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Growth hormone and insulin-like growth factors I and II produce distinct alterations in glucose metabolism in 3T3-F442A adipocytes

(glucose oxidation/lipid accumulation/somatomedin C/multiplication-stimulating activity/recombinant DNA-derived insulin-like growth factor I)

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ABSTRACT **In 3T3-F442A adipocytes, human growth hormone (hGH) stimulates glucose oxidation in 4 hr. A maximal increase is evident at hGH concentrations of 50–100 ng/ml and rarely exceeds 50% above control. The stimulation is transient; after 48 hr of incubation with GH, glucose oxidation is significantly suppressed to 35% below control values. In view of the concept that insulin-like growth factors (IGF) may mediate the effects of GH, we compared the effects of hGH (500 ng/ml) and several preparations of IGF on glucose metabolism in 3T3 adipocytes. After 4 hr of incubation, IGF-I from human plasma stimulated glucose oxidation in a dose-related manner, producing a 10-fold increase at 50 ng/ml. Methionyl-IGF-I produced by recombinant DNA techniques was 85–88% as effective as IGF-I. IGF-II stimulated glucose oxidation 3-fold at 50 ng/ml after 4 hr of incubation. In contrast to the suppression observed with hGH after 48 hr, all three of the IGF preparations stimulated glucose oxidation after 48 hr of incubation and were as effective as they were after 4 hr. When each of the IGF preparations was tested (at 5 ng/ml) in combination with hGH, both the stimulatory and suppressive effects of GH were superimposed on the stimulation by the IGFs. Thus, the stimulatory properties of IGF differed from those of GH in that the maximum extent to which IGF increased glucose oxidation, compared with hGH, was as much as 20-fold greater. Furthermore, all of the IGF preparations stimulated glucose oxidation after 48 hr under conditions in which hGH suppressed glucose metabolism. Thus, it is unlikely that extracellular IGFs mediate the effects of hGH on glucose metabolism in 3T3-F442A adipocytes.**

It is widely recognized that pituitary growth hormone (GH) alters glucose metabolism (1). However, little is understood about the mechanism(s) by which GH exerts its effects. Our lack of understanding of GH actions is in part a reflection of the complexity of its effects on carbohydrate and lipid metabolism. For example, GH produces diabetes when it is chronically elevated in GH-sensitive animals such as dogs and *ob/ob* mice (1, 2). The GH-induced diabetes is believed to be associated with suppression of glucose utilization and interference with the effectiveness of insulin (1, 3). Physiological levels of GH produce qualitatively similar effects in normal animals (4–6). In contrast, an acute exposure to GH stimulates rather than suppresses glucose utilization in GH-deficient subjects (7, 8). Although the physiological importance of the stimulatory actions of GH is not clear, *in vitro* analysis of GH action has focused on these stimulatory effects because they are readily demonstrable in tissue from hypophysectomized rats. In contrast, *in vitro* analysis of the suppressive effects of GH on glucose metabolism has been difficult (9, 10) because the onset of the suppressive effects requires several days and *in vitro* preparations sensitive to GH (e.g., adipocytes) are not viable for extended periods.

Recently, we reported that cultured 3T3-F442A adipocytes could be used to study the metabolic effects of GH (11). The differentiation of 3T3-F442A fibroblasts to adipocytes has been shown to be GH-dependent (12), although GH is not required to maintain the adipocytes. We have found that, once converted, the 3T3 adipocytes are sensitive to both stimulatory and suppressive effects of human GH (hGH) on glucose metabolism (11). GH added directly to the 3T3 adipocytes produced a transient stimulation of the conversion of glucose to carbon dioxide or lipids during the first 4 hr of incubation *in vitro*. Longer exposures to GH (24–48 hr) resulted in a suppression of glucose metabolism, which was sustained for at least 7 days. These results indicate that pituitary GH has direct effects on glucose metabolism in the 3T3 adipocytes. Hence, they provide a useful *in vitro* system for analysis of fundamental aspects of GH action.

