Influence of Exogenous Growth Hormone on Endogenous Growth Hormone Release.* (31384)

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While a feed-back mechanism has been established for the control of some anterior pituitary secretions by specific target gland secretions, for growth hormone such a mechanism is still lacking. This pituitary hormone exerts its action not on a specific target gland or organ, but on the intermediary metabolism of carbohydrates, lipids and proteins. Thus acute insulin-like action(1), reduced glucose tolerance(2), increase in fat mobilization(3), nitrogen retention(4) and hypoaminoacidemia(5) have been reported after growth hormone administration.

The widespread action exerted on intermediary metabolism strengthens the possibility that, in contrast to other pituitary trophic hormones, the secretory rate of growth hormone may be influenced by one or more of the effects elicited on the intermediary metabolism. Recent findings of Knopf *et al* (6) are in keeping with this conception.

In the present study we investigated whether marked fat mobilization observed after growth hormone treatment might modify the release of growth hormone from the piuitary induced by insulin hypoglycemia.

Thus pituitary content of growth hormone was determined in rats submitted to insulin hypoglycemia, with or without pretreatment with growth hormone. Growth hormone was administered in order to increase the plasma levels of non-esterified fatty acid (NEFA).

The adipokinetic effect of growth hormone was preferentially chosen as index of metabolic action, since it has been shown that the stimuli to growth hormone secretion reflect the inadequacy of available carbohydrates and the need for sources of energy other than carbohydrates, namely fatty acids(7-9).

Materials and methods. Growth hormone was measured in pituitaries of Sprague-Dawley male rats (120-140 g b.w.), fasted for 18 hours, and divided into groups of 8 animals each, as follows:

a) Untreated

b) Treated on the 12th hour of fasting with bovine growth hormone‡ (1 mg/100 g body weight, i.p.), and killed on the 18th hour.

c) Treated on the 18th hour with insulin (1 U/kg body weight, i.p.) and killed after 1 hour.

d) Treated both on the 12th hour with bovine growth hormone (1 mg/100 g body weight, i.p.) and on the 18th hour with insulin (1 U/kg body weight, i.p.) and killed after 1 hour.

The growth hormone was determined according to the procedure previously reported (10).

A blood sample was obtained from each animal for evaluation of plasma NEFA levels, according to Dole(11), and of glucose levels, according to Hugget and Nixon(12).

Results. As shown in Table I the animals treated with growth hormone (GH) on the 12th hour of fasting and killed after 6 hours (Group B), showed a slightly increased GH pituitary content as compared with untreated fasting animals (Group A). Growth hormone administration also increased blood glucose and plasma NEFA levels. Administration of insulin to rats fasted for 18 hours and killed after 1 hour (Group C) induced a marked depletion of pituitary growth hormone content and decreased blood glucose and plasma NEFA levels.

As a result of the combined treatment with GH on the 12th hour of fasting and with insulin on the 18th hour (sacrified after one

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‡ Bovine growth hormone ($GH\text{-}B_9$) was kindly supplied by the Endocrinology Study Section (NIH).

TABLE I. Effect of Insulin Hypoglycemia on Growth Hormone Pituitary Content, Plasma Blood Glucose and NEFA Levels of Rats Pretreated or Not with Bovine Growth Hormone.

Groups	Treatments: Bovine growth hormone (BGH)	Treatments: Insulin (I)	Growth hormone pituitary content (*μg/GH/mg) pituitary—(fiducial limits in parentheses)	Plasma NEFA (†μEq/ml) (mean ± S.E.)	Blood glucose (mg/100 ml) (mean ± S.E.)
A	—	—	84.7 (75.7-107.1)	.715 ± .048	66 ± 3.3
B	(1 mg/100 g b.w. i.p.)	—	91.9 (86.6- 97.7)	.918 ± .066	86 ± 12.3
C	—	(1U/kg i.p.)	25.1 (18.3- 33.2)	.272 ± .039	30 ± 2.7
D	(1 mg/100 g b.w. i.p.)	(1U/kg i.p.)	62.3 (50.1- 73.6)	.504 ± .065	29 ± 1.6
E	Basal values in non-fasting rats		92.9 (78.9-102.5)	.430 ± .058	88 ± 7.2

* Growth hormone pituitary content—significance among groups—A–C $p < .001$. D–C $p < .001$.

