Adrenocorticotropic Hormone, Blood Pressure, and Serum Erythropoietin Concentrations in the Rat

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Background: This study was designed to investigate the effects of adrenocorticotropic hormone (ACTH) on systolic blood pressure (BP) and serum erythropoietin (EPO) concentrations in two strains of rats. We hypothesized that ACTH-induced hypertension in the rat is characterized by increased EPO production.

Methods: Male Sprague-Dawley (SD) and Wistar outbred (Wistar) rats were treated with saline or ACTH (0.2 mg/kg/d). Systolic BP was measured using the tail-cuff method. Serum EPO concentrations were assayed using an ELISA kit for human EPO, which cross-reacts with but underestimates rat EPO. Thymus weight was used as a marker of glucocorticoid activity.

Results: In SD rats, ACTH increased systolic BP (from 109 ± 4 to 142 ± 5 mm Hg, \( P < .0005 \)), significantly greater than in saline-treated rats (\( P < .01 \)). Systolic BP in ACTH-treated Wistar increased from 120 ± 3 to 133 ± 4 mm Hg (\( P < .05 \)), but was not significantly different from saline-treated Wistar rats. The ACTH-induced increase in systolic BP was greater in SD than in Wistar rats (\( P < .05 \)). Serum EPO levels were 5.6 ± 0.4 in SD and 5.9 ± 0.3 IU/L in Wistar rats, and decreased to undetectable levels with ACTH treatment in 10 of 10 SD and 7 of 10 Wistar rats. The ACTH treatment increased hemoglobin and hematocrit, and decreased thymus weight in both strains.

Conclusions: 1) ACTH decreased serum EPO concentrations in both strains; 2) EPO is inversely related to ACTH-induced hypertension in the rat; and 3) Wistar rats are relatively resistant to the BP raising effects of ACTH treatment but not to the ACTH-induced decrease in thymus weight. These data suggest that EPO is not causal in ACTH-induced hypertension.

Key Words: Adrenocorticotropic hormone, blood pressure, erythropoietin.
Methods

Animals

This study was approved by the Animal Experimental Ethics Committee of the Australian National University (AECC Protocol No J.HB. 10.02). Male Sprague-Dawley (SD) rats (n = 20, body weight 260 g) (Animal Resources Center, Perth, WA, Australia) and age-matched male Wistar out-bred (Wistar) rats (n = 20) (Animal service ANU, Canberra, ACT, Australia) were housed in plastic cages in the High Blood Pressure Research Unit under controlled lighting (12-h light/dark cycle) and temperature (21°C to 23°C) conditions. The rats had access to tap water ad libitum and were fed with standard rat food. Animals were allowed 10 days for acclimatizing to their surroundings, handling, and BP measuring equipment before any experimental procedure.

Experimental Protocol

Four days of control readings were followed by 13 days of ACTH (0.2 mg/kg/d subcutaneously [sc]) or sham (saline 1 mL/kg/d sc) treatment. The control days were designated “C” and ACTH or saline treatment days as “T.” The rats were randomly assigned to one of four groups: group 1: saline-treated SD rats (n = 10) (an injection of saline [1 mL/kg sc] daily between 11 AM and noon for 13 days (T0 to T12); group 2: ACTH-treated SD rats (n = 10) (ACTH [0.2 mg/kg sc; Novartis Pharmaceuticals, Sydney, Australia] daily injection [1 mL/kg] from T0 to T12; group 3: saline-treated Wistar rats (n = 10; saline treatment from T0 to T12); and group 4: ACTH-treated Wistar rats (n = 10; ACTH treatment from T0 to T12).

At the end of the study, the rats were anesthetized with 60 mg/kg of pentobarbital. Blood samples were collected under anesthesia. The thymus was surgically removed at this time and sections were stained with hematoxylin and eosin.

Systolic BP and Body Weight Measurements

Systolic BP was measured at 10 AM and noon on alternate days using a tail-cuff system (Narco Biosystems, Houston, TX). The rats were restrained on a heating plate (39° to 40°C). The animals were weighed on alternate days after systolic BP measurement and before injection.

