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GROWTH HORMONE DIRECTLY ALTERS GLUCOSE UTILIZATION IN 3T3 ADIPOCYTES

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SUMMARY: 3T3-F442A adipocytes incubated in the presence of growth hormone (GH) showed a transient stimulation of the incorporation of [^{14}C]glucose into cellular lipids, or production of $^{14}CO_2$ in the first 4 hr. By 48 hr, a significant suppression of glucose metabolism was evident, and persisted for 7 days. Both effects resulted from direct in vitro addition of GH to 3T3 adipocytes. This system therefore provides the first appropriate cellular model for examination of both the characteristic acute stimulatory and delayed suppressive actions of GH on glucose metabolism.

Previous studies on the actions of growth hormone (GH) in vivo provide evidence for its wide variety of metabolic effects. These include stimulation of lipolysis (1), exacerbation of diabetes (2,3), and suppression of sensitivity to insulin (2,4). These effects usually require prolonged elevation of GH, for they are delayed in onset. Attempts to study them in vitro in sensitive target tissues (e.g. adipose tissue from hypophysectomized rats) have been difficult, since the delayed effects of GH do not have straightforward in vitro correlates. For example, the lipolytic action of GH has been observed in vitro primarily as a potentiation of other lipolytic stimuli such as dexamethasone or theophylline (5,6). GH does not appear to alter the effectiveness of insulin when the hormones are added in vitro (7,8), and its ability to suppress glucose metabolism requires at least 1-2 days in vitro (7,8), hence is limited by survival of tissue or cell preparations. However, in vitro studies have revealed that GH also has acute and transient "insulin-like" properties (1,9), whose physiological importance is unclear.

ABBREVIATIONS. hGH = human growth hormone, DMEM = Dulbecco's Modified Eagle's Medium, BSA = bovine serum albumin.

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At present, little is known about cellular mechanisms by which GH exerts any of its effects. This is in part due to the lack of a simple and reliable in vitro system for analysis of such mechanisms, particularly with respect to the long-term effects of GH which are of physiological and clinical significance (e.g. insulin antagonism, diabetogenicity).

The present study describes for the first time effects of human pituitary GH on glucose oxidation and incorporation of glucose into lipid in 3T3-F442A adipocytes. This cell line, of embryonic mouse origin, accumulates lipid after the preadipocytes reach confluence, and differentiate to cells with characteristics of adipocytes (10,11). Differentiation of the preadipocytes has been shown to be dependent on GH (12,13), although GH is not required to maintain the cells in the differentiated state. We have used the converted 3T3 adipocytes to demonstrate first, the characteristic transient stimulation of glucose utilization (9). Second, we demonstrate that GH suppresses glucose utilization in a highly reproducible fashion in 24-48 hr. This suppression, which is relevant to the diabetogenic action of GH, lasts for 7 days. Thus, the cultured 3T3 adipocytes provide a useful system for examination of cellular aspects of GH action, and for evaluating biological activity of GH in clinical and physiological contexts.

MATERIALS AND METHODS

Materials: 3T3-F442A cells were generously provided by Dr. H. Green. All media and sera were obtained from Grand Island Biological Co. Human GH (A type monomer, lot K120583A) was kindly provided by Dr. J. L. Kostyo. Uniformly labelled [^{14}C]-D-glucose was purchased from New England Nuclear. Dexamethasone was a gift of W. Henckler of Merck & Co. and crystalline porcine insulin (lot 615-144-2A) was kindly provided by R. Chance of Eli Lilly, Inc. Methylisobutylxanthine and BSA (fatty acid free) were purchased from Sigma. All other materials were of the highest grade available.

Cell culture: Cells were plated at a density of 200/cm^2 in 100 mm Corning dishes and grown to confluence in DMEM containing 4.5 g/L glucose and 10% calf serum at 37°C in an atmosphere of 10% CO_2-90% air. Conversion to adipocytes was initiated by incubation in DMEM containing final concentrations of dexamethasone (0.25 uM), methylisobutylxanthine (0.5 mM), insulin (2 ug/ml), and 10% fetal calf serum. After 48 h, cells were maintained in DMEM containing 8 or 10% fetal calf serum and were used 5-8 days later. Conversion was evaluated by phase contrast microscopy and was estimated at 70-85%. The medium was replaced with DMEM (1 g/L glucose) containing 1% BSA, 22-24 hr prior to experiments

Lipid Accumulation. Monolayers of converted cells were incubated in DMEM containing 1 g/L glucose, 1% BSA, [^{14}C]glucose (0.1 uCi/ml), and hormones, for varying periods of time at 37°C. Incorporation of [^{14}C]glucose was

stopped by aspirating the medium and immediately replacing it with 5 ml Dole's reagent (40 parts isopropyl alcohol, 10 parts heptane, 1 part 1 N H_2SO_4). Cells were scraped off the dishes, and lipids were extracted by the method of Dole and Meinertz (14). The lipids were weighed, and incorporated radioactivity was counted by liquid scintillation spectrometry. Data are expressed as dpm/mg lipid.

