SUMMARY The threshold and dose-response relationships for the blood pressure and metabolic effects of adrenocorticotropic hormone (corticotropin, ACTH) were examined in conscious sheep. Corticotropin was infused at five rates (0.5 μg/kg/day, n = 4; 1 μg/kg/day, n = 4; 2 μg/kg/day, n = 6; 5 μg/kg/day, n = 5; and 10 μg/kg/day, n = 5) for 3 days, and the time of onset of the rise in blood pressure was assessed with a computer-based system. The effects of equimolar infusion of β-endorphin and ACTH at 5 μg/kg/hour also were examined. Corticotropin infusion at 0.5 μg/kg/day had no effect on mean arterial pressure. An ACTH infusion of 1.0 μg/kg/day significantly increased mean arterial pressure (p < 0.001), but the rise was less than that at the three higher doses, all of which produced similar effects. Changes in heart rate were significant at the 10 μg/kg/day level only (p < 0.01). Initial urinary sodium retention was present at the three higher but not the two lower rates of infusion. Corticotropin infusion had no effect on urinary potassium excretion at any rate but produced hypokalemia at rates of 1.0 μg/kg/day and above, which appeared to be dose related. Plasma sodium concentration was increased significantly only at the three higher rates (p < 0.001). Urine volume and water intake were increased at ACTH infusion rates of 2.0 to 10 μg/kg/day. Blood cortisol concentration was increased significantly only at the three higher rates (p < 0.001). Urine volume and water intake were increased at ACTH infusion rates of 2.0 to 10 μg/kg/day. Blood corticosterone levels were maximal at infusion rates of 0.5 μg/kg/day. A well-defined diurnal rhythm of mean arterial pressure was apparent during the 3 control days. At 10 μg/kg/day ACTH produced an increase in mean arterial pressure within 8 hours. Infusion of equipotent amounts of ACTH and β-endorphin did not modify ACTH-induced hypertension. These results indicate that (1) increases in blood pressure can be produced with ACTH infusion at rates that produce plasma ACTH concentrations in the physiological range; (2) the rise in blood pressure occurs within 8 hours of ACTH infusion; (3) ACTH-induced hypertension is unlikely to be due to inhibition of β-endorphin secretion. (Hypertension 7: 287-291, 1985)

KEY WORDS • corticotropin • hypertension • β-endorphin • corticosteroids

The administration of adrenocorticotropic hormone (ACTH or corticotropin) to sheep has been shown to produce an adrenally dependent increase in blood pressure within 24 hours, which is associated with hypokalemia and hypernatremia. After an initial fall in sodium excretion for 24 to 48 hours, sodium excretion returned to preinfusion values. Urine volume and water intake were increased.

In these studies ACTH was injected intramuscularly (Synacthen depot, Ciba-Geigy, Basel, Switzerland) or infused intravenously (Synacthen, Ciba-Geigy) at 20 μg/kg/day, a rate that has been shown to produce maximal adrenocortical steroid output. The aim of the present study was to investigate the threshold and dose-response relationships for the effects of ACTH on blood pressure and other metabolic parameters. Corticotropin was infused at five different rates (range, 0.5–10 μg/kg/day) for 3 days into conscious sheep. The onset of the rise in blood pressure was assessed with a computer-based blood pressure monitoring system. The effect of combined infusion of ACTH (5 μg/kg/day) and β-endorphin (5 μg/kg/day) was also tested to determine whether inhibition of β-endorphin secretion (mediated by ACTH-stimulated cortisol production) is involved in ACTH-induced hy-
HYPERTENSION
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Adult Merino-cross sheep (weight 35–40 kg) were housed in individual metabolism cages and ate 800 g of a lucerne-oaten chaff mixture containing 40 to 80 mmol Na and 130 to 250 mmol K at 1700 hours. Water was offered ad libitum. Food, urine, and water measurements were made at 1100 hours each day. Arterial blood samples were taken for measurement of plasma sodium, potassium, and (at 2 μg/kg/day) ACTH concentrations. Arterial blood for measurement of blood cortisol concentration was taken 24 hours before and after ACTH infusion. Electrolyte levels were determined with a Technicon auto-analyzer (Technicon Corp., Inc., Tarrytown, NY); cortisol levels were determined by a double isotope derivative dilution assay. 3 Corticotropin was measured by radioimmunoassay (International CIS, Saclay, France).

