Effects of Antiglucocorticoids on Glucocorticoid Hypertension in the Rat

JEAN-PIERRE GRÜNFELD, LAURE ELOY, ANNE-MARIE MOURA, DOMINIQUE GANEVAL, BLANCA RAMOS-FRENDO, MANUEL WORCEL

SUMMARY The early phase of hypertension induced in rats by a glucocorticoid agonist RU 26988 was studied. Systolic blood pressure increased by 35 mm Hg. Water and sodium urinary excretion increased transiently, and plasma volume decreased. Total and ouabain-sensitive sodium efflux, as well as rubidium efflux, were enhanced by glucocorticoid administration. Low salt intake did not prevent hypertension. Pretreatment with RU 38486, a steroid with antiglucocorticoid properties, largely prevented the rise in blood pressure (+10 mm Hg) and suppressed transient natriuresis and the decrease in plasma volume. Changes in total and ouabain-sensitive sodium efflux were completely prevented, whereas changes in rubidium efflux were only partly reversed. Similarly, administration of progesterone, a steroid with antiglucocorticoid effects, prevented glucocorticoid hypertension (+11 mm Hg) and vascular ionic changes. In contrast administration of RU 28318, an antimineralocorticoid agent, was without effect on glucocorticoid hypertension (+38 mm Hg). Progesterone or RU 38486 administered after glucocorticoid also decreased blood pressure. Present data indicate that glucocorticoid hypertension may be prevented or reversed in its early phase by steroid drugs with antiglucocorticoid properties. These drugs also appeared to prevent the sodium and rubidium flux abnormalities induced by glucocorticoid. We suggest that activation of the vascular glucocorticoid receptors may be involved in the pathophysiology of glucocorticoid hypertension. (Hypertension 7: 292-299, 1985)

KEY WORDS • hypertension • glucocorticoid • antiglucocorticoid • progesterone • Na⁺-K⁺-ATPase

We therefore initiated a study of the in vivo effects of RU 26988 in the rat. RU 26988 is a specific glucocorticoid receptor agonist that exhibits high affinity for glucocorticoid receptor sites and does not compete with aldosterone for the mineralocorticoid receptor.11, 12 We attempted to antagonize glucocorticoid-induced hypertension by administration of an antimineralocorticoid (RU 28318) and steroid derivatives known to bind competitively at the glucocorticoid receptor — progesterone and RU 38486. The latter steroid has mainly antiprogestosterone properties; in vitro studies have also shown antiglucocorticoid activity.13, 14 Lastly, we investigated the ionic changes induced by glucocorticoid and antiglucocorticoids on the rat tail artery after in vivo administration of the steroid drugs.

Material and Methods

Studies were performed in Wistar rats that were approximately 8 weeks old with initial body weights ranging from 150 to 250 g. The animals were housed in individual metabolic cages. They received water ad...
libitum and were fed either a normal-sodium diet or a low-sodium diet (Extralabo, Longueville, France) containing 143 mmol or 3.5 mmol Na/kg respectively. Urine was collected daily. Sodium concentrations were determined by flame photometry. Approximate sodium balance data were calculated for 24-hour periods from daily intake and urine excretion. Stools were not collected.

Systolic blood pressure (SBP) was measured by the tail-cuff method in trained and prewarmed rats under ether anesthesia. The measurements were performed between 0900 and 1100 hours. Plasma volume was measured by the ¹²⁵I-albumin dilution method.¹⁵

The effects of drugs on transmembrane ²²Na and ⁶⁸Rb effluxes from arterial smooth muscle cells of treated rats were tested ex vivo. The method used for the study of ²²Na and ⁶⁸Rb effluxes from the rat tail artery has been published elsewhere.¹⁶ In brief, following excision and dissection, the rat tail artery was cut in half longitudinally. The tissues were mounted as strips 1 cm long and loaded for 90 minutes at 37°C with ²²NaCl (Radiochemical Centre, Amersham, England) in physiological saline (composed of 136.9 mM Na⁺, 5.9 mM K⁺, 2.5 mM Ca²⁺, 1.2 mM Mg²⁺, 133.5 mM Cl⁻, 1.2 mM H₂PO₄⁻, 15.5 mM HCO₃⁻, and 11.5 mM (glucose) gassed with 95% O₂ and 5% CO₂, pH 7.3. The ²²Na efflux from the strip was studied with a continuous flow technique by superfusion. Before desaturation, the strips were rapidly washed in cold (4°C) physiological solution for 15 minutes to eliminate excess radioactivity on the surface of the strip and in the extracellular space. Thereafter, the flow was adjusted to 2 ml/minute and the entire effluent was collected at 1-minute intervals. The radioactivity left in the tissue at the end of the experiment as well as the activity of the washout tubes was counted in an α-well counter.

