Natriuretic Effect of α-MSH in the Water-Loaded Rat[1] (34499)

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There are a number of observations which suggest the possibility that melanocyte-stimulating hormone (MSH) may be concerned in some way with sodium metabolism. For example, Duchen (1) has observed histological changes produced in the pars intermedia of rats after ingestion of hypertonic saline without detecting any change in its volume (2). Earlier, Legait (3) had reported changes in the weight of this gland in desert rats after prolonged dehydration. Orias (4) reported that water deprivation for 12 hr produced a marked depletion of pituitary MSH but its content was restored to normal after 7 days. Furthermore, intravenous injection of hypertonic NaCl solutions caused a decrease in pituitary MSH content. This effect could not be produced by the intravenous injection of several other hypertonic solutions (KCl, $CaCl_2$, or glucose), thus suggesting the possibility of a relationship between MSH and Na metabolism. Later, Kastin confirmed this result by reporting a depletion of pituitary MSH in rats when NaCl hypertonic solutions were given orally (5).

Materials and Methods. Male albino rats (Holtzman) weighing 200–400 g were trained for intraperitoneal injection and water loading for 4–5 days by several stomach tubings and sham injections. They were maintained in an animal room with controlled temperature and lighting (lights on for 14 hr daily) and given free access to tap water and Purina Lab Chow. At the time of the experiment the rats were given orally via stomach tube a volume of tap water equivalent to 5% of their body weight. They were then placed in separate metabolic cages without access to food or water for collection of urine samples. The sample collected during the first hour (S1) was discarded. One hour later they received a second gavage of the same volume of fluid, and urine samples were collected every 20 min for 120 min. Immediately after collection of the first 20-min sample (S2), the rats were given 0.5 ml (ip) of the substance to be tested. Data from five animals which were stressed by the procedure as indicated by urine volumes of less than 0.5 ml in the first three urine samples were discarded. After a 4-day rest period, the rats were injected with a different substance and the above procedure repeated. Animals were used repeatedly until they reached a weight of 400 g.

The animals were divided into eight groups. The first group was injected with saline and was used as the controls. The second, third, and fourth groups were injected with α-MSH[2] in doses of 90, 50, and 25 μg, respectively. The fifth was injected with 90 μg of α-MSH inactivated by incubation with pepsin (24 hr at room temperature) (enzyme to substrate ratio equal 1:50 by weight). The sixth group was injected with the diluent and pepsin only. The seventh and eighth groups were injected with synthetic oxytocin (Syntocinon)[3] in doses of 100 and 25 mU, respectively.

The concentration of Na^+ and K^+ in urine was determined by use of a Beckman flame photometer.

Duncan's Multiple-Range Test, which involves an analysis of variance, was applied in the statistical analysis of the results. If the

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[2] MSH kindly provided by Dr. P. A. Desaulles and Dr. W. Rittel of the CIBA laboratories. It was a nearly pure preparation of α-MSH (peptide content 95%) which was dissolved in acidified 0.9% NaCl (approximately 0.02 *N* acetic acid).

[3] Syntocinon was provided by Dr. Gerald Q. O'Neill of Sandoz Laboratories.

