Natriuretic Effect of Alpha Melanocyte Stimulating Hormone (α-MSH) in Hypophysectomized or Adrenalectomized Rats (36256)

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We have recently reported a marked natriuretic effect of α-MSH (1, 2) in the rat. Once the effect had been clearly established, experiments were performed to determine if the natriuresis was produced by a direct effect of MSH on the kidney or if it was mediated by a hormone produced by other gland(s). It is well known that the pituitary gland [via its secretion of oxytocin or vasopressin (1–5)] or the adrenal glands [via the secretion of aldosterone (6)] can influence sodium excretion in the rat. In the present series of experiments, we attempted to determine if the pituitary or the adrenals mediated the natriuretic effect of MSH.

Materials and Methods. Hypophysectomized animals. Hypophysectomized rats obtained from Hormone Assay Laboratories, Chicago, were maintained with daily sc injections of 1.5 μg/100 g of body weight of thyroxine and 4 mU of ACTH and were used for experiment 7–10 days postoperatively. They were given a choice of tap water or 5% dextrose to drink, except on the day of the experiment, when only tap water was available. The rest of the experimental procedure remained unchanged from the one reported earlier (2). In brief, the procedure was to give a volume of tap water by stomach tube equivalent to 5% of the body weight. The rats were then placed in separate metabolic cages without access to food or water for collection of urine samples. The urine sample collected during the first hour (S1) was discarded and a second gavage of the same volume was administered 1 hr after the first. Urine samples were collected every 20 min for 120 min. Immediately after collection of the first 20 min sample (S2), one group of animals was injected ip with 10 μg of α-MSH[2] dissolved in 0.5 ml of acidified 0.9% sodium chloride solution and the other group was injected with an equal volume of the acid saline diluent and served as the control group. Completeness of hypophysectomy was evaluated by examination of the hypophyseal capsule with the aid of a dissecting microscope; and one animal, which had a small remnant, was discarded.

Adrenalectomized animals. Male rats (Holtzman), weighing 200–300 g, were adrenalectomized and maintained with daily sc injections of 1 mg of hydrocortisone and 0.1 mg of deoxycorticosterone acetate. They were given a choice of tap water or saline (0.9% NaCl) to drink, except on the day of the experiment, when only tap water was available. The experimental procedure was exactly as described for the hypophysectomized rats. The animals were divided into two groups, one receiving an ip injection of 10 μg of α-MSH and the other receiving ip saline and serving as a control group.

The concentration of Na^+ and K^+ in urine was measured by use of an IL 143 flame photometer using lithium as an internal standard.

The significance of differences between means has been examined by use of Student's t test.

Results. Hypophysectomized animals. Sodium excretion. The sodium excretion is

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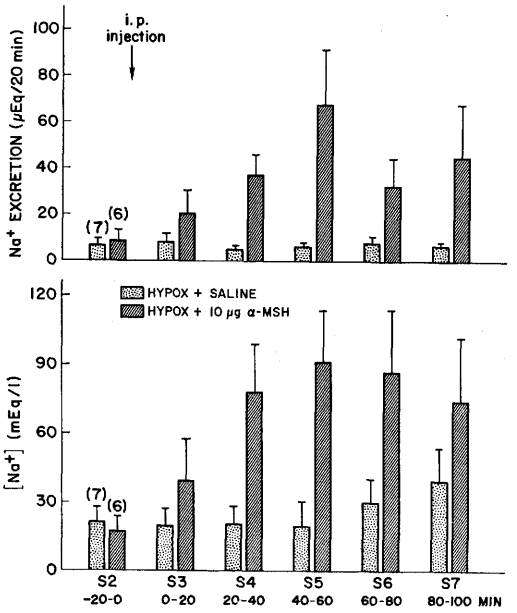

FIG. 1 (top). Effect of α-MSH or saline on the urinary sodium excretion in hypophysectomized rats (μEq/20 min). In Figs. 1–6, each sample represents a collection period of 20 min. The injection of 10 μg of α-MSH and saline was given ip after the collection of the second sample (S2) (—20–0 min). Time zero represents the time of injection; (column) the mean for the group; (vertical bar) one standard error of the mean; and (number on top of each column) the number of animals per group. (bottom) Effect of α-MSH or saline on the urinary [Na+] (mEq/liter) in hypophysectomized rats.

shown in the top half of Fig. 1. It remained approximately constant during the experimental period in the saline-injected controls. There was a highly significant increase in the samples collected from the α-MSH-injected group [$p < .01$ for the 20–40 min sample (S4)] compared with the saline-injected group. The effect reached its maximum in the 40–60 min sample (S5) and represented a 12-fold increase in sodium excretion. When comparison was made with the preinjection sample (S2) the increase in sodium excretion was also significant ($p < .02$ for S4 collected 20–40 min after injection; and $p < .05$ for S5).

