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Chronic ACTH Administration and the Development of Hypertension in Rats (40799)

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Experimental hypertension can be induced by the administration of pharmacologic doses of adrenal mineralocorticoids (1, 2); there is considerable species variation in response, however, and the animal model studied most extensively is the saline-loaded, uninephrectomized rat given deoxycorticosterone (DOC) acetate. Based on previous observations in sheep by Scoggins *et al.* (3, 4) that ACTH administration induced hypertension, it was reasoned that chronic administration of ACTH to rats might stimulate endogenous adrenal steroidogenesis and induce hypertension in this species also. In the present study, the effects of continuous ACTH infusion on blood pressure and electrolyte and water metabolism were evaluated in the rat.

Methods. All rats (Sprague–Dawley males) were housed individually in stainless steel metabolic cages and kept in temperature-controlled rooms which were maintained on a 12-hr light–dark cycle beginning at 5 AM. Distilled water and powdered Purina rat chow (sodium content: 1.37–1.50 meq/10 g; potassium content: 2.45–2.68 meq/10 g) were available *ad libitum*. Daily sodium and potassium balances were estimated from measurements of ingested and excreted (urinary) sodium and potassium. Urine volume and body weight also were measured daily. Systolic blood pressures were measured daily by the tail artery occlusion method; the recorded value was the average of three or four successive determinations.

Hypertensive studies. Both a control group ($n = 7$) and an experimental group ($n = 7$) were studied. Systolic blood pressures were measured for 3 consecutive days in each rat. Immediately after determining blood pressure on the third day, seven of the rats were lightly anesthetized with ether, a small skin incision was made on the dorsal region of the neck, and a minipump (Alza, Model 1701) containing a saline solution of ACTH (Acthar, Armour Pharmaceutical Co.) was inserted subcutaneously. The concentration of ACTH in the infusate was adjusted so that a pharmacologic dose of 1.0 unit per day of ACTH was delivered in a volume of 24 μl (1.0 μl/hr); ACTH was infused subcutaneously for a total of 7 consecutive days. Systolic blood pressures were measured daily in both the control (no drug; $n = 7$) and the ACTH-treated rats ($n = 7$) for the 7 days of ACTH infusion and for an additional 7 days of recovery.

Hormonal studies. Both a control group ($n = 8$) and an experimental group ($n = 8$) of male rats were used in this part of the study also. After 2 days of systolic blood pressure measurements in each rat, eight rats were prepared with minipumps containing ACTH as described above. Blood pressures were followed daily for 5 days of ACTH infusion (1.0 unit per day). The rats were decapitated on the morning of the sixth day (8 AM) after 5 days of ACTH infusion; plasma renin activity (PRA) was determined from blood collected during the first 5 sec after decapitation while plasma concentrations of aldosterone (PAC) and corticosterone (PCC) were determined from a second sample collected during the next 10 to 15 sec.

Analytical methods. Blood samples for determination of PRA, PAC, and PCC were collected in tubes containing disodium ethylenediaminetetraacetate (EDTA). The samples were centrifuged and the plasma was stored frozen until the time of assay for PRA, PAC, and PCC.

Plasma aldosterone concentration was determined by the method of Bühler *et al.* (5). Plasma samples of 1.0 ml. each were extracted with methylene chloride and this

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extract was placed on small celite columns with 40% ethylene glycol–water as the stationary phase. After elution of the other steroids, aldosterone was eluted with ethylacetate:isooctane (60:40). The eluted aldosterone was quantified by radioimmunoassay (Bühler *et al.*) and PAC is expressed as nanograms percent (ng%). Plasma corticosterone concentration was determined with a commercially available radioimmunoassay kit (Endocrine Sciences, Palo Alto, Calif.). Briefly, 0.3 ml plasma samples were extracted first into hexane:benzene (80:20) and then underwent a second extraction with hexane:benzene (20:80). The extracted corticosterone was quantified by radioimmunoassay procedures and PCC is expressed as micrograms percent (μg%). Plasma was prepared for the determination of PRA in the following manner. Plasma samples were dialyzed against phosphate buffer (pH 5.3) for 18 hr (three changes). After sodium chloride and diisopropylfluorophosphate (DFP) were added, the samples were incubated at 37°C for 60 min to generate angiotensin I. Angiotensin I content was quantified by radioimmunoassay (6) and PRA is expressed as nanograms (ng) of angiotensin I per milliliter per hour.

