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ACTH Induced Sodium Appetite in the Rat[1]

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WEISINGER, R. S., D. A. DENTON, M. J. MCKINLEY AND J. F. NELSON. *ACTH induced sodium appetite in the rat.* PHARMAC. BIOCHEM. BEHAV. 8(4) 339–342, 1978. – Subcutaneous injections of long-acting synthetic ACTH (5 U/day) caused a large increase in the intakes of both 0.5 M NaCl and water in rats. By the fifth day of treatment the rats were turning over an amount of sodium approximating their own total body sodium. The mineral appetite was specific for NaCl. Intakes of KCl, $MgCl_2$ and $CaCl_2$ were unchanged. ACTH was ineffective in adrenalectomized rats suggesting that the appetite was dependent on adrenal hormones.

Sodium appetite ACTH Adrenalectomy Specificity

THE ADENOHYPOPHYSEAL hormone ACTH has recently been shown to cause a large specific increase in the intake of NaCl when administered to the wild rabbit [4]. This increase in salt appetite was dependent, in part, on the adrenal gland, but also involved extra adrenal mechanisms. That is, adrenalectomy did not entirely eliminate the effect of ACTH treatment on intake of NaCl solution. In contrast, earlier studies in the rat found that injections of large doses of ACTH (6 mg/day) did not increase NaCl intake [6], although ACTH potentiated the increase of salt intake caused by deoxycorticosterone acetate [5]. Since this early work, a pure synthetic preparation of ACTH has become available. Using the long lasting 1–24 fragment of ACTH, we have re-examined its effect on sodium appetite of rats.

METHOD

Experiment 1: ACTH Induced Na Appetite

Eleven naive male Wistar rats were used. The animals weighed 250–350 g at the start of the experiment and were housed in individual stainless steel cages. The rats had free access to rat chow (Barastock, Na content = 117 mmol/kg), water, and 500 mM NaCl solution. The liquids were available from plastic containers fitted with glass spouts and were hung on the front of the animal's cage. The position of the containers was changed on a random basis daily.

Animals were randomly divided into 2 groups – ACTH and control. After an initial 5 day period of measurement (baseline), animals in the ACTH group (n = 6) received one subcutaneous injection of 5 U ACTH (0.05 ml Synacthen depot, CIBA) daily for 5 consecutive days while the control animals (n = 5) received subcutaneous injections of physiological saline (0.05 ml). A 2 day period similar to that of the baseline period followed this treatment period. Intakes of NaCl solution and water were measured daily. The animals were weighed every 2–3 days. In addition, in ACTH treated rats, food intake and Na excretion were also measured daily. Measurements and injections were made between 1400 and 1500 hr each day. The cages of animals in the ACTH group were fitted with fibreglass collecting pans which allowed for the collection of urine and faeces. Daily Na excretion was measured by homogenizing the urine and faeces together with a known volume of water. Na and K concentrations in the supernatant fluid were determined by Technicon Autoanalyzer.

Experiment 2: Specificity of Appetite

Nine naive male Wistar rats 250–350 g were used. The animals were housed in individual cages and were allowed free access to food, water, and 500 mM solutions of NaCl and KCl and 250 mM solutions of $MgCl_2$ and $CaCl_2$. The liquids were randomly placed on the cage each day.

The animals were randomly divided into ACTH (n = 5) and control (n = 4) groups and the procedure was essentially the same as for Experiment 1, except that food intake and external Na balances were not measured.

Experiment 3: Effect of ACTH on Na Appetite of Adrenalectomized Rats

Seven naive male Wistar rats 200–275 g were used. The animals were adrenalectomized via the dorsal approach while under ether anaesthesia and were maintained in separate metabolism cages with free access to water, 500 mM NaCl and rat chow (Clark-King G2+, Na content = 60 mmol/kg). Two weeks after surgery, after 2–3 days of control observation, four of the rats received

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one subcutaneous injection of 5 U ACTH daily for 5 days which was followed by 2 injection free days. The animals then received 1 injection of physiological saline daily for 5 days. The other 3 rats received the injections in the reverse order, i.e., physiological saline then ACTH. Following the second series of injections, control observations were made for 2–4 days.

The injections and the measurement of food, water and NaCl intake and body weight were made between 1400–1600 hr daily. In addition, the effect of ACTH treatment on NaCl intake was examined in these adrenalectomized rats while they were concurrently being treated with either cortisol (1.25 mg/day hydrocortisone, CIBA, n = 4) or DOCA and corticosterone (0.5 mg of each day, n = 7). Treatment lasted 4–5 days.

