Bufo arenarum, according to the reflectometric method of Shizume et al.[6], adapted for using with the Argentinian toad[7].

Observations. In the figure it can be seen that in the grafted rats urine sodium concentration and total excretion of sodium rose, while urine volume and potassium excretion did not change significatively. Plasma of all the transplanted rats showed the ability to darken the toad skins, whereas darkening was not reflectometrically detectable when the plasma of the controls was assayed.

Discussion. Plasma of the rats bearing ocular transplants darkened the toad skins. Since the plasma of intact normal rats has no darkening activity when tested in skins of B. arenarum[8], it can be assumed that the grafted rats showed raised levels of MSH or MSH-like substances in their plasma. After water loading the rats displaying high levels of circulating MSH excreted significant by larger amounts of sodium when compared with the controls. Urine volume and potassium excretion did not vary, indicating that the natriuresis found was absolute.

These observations allow us to realize that the effect formerly described by Orias and McCann[2,3] could be assigned to a 'physiological' mechanism. This assumption had been previously suggested by us after observing that the MSH-releasing drug, fluphenazine, induced natriuresis[9]. The results herein reported lead us to speculate that the physiological role of MSH shifts to a new function, parallel with phylogenetic evolution. In lower vertebrates, the hormone mediates the well-known mechanisms involving tegumentary colour changes, whereas in mammals it seems to participate in the electrolyte balance. Previous studies showing that the morphology of the pars intermedia varies according to the availability of water[10], and that the injection of hypertonic saline elicits release of MSH[11], support the hypothesis expressed above.

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Comparison of the effectiveness of intramuscular and intraperitoneal ACTH in the rat[1]

N. Yasuda, K. Takebe and M. A. Greer

Division of Endocrinology, Department of Medicine, University of Oregon Medical School, Portland (Oregon 97201, USA), 11 October 1976

Summary. Injection of 1–24 ACTH is more effective by the i.m. than i.p. route. Large doses are required to induce consistent maximal adrenal corticosterone secretion.

ACTH is usually given either i.v. or i.p. to experimental animals[2,3]. In evaluating the adrenal response to ACTH in intact rats, i.v. ACTH may not be appropriate, since it requires stressful maneuvers, such as a skin incision or a tourniquet placed on the root of the tail, which may induce secretion of endogenous ACTH to add to the effect of exogenously administered ACTH. In the present study, seeking the most appropriate route for injection of ACTH in intact rats, we compared the effect of i.m. or i.p. injection of synthetic 1–24 ACTH.

Materials and methods. Male Sprague-Dawley rats weighing 250–300 g were housed 2/cage with controlled lighting (lights on 06.00–18.00 h) and temperature (24 ± 1 °C). Purina Laboratory Chow and tap water were allowed ad lib. The animal quarters were not entered 10 h prior to the experiment to standardize the experimental conditions. 30 ng to 30 μg/0.1 ml 0.9% saline/100 g b.wt of synthetic 1–24 ACTH (Cortrosyn, Organon), or an equivalent volume of saline, was given i.p. or i.m. in the thigh. At various times after injection, 0.3 ml heparinized blood samples for corticosterone measurement were obtained from the subclavian vein via percutaneous venipuncture, under < 3 min ether anesthesia. In some experiments, plasma ACTH as well as corticosterone concentration was measured. In such experiments, dexamethasone phosphate (Decadron, Merck, Sharp & Dohme) 100 μg/0.1 ml/100 g b.wt was given i.p. at 07.00–07.30 h to inhibit stimulation of endogenous ACTH secretion at the time of sampling, and pentobarbital (Nembutal, Abbott) 4 mg/0.5 ml/100 g b.wt was given i.p. 4 h later. 5 min after pentobarbital injection, synthetic 1–24 ACTH or saline was injected. Heparinized blood (1.5 ml) was obtained for both ACTH and corticosterone measurement at various times after ACTH injection. All blood samples were collected between 10.00 and 13.00 h. ACTH was measured by radioimmunoassay[4]; corticosterone was measured by

Table 1. Plasma corticosterone 30 or 45 min after 100 or 300 ng/100 g b.wt i.m. or i.p. 1–24 ACTH injection

Dose and route of ACTH injection	Plasma corticosterone (μg/100 ml) 30 min	45 min
100 ng i.m.	10.1 ± 2.2	13.2 ± 10.1
100 ng i.p.	13.4 ± 6.5	5.6 ± 0.9
300 ng i.m.	14.4 ± 2.2	9.5 ± 2.8
300 ng i.p.	35.4 ± 18.0	5.1 ± 0.3

Mean and SE of 5 rats are shown for each time point.

1 Supported by grants from the National Institute of Arthritis, Metabolism and Digestive Diseases, National Institutes of Health, Bethesda, Maryland, USA.

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Table 2. Temporal change in plasma corticosterone and ACTH after injection of 30 μg/100 g b.wt 1–24 ACTH i.m. or i.p.

Time after injection	Plasma corticosterone (μg/100 ml) i.m.	i.p.	Saline control IP	Plasma ACTH (pg/ml) i.m.	i.p.
6 min	27.0 ± 1.8	19.2 ± 1.8		5904 ± 918	2384 ± 296
15 min	43.0 ± 3.7	39.9 ± 3.5	6.2 ± 1.0	6462 ± 1490	2599 ± 401
30 min	47.4 ± 2.2	53.9 ± 3.1	7.2 ± 0.4	1387 ± 110	1171 ± 217
60 min	57.5 ± 2.7	58.3 ± 2.4		1059 ± 192	586 ± 129

Mean and SE of 5 rats are shown for each time point.

a semi-automated acid fluorescence technique[5]. Statistical comparison was made with Student's t-test and the Newman-Keuls multiple comparison test[6].