Student's t test was used for statistical analysis of the data. Values are presented as means ± SE.

Results. Figure 1 shows the daily systolic blood pressures in both the control (no drug) and the experimental (ACTH-infused) rats. There were no differences ($P > 0.05$) in systolic blood pressure between the control group and the experimental group prior to ACTH infusion or during the last 3 recovery days of the study. The preinfusion systolic blood pressure in the experimental group was 105 ± 3 mm Hg prior to the start of ACTH infusion. Blood pressure did not change significantly during the first 2 days of ACTH infusion but then increased to 125 ± 5 mm Hg ($P < 0.01$) on the third experimental day. Blood pressure increased further to 139 ± 5 mm Hg ($P < 0.01$) on the fourth day of ACTH infusion and plateaued near this higher level for the remainder of the ACTH infusion period (Fig. 1). Following cessation of ACTH infusion, systolic blood pressures remained elevated for 4 days ($P < 0.05$) before returning to the preinfusion levels. Blood pressure in the experimental group was elevated above that in the control group ($P < 0.05$) by Day 2 of ACTH infusion and blood pressure in the ACTH infused rats remained elevated ($P < 0.05$) above the control group's blood pressure until the fifth day after infusion of ACTH was stopped (Fig. 1).

Figure 2 shows the positive cumulative sodium and potassium balances in both the control and the experimental groups during the 7-day period of ACTH infusion into the experimental group. Sodium and potassium balances were not significantly different ($P > 0.05$) between the experimental and the control groups prior to ACTH infusion. During ACTH infusion, both cumulative sodium (1.36 meq) and potassium (4.29

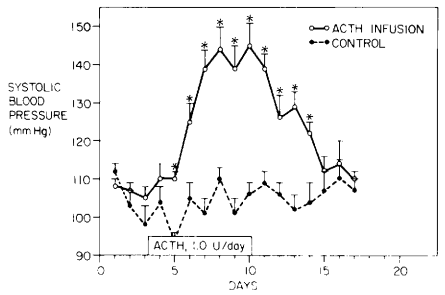

FIG. 1. Daily systolic blood pressures (mm Hg) in control rats ($n = 7$) and in rats receiving continuous infusion of ACTH (1.0 unit/day, sc) ($n = 7$) for 7 days. Asterisks denote significant differences ($P < 0.05$) between the two groups (Hypertensive study).

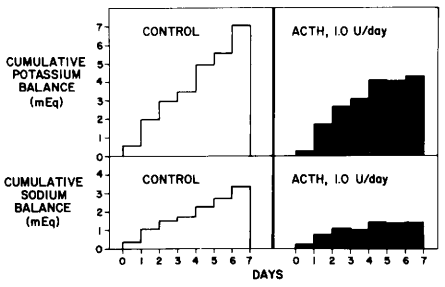

FIG. 2. Cumulative potassium and sodium balances (meq) in control rats ($n = 7$) and in rats ($n = 7$) receiving continuous infusion of ACTH (1.0 unit/day, sc) for 7 days. (Hypertensive study).

meq) balances were positive in the experimental rats for the 7 days of infusion. However, the cumulative sodium (3.52 meq) and potassium (7.26 meq) balances in the control rats over the same period of 7 days exceeded ($P < 0.05$) those of the ACTH-infused rats. Also, weight gain was strikingly greater in the control group than in the experimental group during ACTH infusion (Fig. 3). Daily urine volume increased on the fourth day of ACTH infusion and it remained elevated until Day 4 after ACTH infusion stopped (Fig. 4). These metabolic data demonstrate the striking antianabolic effects of chronic ACTH administration in these young growing rats.