Hematology and Biochemistry

Blood was collected in chilled plain tubes for EPO assay. Serum EPO was measured at St. Vincent’s Pathology, Sydney, Australia using a commercial ELISA kit (Medac Diagnostika, Hamburg, Germany) with antibodies against human EPO, which cross-reacts with but underestimates rat EPO. Minimum detectable value is 5 IU/L. The intra- and interassay coefficient of variations are 3.1% and 5.8% at a level of 56.8 IU/L.

The full blood count was performed by ACT Pathology, The Canberra Hospital, ACT Australia. Tail blood glucose level was measured by Precision Plus Blood Glucose Electrodes (Abbott, MA).

Histology

Thymus glands were fixed in formalin immediately after removal and sections were stained with hematoxylin and eosin.

Statistical Analysis

Data are shown as mean ± SEM. Systolic BP between groups and within each treatment group between different days was analyzed by repeated measures analysis of variance (RM-ANOVA), with the Greenhouse-Geisser adjustment for multivariate sphericity (SPSS v. 11.0, SPSS Inc., Chicago, IL). The Ryan-Holm step-down Bonferroni procedure was applied to the raw P values, to control the family-wise type I error-rate. The adjusted P* = .05 was regarded as significant.

Results

Effects of ACTH or Sham Injection on Systolic BP and Body Weight

Group 1: Saline-treated Sprague-Dawley Rats  Daily saline injections did not alter systolic BP significantly (T0: 118 ± 4, T12: 128 ± 4 mm Hg, P = not significant [ns]; Fig. 1A). Body weight was 277 ± 2 g at T0 and increased to 355 ± 6 g on T6 (P < .0005; Fig. 1C).

Group 2: ACTH-treated Sprague-Dawley Rats  In rats treated with ACTH (0.2 mg/kg/d sc), systolic BP increased (from 109 ± 4 T0 to 142 ± 5 mm Hg T12, P < .0005; Fig. 1A). Body weight did not increase (273 ± 5 g at T0 and 278 ± 9 g at T12, P = ns; Fig. 1C).

Group 3: Saline-treated Wistar Rats  Systolic BP was constant with saline treatment (121 ± 7 T0 and 122 ± 3 mm Hg T12, P = ns; Fig. 1B), whereas body weight increased from 271 ± 12 at T0 to 325 ± 10 g at T6 (P < .0005; Fig. 1D).

Group 4: ACTH-treated Wistar Rats  Systolic BP was 120 ± 2 mm Hg before ACTH administration (T0), and increased to 133 ± 4 mm Hg (T12) (P < 0.05; Fig 1B). Body weight increased from 244 ± 6 g at T0 to 266 ± 10 g at T6 (P < .05; Fig. 1D).
Group 3 (Saline-treated Wistar rats) Compared With Group 4 (ACTH-treated Wistar Rats) There was no significant difference in systolic BP between ACTH-treated (group 4) and saline-treated Wistar rats (group 3) ($P = .17$; Fig. 1B). Body weight was lower in ACTH-treated than saline-treated rats ($P < .0005$; Fig. 1D).

Group 2 (ACTH-treated Sprague-Dawley rats) Compared With Group 4 (ACTH-treated Wistar Rats) In ACTH-treated rats, systolic BP was higher in SD rats than Wistar rats ($P < .05$). There was no difference in body weight between ACTH-treated SD and Wistar rats.

Effects of ACTH or Saline on Serum EPO Concentrations

Serum EPO levels were $5.6 \pm 0.4$ in saline-treated SD (group 1) and $5.9 \pm 0.3$ IU/L in saline-treated Wistar rats (group 3). The ACTH treatment decreased serum EPO concentrations to undetectable levels in 10 of 10 in SD (group 2) and 7 of 10 in Wistar rats (group 4; Table 1). The correlation between changes in systolic BP and serum EPO is depicted in Fig. 2.