Results. Corticosterone response to small doses of 1–24 ACTH. Since 15 ng/100 g b.wt 1–24 ACTH is effective i.v.[2], groups of 6 rats were given 30 ng/100 g b.wt 1–24 ACTH or saline i.m. and sampled 30 or 45 min later. Basal control animals were not injected with either saline or ACTH and were sampled under < 3 min ether anesthesia. Plasma corticosterone increased slightly above baseline at 30 min in both saline and ACTH groups, but this change was not significant ($p > 0.05$, Student's t-test). Plasma corticosterone returned to the basal level by 45 min; there were no significant differences between ACTH- and saline-injected groups. Larger doses of 1–24 ACTH (100 or 300 ng/100 g b.wt) were injected i.m. or i.p. in groups of 5 rats. Blood samples were obtained 30 or 45 min later. No consistent corticosterone response was obtained with either of the i.m. or i.p. doses (table 1).

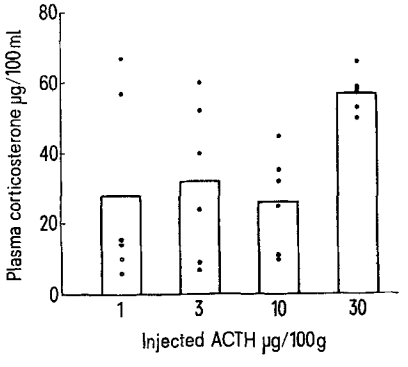

Fig. 1. Plasma corticosterone concentration 30 min after i.p. 1–24 ACTH, 1–30 μg/100 g b.wt. Dots represent individual animals; bars represent means.

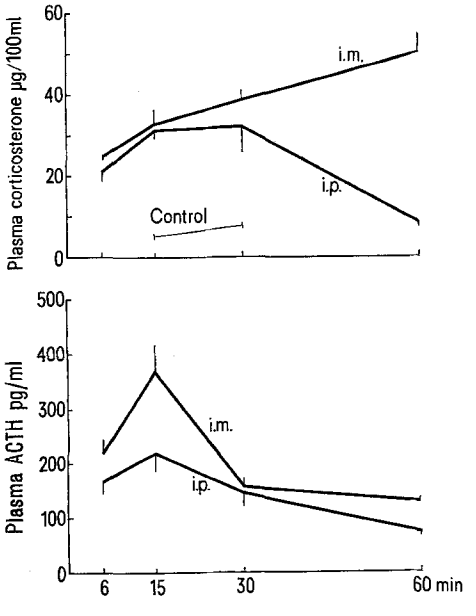

Fig. 2. Temporal change in plasma corticosterone (upper figure) and ACTH (lower figure) after injection of 3 μg/100 g b.wt. ACTH i.m. or i.p. Mean and SE of 5 rats are shown for each time point.

Corticosterone response to larger doses of i.p. 1–24 ACTH. To produce a consistent corticosterone response, larger doses of 1–24 ACTH (1–30 μg/100 g b.wt) were injected i.p. in groups of 6 rats and blood samples were obtained 30 min later. As shown in figure 1, a consistent rise of plasma corticosterone was obtained only with the highest dose of ACTH.

Plasma ACTH and corticosterone after larger doses of i.m. or i.p. 1–24 ACTH. 3 or 30 μg/100 g b.wt 1–24 ACTH were injected i.m. or i.p. in groups of 5 rats and blood samples were obtained 6, 15, 30 and 60 min later. Control animals received saline instead of ACTH and were sampled 15 or 30 min later. Plasma ACTH concentration was significantly higher ($p < 0.05$) in the i.m. than in the i.p. group at 15 and 60 min (3 μg/100 g body weight 1–24 ACTH, figure 2) or at 6, 15 and 60 min (30 μg/100 g b.wt 1–24 ACTH, table 2). ACTH was non-detectable in the plasma of the controls at 15 and 30 min. Usually 3 ml of plasma are required to detect ACTH in the basal state; < half this amount was used in this study. Plasma corticosterone underwent a higher and more prolonged elevation in the i.m. than in the i.p. group after 3 μg/100 g b.wt 1–24 ACTH (figure 2) but was not significantly different between i.m. and i.p. groups after 30 μg/100 g b.wt (table 2).

Discussion. Our results indicate that 1–24 ACTH produces higher plasma ACTH and corticosterone when injected i.m. than i.p. A relatively large dose is necessary to induce a consistent corticosterone response in rats. It was rather surprising that as much as 10 μg/100 g b.wt 1–24 ACTH given i.p. did not induce a consistent corticosterone response. Differences between the absorption of this peptide hormone along its pathway from the injection site and/or in hepatic degradation may account for the difference between the effectiveness of i.m. and i.p. injection. 30 μg 1–24 ACTH/100 g b.wt induced an indistinguishable marked rise in plasma corticosterone after both i.p. and i.m. injection, indicating that this dose induces maximal adrenal activation by either route of administration.