Changes in plasma renin activity (PRA) and plasma aldosterone concentration (PAC) in experimental rats were striking following 5 days of continuous ACTH infusion. After 5 days of ACTH infusion, the PAC of 37.9 ± 8.0 ng% was elevated over control levels of 8.7 ± 1.4 ng% ($P < 0.01$) and the PRA of 0.54 ± 0.23 ng/ml hr^{-1} was decreased from the control levels of 1.67 ± 0.22 ($P < 0.01$). Plasma corticosterone concentration was 20.8 ± 9.6 μg% in the ACTH-infused rats and 1.0 ± 0.3 μg% ($P < 0.1$) in the control rats. Systolic blood pressures in the ACTH infused rats increased from a preinfusion level of 97 ± 3 to 120 ± 6 mm Hg ($P < 0.01$) on the fifth day of infusion.

Discussion. The important new observation in the present study is that continuous administration of ACTH to normal rats induced the development of hypertension. Hormonal analysis showed that PRA was suppressed and that plasma concentrations of aldosterone and corticosterone were elevated on Day 5 of ACTH infusion and in association with the hypertension. Hypertension occurred without prior sensitizing maneuvers such as saline loading and unilateral nephrectomy, and it was entirely reversible after ACTH infusion was stopped. The present results are similar to those reported by Haack *et al.* (7). These investigators reported that daily bolus injections of ACTH (5 mg/kg of body wt, sc) produced hypertension and increased urinary excretion of corticosterone, 18-OH-corticosterone, aldosterone tetrahydroaldosterone, and 18-OH-deoxycorticosterone in the rat (7). Thus, the present results and the report by Haack *et al.* (7) demonstrate that chronic administration of ACTH leads to the development of hypertension in the rat.

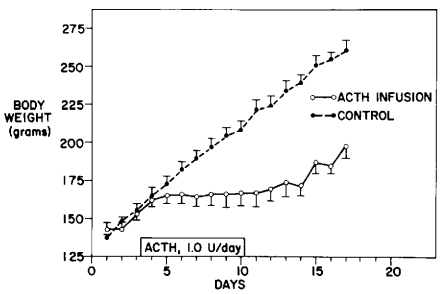

FIG. 3. Daily body weights (g) of control rats ($n = 7$) and rats receiving continuous infusion of ACTH (1.0 unit/day, sc) for 7 days. (Hypertensive study).

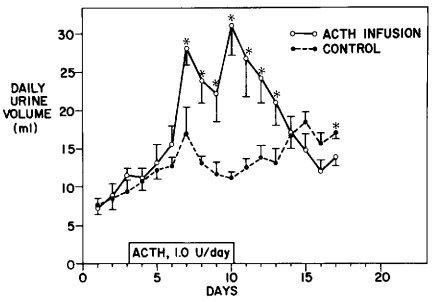

FIG. 4. Daily urine volume (ml) in control rats ($n = 7$) and in rats ($n = 7$) receiving continuous infusion of ACTH (1.0 unit/day, sc) for 7 days. Asterisks denote significant differences ($P < 0.05$) between the two groups. (Hypertensive study).

The normalization of blood pressure and water turnover as well as the reinstitution of growth and gain in body weight during the recovery period with the minipumps still implanted make it unlikely that the observed responses during ACTH infusion were influenced appreciably by the "wearing" of the minimpumps. Moreover, since pharmacologic doses of ACTH were being infused continuously for an entire week, it seems unlikely that any transient nonspecific stimulation of endogenous ACTH release during surgery would change the results substantially.

The results of the present study do not

permit a precise definition of the pathogenic mechanisms which are involved in the hypertensive response to chronic ACTH administration in the rat. However, plasma levels of both aldosterone and corticosterone were elevated in this study and these steroids, either alone or together, might be involved in the development of the hypertension; pharmacologic doses of both DOC or corticosterone have been reported to induce hypertension in the rat (1, 2). Also, other adrenal steroids are increased during chronic administration of ACTH to rats (7) and these may play a role in the hypertensive process.