One issue that remains unclear is whether the effects of GH are produced by mediators such as the insulin-like growth factors (IGFs) (13–15). IGF-I and -II are known to stimulate glucose oxidation acutely in adipose tissue (13, 16). In the present study, we compared acute and delayed effects of pituitary GH to those of IGF-I from human plasma (hIGF-I; also called somatomedin C), bacterially produced methionyl-IGF-I (mIGF-I), and rat IGF-II (rIGF-II; also called multiplication-stimulating activity, MSA) on glucose metabolism in 3T3 adipocytes. In this report, we present evidence that the acute and sustained effects of GH and these preparations of IGF on glucose metabolism in the 3T3 adipocytes are qualitatively different. This makes it highly unlikely that the stimulatory and suppressive effects of GH on glucose metabolism are mediated by IGFs.

MATERIALS AND METHODS

Materials. 3T3-F442A cells were provided by H. Green (Harvard Univ.). Sera and media were purchased from GIBCO. hGH was prepared and provided by J. Kostyo (Univ. of Michigan, lot K120583A, A-type). hIGF-I was a gift from J. J. Van Wyk (Univ. of North Carolina). Bacterially produced mIGF-I (3000 units/mg) was provided by L. Fryklund (KabiVitrum, Stockholm, Sweden). rIGF-II isolated from Buffalo rat liver cell-conditioned medium was a gift from J. A. Florini (Syracuse Univ.). Crystalline porcine insulin was a gift from R. Chance (Eli Lilly), and dexamethasone was provided by W. Henckler of Merck.

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Abbreviations: IGF-I and -II, insulin-like growth factors I and II; mIGF-I, methionyl IGF-I; rIGF-II, rat IGF-II (multiplication-stimulating activity, MSA); hIGF-I, human IGF-I (somatomedin C); GH, growth hormone; hGH, human GH.

D-[^{14}C]Glucose was purchased from New England Nuclear. Bovine serum albumin (fatty acid-free) was purchased from Sigma. All other reagents were of the highest grade available.

Cell Culture. 3T3-F442A cells were grown to confluence in Dulbecco's modified Eagle's medium (DMEM) with 10% calf serum, were converted to adipocytes, and were maintained in DMEM with 10% fetal calf serum until 70–80% conversion was achieved as described (11). Prior to experimentation the cells were deprived of serum for 18–24 hr with DMEM (1 g of glucose per liter) containing 2% bovine serum albumin.

Glucose Oxidation. Cell suspensions were prepared with Joklik's medium with 2 mM EDTA (17) and were brought to a final concentration of 5×10^5 cells per ml in Krebs–Ringer bicarbonate buffer (pH 7.4) containing 1 mM glucose, 1% bovine serum albumin, and D-[^{14}C]glucose (0.1 μCi/ml; 1 Ci = 37 GBq). Hormones or vehicle were present as indicated in legends to figures. Incubation proceeded for 4 hr at 37°C under an atmosphere of 95% O_2/5% CO_2 and was terminated by injection of 1 M HCl into the medium. Evolved $^{14}CO_2$ was collected in phenylethylamine and assayed for radioactivity. Results are expressed as cpm per 10^6 cells. For the 48-hr studies, monolayers of cells were incubated for 44 hr in DMEM with glucose at 1 g/liter, 1% bovine serum albumin, and hormones or vehicle as indicated. Cells were then suspended and were incubated for 4 hr exactly as described above.

Statistics. Data were compared by Student's *t* test when two comparisons were made and by analysis of variance when multiple comparisons were made. A value of *P* less than 0.05 was considered significant.