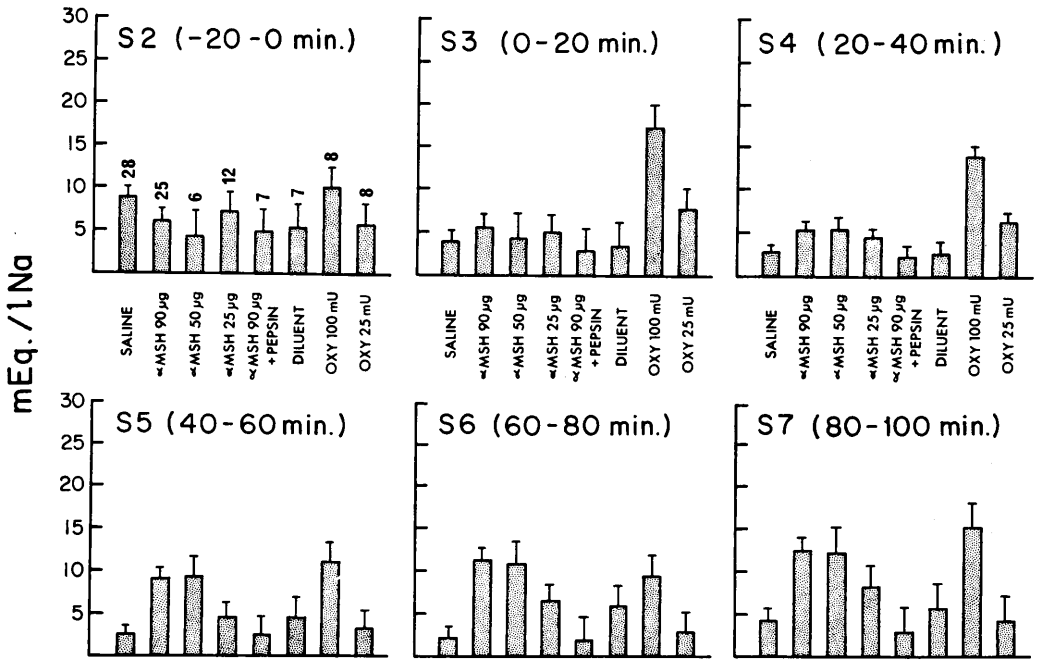

FIG. 1. Effect of α-MSH and other treatments on the urinary sodium concentration (meq/liter). In this and subsequent figures, each sample represents a collection period of 20 min. The injection of the substance to be tested was given intraperitoneally after the collection of the second sample (S2) (—20–0 min). Time zero represents the time of injection. Each column represents the mean, the vertical bar indicates 1 standard error of the mean, and the number on top of each column is the number of experiments.

analysis of variance indicated significant differences between groups, then all possible combinations of treatments were compared. The statistical calculations were performed on an IBM 1800 computer and the statistical advice of Mrs. Joan Reisch is acknowledged.

Results. Sodium concentration [Na^+]. When statistical comparisons were made against the preinjection control sample (S2), the urinary [Na^+] (Fig. 1) of animals injected with either saline or the diluent decreased significantly throughout the collection period [samples 2–7 (S2–7)]. The injection of 90 μg of α-MSH produced an increase in the concentration which became highly significant ($p<.01$) in samples 6 and 7 (S6 and S7) collected during the 60 to 80 and 10 to 100 min periods after injection. The dose of 50 μg increased [Na^+] in S7, but 25 μg did not increase [Na^+] significantly. When the 90-μg dose of α-MSH was inactivated by pepsin, the increase in [Na^+] failed to occur. Oxytocin failed to increase the [Na^+] after the injection of either 100 or 25 mU.

When comparisons were made at the various time intervals between the treated and control groups, a slightly different picture emerged. MSH at the 50- or 90-μg dose gave a [Na^+] significantly higher than that for the saline-injected controls in S6 and S7. On the other hand, only the high dose of oxytocin produced a significant ($p<.01$) elevation in [Na^+] when compared with the saline-treated controls as early as S3 collected 20 min after injection; however, it should be noted that the inital [Na^+] (S2) of this group was higher, but not significantly so, than that of any other group of rats in the series.