A desaturation curve was obtained by adding in reverse order the washout curve to the radioactivity remaining in the tissue. Some strips were exposed to 1 mM ouabain during the last 10 minutes of the initial washout period at 4°C as well as during the entire desaturation period performed at 37°C. In these conditions, the glycoside completely blocks the sodium pump. Consequently it is possible to evaluate the ouabain-dependent (sodium pump) and ouabain-independent fractions of ²²Na efflux. Results are expressed as rate coefficient (min⁻¹), which was calculated as previously described.¹⁶ The effects of the drugs on ²²Na efflux rates were evaluated at the initial 12 minutes of washout at which the rates remain constant. This part of the ²²Na desaturation curve is specifically dependent on transmembrane sodium movements from smooth muscle cells. The evaluation of ⁶⁸Rb efflux was performed with an experimental protocol adapted from Mauger and co-workers.¹⁷

The following drugs were used: RU 26988 [11β, 17β-dihydroxy-17α-(1-propynyl) androsta-1,4,6 trien-3-one], a specific glucocorticoid receptor agonist,¹¹ was administered at a dose of 20 mg/kg/day by stomach tube. RU 38486 [17β-hydroxy-11β-(4-dimethylaminophenyl)-17α-(prop-1-ynyl)-estra-4,9-

The administration of RU 26988, a glucocorticoid agonist, resulted in a prompt rise in SBP (approximately 35 mm Hg) (Figure 1). Body weight decreased slightly (−10 g). On Day 1, urine water and sodium excretion increased transiently. Cumulative sodium balance was negative (−850 μmol on Day 3). Plasma volume (expressed in milliliters per 100 grams of body

Results

In Vivo Studies

Group I

The administration of RU 26988, a glucocorticoid agonist, resulted in a prompt rise in SBP (approximately 35 mm Hg) (Figure 1). Body weight decreased slightly (−10 g). On Day 1, urine water and sodium excretion increased transiently. Cumulative sodium balance was negative (−850 μmol on Day 3). Plasma volume (expressed in milliliters per 100 grams of body
weight, BW) was decreased on the third day of glucocorticoid administration (from 4.42 ± 0.09 to 4.28 ± 0.08 ml/100 g BW). After RU 26988 administration was stopped, blood pressure returned rapidly to a normal level, and growth rate was restored to normal. Urine sodium excretion was depressed transiently, then positive sodium balance was reattained. Plasma volume returned to basal values (Figure 1).

In rats on a low-sodium diet, glucocorticoid administration induced a similar rise in SBP (from 109 ± 4.3 to 142 ± 2.5 mm Hg). Urinary sodium excretion increased dramatically, from 5.8 to 500 µmol/24 hours (on Day 3). Cumulative sodium balance was −1000 µmol on Day 3. Plasma volume did not change appreciably (from 4.3 ± 0.06 to 4.26 ± 0.08 ml/100 g BW). These changes reverted to baseline levels after stopping the glucocorticoid administration.

**Group II**

Administration of antiglucocorticoid (RU 38486) alone for 5 days did not consistently modify body weight or urine water and sodium excretion (Figure 2). The SBP increased slightly from the basal mean value of 106 ± 1.7 to 118 ± 2.5 mm Hg (Day 5), whereas the other parameters measured (including plasma volume) did not change appreciably.

The antiglucocorticoid drug prevented the increase in SBP induced by the glucocorticoid agonist RU 26988 (Figure 3). A slight rise in blood pressure was observed, which averaged 10 mm Hg on Day 5. Weight loss was not prevented by the antiglucocorticoid. In contrast, the increase in urine sodium excretion was completely prevented, and even natriuresis dropped on Days 4 and 5. This decrease probably was due to low food and sodium intake as sodium balance remained positive in these rats throughout the experimental period. Mean cumulative sodium balance on Day 5 (calculated from Day 3 to Day 5) was +550 µmol. Plasma volume did not decrease substantially. After drug administration was stopped, blood pressure returned to basal values, growth rate resumed, urine sodium excretion and sodium intake remained low, and plasma volume was unchanged (Figure 3).