Data Analysis

An analysis of variance and the appropriate t tests were used to compare baseline values with those values obtained during and after ACTH treatment. Data are presented in the text as a mean ± SEM.

RESULTS

Experiment 1: ACTH Induced Na Appetite

Figure 1 shows the results of this experiment. The injections of ACTH caused a marked increase in NaCl solution intake compared to preinjection levels. Intake was significantly elevated from the second day of treatment (i.e., during the 24 hr period subsequent to the second injection) to the first day of the post period (all p's<0.001). On the fifth day, intake ranged from 16.7 to 65.2 ml with a mean intake of 37.6 ± 6.7 ml = 18.8 mmol of NaCl. The level of NaCl intake of these animals returned to baseline by the second day of the post period. Na balance (intake–output) was unchanged from baseline except that on the fifth day of ACTH treatment a small but significant positive Na balance was measured ($p<0.05$). Intake of NaCl by rats in the control group was not changed during the injection period, however there was a small but significant increase during both days of the postinjection period compared to the baseline period ($p<0.001$ and $p<0.05$, respectively).

In ACTH treated animals, water intake was increased on the third day of treatment ($p<0.001$) and thereafter, compared with baseline intake. On the fifth day of ACTH administration, water intake was 75 ± 9 ml (range = 56–116 ml). The ingestion of water decreased during the postinjection period but remained above baseline levels ($p<0.001$ and $p<0.05$, respectively for the 2 days of the postinjection period). Intake of water by animals in the control group did not change over the period of measurement and was 38 ± 7.7 ml/day (range = 27–70 ml, n = 70 observations).

ACTH treated rats lost body weight over the treatment period. Compared to baseline, the loss was significant on the third day of treatment and thereafter (all p's<0.001). Food intake was significantly increased on the first ($p<0.01$) and significantly decreased on the fifth day of treatment ($p<0.05$) relative to pretreatment intake. The rats of the control group gained body weight over the period of measurement ($p<0.05$).

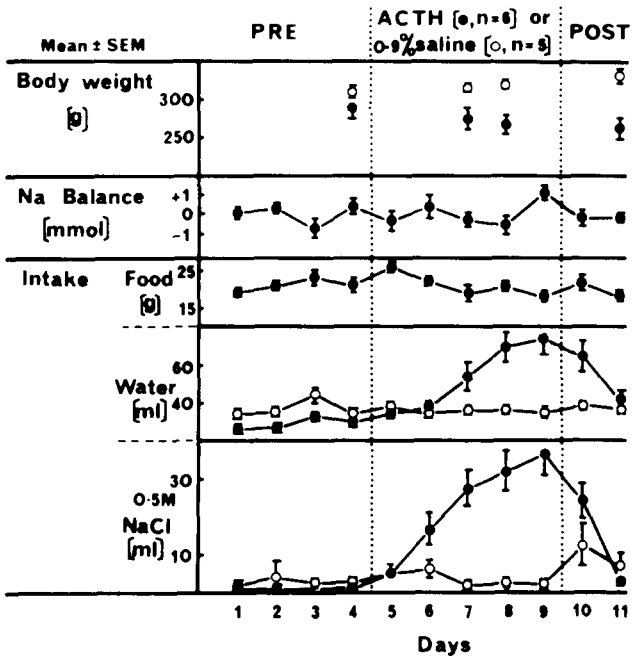

FIG. 1. Mean (SEM) body weight (g), intake of 0.5 M NaCl (ml), water (ml) and food (g) for the ACTH (●) and control (○) groups. Na balance for the ACTH group is also shown.

Experiment 2: Specificity of Appetite

Figure 2 shows the results of this experiment. ACTH treatment caused a marked increase in NaCl intake which was significantly elevated over baseline levels on the second day ($p<0.001$). Maximum intake occurred on the first day of the posttreatment period and was still slightly but significantly increased on the second day of the postinjection period relative to baseline intake. Intake of NaCl solution was unchanged in the control group during the period of measurement.

ACTH treatment also increased the ingestion of water. The increase, relative to baseline levels, was significant on the third day ($p<0.05$) and remained elevated thereafter. Intakes of KCl, $MgCl_2$, or $CaCl_2$ were not significantly changed during the period of measurement in either the ACTH or the control group and intake of water was not significantly changed in the control group.

During the course of the experiment, body weight increased in the rats of the control group and decreased in the rats of the ACTH group compared to baseline levels. The changes were significant by the third day of treatment ($p<0.01$, $p<0.05$, respectively, for the ACTH or control group).