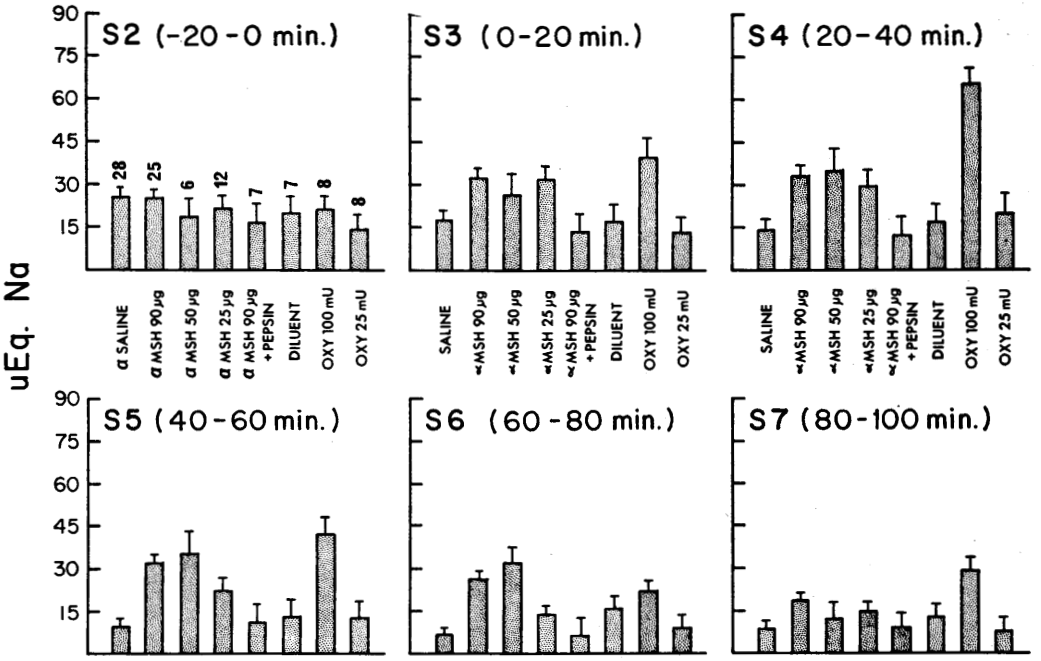

FIG. 2. Effect of α-MSH and other treatments on the urinary excretion of sodium expressed as μeq/20 min. For legend see Fig. 1.

Sodium excretion. The sodium excretion (Fig. 2) of saline-injected animals decreased significantly ($p<.01$) in all postinjection samples on comparison with the preinjection sample (S2), whereas it remained unchanged or increased slightly in the groups injected