Blood pressure was measured with a Tygon cannula inserted through the carotid artery into the aortic arch. The cannula was kept patent for the duration of the experiment by a continuous heparin infusion (10 IU/ml at 3 ml/hr) through an Intraflow device (Sorenson Research Co., Salt Lake City, UT). The Tygon cannula was attached to a Bentley transducer positioned on the animal’s back. The signal was analyzed with a Gould-Brush amplifier-recorder system. An IBM series 1 computer was used to sample blood pressure for 10 seconds at 10-minute intervals throughout the experiment. The system was calibrated every 24 hours.

Intravenous infusion of ACTH through a jugular vein cannula was begun after 3 days of control blood pressure recording. The sheep received an ACTH infusion at one of the five rates of infusion (0.5 μg/kg/day, n = 4; 1 μg/kg/day, n = 4; 2 μg/kg/day, n = 6; 5 μg/kg/day, n = 5; and 10 μg/kg/day, n = 5). The order of ACTH infusions given to any sheep was random with a minimum of 10 days between infusions. Corticotropin and β-endorphin (Pensinula) were infused together at 5 μg/kg/day for 3 days in five sheep. Peptides were infused in physiological saline at 5 ml/hour into the jugular vein. All infusions commenced at 1200 hours and ran for 72 hours.

Results, expressed as mean ± se, were analyzed by analysis of variance (ANOVA) and Student’s t test for paired observations. The mean arterial pressure (MAP) for each animal was analyzed at 2- or 3-hour intervals for the 3 control days and for the first 24 hours of ACTH infusion at each rate. A composite mean control day was then calculated by averaging the values obtained for each time interval for the first 2 control days. The composite mean control values were then subtracted from the corresponding time intervals for the last control day and the first day of ACTH infusion. At 2-hour intervals this produced 12 control day delta values that could be compared with the 12 obtained on the ACTH day. The delta values for the control day should have a mean of approximately zero. The results were then analyzed by a two-way ANOVA.

Results

Dose-Response Relationships Between Corticotropin and Effect on Blood Pressure and Other Parameters

Infusion of ACTH at 0.5 μg/kg/day had no effect on MAP. Administration of ACTH at 1 μg/kg/day caused a significant increase in MAP on all infusion days (p < 0.001). The rise in MAP, however, was less than that seen with the three higher rates of infusion (2, 5, and 10 μg/kg/day), all of which produced similar effects (Figure 1; Table 1). On cessation of ACTH administration MAP fell within 24 hours to preinfusion levels. Changes in heart rate with ACTH infusion were inconsistent and were only significant at a rate of 10 μg/kg/day (p < 0.01).

Initial urinary sodium retention was not observed at the two lowest rates of infusion (Table 1). In the other groups urinary sodium excretion fell for the first 24 to 48 hours of infusion. After 72 hours of ACTH infusion urinary sodium excretion in all groups was equal to or greater than the preinfusion value. On cessation of ACTH infusion there was a natriuresis in all but the 0.5 μg/kg/day group. By the third postinfusion day sodium excretion had returned to preinfusion levels. No effect on urinary potassium excretion was observed at any rate of ACTH infusion.

Infusion of ACTH resulted in a fall in plasma potassium concentration at rates of 1 μg/kg/day and above. The fall in plasma potassium levels appeared to be dose related (Table 1). On stopping ACTH infusion plasma potassium concentration rose to normal levels within 24 hours. Plasma sodium concentration was increased significantly (p < 0.001) only at the three highest rates of infusion. Changes in plasma osmolality followed closely those observed for plasma sodium concentration.

Urine volume was increased with rates of infusion of 2 to 10 μg/kg/day ACTH (Table 1); however, there was a post-ACTH diuresis at 1 μg/kg/day rates as well as at the three highest rates of infusion. Changes

FIGURE 1. Effect of ACTH, infused at five different rates (0.5–10 μg/kg/day for 3 days), on MAP.
### TABLE 1. Effect of Intravenous Infusion of ACTH at Varying Rates of Infusion (0.5–10.0 μg/kg/d) in Sheep