Sodium concentration [Na^+]. Figure 1 (bottom half) shows that the [Na^+] in the urine samples collected from the α-MSH-injected group increased significantly in the 20–40 and 40–60 min samples (S4 and S5) collected after injection compared with that of the saline-injected group ($p < .02$). The increase was also significant compared with the preinjection sample (S2) ($p < .02$ for S4 and S5; and $p < .05$ for S6). The [Na^+] in the urine of the saline-injected rats remained unchanged throughout the 100 min of the experiment.

Potassium excretion. Control potassium excretion remained approximately constant (Fig. 2, top). There was a highly significant increase in potassium excretion in the samples collected from the α-MSH-injected group ($p < .02$ for S3; and .001 for S4) compared with the saline-injected group and on comparison with the preinjection sample ($p < .02$ for S3 and S4).

Potassium concentration [K^+]. The [K^+] remained unchanged in the α-MSH-injected group compared with the saline-injected animals or the preinjection sample (S2), except for the sample collected 0–20 min after injection, which showed a borderline significant increase ($p < .05$) (Fig. 2, bottom).

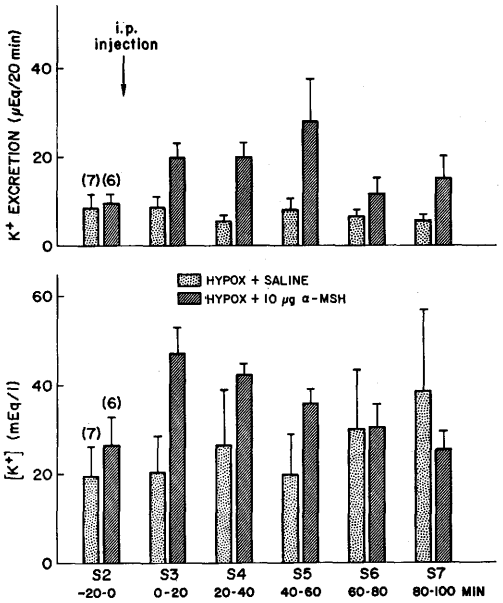

FIG. 2 (top). Effect of α-MSH or saline on the urinary potassium excretion in hypophysectomized rats: For legend see Fig. 1. (bottom) Effect of α-MSH or saline on the urinary [K^+] in hypophysectomized rats.

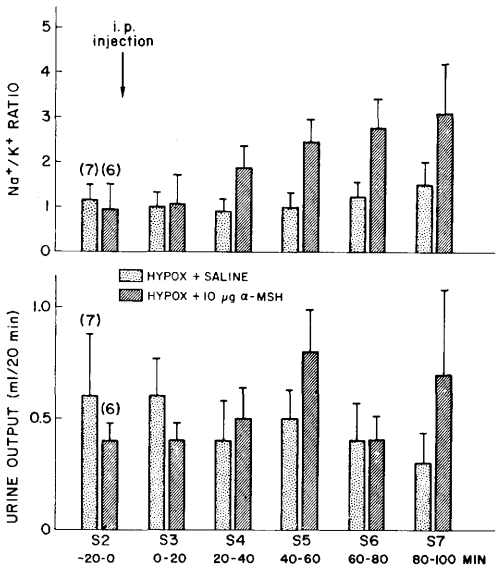

FIG. 3 (top). Effect of α-MSH or saline on the Na^+/K^+ ratio in hypophysectomized rats: For legend see Fig. 1. (bottom) Effect of α-MSH or saline on the urine output in hypophysectomized rats (ml/20 min).

Na^+/K^+ ratio. The ratio increased in the samples collected from the α-MSH-injected group and this change was significant in S5 ($p < .05$) compared with the ratio in the saline-injected group; however, there was no change compared with the preinjection sample (S2) (Fig. 3, top).

Urine output. The urine output of the α-MSH-injected group remained unchanged compared with either the saline-injected group or the preinjection sample (S2) (Fig. 3, bottom).