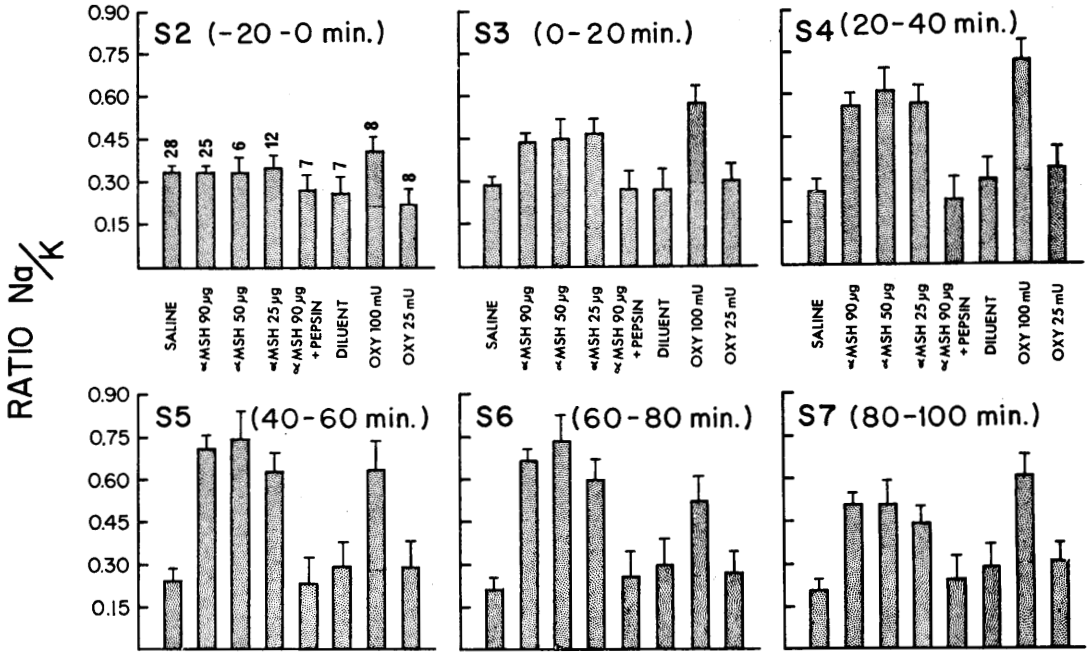

FIG. 3. Effect of α-MSH and other treatments on the Na/K ratio. For legend, see Fig. 1.

with 90 or 50 μg of α-MSH. There was a highly significant increase in sodium excretion of the groups injected with either 50 or 90 μg of MSH when compared with the saline-injected rats. The increase appeared in the first postinjection sample (S3) collected 20 min after injection, and it persisted for 80 min. When the α-MSH was inactivated by pepsin, the natriuretic effect did not appear. Excretion obtained when the diluent was injected did not vary significantly from that of the saline-injected groups. Oxytocin in doses of 100 mU also produced a natriuretic response ($p<.01$) when compared with either the preinjection sample (S2) or the saline-treated controls, but the 25 mU dose proved ineffective.

Potassium concentration [K^+]. As in the case of [Na^+], [K^+] decreased after the injection of saline (S3–S6) (Table I). A similar decrease occurred in rats injected with α-MSH, and in contrast to the situation with [Na^+], there was no increase in [K^+] when values for MSH-treated rats were compared with those injected with saline. MSH inactivated with pepsin and the diluent likewise were ineffective in altering [K^+] from the control level. On the other hand, both doses of oxytocin prevented the decline in [K^+] which occurred in the other groups, and there was an immediate highly significant or significant (versus diluent) increase (S3–S4) in [K^+] on comparison to any of the other groups.

Potassium excretion. The pattern of changes observed was similar to that obtained with [K^+] (Table II). The only difference was that the level of significance of the changes obtained with oxytocin was less, such that only the increase in potassium excretion (S4) in the animals injected with 100 mU dose of oxytocin was significantly different ($p<.01$) from that in the pretreatment sample (S2).

Na/K ratio. The most striking changes were observed when the Na/K ratio was calculated. As can clearly be seen in Fig. 3, the Na/K ratio decreased significantly in the saline-injected group, whereas it increased significantly ($p<.05$ for 25 and 50 μg and

TABLE I. Effect of α-MSH and Other Treatments on the Urinary [K^+] (meq/liter).

Treatment	No. of expt.	Time after injection (min)					
		−20–0 (S2)[a]	0–20 (S3)	20–40 (S4)	40–60 (S5)	60–80 (S6)	80–100 (S7)
Saline	27	25.14 ± 2.97[b]	13.76 ± 2.09	11.79 ± 1.59	12.03 ± 1.92	12.72 ± 1.97	24.14 ± 3.79
α-MSH 90 μg	25	18.65 ± 3.09	13.99 ± 2.17	11.15 ± 1.65	13.10 ± 2.00	16.16 ± 2.05	23.68 ± 3.79
α-MSH 50 μg	6	13.93 ± 6.30	10.49 ± 4.43	9.49 ± 3.38	12.86 ± 4.08	14.73 ± 4.11	25.82 ± 7.74
α-MSH 25 μg	12	18.25 ± 4.46	11.23 ± 3.13	8.79 ± 2.39	7.90 ± 2.89	10.67 ± 2.91	19.84 ± 5.48
Pepsin-inactivated MSH	7	18.18 ± 5.83	11.21 ± 4.10	9.94 ± 3.13	10.86 ± 3.78	7.23 ± 4.11	15.54 ± 7.17
Diluent	7	21.31 ± 5.83	13.56 ± 4.10	10.60 ± 3.13	14.13 ± 3.78	15.80 ± 3.81	17.39 ± 7.17
Oxytocin 100 mU	8	25.12 ± 5.46	27.66 ± 3.84[c]	20.78 ± 2.93[c]	17.21 ± 3.54	17.60 ± 3.56	22.92 ± 6.71
Oxytocin 25 mU	8	25.71 ± 5.46	28.63 ± 3.84[c]	25.08 ± 2.93[c]	13.35 ± 3.54	11.47 ± 3.56	15.04 ± 6.71