<table>
<thead>
<tr>
<th>Rate of infusion (μg/kg/d)</th>
<th>Mean arterial pressure (mm Hg)</th>
<th>Plasma (K) (mmol/L)</th>
<th>Urine volume (ml/d)</th>
<th>Urinary Na excretion (mmol/d)</th>
<th>Heart rate (beats/min)</th>
<th>Plasma (Na) (mmol/L)</th>
<th>Water drunk (ml/d)</th>
<th>Urinary K excretion (mmol/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>4</td>
<td>69 ± 1</td>
<td>68 ± 4</td>
<td>4.5 ± 0.1</td>
<td>38 ± 9</td>
<td>40 ± 6</td>
<td>144 ± 1.0</td>
<td>138 ± 10</td>
</tr>
<tr>
<td>1.0</td>
<td>4</td>
<td>68 ± 2</td>
<td>72 ± 0</td>
<td>4.4 ± 0.0</td>
<td>41 ± 12</td>
<td>63 ± 2</td>
<td>145 ± 1.0</td>
<td>138 ± 10</td>
</tr>
<tr>
<td>2.0</td>
<td>6</td>
<td>68 ± 2</td>
<td>82 ± 4*</td>
<td>3.9 ± 0.1*</td>
<td>69 ± 12</td>
<td>145 ± 1.0</td>
<td>145 ± 1.0</td>
<td>138 ± 10</td>
</tr>
<tr>
<td>5.0</td>
<td>5</td>
<td>65 ± 1</td>
<td>78 ± 2*</td>
<td>3.0 ± 0.2*</td>
<td>62 ± 12</td>
<td>145 ± 1.0</td>
<td>145 ± 1.0</td>
<td>138 ± 10</td>
</tr>
<tr>
<td>10.0</td>
<td>5</td>
<td>69 ± 2</td>
<td>82 ± 2*</td>
<td>3.8 ± 0.1*</td>
<td>63 ± 12</td>
<td>145 ± 1.0</td>
<td>145 ± 1.0</td>
<td>138 ± 10</td>
</tr>
</tbody>
</table>

* +p < 0.001, †p < 0.01, ‡p < 0.05.
in water intake paralleled those observed for urine volume.

Blood cortisol concentration was increased in the 1 μg/kg/day group and maximal in the 2 μg/kg/day group (Figure 2).

Infusion of 2 μg/kg/day, achieved a plasma ACTH concentration of 257 ± 32 pg/ml (n = 5). The basal level of ACTH for sheep in this laboratory was 40.6 ± 2.1 pg/ml (n = 187).

**Combined Infusion of Corticotropin and β-Endorphin**

Infusion of β-endorphin (5 μg/kg/day) had no effect on either the blood pressure (MAP: 66 ± 2 mm Hg control, 87 ± 1 mm Hg, Day 3) or metabolic responses to ACTH.

**Onset of Rise in Mean Arterial Pressure with Corticotropin Infusion**

The MAP of an individual sheep receiving ACTH (5 μg/kg/day) is shown on Figure 3. Results are shown for the 3 control days, the 3 days of ACTH infusion, and for the 3 postinfusion days. A diurnal rhythm of MAP was apparent during the control and postinfusion days. Infusion of ACTH resulted in a rapid (within 12 hours) increase in MAP. The rise in MAP was sustained for the duration of infusion and fell rapidly on cessation of ACTH administration.

From data of this type for all sheep at the three highest rates of ACTH infusion it was possible to calculate statistically (see Methods) the time of onset of MAP rise. Figure 4 shows the results for the 10 μg/kg/day group. The MAP was significantly increased above the corrected preinfusion value by 8 hours (p < 0.001). Similar times of onset (8–10 hours) of MAP rise were observed in both the 2 and 5 μg/kg/day groups.

**Discussion**

The results of this study indicate two important parameters of ACTH-dependent hypertension in sheep. (1) Significant increases in blood pressure can be produced with rates of ACTH infusion as low as 1 μg/kg/day. (2) The rise in blood pressure with ACTH occurs within 8 to 10 hours of the start of the infusion.

In all other published studies on ACTH-induced hypertension 20 μg/kg/day of ACTH was used. The increases in MAP of 15 to 20 mm Hg observed in the 2 to 10 μg/kg/day groups after 3 days of ACTH infusion are similar to those found with the 20 μg/kg/day rate of infusion. Thus the threshold for the effect on MAP appears to be between 0.5 and 1 μg/kg/day. From this threshold value the dose-response curve is steep, and for these relatively short-duration infusions the plateau effect was reached between 2 and 5 μg/kg/day.