**Group III**

Progesterone administration alone had no appreciable effect on blood pressure, weight, plasma volume, or urinary sodium excretion. When glucocorticoid was added (Figure 4) progesterone blunted the rise in SBP: SBP rose slightly, from 109 ± 1.8 to 115 ± 2.4 mm Hg (Day 5). The protective effect was similar to that afforded by the antiglucocorticoid compound. In con-

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**Figure 1.** Changes in SBP, weight, diuresis, natriuresis, and plasma volume in Group Ia rats (n = 17; normal sodium intake) before, during, and after administration of the glucocorticoid RU 26988. Values are means ± SEM. * = p < 0.05 versus controls (Dunnett's method).

**Figure 2.** Changes in SBP, weight, diuresis, natriuresis, and plasma volume in Group II rats (n = 5) before, during, and after administration of the antiglucocorticoid RU 38486. * = p < 0.05 versus controls (Dunnett's method).
trast, progesterone administration did not prevent and even magnified the transient natriuretic effect of RU 26988 (3662 ± 165, Group III, versus 2992 ± 106 μmol/24 hr, Group I; p < 0.01, Student's t test). In Group III rats urinary sodium excretion increased slightly (p < 0.05, Dunnett's method) on Day 2 when progesterone alone was administered. On Days 4 and 5 (Figure 4) urine sodium excretion dropped, which contrasts with the data obtained in Group I (Figure 1). On Day 5 the mean cumulative sodium balance was −800 μmol. Plasma volume did not change significantly on Day 5, but the basal value (Day 2) was lower than in Group I (4.05 ± 0.08 versus 4.42 ± 0.09 ml/100 g BW). This decrease was possibly related to the natriuretic effect of progesterone.

**Group IV**

Administration of the antimineralocorticoid alone did not change SBP (Figure 5). Urine water and sodium excretion increased slightly but not significantly in the rats on a normal-sodium diet. Glucocorticoid administration resulted in a rapid and more pronounced rise in SBP, from 113.4 ± 2.9 to 151 ± 3.5 mm Hg, and a weight loss. A transient increase in urine water and sodium excretion persisted and was followed by an abrupt drop in urine sodium excretion, as in Group III. On Day 5, cumulative sodium balance was close to 0. Surprisingly, plasma volume increased on Day 5 (Figure 5). It returned to basal value on Day 11, as did blood pressure.

**Group V**

Glucocorticoid administration for 5 consecutive days induced an increase in SBP that was maximal on Day 2 and subsequently remained stable (Figure 6). In Groups Va and Vb, after hypertension was induced the antagonist, either RU 38486 or progesterone, was administered for 3 days (Figure 6). This resulted in a fall in blood pressure that was slightly more pronounced with RU 38486. Urine sodium excretion was not modified (except on Day 1 when the transient rise in urine sodium excretion occurred). On Day 5 cumulative sodium balance was 1250 μmol and 250 μmol in Groups Va and Vb respectively.

**Ex Vivo Studies**

Results of ex vivo studies are summarized in Table 1. The glucocorticoid agonist RU 26988 increased to-
tal sodium and rubidium efflux, stimulated the sodium pump, and did not affect passive sodium permeability (ouabain-insensitive).

The antiglucocorticoid drug RU 38486 decreased total sodium efflux and passive sodium permeability, but did not alter sodium pump activity and rubidium efflux. The concomitant administration of RU 26988 and RU 38486 reversed some changes induced by RU 26988 and restored total sodium efflux and sodium pump activity to control values. Passive sodium permeability was, however, significantly depressed when compared with controls (p < 0.05). This finding might be related to the effect of RU 38486. The rubidium efflux was depressed when compared with the results of administration of RU 26988 alone, but was slightly higher than in controls. Progesterone administration decreased total sodium efflux by depressing both passive permeability and sodium pump activity. The rubidium efflux was slightly enhanced. The concomitant administration of RU 26988 and progesterone strikingly depressed total sodium efflux, passive permeability, and sodium pump activity below control values. The rubidium efflux in this group was decreased when compared with that of RU 26988-treated rats.

Discussion

The present results indicate that in the rat the early phase of glucocorticoid hypertension can be prevented or reversed by steroids with antiglucocorticoid activ-

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**Table 1.** $^{22}$Na and $^{86}$Rb Efflux from Tail Arteries of Control Rats and Rats Treated with Glucocorticoid Agonists, Antagonists, or Both