RESULTS

Acute Stimulation of Glucose Oxidation by hGH and IGF. Pituitary hGH stimulated glucose oxidation 30–35% above control values after 4 hr of incubation (Fig. 1). This modest but significant response was the maximum achieved in most experiments and was evident at concentrations ≥ 50 ng/ml. Prolonging the incubation period to 8 hr (data not shown) did not increase the effectiveness of hGH at 500 ng/ml in stimulating glucose oxidation in 3T3 adipocytes. After 4 hr,

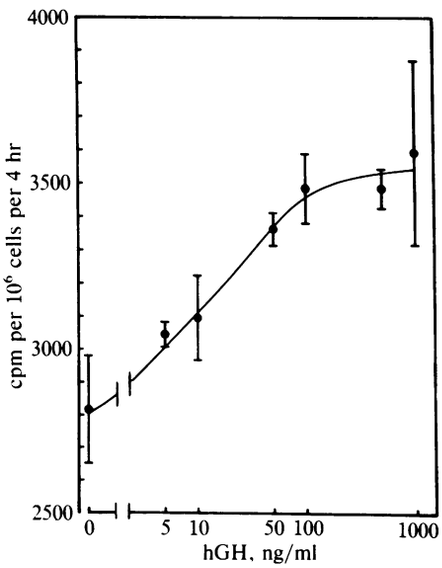

FIG. 1. Stimulation of glucose oxidation in 3T3-F442A adipocytes by 4-hr-total incubation with hGH. Each point represents the mean ± SEM of four observations in a single experiment. Similar results were obtained in four other experiments. Values were significantly greater than control at hGH concentrations of 50 ng/ml and above.

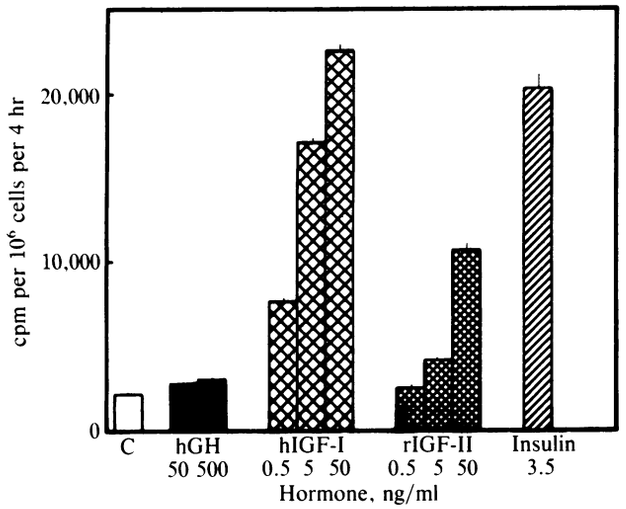

FIG. 2. Effect of 4-hr-total incubation with hGH, hIGF-I, rIGF-II, or insulin on glucose oxidation in 3T3-F442A adipocytes. Each bar represents the mean + SEM of four observations in a single experiment. Similar results were obtained in two other experiments. The stimulation by hormone was significantly greater than control (C) for all conditions except for rIGF-II at 0.5 ng/ml.

in contrast to GH, insulin (3.5 ng/ml) was able to stimulate glucose oxidation 2- to 10-fold (ref. 11; Fig. 2).

The stimulation of glucose oxidation by a variety of IGF preparations was compared to that of hGH after 4 hr of incubation with the 3T3 adipocytes (Fig. 2). hIGF-I derived from human serum produced a 10-fold stimulation of glucose oxidation at 50 ng/ml (Fig. 2), which was similar to the stimulation produced in these cells by insulin at 3.5 ng/ml. hIGF-I stimulated glucose oxidation 3-fold at a concentration of 0.5 ng/ml and 8-fold at 5 ng/ml. Thus, hIGF-I was significantly more effective than hGH in stimulating glucose oxidation. In the same experiment, rIGF-II also stimulated glucose oxidation at 5 and 50 ng/ml (Fig. 2) but was not effective at 0.5 ng/ml. Although the stimulation by rIGF-II at 50 ng/ml was only half that induced by the same concentration of hIGF-I, it was still 3 times that induced by hGH at 50 ng/ml.