Adrenalectomized animals. Sodium excretion. The sodium excretion for both groups is shown in the top half of Fig. 4. There was a highly significant increase in the excretion of the ion in the α-MSH-injected group compared with the excretion in either the saline-injected group ($p < .01$ for S4, S5, and S7; and $p < .02$ for S6) or the sodium excretion in the preinjection sample (S2) of the MSH-treated group ($p < .02$ for S4; and $p < .05$ for S5). The excretion reached its maximum in the sample collected 20–40 min after the injection (S4) and represented a 12-fold increase compared with the excretion in the same sample of the control group, and this natriuresis was larger than that obtained with the same 10 μg dose in intact rats (2). In striking contrast to the MSH-injected rats, the Na^+ excretion of the saline-treated controls declined gradually throughout the experiment.

Sodium concentration [Na^+]. The [Na^+] of the α-MSH-injected group increased significantly in every urine sample collected after the injection ($p < .02$ in S3; $p < .01$ in S4, S5 and S6; $p < .001$ in S7), while the concentration in the saline-injected group decreased slightly in the first postinjection sample (S3) and remained unchanged thereafter (Fig. 4, bottom). The increase in the MSH-injected rats was also significant compared with the preinjection sample of the group ($p < .01$ for S4 and S5; and $p < .05$ for S6).

Potassium excretion. The potassium excretion in the samples collected from the α-MSH-injected group increased significantly only in the sample collected 40–60 min after injection ($p < .02$ for S5) compared with the

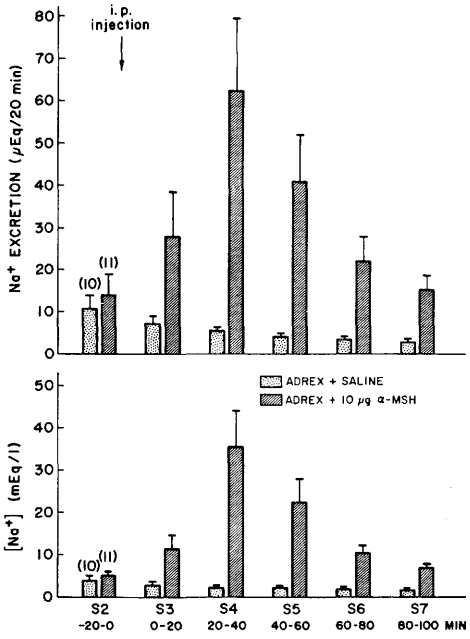

FIG. 4 (top). Effect of α-MSH or saline on the urinary Na^+ excretion in adrenalectomized rats: For legend see Fig. 1. (bottom) Effect of α-MSH or saline on the urinary [Na^+] in adrenalectomized rats.

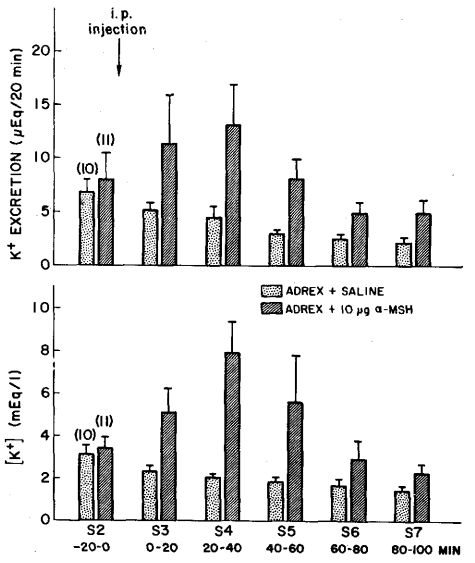

FIG. 5 (top). Effect of α-MSH or saline on the urinary K^+ excretion (μEq/20 min) in adrenalectomized rats. For legend see Fig. 1. (bottom) Effect of α-MSH or saline on the urinary $[K^+]$ in adrenalectomized rats.

saline-injected control group but remained unchanged compared with the preinjection sample (S2) (Fig. 5, top).

Potassium concentration $[K^+]$. The $[K^+]$ in the samples collected from the α-MSH-injected group (Fig. 5, bottom) showed a significant increase only in the samples collected 0–20 and 20–40 min after injection ($p < .05$ for S3; and $p < .02$ for S4) compared with that of the saline-injected group and only in S4 ($p < .02$) compared with the preinjection sample.

Na^+/K^+ ratio. The Na^+/K^+ ratio is shown in the top half of Fig. 6. As could be expected from the changes in Na^+ and K^+ excretion, there was a highly significant increase in the α-MSH-injected group compared with the saline-injected control group ($p < .001$ for S4, S5, S6, and S7) or the preinjection sample S2 ($p < .001$ for S4 and S5; $p < .01$ for S6 and S7).

