Vol. 217, No. 1, 1995 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
December 5, 1995 Pages 28-33

22-kDa AND 20-kDa hGH ISOFORMS SHOW DIFFERENTIAL EFFECTS WHEN ASSAYED IN 3T3-F442A AND 3T3-F442A/C4 ADIPOCYTES

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Received September 27, 1995

Summary: To know whether 22.0-kDa human growth hormone (hGH22K) and the 20.0-kDa isoform (hGH20K) show different activities, we used 3T3-F442A and 3T3-F442A/C4 cells to evaluate their adipogenic and metabolic effects. Both isoforms had similar adipogenic and insulin-like activities. Lipolytic and diabetogenic effects of hGH22K were, respectively, 12.5 and 1.7-fold higher than those found for hGH20K. The 3T3-F442A/C4 clone was not responsive to insulin-like and diabetogenic effects of hGH. The results suggest that adipogenic and lipolytic effects of hGH are mediated by mechanisms different from those involved in insulin-like and diabetogenic activities. © 1995 Academic Press, Inc.

In vivo, human growth hormone (hGH) exerts a wide variety of effects. Some of them are indirect and mediated by IGF-I (1). Others are direct, such as regulation of glucose metabolism in adipocytes (2). Short-term stimulation with hGH increases glucose transport and lipid synthesis (insulin-like effect). Chronic treatment inhibits insulin-stimulated glucose transport (diabetogenic effect), and induces glycerol release (lipolytic effect)(2). The mechanisms mediating such opposite effects are still unknown.

Human GH preparations comprise heterogeneous isoforms that could have different functions (3). Major isoforms (hGH22K and hGH20K) result from alternative splicing of a mRNA precursor (4), and possible differences in their activities are uncertain, since their somatogenic and lactogenic activities seem not to be different, but their effects on metabolism are quite variable (5,6,7).

To know whether hGH22K and hGH20K show different biological activities, we studied their effects as stimulators of adipocyte differentiation, and on glucose and lipid metabolism, using 3T3-F442A cells (8,9) and 3T3-F442A/C4 cells (10) as bioassay systems. The isoforms showed

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0006-291X/95 $12.00
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similar adipogenic activity, but lipolytic and diabetogenic effects of hGH22K were, respectively, 12.5 and 1.7-fold higher than those found for hGH20K.

MATERIALS AND METHODS

Materials: High glucose (4.5 g/L) and low glucose (1.0 g/L) Eagle's medium modified by Dulbecco-Vögt (DMEM) were from Gibco (Grand Island, NY), calf serum was from HyClone Labs. (Logan, Utah). Cat serum was obtained by bleeding of adult domestic cats. hGH isoforms were provided by the NIDDK. D-[^{14}C]-glucose (55.4 mCi/mmol) was from New England Nuclear (Boston, MA). All other reagents were analytical grade.

Cell culture. 3T3-F442A, kindly provided by Dr. H. Green (Harvard Medical School), or 3T3-F442A/C4 preadipocytes, kindly provided by Dr. Walid Kuri-Harcuch, were plated and maintained as previuosly described (10). To assay hGH adipogenic activity, one day confluent cultures were fed with DMEM plus 1.7%(v/v) cat serum, 0.25%(v/v) calf serum, 5 µg/ml insulin, 5 µg/ml transferrin, 1 x10^{-9} M L-T_3, 1.0 µM d-biotin, 40 µM β-mercaptoethanol, 0.01 ng/ml EGF (10,11) and hGH, as indicated. For metabolic experiments, cells were grown with adipogenic medium (10); when adipose conversion was about 80-90%, cells were incubated in low glucose serum-free DMEM plus 2%(w/v) bovine serum albumin (BSA) for 24h before hGH activity testing.

Enzyme assay. Adipose conversion was quantitated by glycerophosphate dehydrogenase (EC 1.1.1.8) activity (12,13).

Lipid accumulation. Short- and long-term effects of hGH were assayed by incorporation of [^{14}C]-glucose (0.2 µCi/ml) into total lipids, for 4 or 48 hours respectively (9).

Lipolysis. Lipolysis was quantitated as glycerol release into culture medium (14) using a commercial kit (Sigma, Cat. No. 337).

Statistics. Data were expressed as the mean ± SE from duplicate dishes for 4 experiments, and evaluated by Student's T test. $P<0.05$ was considered significant.

RESULTS

Green et al., showed that hGH22K and hGH20K had similar adipogenic activity in 3T3-F442A cells (8). Since 3T3-F442A/C4 preadipocytes show a 4-5 fold higher responsiveness to hGH (10), we assayed both isoforms in one day confluent cultures of 3T3-F442A/C4 cells. Both hormones stimulated adipose conversion in a dose dependent manner. Similarly to previous results (8), the hGH22K concentration necessary to promote half of the maximal response (ED_{50}) was about 2.0 x 10^{-10} M (4-5 ng/ml) (Fig. 1); ED_{50} for hGH20K was similar (Fig. 1). Both isoforms led to maximal response at about 1.0 x 10^{-9} M (20 ng/ml) (Fig. 1).