The threshold for the ACTH effect on plasma potassium concentrations was similar to that for MAP; however, the hypokalemia increased in a dose-dependent manner. The fall in plasma potassium concentrations of 1.4 mmol/liter in the 10 μg/kg/day infusion group is similar to that obtained in 20 μg/kg/day group. In sheep plasma potassium concentration is a sensitive in...
vivo index of "mineralocorticoid" activity and can change without marked alteration in urinary potassium excretion. This finding was also observed in the present study. Changes in plasma sodium concentration and urinary sodium excretion were less consistent than those for plasma potassium concentration but were in close agreement with those expected following ACTH administration.

An increase in urine volume is a feature of ACTH and "glucocorticoid" administration in sheep. In the present study dose-dependent increases in urine volume were observed at rates of ACTH infusion of 2 μg/kg/day and above.

The threshold for the effects of ACTH on blood cortisol levels was similar to that for blood pressure. No changes were seen at 0.5 μg/kg/day; with ACTH infusion rates of 1.0 μg/kg/day both cortisol levels and blood pressure started to increase. Maximal concentration of cortisol was seen at 2 μg/kg/day, a rate of infusion that also produced a maximal rise in blood pressure.

Infusions of 2 μg/kg/day produced plasma ACTH concentrations of approximately 250 pg/ml, a value much higher than the basal ACTH level in the sheep, similar to that obtained following corticotropin-releasing factor infusion, and substantially less than that produced by a "stressful" procedure such as exposure to cold (unpublished observations). In other experiments ACTH infusion at 0.5 μg/kg/day increased blood pressure when infused for 24 to 48 hours into the lateral cerebral ventricle of conscious sheep. This rate of ACTH infusion had no systemic effect on blood pressure or on steroid secretion, which suggests that ACTH may have both a central non-adrenally dependent effect as well as an adrenally dependent effect on blood pressure. These results suggest that the hypothalamic-pituitary-adrenal axis may play a role in the normal day-to-day regulation of blood pressure.

Overall, there was a close relationship between the effects of ACTH on blood pressure and on the other parameters measured. Although the effects on blood pressure may appear to be related to the effects of "mineralocorticoid" or "glucocorticoid" activity, other studies have demonstrated that the effects of ACTH on blood pressure in sheep are due primarily to a "hypertensinogenic" class of steroid action and are not simply related to "glucocorticoid" or "mineralocorticoid" effects (or both).

As infusion with β-endorphin did not modify the blood pressure and metabolic response to ACTH, ACTH-induced hypertension is unlikely to be due to an imbalance between circulating levels of β-endorphin and ACTH. These two peptides are derived from a "hypertensinogenic" class of steroid action and are usually secreted together in response to a variety of stimuli.

The rapid rise in blood pressure following ACTH administration is of particular interest. In previous studies we did not measure blood pressure for the first 24 hours of ACTH infusion; however, at all rates of infusion in the present study MAP was significantly increased within 8 to 10 hours (p < 0.001). The increase in blood pressure in previous studies usually was associated with an increase in cardiac output. Although not measured as part of this study, recent experiments suggest that the rise in cardiac output may precede the rise in MAP (unpublished observations). The mechanism of the rise in MAP with ACTH has been comprehensively studied, but is still not completely understood. The rapidity of the rise in blood pressure is much faster than is usually reported for other types of adrenocortical steroid hypertension; however, blood pressure rarely is measured in the first few hours after the start of the steroid infusion.

All sheep in the present study had a diurnal rhythm for blood pressure. Blood pressure peaked early in the morning before the animals were disturbed by the arrival of staff. All the animals were fed in the evening at 1700 hours, and although this increased heart rate, it had little effect on their MAP. The technique used to derive the onset of the ACTH blood pressure rise took into account this diurnal pattern for MAP and enabled the increase to be precisely determined.

**Conclusion**

The present study indicates that (1) blood pressure increases within 8 to 10 hours after ACTH administration is commenced, (2) the rate of ACTH infusion can be as low as 1 μg/kg/day (one-twentieth the rate previously used to produce this type of hypertension in sheep and within the physiologic range for plasma ACTH concentration), and (3) ACTH-induced hypertension is unlikely to be due to an imbalance between circulating β-endorphin and ACTH.

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Onset and dose relationships of ACTH effects on blood pressure in sheep.

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