† Plasma NEFA levels—significance among groups— A–B $p < .05$. D–C $p < .02$.

hour) (Group D) pituitary GH content appeared only slightly reduced when compared to fasting untreated rats; blood glucose levels were very low, while plasma NEFA levels were in the range of the values found in non-fasting untreated rats (0.504 μEq/ml *vs.* 0.430 μEq/ml).

Discussion. Growth hormone content of the pituitary of rats after 18 hours fasting appears to be slightly reduced when compared with non-fasting rats. This finding is in agreement both with the results reported by Friedman and Reichlin(13) who observed a decline of pituitary growth hormone in rats only after 24 hours starvation, and with those of Roth *et al*(7) who observed in the human a progressive rise in level of the hormone in plasma only after 20 hours fasting. Thus values of growth hormone in rats fasted for 18 hours may be reasonably taken as reference for the other experimental groups.

The administration of growth hormone, which in itself does not modify the growth hormone content in the pituitary, causes, as was expected, a remarkable increase of NEFA levels(3) and restores blood glucose levels to values similar to those present in non-fasting animals. While in untreated animals insulin-induced hypoglycemia elicits a striking depletion of pituitary growth hormone(14,15), a hypoglycemic stimulus of the same magnitude is less effective in rats pretreated with growth hormone. Thus the reduction of blood glucose levels to values which usually stimulate growth hormone release(7) does not completely maintain its effectiveness after administration of growth hormone. Exogenous growth hormone is therefore capable of inhibiting endogenous growth hormone release, suggesting the existence for this pituitary hormone, of an "auto" feed-back mechanism, as has already been shown for ACTH(16).

It seems probable that this suppressive action of exogenous growth hormone on growth hormone secretion may result from the enhanced mobilization of fatty acids present in growth hormone-treated animals.

The inadequacy of available carbohydrates and the need for sources of energy other than carbohydrates, namely non-esterified fatty acids(17), rather than the absolute hypoglycemia, seem to be the physiological state leading to stimulation of growth hormone secretion. Whenever an oxidizable substrate like fatty acid is required, either because of carbohydrate deficiency or because of a preferential demand for lipids as metabolic substrates, an enhanced secretion of growth hormone from the pituitary could meet this request. In the present experiments (with plasma NEFA levels significantly higher in GH-treated than in control rats), the influence exerted by the availability of plasma lipids on insulin-induced growth hormone release was examined. The postulated suppressive action of high plasma NEFA levels on the release of growth hormone induced by

insulin hypoglycemia also seems supported by the observations that in obese subjects, in whom fasting NEFA levels are consistently elevated(18) and growth hormone levels are low(19), further fasting or hypoglycemia fails to increase the fasting value of growth hormone(19). The close interrelationship between NEFA plasma levels and the secretion of growth hormone deserves close attention even if, in this regard, other indices of the metabolic action of growth hormone are investigated.

Summary. Pituitary content of growth hormone was determined in rats submitted to insulin hypoglycemia, pretreated or not treated with bovine growth hormone. Exogenous growth hormone was capable of inhibiting endogenous growth hormone release, suggesting the existence of an "auto" feed-back mechanism. A close interrelationship was also observed between plasma NEFA levels and growth hormone secretion.

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Electrophoretic Behavior of Serum Amylase in Various Mammalian Species.* (31385)

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At least 30 enzymes are now recognized to exist in multimolecular forms. Such polymorphism may reflect differences in the evolutionary development of certain functionally related proteins. Attempts have been made to extend the concept of enzyme multiplicity to include amylase in human serum, but these have met with only limited success to date (1,2). It seems reasonable to presume that the starch-hydrolyzing activity in the serum of man as well as other species is derived from various tissues which have been demonstrated to contain amylase. With a view toward broadening our fundamental knowledge of the

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