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Nature of the Antinatriuretic Action of Growth Hormone

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Abstract

Three interrelated experiments were initiated to study growth hormone's stimulation of urinary sodium retention. First a dose-response curve was determined by administration of known dosages of growth hormone or the vehicle to groups of rats. Urine was collected from four to six hours after administration.

In a second experiment, the relationship between time and degree of response to growth hormone was determined. Urine was collected from 0-2, 2-4, 4-6, 6-8, 8-10, 12-14, 16-18, and 20-22 hours after administration. Sodium excretion was not altered in the 0-2 hour collection period. Significant retention took place in both the 2-4 and 4-6 hour collection periods. The 6-8 and the 8-10 hour collections showed a gradual return to normal levels of sodium excretion. The 12-14 hour collection showed a significant increase in sodium excretion while the 16-18 and 20-22 hour collections returned to normal levels.

The RNA inhibitor actinomycin D was used to determine if de novo synthesis of RNA might be involved in growth hormone's mechanism of sodium retention. Actinomycin D was given one-half hour before growth hormone. Urine was collected from four to six hours after administration. Actinomycin D alone did not alter sodium excretion and when administered with growth hormone, it blocked the antinatriuretic action.

The relationship between the dose of growth hormone and the antinatriuretic response was found to be linear. Both the delay of antinatriuresis and the inhibition of this action by actinomycin D suggest that the antinatriuretic action of growth hormone is mediated via RNA synthesis.

Introduction

It has been shown that the intramuscular injection of bovine growth hormone significantly decreases urinary sodium excretion in rats, but very little is known about the mechanism of this antinatriuretic action. Therefore, research was initiated to investigate this action in detail.

It is hypothesized that growth hormone initiates its action of antinatriuresis via the mechanism of DNA-RNA initiated enzyme synthesis as proposed by Karlson.[1] He theorizes that hormones, in general, exert their action by promoting enzyme synthesis *de novo*. A hormone stimulates the synthesis of messenger RNA via DNA. The messenger RNA, in turn, stimulates the ribosomal synthesis of a specific enzyme.

Previous studies *in vitro*, using the diaphragm of hypothesectomized rats,

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have shown that growth hormone stimulates an uptake of some but not all amino acids.[2] These experiments give support to the theory of Karlson[1] in relation to the overall action of growth hormone *in vitro*. However, *in vivo* evidence concerning its mechanism of antinatriuresis remained to be shown. Therefore, the purpose of this project was to investigate the *in vivo* mechanism of action of growth hormone.

Methodology I

Thirty-one male Sprague-Dawley rats were adrenalectomized bilaterally five days before the experiment to eliminate the endogenous source of aldosterone, an antinatriuretic hormone. The animals were then maintained on a 0.8 per cent NaCl, 0.1 per cent KCl solution for drinking water and "Purina" lab chow.

The protocol of the experiment is the following:

0 time—All rats received a 17 ml/kg subcutaneous injection of 0.9 per cent NaCl.
2 hours—The rats were divided into four groups. Each received one of the following injections intramuscularly:

1. 1 mg/kg of growth hormone (bovine)
2. 0.1 mg/kg of growth hormone (bovine)
3. 0.01 mg/kg of growth hormone (bovine)
4. saline vehicle

4 hours—Start urine collection by the method of Kagawa, *et al.*[3]
6 hours—Collect urine

The sodium content was analyzed by means of flame photometry and calculated as uEq/kg x 10^2. The results are shown in Figure I.

Conclusions

This experiment showed a linear relationship between the dose and the antinatriuretic response to growth hormone in adrenalectomized rats. This information is useful in determining a maximum effective dose of growth hormone. It is also useful for further experimentation to know that there is a relatively constant relationship between dose and response.

Methodology II

A series of eight experiments was performed to examine the effect of time on the antinatriuretic response to growth hormone in adrenalectomized rats. The protocol of the experiment is the following:

0, 2, 4, 6, 8, 12, 16, or 20 hours befor the start of the urine collection—The rats were divided into two groups. One received a 1 mg/kg intramuscular injection of growth hormone (bovine). The other was given the saline vehicle.
-4 hours—All rats received a 17 ml/kg subcutaneous injection of 0.9 per cent NaCl.
0 time—Start urine collection by the method of Kagawa, *et al.*[3]
6 hours—Collect urine

Urine was collected from 0-2, 2-4, 4-6, 6-8, 8-10, 12-14, 16-18, or 20-22 hours after growth hormone administration. Urinary sodium was analyzed by means of flame photometry and calculated as uEq/kg x 10^2.