Glucose oxidation and incorporation into lipid. Suspensions of converted adipocytes were prepared using Joklik's medium in the presence of EDTA, as described by Deutsch and Rosen (15). They were resuspended to a final concentration of 2.5 x 10^5 cells/ml in Krebs Ringer bicarbonate buffer containing 1 mM glucose, 1% BSA, [^{14}C]glucose (0.1 uCi/ml), and hormones as indicated, and were incubated at 37°C for 1 or 4 hr, as indicated, under an atmosphere of 95% O_2-5% CO_2. Each condition was tested in quadruplicate. Evolved $^{14}CO_2$ was collected and the amount of radioactivity incorporated into cellular lipids was determined, as described previously (16). Data are expressed as dpm/mg lipid x hr. Statistical comparisons were by Student's t-test.

RESULTS

Table 1 illustrates that continuous exposure to GH produces both an early stimulatory and subsequent inhibitory effect on the incorporation of glucose into lipid in converted 3T3 adipocytes. In this experiment, a significant stimulation of lipid synthesis by GH was evident at 1 and 4 hr, but had subsided by 22 hr, despite the continued presence of GH. This is consistent with the previously described transient nature of "insulin-like" responses to GH observed in adipose tissue. When the incubation time was extended, 48 hr of continued exposure to GH resulted in a sustained suppression of lipid accumulation, as shown in Fig. 1. The suppression persisted for at least 7 days, since the incorporation of label into lipid in the cells incubated without GH continued to increase, while that in cells incubated with GH was markedly attenuated. The viability of the cells, estimated by trypan blue

Table 1. Effects of various periods of incubation with GH on lipid accumulation in 3T3-F442A adipocytes

	[^{14}C] Glucose incorporation (dpm/mg lipid)		
	1 hr	4 hr	22 hr
C	353 ± 13*	678 ± 40	3552 ± 473
GH	472 ± 14	808 ± 18	2522 ± 358
p	0.001	0.05	ns

*mean ± standard error, n=4. Human GH was present at 1 ug/ml

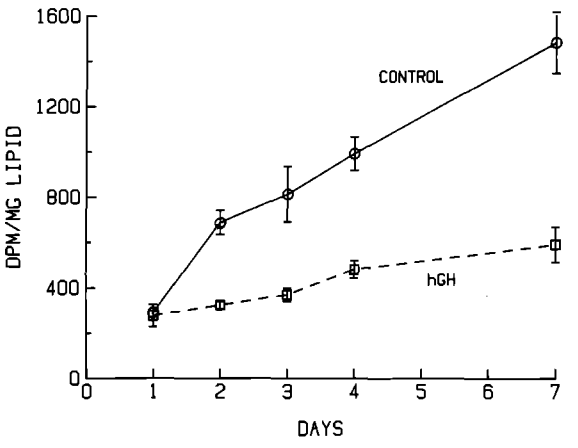

Figure 1. Lipid accumulation in 3T3-F442A adipocytes incubated in the absence (○) or presence (□) of GH. Day 0 represents the initiation of incubation with [^{14}C]glucose, in the absence (control) or presence of hGH (1 ug/ml). Each point represents the mean ± standard error of quadruplicate observations.

exclusion (16-18% non-viable), was not appreciably changed during prolonged incubation. The transient nature of the stimulatory response to GH observed in the 3T3 adipocytes is specific to GH and characteristic of its effects on adipose tissue (9). The chronic (7 day) suppression of lipid synthesis by GH in the 3T3 adipocytes has not been demonstrated before.