Methionyl-IGF-I prepared by recombinant DNA techniques was compared to hIGF-I and rIGF-II. At 5 ng/ml, the bacterial mIGF-I was 85% as effective as hIGF-I in stimulating glucose oxidation (Table 1). The response achieved with mIGF-I at 50 ng/ml was 88% of that produced by insulin at 3.5 ng/ml (data not shown). Thus, although responses to the preparations of IGF varied, they all produced a greater

Table 1. Glucose oxidation in 3T3 adipocytes after a total incubation of 4 or 48 hr with hormones

IGF added, ng/ml	CO_2 production in final 4 hr of incubation, cpm per 10^6 cells	
	4-hr-total incubation	48-hr-total incubation
None	1982 ± 34	1136 ± 42
mIGF-I		
0.5	2688 ± 160	1802 ± 62
5.0	3784 ± 234	3002 ± 136
50.0	4294 ± 66	6412 ± 48
hIGF-I 5	4306 ± 128	4500 ± 130
rIGF-II 5	2368 ± 116	1770 ± 78

Values shown are means ± SEM of quadruplicate observations. Similar results were obtained in two other experiments.

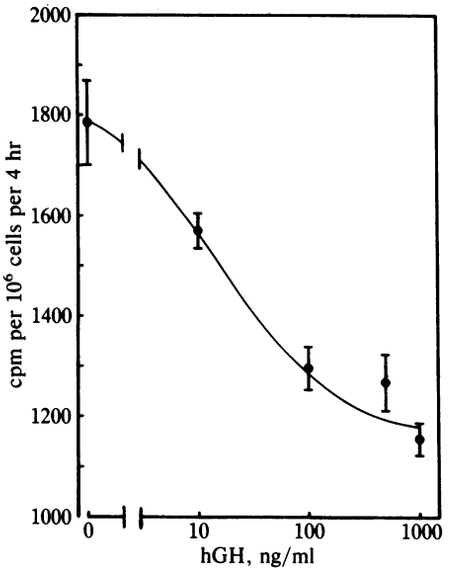

FIG. 3. Suppression of glucose oxidation in 3T3-F442A adipocytes by 48-hr-total incubation with hGH. Glucose oxidation was measured during the final 4 hr of incubation. Each point represents the mean ± SEM of four observations in a single experiment. Similar results were obtained in four other experiments. Values are significantly lower than control at hGH concentrations of 10 ng/ml and above.

stimulation of glucose oxidation in 4 hr than did the maximum concentration of hGH in the same cell suspensions.

Delayed Responses to hGH and IGF Differ. Suppression of glucose oxidation resulted from incubation of 3T3 adipocytes with hGH for 48 hr (Fig. 3). A maximum suppression was evident with hGH at 100 ng/ml. When the accumulation of [^{14}C]glucose into lipid, rather than glucose oxidation, was measured as an indicator of glucose utilization, a significant suppression (6%) was evident with hGH at 2 ng/ml after 48–72 hr and was maximal (35%) at 50 ng/ml (C.M.F. and J.S., unpublished data).

