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THE 20,000-DALTON STRUCTURAL VARIANT OF HUMAN GROWTH HORMONE: LACK OF SOME EARLY INSULIN-LIKE EFFECTS

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SUMMARY: In contrast to the major form of human growth hormone the 20,000-dalton (20K) variant of the hormone produced no decrease in either serum glucose or free fatty acids one hour after injection into fasted, hypophysectomized rats. Furthermore, the variant caused no rise in serum free fatty acids after 5 hours. In vitro experiments utilizing epididymal adipose tissue from hypophysectomized rats indicated that 20K was unable to accelerate glucose utilization as measured by glucose uptake and CO_2 formation. The data show that this form, even though growth promoting, lacks some of the metabolic properties attributed to growth hormone. We conclude that an insulin-like effect is not necessarily a prerequisite for growth promoting activity.

A naturally occurring structural variant of human growth hormone was found to differ from the major form of the hormone by having some 15-20 fewer amino acid residues (1). The molecular weight of the newly recognized variant was estimated to be near 20,000 whereas the major form (hGH) has a value of 22,000. There was no indication that the form was a proteolytic modification of hGH. The growth promoting properties of the 20,000-dalton form (20K) was found (1) to be essentially the same as that of hGH. The work to be reported here was carried out to determine whether the structural modifications of 20K altered certain metabolic activities attributed to hGH even though the growth activity was uneffected. Because the insulin-like properties of growth hormone are well documented by both in vivo and in vitro assays (2, 3), we felt these would be important tests to do with 20K.

MATERIALS AND METHODS

Male, Long Evans hypophysectomized rats (130-160 g) that had been maintained on a high sucrose, fat-free diet (NCI, Indiana) were fasted overnight for 18 hours and injected intraperitoneally on the next morning with either 50 or 100 µg of hormone (in 0.5 ml of saline) depending on the experiment (See Tables).

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All samples were administered on the basis of protein (4). At the desired time (½, 1 or 5 hours) the rats were decapitated and blood collected. Control rats were injected with 0.5 ml of saline.

The 20,000-dalton variant (20K) was prepared as described previously (1). The growth promoting activity (5) was 1.7 ± 0.3 IU/mg (mean ± SEM). The purified hGH was prepared by DEAE-cellulose chromatography, a process that removes the hyperglycemic-hyperinsulinemic factor (6). The growth promoting activity was 2 ± 0.2 IU/mg (mean ± SEM).

Serum levels of free fatty acids were titrated by the method of Dole (7); glucose was measured by the o-toluidine method (8); and glycerol levels were assayed after deproteinization (5 min in boiling water) with an ATP regenerating system (9). Serum insulin was determined by the double antibody technique (10). Production of $^{14}CO_2$ was measured after incubating epididymal adipose tissue in Krebs-Ringer buffer (pH 7.5) which contained 1% bovine serum albumin and which was gassed with O_2-CO_2 (95%-5%). The procedure was essentially as described by Rodbell (11). Glucose uptake was determined by measuring changes in glucose concentration (12) in the same medium with the glucose oxidase method (9) after 1 hour incubation at 37°C.

RESULTS

Table I summarizes the time course of the *in vivo* effects that purified human growth hormone (hGH) and the 20,000-dalton variant (20K) had on serum

Table I
Serum levels of glucose and free fatty acids (FFA) in fasted hypophysectomized rats at various times after injection of hGH and 20K

	TREATMENT	DOSE mg/rat	SERUM GLUCOSE (Mean ± SEM) mg/dl	Δ (%)	P	SERUM FFA (Mean ± SEM) (μEq/ml)	Δ (%)	P
I.	½ h*							
	SALINE	--	66.0 ± 3.2	--	--	0.719 ± 0.021	--	--
	hGH	0.05	57.5 ± 4.2	--	NS	0.419 ± 0.035	-42	<0.005
	20K	0.05	62.0 ± 3.2	--	NS	0.652 ± 0.019	-9	<0.025
II.	1 h*							
	SALINE	--	78.0 ± 5.3	--	--	0.786 ± 0.074	--	--
	hGH	0.05	52.6 ± 4.9	-33	<0.01	0.552 ± 0.074	-30	<0.05
	SALINE	--	65.5 ± 2.5	--	--	0.722 ± 0.024	--	--
	20K	0.05	57.0 ± 3.1	--	NS	0.759 ± 0.052	--	NS
III.	5 h*							
	SALINE	--	46.0 ± 3.0	--	--	0.639 ± 0.021	--	--
	hGH	0.05	48.8 ± 1.4	--	NS	0.892 ± 0.046	+40	<0.005
	SALINE	--	53.7 ± 2.3	--	--	0.726 ± 0.065	--	--
	20K	0.05	58.7 ± 4.2	--	NS	0.734 ± 0.063	--	NS

*Time Post-Injection.
Data were analyzed with a Student's t-test for random sampling. (NS = No Significant Difference). Values represent the average of 7 animals except for the 1 hour experiment. There the values are the means of 3 experiments obtained with 24 animals each for 20K and hGH and 30 animals for the saline control.