<table>
<thead>
<tr>
<th>Efflux rate</th>
<th>Controls</th>
<th>RU 26988</th>
<th>RU 38486</th>
<th>RU 26988 + RU 38486</th>
<th>Progesterone</th>
<th>RU 26988 + progesterone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total $^{22}$Na efflux (min$^{-1}$)</td>
<td>0.135 ± 0.003</td>
<td>0.155 ± 0.004*</td>
<td>0.124 ± 0.003*</td>
<td>0.127 ± 0.003</td>
<td>0.107 ± 0.005*</td>
<td>0.110 ± 0.004†</td>
</tr>
<tr>
<td>(n = 10)</td>
<td>(n = 11)</td>
<td>(n = 8)</td>
<td>(n = 10)</td>
<td>(n = 10)</td>
<td>(n = 10)</td>
<td>(n = 10)</td>
</tr>
<tr>
<td>Ouabain insensitive</td>
<td>0.100 ± 0.003</td>
<td>0.096 ± 0.002</td>
<td>0.086 ± 0.003*</td>
<td>0.089 ± 0.003†</td>
<td>0.084 ± 0.003*</td>
<td>0.083 ± 0.003†</td>
</tr>
<tr>
<td>Ouabain sensitive</td>
<td>0.035 ± 0.003</td>
<td>0.059* ± 0.003</td>
<td>0.038 ± 0.002</td>
<td>0.038 ± 0.002†</td>
<td>0.023 ± 0.003*</td>
<td>0.027 ± 0.004†</td>
</tr>
<tr>
<td>$^{86}$Rb efflux (min$^{-1}$)</td>
<td>0.0103 ± 0.0001</td>
<td>0.0128 ± 0.0004*</td>
<td>0.0099 ± 0.0002</td>
<td>0.0111* ± 0.0002</td>
<td>0.0110 ± 0.0002*</td>
<td>0.0105 ± 0.0002†</td>
</tr>
<tr>
<td>(n = 10)</td>
<td>(n = 10)</td>
<td>(n = 13)</td>
<td>(n = 11)</td>
<td>(n = 8)</td>
<td>(n = 11)</td>
<td>(n = 11)</td>
</tr>
</tbody>
</table>

*p < 0.05 versus controls.
†p < 0.05 versus RU 26988.
All values are means ± SEM.
ity, such as RU 38486 and progesterone. In vitro studies have shown that the steroid RU 26988 has a high affinity for the glucocorticoid receptor and is devoid of any affinity for the mineralocorticoid receptor.11,12 Our in vivo study shows that RU 26988 at high doses (20 mg/kg/day) induces glucocorticoid-type hypertension. The rise in blood pressure was of rapid onset and reversed rapidly after administration was stopped. Low sodium intake did not prevent hypertension. Hypertension was associated with a transient increase in urine water and sodium excretion. The weight loss was moderate, possibly related both to the hypercatabolic state owing to glucocorticoid excess and to negative sodium balance. Plasma volume decreased significantly on the third day of RU 26988 administration ($p < 0.05$). This decrease clearly suggests that the early rise in blood pressure cannot be ascribed to an increase in plasma volume.

Haack and colleagues1 found that plasma volume increased in another model of glucocorticoid hypertension in the rat. They observed this increase, however, on the fifth day of corticosterone administration. Ramos-Frendo and co-workers13 showed that plasma volume did not change significantly in the very early phase (second day) of glucocorticoid (namely, methylprednisolone) hypertension and subsequently increased only on the fifth day following methylprednisolone administration.14

The steroid derivative RU 38486 exerts potent anti-gluco-corticoid activity in vitro and in vivo,15,16,19 in addition to its antiprogestrone properties. Its effect on glucocorticoid hypertension has not been examined in depth. Preliminary experiments indicated that the dose of 20 mg/kg/day of RU 26988 was the most appropriate for inducing reproducible hypertension in the rat. Detailed dose-response studies were not performed. On the second day of RU 26988 administration, however, SBP was $109 \pm 9, 119 \pm 6, 130 \pm 4, \text{and } 132 \pm 2$ mm Hg in the rats who received 5, 10, 15, and 20 mg/kg respectively. The dose of RU 26988 necessary to rapidly increase blood pressure is high. This derivative has a greater glucocorticoid activity in vitro than in vivo, when compared with other agonists such as demethasone.3,11 High doses of RU 38486 (100 mg/kg/day) had to be used to prevent the rise in blood pressure. Doses of 20 and 50 mg/kg/day of RU 38486 had no effect on the hypertension induced by RU 26988 at a dose of 20 mg/kg/day (results not shown).

The glucocorticoid-induced increase in blood pressure was not completely prevented by administration of RU 38486 at high doses. Moreover, administration of this steroid derivative alone for 5 days produced a slight increase in blood pressure. This increase may be explained by a slight glucocorticoid agonistic activity (not found at lower doses)14 or by the stimulatory effect of the drug on adrenocorticotropic hormone and endogenous corticosterone secretion or by both.20 As previously shown by Philibert and colleagues,13 the antiguocorticoid compound suppressed glucocorticoid-induced diuresis as well as natriuresis. RU 38486 administration did not prevent the weight loss induced by the glucocorticoid agonist, which demonstrates that weight loss is not dependent on the transient increase in natriuresis. Similarly, the decrease in plasma volume was not suppressed by RU 38486 administration. This finding is surprising because cumulative sodium balance was positive during concomitant glucocorticoid and antiglucocorticoid administration (Group II).