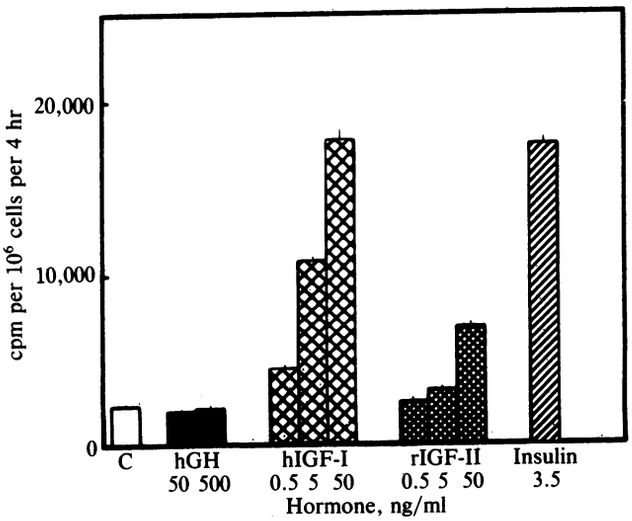

FIG. 4. Effect of 48-hr-total incubation with hGH, hIGF-I, rIGF-II, or insulin on glucose oxidation in 3T3 adipocytes. Glucose oxidation was measured during the final 4 hr of incubation. The cells were from the same preparation as those used in the experiment in Fig. 2. Each bar represents the mean + SEM of four observations. Similar results were obtained in two other experiments. The suppression by hGH and the stimulation by hIGF-I, rIGF-II, and insulin were significant except for rIGF-II at 0.5 ng/ml.

In marked contrast to hGH, all of the IGF preparations stimulated glucose oxidation after 48 hr (Fig. 4; Table 1). Thus, the stimulatory properties of the IGFs were not transient, although those of GH were. Furthermore, the stimulatory effects of each IGF preparation were similar in magnitude after 48 hr and 4 hr of incubation. The relative potencies of the IGFs in stimulating glucose oxidation after 48 hr were comparable to their relative potencies after 4 hr. hIGF-I was the most effective of the IGF preparations in stimulating glucose oxidation after 48 hr (Fig. 3), producing an almost 8-fold stimulation at 50 ng/ml. The same concentration of rIGF-II stimulated glucose oxidation only 3-fold. The bacterial mIGF-I was slightly less effective than hIGF-I and produced a 6-fold stimulation at 50 ng/ml (Table 1).

Effects of GH Prevail in the Presence of IGF. To examine further the dynamics of hGH and IGF actions on glucose metabolism in 3T3 adipocytes, we explored the combined effects of hGH and each of the various IGF preparations (Fig. 5). The IGFs were each tested at 5 ng/ml and were stimula-