When insulin-like activity associated to hGH22K and hGH20K was studied, we found that 3T3-F442A adipocytes, but not 3T3-F442A/C4 adipocytes, presented a typical insulin like response after stimulation with 500 ng/ml of both isoforms (Table I). Glucose incorporation into total lipids was increased 36% when cultures were stimulated with hGH22, and 55% when treated with hGH20K. Differences in stimulatory effects between both hormones were not significant ($p> 0.05$). Next, we assayed diabetogenic activity of hGH22K or hGH20K in 3T3-F442A and 3T3-F442A/C4 adipocytes. Table II shows that 50 ng/ml of hGH22K or hGH20K suppressed lipid synthesis in 3T3-F442A cell cultures, but not in the 3T3-F442A/C4 subclone. Whereas hGH22K

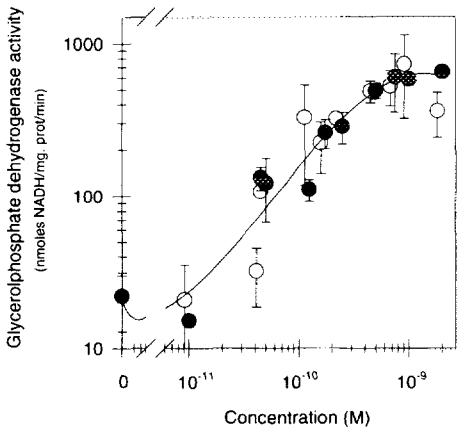

Figure 1. Stimulation of the adipose conversion of 3T3-F442A/C4 preadipocytes by hGH22K or hGH20K. One day confluent 3T3-F442A/C4 preadipocytes were stimulated to differentiation with the indicated concentrations of hGH22K (○) or hGH20K (●). After seven days, cultures were extracted and adipose differentiation was quantitated by determining GPDH enzyme activity (see Materials and Methods).

inhibited lipid synthesis about 36.32% ($p< 0.05$), hGH20K only showed a weak diabetogenic activity (21.47%, $p<0.05$). The results demonstrate that hGH20K was 1.7 fold lower than hGH22K ($p<0.05$).

Finally, when 3T3-F442A adipocytes were used to assay lipolytic effects of hGH, hGH22K induced a significant release of glycerol at concentrations as low as 1 ng/ml ($p<0.05$). In contrast, hGH20K lead to lipolysis when 10 ng/ml or higher concentrations were used ($p<0.05$)

Table I. Insulin-like activity of hGH22K and hGH20K on 3T3-F442A and 3T3-F442A/C4 adipocytes

Added Hormone (ng/ml)		[^{14}C]-glucose incorporated into lipids (cpm/35-mm dish)	
		3T3-F442A/C4	**3T3-F442A**
0.0 (Control)		12,242 ± 1,129	8,392 ± 1031
hGH22K	50.0	12,455 ± 1,486	ND
	100.0	12,675 ± 242	ND
	500.0	13,198 ± 7	10,900 ± 611
hGH20K	50.0	13,894 ± 1,249	ND
	100.0	13,752 ± 349	ND
	500.0	13,554 ± 1,225	11,844 ± 128

Lipid accumulation is expressed as the incorporation of [^{14}C]-glucose into total lipids. ND, not determined. Stimulation of glucose incorporation was significant for both isoforms ($p<0.05$), but diferences between hGH22K and hGH20K activities were not significant ($p>0.05$).

Table II. Diabetogenic activity of hGH22K and hGH20K on 3T3-F442A and 3T3-F442A/C4 adipocytes

Added hormone (ng/ml)	[^{14}C]-glucose incorporated into lipids (cpm/dish)	
	3T3-F442A/C4	**3T3-F442A**
0.0 (Control)	56,298 ± 4,250	55,212 ± 3,708
hGH22K		
50.0	59,491 ± 1,295	35,162 ± 1,558
100.0	56,395 ± 7,344	ND
500.0	55,735 ± 6,915	ND
hGH20K		
50.0	61,119 ± 434	43,359 ± 3,129
100.0	63,389 ± 3,280	ND
500.0	55,896 ± 0	ND

Lipid accumulation is expressed as the incorporation of [^{14}C]-glucose into total lipids. ND, not determined.