During recent years, various antiglucocorticoid derivatives have been synthesized and tested in vitro.21,22 Few compounds, however, have been shown to exert antiglucocorticoid activity in vivo in intact animals.23,24 In addition, they have not been used so far to antagonize the vascular and pressor effect of a glucocorticoid.

Several lines of evidence indicate that RU 38486 exerts a protective effect against glucocorticoid hypertension through its antiglucocorticoid action. (1) The preventive effect was evident despite a similar fall in plasma volume and despite a positive sodium balance. This finding clearly indicates that volume changes are not involved in this hypertension model. (2) A mineralocorticoid antagonist, RU 28318, at a dose that decreased blood pressure in mineralocorticoid hypertension,25 did not prevent glucocorticoid hypertension. (3) Administration of progesterone, which has antiglucocorticoid activity,26 prevented glucocorticoid hypertension, as did administration of RU 38486. The kidney, however, was not affected in a similar manner by the two antiglucocorticoid drugs. RU 38486 administration prevented the transient diuresis and natriuresis induced by the glucocorticoid, whereas progesterone, probably through its antimineralocorticoid action, tended to magnify them. Neither drug prevented the weight loss caused by the glucocorticoid.

The antiglucocorticoid drugs RU 38486 and progesterone exerted not only a preventive effect but also a curative effect on glucocorticoid hypertension in its early phase. This effect could not be ascribed to changes in sodium balance. Progesterone has been shown to decrease blood pressure in some hypertensive patients and animals.27,28 and it has been suggested that its blood pressure lowering effect may not depend on its antimineralocorticoid properties.28

Arterial hypertension induced by the glucocorticoid RU 26988 was accompanied by an increase in total Na efflux from vascular smooth muscle that appears to be caused exclusively by a stimulation of the sodium pump. Simultaneously, RU 26988-induced hypertension was characterized by an increase in 86Rb efflux. This latter effect could result from an enhancement of transmembrane permeability to potassium or be secondary to the activation of the sodium pump. Indeed, glucocorticoids have been shown to increase the Na+-K+-stimulated ATPase activity in various tissues, including the rat kidney and liver29 and human erythrocytes.30 Furthermore, Pannini and co-workers31 found increased activity of the sodium pump in vessels excised from dexamethasone-treated rats. The changes in ionic fluxes from smooth muscle observed during the hypertension induced by RU 26988 may result from the long-term effects of the glucocorticoid. In fact, short-term administration of RU 26988 to adrenalecto-
zized rats has negligible effects on the $^{22}Na$ efflux from rat tail artery. In any case, the increase in sodium pump activity and $^{86}Rb$ efflux observed during the hypertension induced by long-term administration of RU 26988 appears to be glucocorticoid specific. Indeed, both effects are suppressed by the antiglucocorticoid compounds RU 38486 and progesterone, the antimineralocorticoid RU 28318 being without effect. It is of interest that, at the high doses used to counteract the hypertension induced by the glucocorticoid RU 26988, the antagonist RU 38486 had an inhibitory action on passive $^{22}Na$ efflux that cannot be explained by a partial agonist glucocorticoid activity, as the pure glucocorticoid does not exert it. By itself, long-term administration of progesterone has been shown to inhibit both ouabain-insensitive and sodium-pump-induced $^{22}Na$ efflux. In this respect, this inhibitory action is similar to the reported effects of the natriuretic factor.

It is worth noting that some compounds described as natural ligands of the sodium pump have a progesterone-like structure.

In other animal models of hypertension, such as spontaneously hypertensive rats and sodium-sensitive Dahl rats, the sodium pump has been shown to be activated. This activation might be either primary or secondary, caused by an increased inward leak of sodium. Conflicting results have been reported in deoxycorticosterone-acetate-salt hypertension in which the sodium pump was either stimulated or depressed. The discrepancy may be explained by methodological differences.

**Conclusion**

The present results indicate that the increase in blood pressure induced by a glucocorticoid is accompanied by an activation of the vascular smooth muscle sodium pump and by an increase in $^{86}Rb$ efflux (an index of potassium efflux). It is not presently possible to ascertain whether these changes are a causal factor of hypertension in this animal model.

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