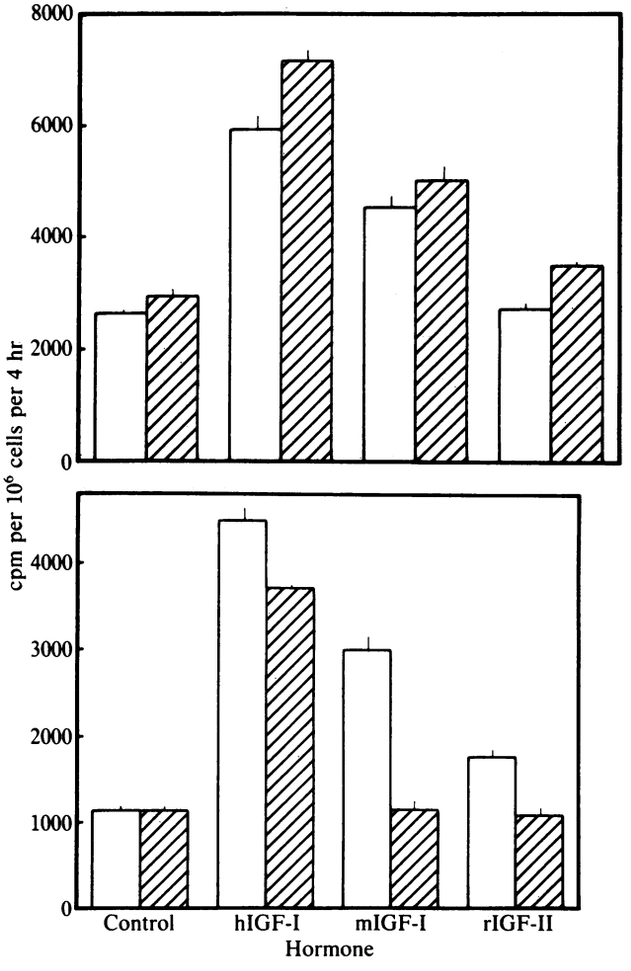

FIG. 5. Combined effects of hGH with hIGF-I, bacterial mIGF-I, or rIGF-II on glucose oxidation in 3T3-F442A adipocytes. hIGF-I, mIGF-I, and rIGF-II were tested at 5 ng/ml; hGH was tested at 500 ng/ml, as indicated by the hatched bars. Each bar represents the mean + SEM of four observations. (*Upper*) Effect of 4-hr incubation. Glucose oxidation in cells incubated with hGH was significantly greater than that in the respective controls for all conditions except with mIGF-I. (*Lower*) Effect of 48-hr-total incubation. Glucose oxidation was measured during the final 4 hr of incubation. The cells were from the same preparation as those used in the experiment in Table 1. Glucose oxidation in cells incubated with hGH was significantly lower than that in the respective controls except for those incubated without IGFs. Similar results were obtained in two other experiments.

tory after both 4 and 48 hr of incubation. After 4 hr of incubation, glucose oxidation in the samples incubated with hGH at 500 ng/ml was 10–29% higher than that in the respective samples incubated without hGH, whether or not IGF was present. This finding indicates that the acute stimulatory effect of hGH was superimposed on the effect of the IGF. After 48 hr of incubation, hGH suppressed glucose oxidation significantly in the presence of the IGFs. The suppression was evident in the stimulated cells, even though hGH alone did not alter glucose metabolism in the nonstimulated (control) cells in this experiment. Thus, the stimulatory and suppressive effects of hGH persisted in the presence of IGFs.

DISCUSSION

Although both hGH and the IGFs stimulated glucose oxidation in 3T3-F442A cells during a 4-hr incubation, their effects were qualitatively different. The stimulation produced by hGH never exceeded 50% above control values when maximal concentrations (e.g., 500 ng/ml, 23 nM) were tested. In contrast, at 50 ng/ml (7 nM), hIGF-I stimulated glucose oxidation 10-fold. Even rIGF-II, the least effective of the IGF preparations tested, stimulated glucose oxidation 2-fold at 5 ng/ml and elicited a 5-fold stimulation at 50 ng/ml. Bacterially produced mIGF-I was consistently about 85% as effective as hIGF-I. Insulin (3.5 ng/ml) produced the greatest stimulation (10-fold) when tested simultaneously with hGH or the IGF preparations. Hence, the maximum extent to which glucose oxidation could be stimulated by hGH was quite limited relative to the stimulation by the IGF preparations or by insulin.

The stimulation of glucose metabolism by GH is transient and is no longer evident in 3T3-F442A adipocytes after 24 or 48 hr of incubation (11), even though the GH is continually present. This also distinguishes the effect of GH from the stimulation by IGF, which persists when the hormone is present for 24 or 48 hr. It is unlikely that the transitory nature of the stimulation by GH reflects more rapid disappearance of hGH than IGF or insulin because GH actually suppressed glucose oxidation after 48 hr, suggesting that it was not cleared from the system. Furthermore, all of the hormones were replenished in fresh medium during the last 4 hr of incubation, when glucose oxidation was measured. If the GH were cleared from the system during the prior 44-hr incubation, an increase rather than a decrease in glucose oxidation would be expected during the last 4 hr.

The possibility that down-regulation of GH receptors contributes to the transitory nature of the stimulation by GH is also unlikely. It has been shown that GH binding and properties of the GH receptor do not change when the ability of GH to stimulate glucose oxidation is altered by prior exposure to GH in rat adipocytes (18,*). Moreover, the binding of ^{125}I-labeled hGH and the properties of crosslinked GH receptors in 3T3-F442A adipocytes appear to be identical to those in rat adipocytes (19, 20,†). The effects of GH in the 3T3-F442A adipocytes appear to be mediated through the GH receptor, since neither insulin nor any of the IGF preparations used in the present study competed with ^{125}I-labeled hGH for binding sites on the 3T3 adipocytes (C.M.F. and J.S., unpublished data).