(Figure 2A, B). ED_{50} for hGH22K was 3-4 ng/ml and maximal response was obtained with 100 ng/ml, whereas ED_{50} for hGH20K was 12.5-fold higher (about 50 ng/ml) with a maximum effect at 1000 ng/ml (Figure 2B). Interestingly, although 3T3-F442A/C4 cells were characterized as a

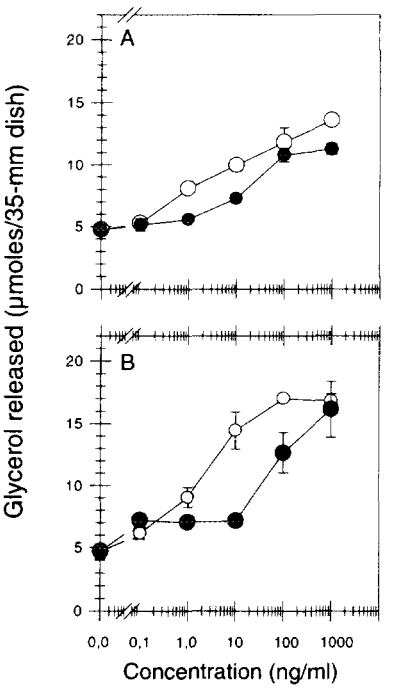

Figure 2. Glycerol realease induced by hGH22K or hGH20K on **(A)** 3T3-F442A/C4 and **(B)** 3T3-F442A adipocytes. Adipocyte monolayers were incubated in Krebs Ringer Bicarbonate buffer (pH 7.4) plus 4% (w/v) BSA, 1 mg/ml glucose, and the indicated hormone concentrations; after 48 h, glycerol released was quantitated. (○) hGH22K; (●) hGH20K.

highly responsive clone to hGH adipogenic activity (10), we found that 3T3-F442A adipocytes were more sensitive to hGH lipolytic activity (compare Figure 2A and B). In conclusion, our results demonstrate that hGH22K is 12.5-fold more lipolytic than hGH20K.

DISCUSSION

Human GH heterogeneity has received increased attention during the last few years (15) since existence of isoforms raised the possibility that each could be playing specific functions. Major hGH isoforms constitute 85% (hGH22K) and 15% (hGH20K) of total hGH synthesized by pituitary (16), but results about some of their biological effects are uncertain. Thus, we looked for possible differences in their activity taking advantage of the availability of 3T3-F442A and 3T3-F442A/C4 cells, which are responsive to hGH effects on differentiation and metabolism (2, 8, 10).

Here, we demonstrate that hGH20K and hGH22K exert differential effects in terminally differentiated 3T3-F442A or 3T3-F442/C4 adipocytes, but not in preadipocytes. First, when adipogenic activity of these isoforms was assayed in preadipocytes, both hGH isoforms showed similar ED_{50}, in accordance with those results showing similar activities for both isoforms in 3T3-F442A cells (8). 3T3-F442A/C4 cells, enabled us to assay hGH adipogenic activity with higher sensitivity, but we did not found any differential adipogenic effect of the isoforms. Second, whereas hGH20K and hGH22K had similar effectiveness when used to stimulate insulin-like effects, we found that hGH20K was 1.7-fold less diabetogenic, as reported by Foster et al (17). Our results show, for the first time, that hGH20K has 12.5-fold lower lipolytic activity than hGH22K.

The differential effects found in this study could be explained through differences in the hGH20K/GH receptor (GHR) interaction. It is assumed that hGH20K and hGH22K have similar 3-D structures, since binding studies indicated similar affinities to rabbit liver receptors (18). However, it has been reported that hGH20K is poorly recognized by polyclonal antibodies against hGH22K (19), and it is also described that GH receptors have 3-10 times lower affinity to hGH20K than to hGH22K (20). Thus, we suggest that the sequence deleted in hGH20K could be essential to support some biological activities of the hormone, in spite of those results which show that mutation of those residues non-present in hGH20K has little or non-consequence in binding (21,22). Reinforcing this view, Keda et al. reported that an oligopeptide comprising aminoacids 31-44 of hGH is 500-fold more lipolytic than hGH22K in rat epididymal fat (23). The results could also be explained through the presence of specific receptors, such as those found in IM-9 lymphoblasts, which exhibit high affinity for hGH20K (24).

Interestingly, we found that 3T3-F442A/C4 cells did not respond to insulin-like and diabetogenic effects of hGH, and they showed a higher sensitivity to adipogenic (10) and a lower lipolytic response to hGH than 3T3-F442A cells. Mechanisms underlying responsiveness of 3T3-F442A/C4 cells to hGH are unknown. Is quite possible that 3T3-F442A/C4 cells underwent alterations in signal transduction pathways during experiments leading to their isolation (10,25); however, results in this paper and the accumulated evidence suggest that insulin-like and diabetogenic effects are separate but related events (26,27). In contrast, lipolytic activity seems to be independent from insulin-like and diabetogenic activities (28,29). Further studies, with 3T3-F442A and 3T3-F442A/C4 cells as bioassays, will give new insights in the mechanisms of hGH action.

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