Urine output. There was no significant difference in the urine output from the α-MSH-injected group compared with the output of either the saline-injected control group or the preinjection output of the MSH-treated rats (S2) (Fig. 6, bottom).

Discussion. The present results not only confirm our previous observations (1, 2) but also throw some light on the site of action of α-MSH in eliciting natriuresis. Although lower doses of MSH produced natriuresis without antidiuresis in intact rats, the 10 μg dose used here significantly increased Na^+ and K^+ excretion and also produced a significant antidiuresis in our earlier studies (2). According to the present results, neither hypophysectomy nor adrenalectomy were able to prevent the natriuretic effect; but, on the contrary, the natriuresis was even greater for the same 10 μg dose than that produced in the intact animals. The increased natriuresis in the adrenalectomized animals could be explained by the lack of aldosterone which could counteract partially the effect produced by α-MSH in an intact animal. The increased natriuresis in the hypophysectomized animals cannot be explained, although it should be kept in mind that the hypophysectomized rat is not an ideal animal to use, especially in water-loaded conditions,

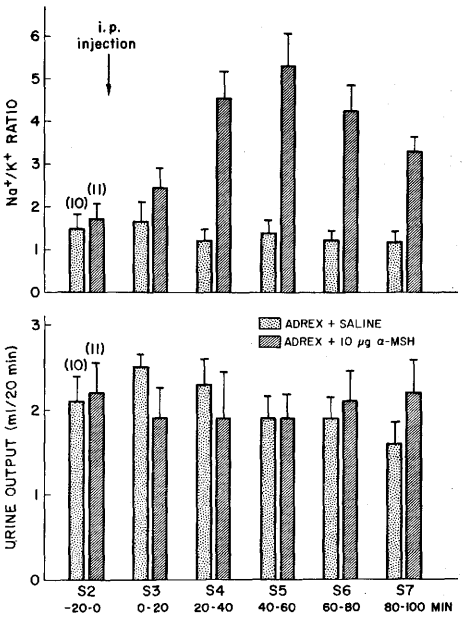

FIG. 6 (top). Effect of α-MSH or saline on the urinary Na^+/K^+ ratio in adrenalectomized rats: For legend see Fig. 1. (bottom) Effect of α-MSH or saline on the urine output (ml/20 min) in adrenalectomized rats.

due to its very low urine output. The kaliuresis produced in the intact animals by the same dose of MSH was also present in both the hypophysectomized and the adrenalectomized rats, although to a lesser degree. As in intact rats, kaliuresis was less pronounced than natriuresis resulting in an increased Na^+/K^+ ratio. The kaliuretic effect is probably caused by contaminating vasopressin, since treatment with thioglycolate to inactivate neurohypophyseal peptides eliminated it (2).

The failure of MSH to produce an antidiuresis in the hypophysectomized or adrenalectomized rats contrasts with the antidiuresis observed in intact rats with this dose of the hormone (2). The finding reinforces our previous conclusion that the natriuretic effect is not caused by antidiuretic hormone (ADH). The antidiuresis observed in the normal rats was produced by the slight contamination of the hormone with ADH (7) since it was eliminated by inactivation of ADH with thioglycolate (2), which did not affect the natriuresis. We would postulate that a decreased sensitivity to ADH was responsible for the failure of antidiuresis to occur in the adrenalectomized and hypophysectomized rats. The low urine volumes of the hypophysectomized rats may be explained on the basis of a reduced glomerular filtration rate and diminished adrenal cortical function in spite of the replacement therapy given. The natriuresis produced by MSH could not have been produced by contamination of the MSH preparation with oxytocin since the highly purified MSH (7) used should have been free of oxytocin contamination and since treatment of the MSH with thioglycolate failed to alter the natriuretic effect (2).

Although it is still premature to make any statement regarding the site of the natriuretic action of MSH, the fact that the natriuretic effect persisted in the absence of the pituitary gland or the adrenals suggests that the kidneys are the most probable site of action. It is still possible that MSH may induce release of the postulated CNS natriuretic factor (8) or even oxytocin since some oxytocin release could still occur from the median eminence stalk region of the hypophysectomized rats. Experiments are presently being planned to elucidate the role of the kidneys in the natriuresis induced by MSH.

Summary. A 12-fold increase in sodium excretion occurred in hypophysectomized or adrenalectomized water-loaded rats after ip injection of 10 μg of α-MSH compared with the low sodium excretion in saline-injected control rats. Potassium excretion also increased significantly but to a much lesser degree. There was no change in the urine output. The results indicate that neither the pituitary gland nor the adrenals mediate the natriuresis produced by MSH.

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