The suppression of glucose oxidation elicited in the 3T3-F442A adipocytes after 48 hr of incubation also distinguishes GH from the IGFs and insulin. This suppression was clearly different from the persistent stimulation produced by all of the IGF preparations tested in the 3T3 adipocytes. A similar distinction was made based on experiments comparing delayed (4 hr) effects of ovine GH, rat somatomedin C, and insulin in adipose tissue from hypophysectomized rats (16). Although GH induced refractoriness to its own stimulatory properties, it failed to interfere with the stimulation by somatomedin C. Furthermore, exposure to somatomedin C did not induce refractoriness to GH or to itself. It remains to be determined whether refractoriness, a delayed effect of GH in rat adipose tissue, is related to the suppression of glucose metabolism by GH in the 3T3 adipocytes. The distinction between suppression by GH and stimulation by IGFs persisted when the hormones were present in combination for 48 hr as well as when they were present individually. Taken together, these findings indicate that IGFs cannot mediate the suppressive effect of hGH on glucose metabolism in 3T3-F442A adipocytes. Since this suppression is consistent with the diabetogenic properties of GH, our studies also suggest that IGFs may not mediate the diabetogenic effect of GH. The present findings are supported by *in vivo* studies by Schoenle *et al.* (21) demonstrating that 6-day infusion of GH reduced the elevated levels of glucose transport in adipocytes from hypophysectomized rats, whereas similar infusions of IGF-I did not alter glucose transport. The hormones were not compared after shorter infusion periods.

It has been reported that IGF-I can be synthesized in cultured fibroblasts as well as in several rat tissues after exposure to GH (22). Furthermore, the effects of IGF-I on glucose metabolism most likely occur through the insulin receptor (23), resulting in internalization of IGF-I. It is not known whether internalized IGF-I is similar to that which is synthesized intracellularly in the presence of GH. Thus, the intracellular mediation of GH effects by IGF is still subject to question. Our data showing qualitative differences in the metabolic effects of GH and the IGFs in 3T3-F442A adipocytes make it unlikely that the IGFs mediate these effects of GH. Rather, GH appears to have direct and distinct metabolic effects on these cells. A dissociation between cellular effects of GH and IGF-I has also been noted with respect to the ability of hGH to promote differentiation of several cell types. IGF-I fails to convert 3T3-F442A fibroblasts to adipocytes under conditions in which hGH is obligatory for conversion (24). Furthermore, IGF-I could not substitute for hGH in promoting differentiation of myoblasts in azacytidine-treated 10T½ cells (25), indicating that the dissociation of effects of hGH and IGF-I is not unique to 3T3-F442A cells. This raises still further a reconsideration of the role of IGFs in mediating the effects of GH, as has been suggested by others (15, 16, 24–27, ‡).

In summary, the results of this study indicate that pituitary hGH and several IGF preparations, including human IGF-I, bacterial mIGF-I, and rIGF-II, produce distinctly different effects on glucose metabolism in 3T3 adipocytes. The acute stimulation by hGH is transitory, while that of IGFs is not, and the maximum stimulation achieved by hGH is only a fraction of that produced by the IGFs or insulin. The suppression of glucose metabolism that occurs in 3T3 adipocytes upon sustained exposure to hGH is not produced by the IGFs. Rather, both IGF-I and -II continued to stimulate the 3T3-F442A adipocytes throughout prolonged incubations. The results of these studies indicate that extracellular IGFs do not mimic the effects of hGH on glucose metabolism in 3T3-F442A adipocytes and suggest that their role as mediators of the metabolic actions of GH should be reevaluated.

*Schwartz, J. & Carter-Su, C., Program of the Seventh International Congress on Endocrinology, July, 1984, Quebec, Canada, p. 1476 (abstr.).

†Carter-Su, C. & Schwartz, J. (1985) Program of the Sixty-Seventh Meeting of the Endocrine Society, June, 1985, Baltimore, MD, p. 222 (abstr.).

‡Green, H. Proceedings of the International Symposium on Growth Hormone, November, 1983, Baltimore, MD.

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