The results are shown in Figure II.
Conclusions:

Results indicate that a delay in the

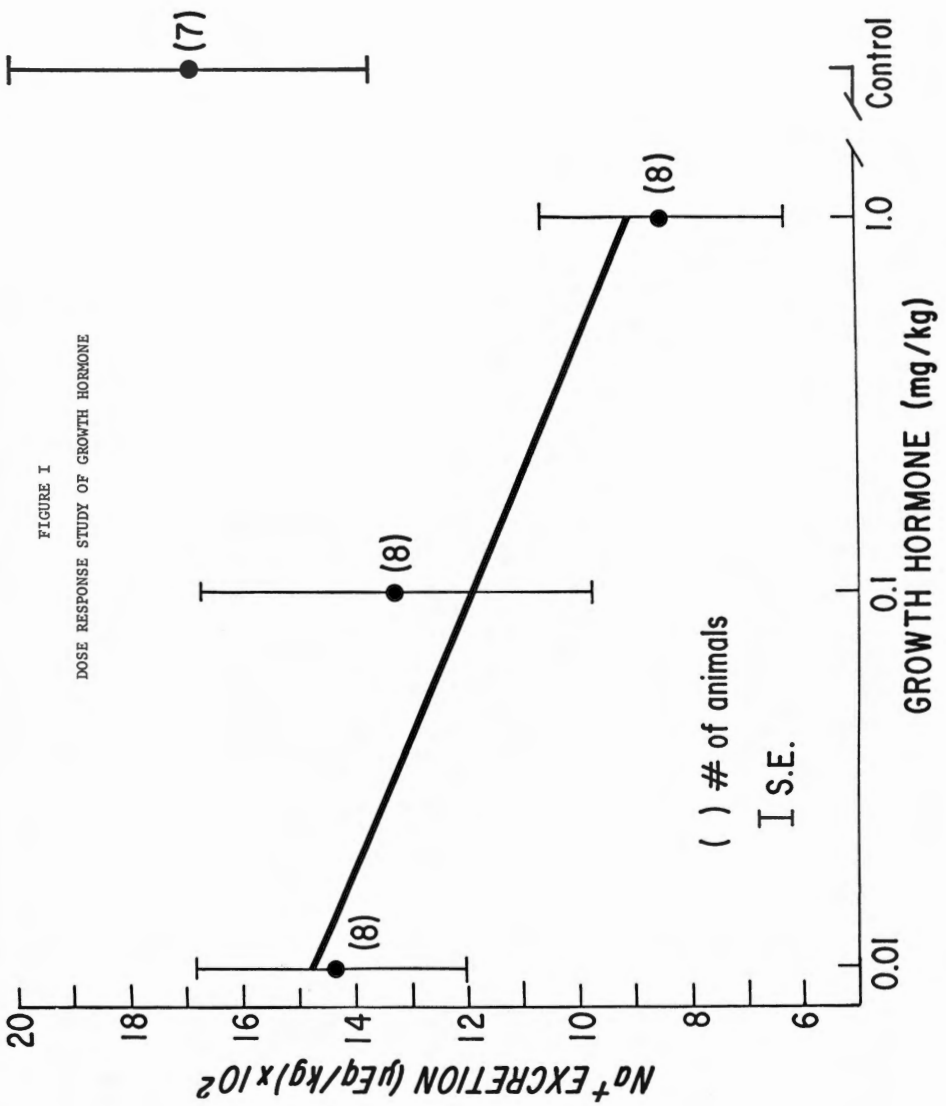


FIGURE I

DOSE RESPONSE STUDY OF GROWTH HORMONE

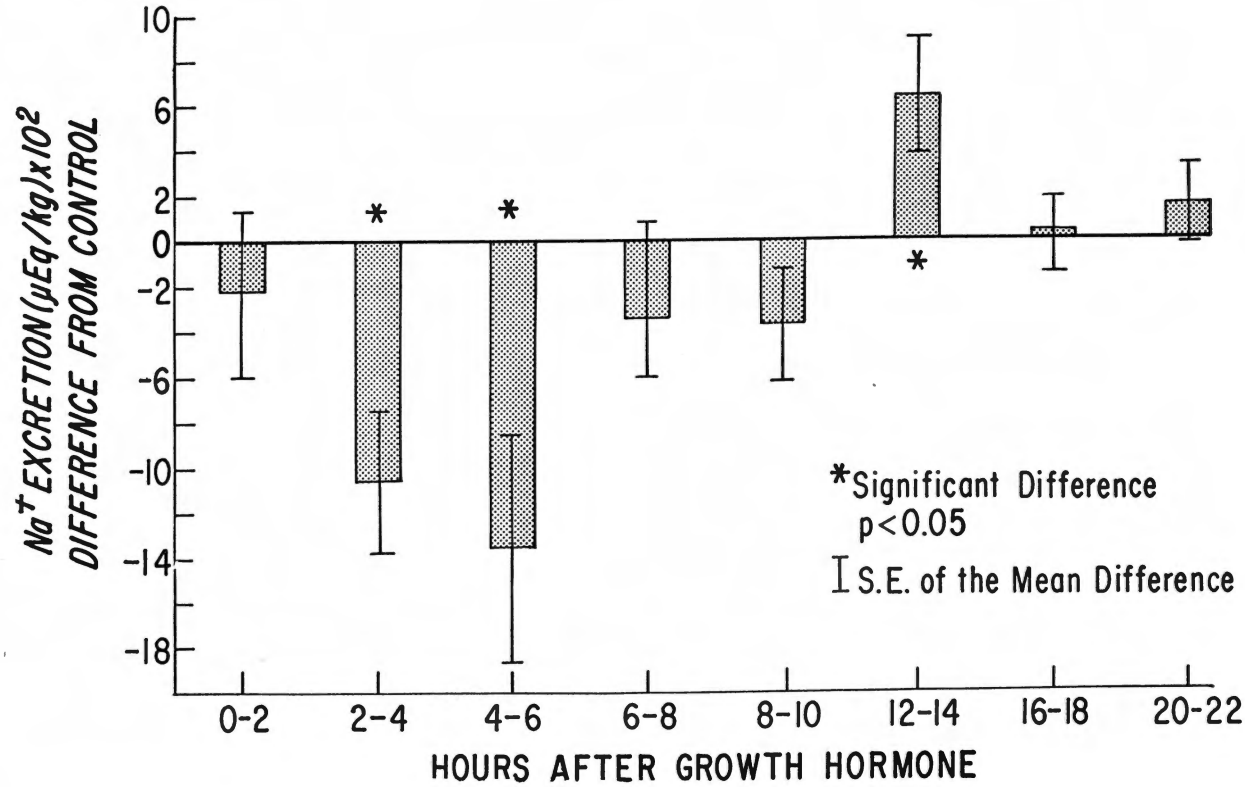
onset of antinatriuresis takes place after growth hormone administration. This is evidenced by the fact that no significant retention of sodium takes place in the 0-2 hour group. Evidence here is in agreement with Karlson's theory. These data are also useful in determining the duration of the antinatriuretic response.