Table II
Serum glycerol levels in fasted hypophysectomized rats 1 hour after injection of purified human growth hormone

TREATMENT	DOSE (mg/rat)	SERUM GLYCEROL (Mean ± SEM) µmol/ml	Δ (%)	P	SERUM GLUCOSE (Mean ± SEM) mg/dl	Δ (%)	P
Experiment I							
SALINE	--	0.295 ± 0.018	--	--	--	--	--
hGH	0.1	0.217 ± 0.0178	-26.5	<0.01	--	--	--
Experiment II							
SALINE	--	0.349 ± 0.024	--	--	79.9 ± 2.4	--	--
hGH	0.1	0.231 ± 0.022	-34	<0.005	45.3 ± 7.2	-43	<0.005

Seven animals per group in each experiment. Data were analyzed with a Student's t-test for random sampling.

free fatty acids and glucose levels in fasted, hypophysectomized rats. It can be seen that the treatment with hGH caused a significant lowering of the blood sugar at 1 hour but not at ½ hour. After 5 hours the glucose returned to a normal value. Serum levels of insulin were determined and the decrease in glucose at 1 hour was not accompanied by an increase in insulin. These data are not shown in the table since they were negative results and similar conclusions have been made by others (2). The concentrations of free fatty acids (FFA), determined on the same samples of serum, showed a drop at both ½ hour and 1 hour and a rise at 5 hours. That the decrease in free fatty acids produced by hGH was associated with a decrease in glycerol is shown in Table II. Note that again a lowering of glucose was seen 1 hour after injection of hGH.

In similar experiments 20K behaved quite differently. The hormone produced no early decrease in glucose levels. There was only a slight lowering of serum free fatty acids at ½ hour and no change at 5 hours. Serum insulin was uneffected at ½ hour and 1 hours.

The absence of an insulin-like activity when 20K was tested *in vivo* was confirmed by an *in vitro* study. The effects that 20K, hGH and insulin had on production of $^{14}CO_2$ and uptake of glucose by the rat epididymal fat pad are shown in Table III. It can be seen that hGH and insulin produced significant increases of glucose utilization whereas 20K was without effect.

Table III

Effect of hGH and 20K on CO_2 production and glucose uptake by adipose tissue of fasted hypophysectomized rats

HORMONES	CONCENTRATION (µg/ml of medium)	CO_2 PRODUCTION (Mean ± SEM) (dpm/mg/h)	Δ (%)	P	GLUCOSE UPTAKE (Mean ± SEM) (nmol/100 mg/h)	Δ (%)	P
CONTROL	--	43.1 ± 4.4	--	--	429 ± 37.8	--	--
hGH	0.2	86.7 ± 11.3	+101	<0.005	640 ± 50.6	+49	<0.005
20K	0.2	47.9 ± 5.3	NS		437 ± 25.3	NS	
INSULIN	0.05	100.7 ± 12.4	+134	<0.005	723 ± 64.9	+69	<0.01

Eight pieces of epididymal fat tissue were used per treatment. Total incubation volume was 1 ml. NS = No Significant change. Data were analyzed with a Student's t-test for random sampling.

DISCUSSION

The ability to lower blood levels of glucose and free fatty acids within 1 hour has been considered an intrinsic property of growth hormone (13). We were surprised therefore to find that 20K produced neither of these effects even though this variant is as good a growth promoting substance in rats as is hGH. The substance stimulated both an increase in body weight and widening of the epiphyseal plate. We have to conclude therefore that the insulin-like effects are not requirements for production of growth and that further study of the actions of 20K may help define metabolic actions needed for growth. In this respect it will be most interesting to determine if 20K can generate the somatomedins, growth hormone-dependent substances which are insulin-like but which have not been shown to produce body growth (14).

We confirmed the insulin-like activity of hGH with a preparation that was free of *in vitro* lipolytic effects on rat adipose tissue (15) and lacked hyperglycemic and hyperinsulinemic activity in the dog (6). To our knowledge the dosage of 50 µg/rat that was used to test for early insulin-like effects is lower than reported in the literature. When we increased the dosage to 100 µg/rat, death by hypoglycemic shock occurred in some of the animals. The mechanism through which hGH affects the serum levels of FFA at early time is not yet fully understood. We have measured serum levels of insulin during the acute decrease

of serum glucose and FFA without noticing any significant variation. This is in agreement with the observation of Swislocki and Szego (2) who were able to produce decrease of FFA in depancreatized rats after injection of growth hormone.

It is well known that in adipose tissue the amount of fatty acids released from triglycerides is a dynamic process resulting from the equilibrium of lipase-stimulated lipolysis and fatty acid reesterification. An increased glucose utilization due to the hGH administration could have increased the intracellular levels of glycerol phosphate and consequently enhanced reesterification with decreases of serum free fatty acid. In such a case the serum levels of glycerol should have remained unchanged since reesterification requires glycerol phosphate present in the cell. Table II clearly indicated that serum glycerol is decreased and therefore the rate of lipolysis of the adipose tissue must have been decreased by hGH administration. In addition the data of Table I shows that half an hour after hGH administration, a decrease in free fatty acid takes place before any significant change of serum glucose occurred.

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