The time course of the effect of GH on the rate of glucose metabolism was evaluated over a 24 hr period. In the experiment shown in Table 2, adipocytes were incubated with the indicated hormones for a total of 1, 4 or 24 hr. For the 1 and 4 hr incubations, suspensions of cells were incubated with [^{14}C]glucose, and glucose utilization was measured, for the total period of exposure to the hormones. For the 24 hr incubation, monolayers of adipocytes were exposed to hormone continuously for 20 hr, and then were suspended in the presence of the same hormones and [^{14}C]glucose for measurements of glucose utilization during the final 4 hr of incubation. In cells exposed to GH for the first 1 or 4 hr, the rate of incorporation of glucose into lipid or of glucose oxidation was significantly greater than control. After a total of 24 hr of incubation with GH, however, both indicators of glucose utilization were significantly lower than control in GH-treated cells. In separate experiments (not shown), the rate of glucose metabolism tested after 48 hr also remained

Table 2. Effect of GH or insulin on rates of glucose utilization in 3T3 adipocytes

Treatment	Duration of total incubation (hours)		
	1	4	24
A. $[^{14}C]O_2$ Production (dpm/mg lipid x hr)			
Control	1179 ± 18	1265 ± 45	7382 ± 252
GH	1773 ± 33[a]	1905 ± 48[a]	2857 ± 534[a]
Insulin	2359 ± 16[a]	4249 ± 72[a]	16420 ± 305[a]
B. $[^{14}C]$ Glucose incorporation (dpm/mg lipid x hr)			
Control	316 ± 19	181 ± 8	457 ± 13
GH	559 ± 71[b]	446 ± 76[b]	203 ± 13[a]
Insulin	897 ± 49[a]	1158 ± 6[a]	3220 ± 97[a]

*mean ± standard error, n=4. Human GH was present at 0.5 ug/ml, insulin at 100 uU/ml, for the total period indicated. The $[^{14}C]$glucose was also present, and glucose oxidation and incorporation into lipid were measured, during the first hr for the 1 hr interval, during the first 4 hr for the 4 hr incubation period. For the 24 hr incubation, monolayers of cells were exposed to the indicated hormones for 20 hr, then were suspended and incubated with the same hormones and $[^{14}C]$glucose, and glucose utilization was measured for the final 4 hr. Data are normalized as dpm/mg lipid x hr for comparative purposes. [a] $p<0.001$ vs control, [b] $p<0.02$ vs control.

lower in GH-treated cells as compared to control cells. Note that glucose oxidation and lipid synthesis in cells incubated with insulin were greater than control values throughout the 24 hr incubation. This clearly distinguishes the transient stimulation by hGH from the continually stimulatory effect of insulin.

DISCUSSION

This study provides the first *in vitro* evidence of a direct suppression of glucose metabolism by GH in 3T3 adipocytes. This is likely to be a component of the diabetogenic action of GH. While the induction of diabetes by GH is evident *in vivo* in some species (2), a suitable *in vitro* indicator of this effect of GH has not been available. In the converted 3T3 adipocytes, the suppression of glucose oxidation or incorporation into lipid was significant in 24-48 h in every experiment (n=5), and was sustained for 7 days. Thus,

these studies also establish that 3T3 adipocytes are a reliable *in vitro* system for examination of cellular aspects of GH action. It is noteworthy that an acute stimulation of glucose metabolism by GH was also evident in these cells. This insulin-like action was transient, in contrast to the continual stimulation caused by insulin. In comparison to studies in adipose tissue from hypophysectomized rats, the time course of the acute response to GH is somewhat delayed (17). The reasons for this difference remain to be established. It should also be recognized that lipolytic properties attributable to GH may contribute to the overall changes in lipid accumulation observed in these experiments.

These studies do not address the issue of whether GH alters sensitivity to insulin in the 3T3 adipocytes. Such changes could be an important component of the diabetogenic action of GH in intact animals. However, in a study by Maloff *et al.* (7) using rat adipose tissue explants in culture, 48 hr exposure to GH also suppressed basal glucose transport, but did not alter insulin binding or the ability of insulin to stimulate transport. Similar findings were reported by Nyberg and Smith (8), using cultured human adipose tissue. The 3T3 adipocyte system is considerably simpler than either of these preparations. It does not require exposure of cells to collagenase, which interferes with metabolic effects of GH (16). In the present study, qualitatively similar responses to GH were obtained whether cells were studied in monolayer or in suspension. Hence, the suspension procedure used does not appear to interfere with the biological responses to GH, as does collagenase digestion.

While direct addition of GH to the incubation medium clearly elicits the stimulation and subsequent suppression of glucose metabolism, it is not yet known what events transpire between initial exposure to GH and these responses. GH binds to the 3T3-F442A cells rapidly and specifically under the conditions of these experiments (Schwartz, unpublished observations). Nixon and Green made similar observations (18), and reported that binding was comparable in preconverted and converted cells. It is of interest that our

preliminary data indicate that the preconverted cells do not exhibit metabolic responses to GH. Although purified rat somatomedin affected rat adipose tissue comparably to insulin rather than to GH (19), the possibility remains that some intracellular mediator may be generated in the 3T3 adipocytes in response to GH.

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