Methodology III

Sixty Sprague-Dawley rats were adrenalectomized bilaterally five days before the experiment to eliminate the endogenous source of aldosterone. The animals were maintained on a 0.8 per cent NaCl, 0.1 per cent KCl solution for drinking water and "Purina" lab chow.

FIGURE II

TIME STUDY OF GROWTH HORMONE



The protocol of the experiment is the following:

0 time—All animals received a 17 ml/kg subcutaneous injection of 0.9 per cent NaCl.

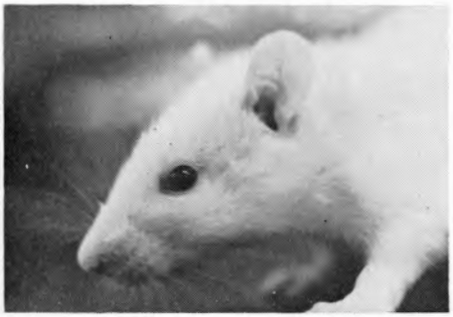
1½ hours—The animals were divided into two groups. One received a 400 ug/kg subcutaneous injection of actinomycin D (Merk, Sharp, and Dohme). The other group received the vehicle.

2 hours—One-half of each group received a 1 mg/kg intramuscular injection of bovine growth hormone while the other half received the saline vehicle. Thus four distinct groups (actinomycin D and growth hormone, growth hormone, actinomycin D, and the control) were determined.

4 hours—Start collection by the method of Kagawa, *et al.*[3]

6 hours—Collect urine

Sodium content was analyzed by



This is one of the white rats used in kidney research at The University of Iowa. Such animals are employed because they are relatively cheap and easy to handle.

This is the lab of Dr. Harold Williamson, renal pharmacologist at The University of Iowa Medical School. Here is where most of Ron Bach's research on growth hormone was conducted.

This building, the east wing of the Medical Laboratories, is where a great deal of medical research is conducted. It also serves as part of the teaching facilities for the medical school.

Ron Bach is in the process of weighing out a chemical agent for his study of growth hormone. Such delicate instruments as the above analytical balance (accurate to 1/10 milligram) were required to achieve significant results in his experiments concerning growth hormone.

means of flame photometry and calculated as uEq/kg. The results are shown in the following table.

Table I

Effect of Actinomycin D on the Sodium Retaining Action of Growth Hormone

Treatment	*Total Sodium Excretion* (uEq/kg/2 hrs)
Growth Hormone	489*
Control	818
Actinomycin D and Growth Hormone	875
Actinomycin D	923
Coefficient of Variability	44.8%

*Values for each treatment represent the means of twelve animals. Any two means joined by a vertical line are not significantly different. Any two means not so joined are significantly different (5 per cent level).

Conclusion:

The level of sodium excretion in the "actinomycin D and growth hormone" group is the same as the controls. This indicates that the blockade of growth hormone activity was accomplished by inhibiting the synthesis of RNA. Therefore, the evidence is found to be in support of Karlson's theory.

Summary

The purpose of this project was to investigate the mechanism of the antinatriuretic action of growth hormone *in vivo*. It was proposed that this mechanism might be mediated in the manner theorized by Karlson.[1] Both growth hormone's delayed onset of action after administration and the blockage of its action by actinomycin D, an RNA inhibitor, give support to this theory. Therefore, it appears that the mechanism of the antinatriuretic action of growth hormone *in vivo* could be due to enzyme synthesis *de novo*.

FOOTNOTES

[1] P. Karlson, *Perspect. Biol. Med.*, 6, 202 (1963).

[2] Knobil, E. and Hotchkiss, J., *Am. Rev. Physiol.* 26, 47 (1964).

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