[a] S = Sample number.
[b] Mean ± SEM.
[c] $p<.01$ versus saline controls.

TABLE II. Effect of α-MSH and Other Treatments on the Urinary K Excretion (μeq/20 min).

Treatment	No. of expt.	Time after injection (min)					
		−20–0 (S2)[a]	0–20 (S3)	20–40 (S4)	40–60 (S5)	60–80 (S6)	80–100 (S7)
Saline	27	70.81 ± 5.95[b]	55.45 ± 5.50	53.45 ± 5.48	45.16 ± 4.88	32.69 ± 4.06	42.89 ± 5.90
α-MSH 90 μg	25	70.98 ± 6.29	70.87 ± 5.82	59.84 ± 5.80	44.65 ± 5.17	39.39 ± 4.30	37.78 ± 6.02
α-MSH 50 μg	6	60.83 ± 12.85	61.39 ± 11.89	57.47 ± 11.84	47.44 ± 10.55	40.26 ± 8.61	19.83 ± 12.29
α-MSH 25 μg	12	58.02 ± 9.09	67.18 ± 8.41	53.87 ± 8.37	39.86 ± 7.46	26.33 ± 6.09	32.63 ± 8.69
Pepsin-inactivated MSH	7	60.76 ± 11.90	50.59 ± 11.01	49.75 ± 10.96	42.87 ± 9.77	26.90 ± 8.61	39.49 ± 11.38
Diluent	7	80.43 ± 11.90	65.55 ± 11.01	57.90 ± 10.96	46.85 ± 9.77	52.57 ± 7.97	43.13 ± 11.38
Oxytocin 100 mU	8	51.73 ± 11.13	68.34 ± 10.30	90.30 ± 10.25[c]	67.81 ± 9.14	40.00 ± 7.46	41.85 ± 10.65
Oxytocin 25 mU	8	56.27 ± 11.13	44.42 ± 10.30	61.28 ± 10.25	49.14 ± 9.14	35.24 ± 7.46	23.62 ± 10.65

[a] S = Sample number.
[b] Mean ± SEM.
[c] $p < .01$ versus saline controls.

$p < .01$ for 90 μg) immediately after the injection in all three groups treated with MSH on comparison with the preinjection ratio and remained high throughout the experiment. In S4 (20–40 min post injection) the increase was highly significant for all doses of MSH. Oxytocin also significantly increased ($p < .01$) the ratio but only when the 100 mU dose was injected. The ratio did not significantly vary from that obtained in the saline-injected group when α-MSH was inactivated by pepsin, or in the groups injected with diluent.

Urine output. In the saline-treated rats urine output increased as the experiment progressed and then declined slightly 40–100 min after injection (S5–S7) (Table III). On comparison with the output of the saline-injected controls (S3), the urine output increased significantly ($p < .01$ for 90 and 25 μg and $< .05$ for 50 μg) (S3) when α-MSH was injected. This effect lasted only for 20 min after injection of each of the three different doses. Output was not significantly different in the rest of the samples from the MSH-treated rats from that of the saline-injected group. The groups injected with inactivated α-MSH or diluent showed no significant alteration in output when compared with the saline-treated rats. Oxytocin, on the other hand, had a significant antidiuretic effect only at the 25 mU dose which lasted for 40 min [S3 ($p < .01$) and S4 ($p < .05$)] when the output was compared to that of the saline-treated controls, but it should be noted that the initial output (S2) in this group was also lower.