Experiment 3: Adrenalectomy

ACTH treatment did not alter the intake of NaCl solution, nor affect any of the other parameters measured in the adrenalectomized rats (see Fig. 3). For example, during the baseline period, intake of NaCl solution was 9.3 ± 0.9 mmol (n = 14, i.e., n = 2 observations on each of the 7 rats) while during ACTH treatment, intake of NaCl solution was 10.2 ± 0.4 mmol/day (n = 35 ob-

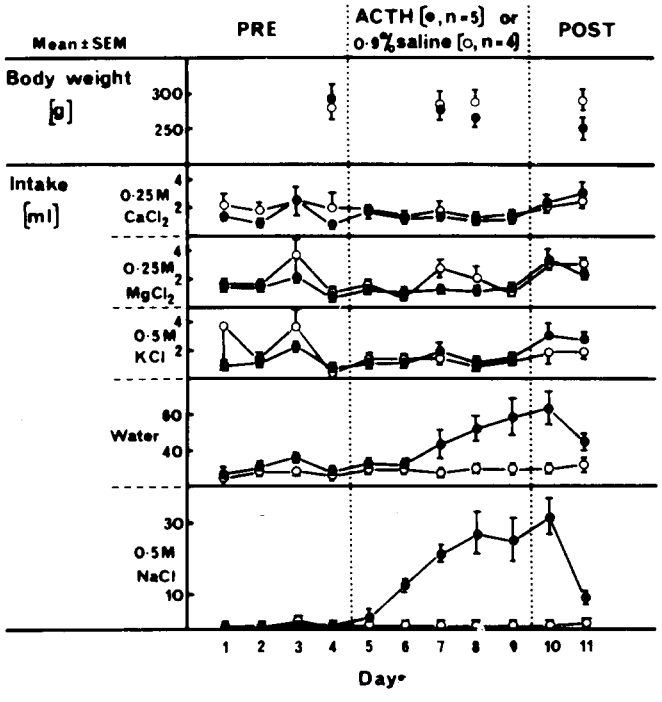

FIG. 2. Mean (SEM) body weight (g), intake of water (ml) and 500 mM solutions of NaCl and KCl (ml) and 250 mM solutions of $MgCl_2$ (ml), and $CaCl_2$ (ml) for the ACTH (●) and control (○) groups.

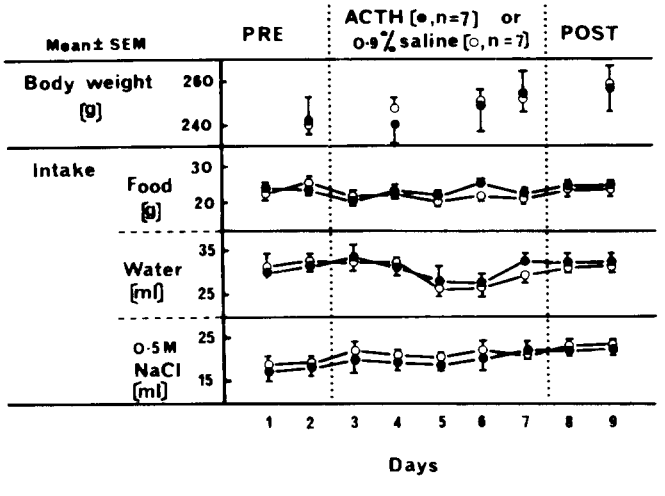

FIG. 3. Mean (SEM) body weight (g), intake of 0.5 NaCl (ml), water (ml), and food (g) for the ACTH (●) and control (○) groups in adrenalectomized rats.

servations). Similarly, the control injections were without significant effect.

ACTH treatment did not alter the intake of NaCl solution in the cortisol treated adrenalectomized rats. For example, during the baseline period, intake of NaCl solution was 12.1 ± 0.8 mmol/day (n = 4), while during ACTH treatment, intake of NaCl solution was 11.4 ± 0.4 mmol/day (n = 16 observations).

The adrenalectomized rats treated with both DOCA and corticosterone ingested 4.3 ± 0.4 mmol of NaCl solution daily during the baseline period. ACTH administration did not increase the intake of NaCl solution (4.6 ± 0.3 mmol/day).

