Characteristics of the Antinatriuretic Action of Growth Hormone* (33741)

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Growth hormone has been shown to produce antinatriuresis in normal animals. Surtshin *et al.* (1) administered growth hormone to trained mongrel female dogs and found a decrease in sodium excretion and a decrease in the ratio of sodium output to intake when a daily dose of 5.0 mg of the hormone was given. Whitney *et al.* (2) studied the effect of growth hormone on electrolyte excretion in rats. The administration of hypophyseal growth hormone to normal female rats produced a retention of both sodium and potassium. Investigations to delineate the antinatriuretic action revealed at least two mechanisms through which growth hormone could produce antinatriuresis. It was demonstrated that growth hormone has an effect upon the adrenal cortex and could thereby indirectly produce sodium retention. Selye (3) demonstrated that growth hormone caused an enlargement of adrenal cortical tissue. Venning and Lusis (4) found that growth hormone increased the amount of aldosterone produced by adrenal tissue. However, it has also been found that growth hormone produces sodium retention in adrenalectomized animals. Surtshin *et al.* (1) found that growth hormone produced a decrease in sodium excretion in adrenalectomized dogs and Stein *et al.* (5) reported that growth hormone produced retention of sodium as well as potassium and chloride in adrenalectomized rats. It was suggested that this antinatriuretic action of the hormone is due to a direct effect upon the renal tubules (5).

The present investigation was concerned with the nature of the antinatriuretic action of growth hormone which is not mediated via the adrenal cortex, therefore adrenalectomized animals were used. The study was designed to: (i) determine a dose–response relationship for the antinatriuresis produced by growth hormone, (ii) determine the onset and duration of the antinatriuretic action of growth hormone, and (iii) determine the effect of actinomycin D on the antinatriuretic action of growth hormone.

Methods and Materials. Male rats (Sprague-Dawley) weighing from 120–150 g were adrenalectomized bilaterally 5 days before experimentation. Following adrenalectomy, the animals were maintained on a saline solution (0.8% NaCl–0.1% KCl) for drinking water and Wayne lab-block. On the day of experimentation, drinking water and food were removed. Bovine growth hormone (purchased from Calbiochem, Los Angeles, Calif.) was administered intramuscularly as a suspension in 0.9% saline. One-half of the dose was injected into each hindlimb. Urine samples were collected for 2 hr by the method of Kagawa and co-workers (6). A 0.9% saline load of 17 ml/kg was given subcutaneously 4 hr before the beginning of the urinary collection period. Urinary sodium was measured with a Coleman flame photometer. For all statistical analyses, the 0.05 level of probability was the criterion of significance.

Dose–response study. Three doses of growth hormone (0.01, 0.1, and 1.0 mg/kg) were given and urine samples were collected from 2 to 4 hr after growth hormone administration. The data were analyzed statistically by analysis of variance to test for a significant regression and to determine if the regression was linear (7).

Time study. To determine the time sequence of the antinatriuretic action of growth hormone, urine samples were collected from 0–2, 2–4, 4–6, 6–8, 8–10, 12–14, 16–18, and 20–22 hr after administration of 1 mg/kg of the agent. A control group of animals was run for each of the various collection times and these animals received an intramuscular in-

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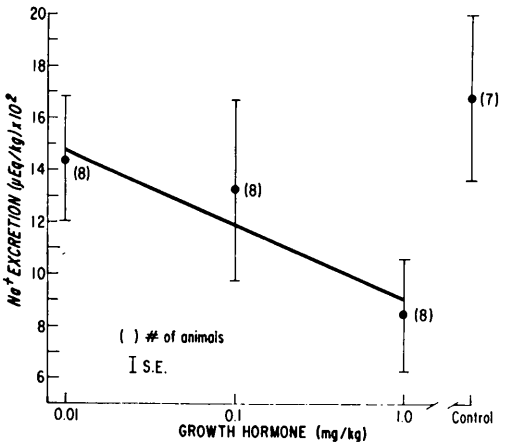

FIG. 1. Dose–response relationship for growth hormone induced antinatriuresis in adrenalectomized rats: the dose of growth hormone administered is shown on the abscissa; the amount of sodium excreted during the 2-hr collection period (collected between 2 and 4 hr after administration of hormone) is shown on the ordinate.

jection of saline. The data were anlayzed statistically by means of the Student's *t* test, group comparisons (7).

Effect of actinomycin D on growth hormone induced antinatriuresis. Actinomycin D (400 μg/kg) or the vehicle was administered subcutaneously 0.5 hr before growth hormone (1 mg/kg). The agents were administered such that the following combinations of treatments were obtained: (i) growth hormone, (ii) control, (iii) actinomycin D and growth hormone, and (iv) actinomycin D. Urine samples were collected from 2–4 hr after growth hormone administration. An analysis of variance and Duncan's new multiple range test were used to analyze the data statistically (7).

Results and Discussion. The results illustrated in Fig. 1 show that a dose–response relationship exists. By increasing the dose administered the antinatriuretic response of growth hormone can be increased. At $p<0.05$, the dose–response relationship was significant, and the regression was linear. It appeared that the sodium retention produced by the administration of 1 mg/kg of growth hormone was maximal since doses greater than 1 mg/kg did not cause any greater retention of sodium.