Effects of ACTH or Saline on Hemoglobin and Hematocrit

In SD rats, hemoglobin (Hb) in ACTH-treated animals (group 2) was higher than in saline-treated rats (group 1) ($P < .0005$). The ACTH also increased Hb in Wistar rats (group 4) compared with saline (group 3) ($P < .001$; Table 1).

The effect of ACTH on hematocrit (Hct) paralleled that on Hb. The ACTH-treated SD rats had higher Hct than saline-treated SD rats ($P < .0005$). In Wistar rats, Hct was also higher with ACTH (group 4) than saline (group 3) ($P < .001$; Table 1).

Effect of ACTH or Saline on Blood Glucose Levels

Basal blood glucose levels were $6.3 \pm 0.2$ (group 1) and $6.9 \pm 0.3$ mmol/L (group 2) for SD rats and $6.7 \pm 0.2$ (group 3) and $7.2 \pm 0.3$ mmol/L (group 4) for Wistar rats. Neither ACTH nor saline treatment affected blood glucose concentrations in either strain (Table 1).

Table 1. Biological measurements at the end of experiments (T12)

<table>
<thead>
<tr>
<th>Groups</th>
<th>EPO (IU/L)</th>
<th>Hb (g/L)</th>
<th>Hct (L/L)</th>
<th>Glucose (mmol/L)</th>
<th>Thymus (mg/100 g BW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SD saline</td>
<td>5.6 ± 0.4</td>
<td>142 ± 3</td>
<td>0.40 ± 0.01</td>
<td>6.5 ± 0.3</td>
<td>167 ± 8</td>
</tr>
<tr>
<td>SD ACTH</td>
<td>Undetectable</td>
<td>165 ± 3†</td>
<td>0.47 ± 0.01†</td>
<td>5.3 ± 0.5</td>
<td>33 ± 5†</td>
</tr>
<tr>
<td>Wistar saline</td>
<td>5.9 ± 0.3</td>
<td>146 ± 2</td>
<td>0.43 ± 0.01</td>
<td>6.3 ± 0.3</td>
<td>137 ± 23</td>
</tr>
<tr>
<td>Wistar ACTH</td>
<td>Undetectable</td>
<td>155 ± 3*</td>
<td>0.46 ± 0.01*</td>
<td>6.1 ± 0.6</td>
<td>35 ± 5†</td>
</tr>
</tbody>
</table>

* $P < .01$, † $P < .001$ ACTH v saline treatment.
BW = body weight.
Effect of ACTH or Saline on Thymus Gland

Thymus wet weight was expressed as milligrams of 100 grams of body weight. The ACTH treatment significantly decreased thymus weight in both strains ($P < .0001$ ACTH- versus saline-treated rats; Table 1). There was no difference in thymus weight after ACTH in the two different strains. Thymus histology in saline and ACTH-treated SD and Wistar rats showed a significant decrease in thymocyte number.

Discussion

In the present study, we investigated the effects of ACTH in two strains of rats. The ACTH increased systolic BP in SD rats but Wistar rats were relatively resistant to the BP raising effect of ACTH treatment. Hypertensive effects of ACTH have been demonstrated in SD, spontaneously hypertensive, Wistar-Kyoto, Brattleboro, and Long Evans rats. The BP raising effect of ACTH in SD rats in the current study was consistent with previously reported data. However, the effect of ACTH on Wistar out-bred rats did not reach statistical significance, indicating that this strain is relatively resistant to the BP-raising effects of ACTH.