The mechanisms by which the adrenal steroids exert their pressor actions are still poorly understood; however, it is possible that one component of the hypertension is related in part to expansion of the extracellular fluid and blood volumes (1, 2, 7). Increased blood volume could occur secondary to sodium retention or via internal redistribution of body fluids (2, 7). In the present study, both the experimental and the control animals were in positive sodium balance as would be expected for young growing rats; however, the control animals were in greater positive sodium balance than the experimental group and this observation suggests that blood volume expansion per se was not critically involved in the development of the hypertension. The affects of ACTH on electrolyte balance and body weight probably reflect the catabolic actions of the glucocorticoids in the growing animal.

Another mechanism by which the adrenal steroids might exert their hypertensive actions is to increase the reactivity of vascular smooth muscle to other vasoconstrictor substances (8, 9). DOC hypertensive rats exhibit increased vascular reactivity and blood pressure responses to norepinephrine, tyramine, angiotensin, and sympathetic nerve stimulation (8). *In vitro* studies of vascular smooth muscle strips from the DOC hypertensive rat have documented a decrease in the threshold to epinephrine stimulation (9). Interestingly, McDougall *et al.* (10) reported an increased pressor response to norepinephrine in conscious ACTH hypertensive sheep after 5 days of ACTH administration, but the change in pressor sensitivity was small.

The marked suppression of plasma renin activity (PRA) during chronic ACTH infusion in the present study may be related to the high blood pressure per se (11) or to the elevation in aldosterone and the positive sodium balance (12). Again, it should be noted that in the present study the control rats were in greater positive sodium balance than the ACTH-infused rats and this suggests that suppression of PRA is not related entirely to the positive sodium balance. A similar depression in PRA has been observed in response to deoxycorticosterone administration in rats (13, 14). Also, chronic administration of ACTH suppressed PRA both in the dog (15, 16) and in man (17) and this effect appeared to be secondary to expansion of the extracellular fluid volume (15). Changes in PRA in ACTH hypertensive sheep were not reported (3, 4).

In the present study, plasma aldosterone concentration (PAC) was elevated approximately four-fold on the fifth day of continuous ACTH infusion. Other preliminary reports in dogs (16) and rats (18) indicate that continuous infusions of ACTH produced increases in PAC which were sustained for 3 days before returning toward control levels on the fourth day of infusion. Previous studies in sheep (3, 4) and man (17, 19) have failed to document a sustained increase in plasma aldosterone during chronic ACTH administration. Also, daily subcutaneous injections of ACTH increased urinary excretion of aldosterone in the rat for 2 days (7); urinary excretion of aldosterone returned to normal on the 10th day of ACTH injection but urinary excretion of tetrahydroaldosterone remained elevated some three-fold above controls (7). The return of aldosterone to normal levels during chronic ACTH administration might be related to the renin-suppressing actions of ACTH. Ganong (15) has postulated that a decrease in PRA decreases the sensitivity of the zona glomerulosa to ACTH and this could lead to a fall in aldosterone despite continuation of ACTH administration.

Continuous infusion of ACTH increased

water turnover and decreased cumulative sodium and potassium balances in the present study. Qualitatively, these results are quite similar to those observed during chronic ACTH administration to the dog (20). In the dog (20), chronic ACTH or cortisone administration also increased water turnover and decreased cumulative sodium and potassium balances; indeed, sodium and potassium balances actually became negative. Chronic ACTH administration to sheep (3) and to rats (7) also increased water turnover; sodium and potassium excretion were unchanged during ACTH administration in the sheep (3). Thus, the metabolic responses to chronic ACTH administration in the rat are similar to those observed in other species.

Summary. The results of the present study demonstrate that the chronic infusion of ACTH stimulates endogenous steroidogenesis, suppresses PRA, and leads to the development of hypertension in normal rats; the hypertension is reversible upon cessation of ACTH infusion. This experimental model should prove useful for studying adrenal mechanisms involved in the pathogenesis of hypertension.

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