Discussion. The present findings provide further evidence for a possible relationship between MSH and Na metabolism. α-MSH exhibited a natriuretic effect in the rat when doses of 90, 50, or 25 μg were injected intraperitoneally into trained water-loaded animals. The excretion of K was not modified by any of the doses injected which resulted in an increase in the urinary Na/K ratio. It remains to be determined if this natriuretic effect is a physiological or pharmacological effect of the hormone, and whether it is produced by a direct action of α-MSH on the kidney or by some indirect effect which, in turn, affects sodium excretion.

Oxytocin also produced an increase in Na

TABLE III. Effect of α-MSH and Other Treatments on the Urine Output (ml/20 min).

Treatment	No. of expt.	Time after injection (min)					
		−20–0 (S2)[a]	0–20 (S3)	20–40 (S4)	40–60 (S5)	60–80 (S6)	80–100 (S7)
Saline	27	3.54 ± 0.24[b]	4.25 ± 0.25	4.64 ± 0.25	4.26 ± 0.26	3.01 ± 0.27	2.68 ± 0.23
α-MSH 90 μg	25	3.89 ± 0.25	5.53 ± 0.26[c]	5.65 ± 0.27	4.33 ± 0.27	3.03 ± 0.29	2.01 ± 0.24
α-MSH 50 μg	6	4.45 ± 0.52	5.71 ± 0.54[d]	6.04 ± 0.56	4.61 ± 0.56	3.29 ± 0.58	0.94 ± 0.49
α-MSH 25 μg	12	4.04 ± 0.37	5.98 ± 0.38[c]	6.03 ± 0.39	4.92 ± 0.40	2.79 ± 0.41	1.89 ± 0.35
Pepsin-inactivated MSH	7	3.61 ± 0.48	4.41 ± 0.50	5.01 ± 0.51	3.92 ± 0.52	3.68 ± 0.58	3.44 ± 0.45
Diluent	7	3.62 ± 0.48	4.68 ± 0.50	5.31 ± 0.51	3.97 ± 0.52	4.07 ± 0.54	2.91 ± 0.45
Oxytocin 100 mU	8	2.87 ± 0.45	3.02 ± 0.46	4.47 ± 0.48	3.79 ± 0.49	2.54 ± 0.50	2.20 ± 0.42
Oxytocin 25 mU	8	2.53 ± 0.45	2.23 ± 0.46[c]	3.21 ± 0.48[d]	3.92 ± 0.49	3.51 ± 0.50	1.78 ± 0.42

[a] S = Sample number.
[b] Mean ± SEM.
[c] $p < .01$ versus saline controls.
[d] $p < .05$ versus saline controls.

excretion, a fact reported earlier by Croxatto *et al.* (6) and others. When 100 mU were injected, it also increased potassium excretion significantly. Only the 25-mU dose of oxytocin produced an antidiuretic effect that lasted for 40 min after it was injected. This effect has been observed previously and has been attributed to the similarity in chemical structure between oxytocin and ADH (7). The higher dose of oxytocin had no significant antidiuretic effect possibly because of the enhanced electrolyte excretion at this dose.

Summary. α-MSH in doses of 50 and 90 μg showed a significant natriuretic effect when injected intraperitoneally into trained water-loaded rats. This effect did not appear with the administration of either α-MSH inactivated with pepsin or the diluent alone. No changes were observed in $[K^+]$ or excretion with any of the doses used. Oxytocin in doses of 100 mU showed not only a natriuretic effect but also increased the K^+ excretion when compared with the preinjection urine sample. α-MSH increased significantly the urine output but only in the sample after the injection of all three doses used, while 25 mU of oxytocin produced an antidiuretic effect in the first two 20-min urine samples after its administration.

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