The sequence of human EPO is 80% identical to rat EPO. The ELISA kit used in this study is designed for assay of human serum EPO concentrations, but has been used previously to measure rat EPO as the antibody cross-reacts. Plasma EPO concentrations were reported as 2.1 ± 0.2 mIU/L in Wistar rats, and 14.8 mIU/mL in male SD rats. In the present study, serum EPO concentrations were 5.6 ± 0.4 and 5.9 ± 0.3 mIU/L for saline-treated SD and Wistar rats, respectively. A limitation of this study is that serum EPO levels are at the lower levels of sensitivity of the assay; and linearity was not confirmed for rat sera. The samples were taken 24 h after the last dose of saline/ACTH injection. We have also assayed serum EPO level of samples taken 1 h after the last dose of saline/ACTH treatment. These EPO levels were higher (9.0 ± 1.0 and 6.4 ± 0.4 mIU/mL for saline and ACTH-treated rats, respectively) (data not shown in text), but the effect of ACTH in decreasing serum EPO concentration was consistent.

In previous studies, we have demonstrated that urine volume is increased, whereas body weight is decreased in rats treated with ACTH compared with saline. In the present study, a decrease of serum EPO concentration was associated with increased blood Hb and Hct. This suggests a decrease in plasma volume. The increased Hb and Hct might inhibit EPO production/release through negative feedback. In contrast, glucocorticoid (cortisol) treatment increased body weight, plasma volume, and serum EPO concentrations, but decreased Hct in humans. In the present study, ACTH decreased serum EPO concentrations in both SD and Wistar rats, but only in SD was systolic BP increased compared with saline control. The EPO levels were inversely related to ACTH-induced hypertension in the rat, in contrast to the positive relationship seen in humans. These data indicate that EPO increases are not causal in ACTH hypertension in SD rats.

Thymus weight is a good in vivo marker of GC activity. The ACTH significantly decreased thymus weight in both SD and Wistar rats. In contrast, blood glucose levels were not affected by ACTH treatment. Thus, thymus weight appears to be a better marker than blood glucose for GC activity in the rat. The GC (cortisone-acetate) induced thymolysis has been reported in male Long Evans rats. Nivazol, a synthetic GC, reduced thymus weight in rats with or without adrenalectomy, and dexamethasone reduced thymus weight in castrated and adrenalectomized male rats. Hydrocortisone treatment led to a significant decrease in thymic weight and even greater decrease in thymocyte number in mice and this thymolytic effect of GC was associated with an increase in programmed cell death. The effects of ACTH on thymus weight in the present study are consistent with previous findings. We have previously reported that 0.5 mg/kg ACTH daily increased plasma glucose concentration (7.6 ± 0.7 and 9.3 ± 0.8 mmol/L in sham and ACTH-treated rats, respectively) in SD rats. However, in the current study, ACTH (0.2 mg/kg/d) did not alter tail blood glucose concentration in either strain of rats. Increased plasma glucose concentrations after GC administration have been reported. Thus, thymus weight seems to be a better marker of GC activity in the rat. ACTH (0.2 mg/kg/d sc) increased BP in SD rats but failed to induce a significant increase in BP in the Wistar out-bred rats. Spirolocholone, an aldosterone receptor blockade, did not block ACTH-induced hypertension in SD rats. The ACTH-induced hypertension in SD rats is associated with decreased plasma NOX and renal eNOS and iNOS mRNA expression. The l-arginine supplementation partially reverses and prevents the ACTH-induced hypertension, and the effect of l-arginine was abolished by NOLA, all suggesting a role for the NO system in ACTH-induced hypertension. There is no evidence of NO overproduction in Wistar rats com-
pared with SD rats. The mechanism by which Wistar out-bred rats are resistant to ACTH BP raising effects is unclear. As ACTH decreased thymus weight and body weight in both strains, the resistance is independent of GC activity.

In conclusion, this study has demonstrated that: 1) ACTH decreases serum EPO concentrations in both SD and Wistar rats; and 2) Wistar rats are relatively resistant to the BP raising effects of ACTH treatment. These data suggest that EPO is not causal in ACTH-induced hypertension.

Acknowledgments

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References

27. Fenske M, Fuchs E, Probst B: Corticosteroid, catecholamine and glucose plasma levels in rabbits after repeated exposure to a novel environment or administration of (1-24) ACTH or insulin. Life Sci 1982;31:127–132.