Figure 2 shows the time sequence following the administration of growth hormone. Growth hormone did not produce a significant antinatriuresis when urine was collected from the time of administration until 2 hr after administration. A significant antinatriuresis was observed when urine samples were collected from 2 to 4 and 4 to 6 hr after injection of the agent. The maximal response appeared to be during the 4–6-hr collection period. The antinatriuretic response was diminished by the 6–8 and 8–10-hr collection periods to the point where the retention was no longer significant. During the 12–14 hr collection period, rats treated with growth hormone excreted more sodium than the corresponding control animals. Beyond 16 hr, the amount of sodium excreted by treated and control animals was observed to be essentially the same. The duration of the retention of sodium produced by a single injection of 1 mg/kg of growth hormone was approximately 4 hr. A significant antinatriuresis was observed from approximately 2 hr to 6 hr after administration of the agent (Fig. 2).

The observed lag in onset of action following administration of the hormone is not a unique characteristic of growth hormone. Such a delayed onset of action has also been observed with other hormones. Karlson (8) has suggested that many hormones act via

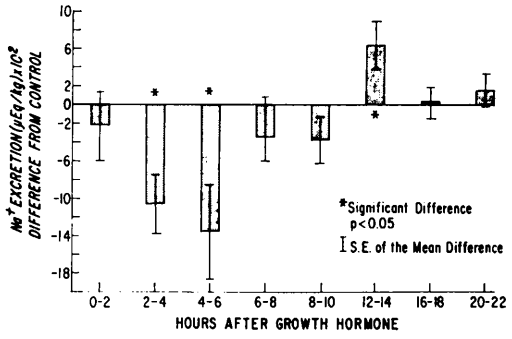

FIG. 2. Time sequence of the antinatriuresis produced by growth hormone in adrenalectomized rats; the bars indicate the difference in sodium excretion between growth hormone treated animals and corresponding control animals; the numbers directly under the bars indicate the interval of time after the administration of growth hormone during which urine was collected.

TABLE I. The Effect of Actinomycin D on Growth Hormone Induced Antinatriuresis in Adrenalectomized Rats.

Treatment	Total sodium excretion[a] (μeq/kg/2 hr)
Growth hormone	489
Control	818
Actinomycin-D and growth hormone	875
Actinomycin-D	923
Coefficient of variability 44.8%	

[a] Values for each treatment represent the means of 12 animals. Any two means joined by the vertical line are not significantly different; any two means not so joined are significantly different (5% level).

RNA and that the delayed onset of action is due to the time necessary to initiate RNA synthesis. The lag in onset of action of growth hormone suggested that the antinatriuretic action of this hormone might be mediated in this manner. To test this hypothesis, actinomycin D was administered prior to growth hormone. The effect of actinomycin D on growth hormone induced antinatriuresis is illustrated in Table I. Control animals excreted 818 μeq/kg during the 2-hr collection period. Growth hormone significantly depressed sodium excretion to 489 μeq/kg. Sodium excretion was not altered significantly by actinomycin D alone, while antinomycin D given prior to growth hormone completely blocked the antinatriuretic response of the agent. The results, demonstrating that actinomycin D blocked antinatriuresis produced by growth hormone, suggest that RNA is in some way involved in the antinatriuretic action of growth hormone. The duration of the observed lag in the onset of action of growth hormone is also consistent with this suggestion. Although other factors such as absorption from the site of injection may have contributed somewhat to the lag, it seems likely that the main reason for the delay in the onset of action is due to the time involved for growth hormone to act via RNA.

Summary. The antinatriuretic action of growth hormone (bovine) was studied in adrenalectomized rats. A dose–response relationship and a time sequence were determined for the antinatriuretic action of growth hormone. Actinomycin D was administered prior to the hormone to determine the effect of actinomycin D on growth hormone-induced antinatriuresis. The results indicated that the antinatriuretic response of growth hormone was dose related. It was found that a 2-hr delay in onset of action followed the administration of growth hormone. The duration of antinatriuresis was approximately 4 hr and this was observed between 2 and 6 hr after administration. The data show that actinomycin D administered prior to growth hormone completely blocked the antinatriuretic action of this agent. The blockade by actinomycin D suggests that RNA synthesis is involved in this action of growth hormone. The observed delay in onset of action of growth hormone is also consistent with the suggestion that growth hormone induced antinatriuresis is mediated through RNA synthesis.

1. Surtshin, A., Rolf, D., and White, H. L., Am. J. Physiol. 165, 429 (1951).

2. Whitney, J. E., Bennett, L. L., and Li, C. H., Proc. Soc. Exptl. Biol. Med. 79, 584 (1952).

3. Selye, H., Proc. Soc. Exptl. Biol. Med. 76, 510 (1951).

4. Venning, E. H. and Lucis, O. J., Endocrinology 70, 486 (1962).

5. Stein, J. D., Bennett, L. L., Batts, A. A., and Li, C. H., Am. J. Physiol. 171, 587 (1952).

6. Kagawa, C. M., Shipley, E. G., and Meyer, R. K., Proc. Soc. Exptl. Biol. Med. 80, 281 (1952).

7. Steel, R. G. D. and Torrie, J. H., "Principles and Procedures of Statistics." McGraw-Hill, New York (1960).

8. Karlson, P., Perspectives Biol. Med. 6, 202 (1963).

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