DISCUSSION

The results of these experiments establish several facts concerning the effect of ACTH on Na appetite in rats. Experiment 1 showed that ACTH caused a large increase in Na intake. Indeed, by the fifth day of ACTH treatment the animals were turning over in one day an amount of Na approximating their own total body Na, i.e., 18 mmol [9]. Compared to results of similar studies, i.e., rats with continuous access to water, food and approximately 500 mM NaCl, the level of NaCl intake observed in the present experiments is greater than that reported following adrenalectomy ([8,18], (Experiment 3), Formalin treatment (2.5 ml of 1.5% Formalin [10], or DOCA administration (5 mg/rat) [23].

The fact that ACTH treatment caused a large appetite for NaCl solution in rats is consistent with the results reported by Blaine *et al.* [4] for the rabbit but inconsistent with those in the rat reported by Braun-Menendez and Brandt [6]. The failure of these latter investigators to observe an enhanced Na appetite after ACTH treatment was most likely due to their use of a short acting preparation of ACTH since the dose of ACTH they used (6–12 mg) was greater than that used in the present experiments, based on an estimated ACTH potency of 1.14 IU/mg [2].

Experiment 2 established that the mineral appetite induced by ACTH administration was specific for NaCl solution. Intakes of KCl, $CaCl_2$, and $MgCl_2$ solutions were unaltered. ACTH also increased water intake, which is similar to its effects in both sheep [20], and rabbits [4]. In the present experiments, it is not clear whether the increased water intake was caused by hypertonicity of body fluids secondary to the ingestion of large amounts of concentrated NaCl or by a direct effect of ACTH on the thirst mechanism.

ACTH treatment failed to induce Na appetite in adrenalectomized rats without steroid replacement therapy (Experiment 3). This suggests that, unlike rabbits [4], ACTH induced Na intake in the rat is entirely determined by adrenal hormone secretion. However, it could be argued that the effects of ACTH on Na appetite were attenuated or muted by the high levels of endogenous ACTH already present in adrenalectomized rats [16]. In adrenalectomized rats treated with cortisol, ACTH treatment failed to induce Na appetite. As glucocorticoid administration suppresses endogenous ACTH secretion [12, 19, 24], such an explanation seems unlikely. It is possible that the failure of ACTH to induce Na appetite in adrenalectomized rats was due to already high levels of NaCl intake (e.g. 9.3–12.1 mmol/day, Experiment 3) which may have masked the effects of ACTH treatment. ACTH treatment did not increase Na intake when baseline Na intake was reduced by DOCA and corticosterone administration, suggestive that the high Na intake resulting from adrenalectomy did not mask an effect of ACTH on Na appetite. However, we were unable to reduce the baseline NaCl intake to that of intact rats, leaving this question not entirely resolved.

The contribution of specific mineralocorticoid and/or

glucocorticoid hormones to the ACTH-induced sodium appetite of rats remains to be determined. As previously noted, ACTH-induced sodium appetite is reduced, but not eliminated by adrenalectomy in rabbits. That is, only part of the ACTH-induced appetite is dependent on adrenal hormones. In regard to the contribution of specific hormones to the appetite, it has been shown that elevated levels of mineralocorticoid hormones are not essential. For example, treatment of the adrenalectomized rabbits with high, physiological levels of cortisol and corticosterone, together with ACTH and a maintenance level of DOCA increased sodium appetite to the level observed in intact ACTH-treated rabbits [4].

A striking observation of the present experiments was that ACTH-treated intact rats lost body weight. Since loss of body Na (i.e., negative Na balance) was not observed, the loss of body weight was most likely due to increased protein catabolism or reduced GH release observed in ACTH or glucorcorticoid treated rats [7, 17, 22]. Although the animals consistently lost body weight, they did not consistently increase food intake (Experiments 1, 2). This is a paradoxical finding in that rats have been shown to regulate body weight and readily adjust intake when given diets of different caloric value [1, 14, 15]. One possible explanation for the failure of ACTH treated rats to increase their food intake consistently is that ACTH treatment elevates both blood glucose levels [3] and free fatty acids [21] – both of which have been implicated in food appetite or body weight regulating systems, e.g. glucostats, lipostats [13].

In conclusion, the results of these experiments clearly establish that ACTH induces a large and specific appetite for NaCl in the rat. The effect of ACTH on appetite appears to be dependent on adrenal hormones. Daily Na balances showed that ACTH treated rats were not in negative Na balance (Experiment 1) suggestive that the ACTH induced Na appetite was not secondary to an induced loss of Na. However, in the short term, such effects cannot be completely excluded. Further research will be necessary to clarify this point and to determine whether ACTH has a physiological role in the NaCl appetite of rats.

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