosterone and corticosterone in combination most closely resembled those in sham adrenalectomized rats. Renin activity and fluid intake were significantly greater than in rats given aldosterone alone (\( P < 0.05 \) and \( P < 0.001 \) respectively). Blood pressure and plasma sodium levels with aldosterone + corticosterone were higher than in sham adrenalectomized rats (\( P < 0.02 \) and \( P < 0.001 \)) and similar to those in rats given corticosterone alone.

Plasma renin activities in these infusion experiments were closely correlated with urine osmolarity (\( r = 0.76; P < 0.001 \)) and fluid intake (\( r = 0.84; P < 0.001 \), see Fig. 3) but not with blood pressure.

Discussion

Corticosterone effects on acute responses to aldosterone

Conventionally the ability of a steroid to lower urinary Na⁺/K⁺ ratios acutely is used to assess mineralocorticoid potency. By this criterion corticosterone appeared to be 1000 times less active than aldosterone, which agrees with previously reported values [5]. However, unlike those of aldosterone, the effects of corticosterone on Na⁺/K⁺ ratios were due solely to an increased kaliuresis. When administered in combination with aldosterone, corticosterone appeared to inhibit antinatriuretic activity. Similar observations were made by Uete & Venning [13]. Although it has been demonstrated that mineralocorticoid effects on different cations may not be linked (for a review see [14]), it is probable that the renal mechanisms of glucocorticoid and mineralocorticoid action are different [15, 16]. For example, glucocorticoids but not mineralocorticoids are known to increase GFR, which could cause a kaliuresis and might also counter the antinatriuretic effects of aldosterone [6]. This would also explain why rats given corticosterone excreted more creatinine and were less sensitive to aldosterone. However, corticosterone alone did not cause a significant acute change in creatinine excretion nor did it promote a natriuresis.

There are at least two other reasons why mineralocorticoid responsiveness might be reduced. Firstly, the plasma half-life of aldosterone is extended by adrenalectomy, presumably because in the absence of corticosterone aldosterone is more readily bound to transcortin and other serum binding proteins, and therefore protected from metabolism [17, 18]. A shorter half-life in rats infused with corticosterone may in part account for the reduced response seen in the present study and in a previous study which compared aldosterone responses in intact and adrenalectomized animals [19].

A second possibility is that corticosterone competes for renal mineralocorticoid binding sites. Two classes of receptors for adrenal steroids have been identified in the rat kidney [3, 20]: type 1 receptors show a greater affinity for aldosterone than corticosterone, whereas type 2 receptors have a higher affinity for glucocorticoids, but may also bind mineralocorticoids in the absence of corticosterone. However, when corticosterone and aldosterone are administered together, aldosterone...
cannot bind to type II sites and, depending on the amount of corticosterone, may be partly displaced from its own type I receptor by ‘illicit’ corticosterone binding [4]. Synthetic glucocorticoids such as dexamethasone are more selectively bound to type II sites and have been shown to have kaliuretic but not antinatriuretic properties [16]. It could be argued therefore, that in corticosterone-treated rats, antinatriuretic properties of aldosterone might be slightly diminished by ‘illicit binding’ but that prior occupancy of type II sites by corticosterone would prevent the kaliuretic actions of aldosterone.

**Long term effects of aldosterone and corticosterone**

A continuous steroid infusion at a rate similar to that produced endogenously [21] is likely to elicit a more physiological response than a single dose equivalent to that produced in vivo during a 24 h period. Long term experiments allowed a comparison of glucocorticoid and mineralocorticoid effects on dietary intake and plasma composition which could not easily have been revealed using the protocol for testing acute effects. It is suggested that, unlike short term renal effects, apparent inhibition by corticosterone of the long term effects of aldosterone is not due to competitive antagonism but rather that mineralocorticoids and glucocorticoids have separate effects on related endocrine systems [11]. For example plasma renin activity is decreased by aldosterone but corticosterone, by increasing the supply of renin substrate, increases plasma renin activity [22]. Similarly glucocorticoids and mineralocorticoids affect water metabolism differently. Glucocorticoids, but not mineralocorticoids, promote the excretion of a water load in adrenal insufficiency [7], possibly by increasing GFR or by inhibiting antidiuretic hormone activity [6]. In contrast, replacement doses of aldosterone in adrenalectomized rats have been shown to decrease drinking rate [10, 11, 23]. The close correlation between fluid intakes and plasma renin activities (Fig. 3) suggests that steroid-induced changes in thirst and/or sodium appetite may be due to changes in angiotensin II levels [24]. Alternatively aldosterone may increase urine concentration by potentiating the renal actions of antidiuretic hormone [25].

Changes in sodium and potassium excretion in the long term are similar to those seen acutely. However, changes in urinary electrolyte excretion and plasma electrolyte concentration were inconsistent. For example, the hypokalaemic actions of corticosterone were less than those of aldosterone despite a greater kaliuretic effect. Also aldosterone caused a marked antinatriuresis yet had no effect on blood pressure or plasma sodium levels. In contrast corticosterone-treated rats excreted more sodium whilst showing higher blood pressures and plasma sodium levels. In explanation of these anomalous effects, it has been suggested that glucocorticoids cause an internal redistribution of water and electrolytes which is independent of renal function [8, 26].

In summary, both acute and long term studies indicate that the effects of mineralocorticoids and glucocorticoids on water and electrolyte metabolism are different. Because of the normally large excess of plasma glucocorticoid over plasma mineralocorticoid levels, it is possible that inhibition by corticosterone of the acute antinatriuretic and kaliuretic actions of aldosterone which we observed in adrenalectomized rats are of physiological importance in the regulation of fluid and electrolytes in intact rats. However, interactions between these two hormones in long term infusion experiments are more conveniently explained by their opposing affects on related endocrine systems than by direct competition for renal receptors.

**References**