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Regulation of glutamine synthetase in cultured 3T3-L1 cells by insulin, hydrocortisone, and dibutyryl cyclic AMP

(3T3-L1 adipocytes/hexokinase/glucose-6-*P* dehydrogenase)

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ABSTRACT **The 3T3-L1 mouse fibroblast cell line develops morphological and biochemical characteristics of adipocytes when maintained at confluence. This conversion to adipocytes is accelerated by addition of insulin to the culture medium [Green, H. & Kehinde, O. (1975) *Cell* 5, 19–27]. During the course of the insulin-mediated adipocyte conversion, the specific activity (units/mg of protein) of glutamine synthetase [L-glutamate:ammonia ligase (ADP-forming), EC 6.3.1.2] increases more than 100-fold. The specific activities of hexokinase (ATP:D-hexose 6-phosphotransferase, EC 2.7.1.1) and glucose-6-*P* dehydrogenase (D-glucose-6-phosphate:NADP$^+$ 1-oxidoreductase, EC 1.1.1.49) also increase but less dramatically (1.5- to 3-fold). In contrast, confluent cells maintained in the absence of insulin for the same time (12–20 days after confluence) display only minimal increases in the activity of these enzymes. Maintenance of confluent cells in culture medium lacking added L-glutamine has little, if any, effect on glutamine synthetase activity in either control or insulin-treated cultures. Treatment of confluent 3T3-L1 cultures with hydrocortisone (1 μg/ml) for 3 days prior to harvesting results in an increase in glutamine synthetase specific activity of 12-fold for control cultures maintained for 13 days in the absence of insulin and 1.4-fold for adipocyte cultures maintained for 13 days in the presence of insulin (10 μg/ml). Treatment of 3T3-L1 control cells and adipocytes with dibutyryl cyclic AMP (1 mM) plus theophylline (1 mM) decreases the glutamine synthetase specific activity and almost completely reverses the insulin- and hydrocortisone-mediated increases in enzyme activity. In contrast, treatment with dibutyryl cyclic AMP plus theophylline has relatively little effect on the specific activities of hexokinase or glucose-6-*P* dehydrogenase or on the protein content of the cultures. These data indicate that glutamine synthetase activity is hormonally regulated in 3T3-L1 cells.**

When cells of the 3T3-L1 Swiss mouse fibroblast line are maintained at confluence, they accumulate large amounts of triglyceride and acquire the morphological and biochemical characteristics of mature adipocytes (1–4). This conversion to adipocytes is facilitated by insulin (2) and is accompanied by a 50-fold increase in the rate of triglyceride synthesis and an increase in the activities of several enzymes involved in triglyceride and fatty acid biosynthesis (4, 5). The work of Green and Kehinde (1–3) and others (4–6) strongly suggests that the 3T3-L1 adipocytes are morphologically and biochemically similar to *in vivo* adipocytes and, as such, provide the opportunity for long-term *in vitro* studies of the regulation of adipocyte intermediary metabolism. Studies of adipocyte metabolism in cultured cells derived from adipose tissue have been frustrated by at least a partial failure of the cells to retain the characteristics of highly differentiated adipocytes (7). Similarly, such studies performed with rat epididymal adipose tissue or isolated adipocytes have been limited primarily because of the difficulty of maintaining the adipose tissue or adipocytes *in vitro* for long periods of time (8).

Glutamine synthetase [L-glutamate:ammonia ligase (ADP-forming), EC 6.3.1.2] is a potentially strategic target for the hormonal regulation of nitrogen metabolism because the amide nitrogen of glutamine can be utilized for the biosynthesis of a wide variety of compounds including purine and pyrimidine nucleotides, pyridine nucleotides, amino sugars, and various amino acids (9). These considerations and the results of our previous studies of rat adipose tissue glutamine synthetase (10, 11) led us to the studies reported here.

MATERIALS AND METHODS

3T3-L1 cells were obtained from Howard Green, Massachusetts Institute of Technology. Porcine crystalline zinc insulin (lot 615-D63-10) was provided by R. J. Hosley, Eli Lilly Research Laboratories.

Growth and Maintenance of 3T3-L1 Cells. Except for the indicated modifications, 3T3-L1 cells were grown and maintained after confluence in Dulbecco's modified Eagle's medium containing 10% calf serum (Flow Laboratories), penicillin (100 units/ml), and streptomycin (100 μg/ml). The final concentrations of *d*-biotin (8 μg/ml) and calcium pantothenate (8 μg/ml) in the medium were those used by Mackall *et al.* (4). In addition, L-asparagine (0.5 mM) and L-glutamate (0.5 mM) were included in the medium, and $NaHCO_3$ was decreased to 2.2 g/liter. Cells were grown and maintained at 37° in a humidified 5% CO_2/95% air atmosphere. Medium was changed every 2 days.

The undiluted calf serum used for medium preparation contained <0.5 mM glutamine, <0.3 ng of insulin per ml, and <35 ng of hydrocortisone per ml. Glutamine was determined by automated amino acid analysis (12). Insulin (13) and hydrocortisone (14) were determined by modifications of published radioimmunoassay procedures. Bovine insulin was used as standard for insulin assays.

Assays. cells were plated at a density of $0.5–3.5 \times 10^4/cm^2$, and the degree of confluence was determined by phase-contrast microscopy. Because of the difficulty in determining the degree of confluence with precision in these cultures, the assignment of confluence should be considered to include an error of ±1–2 days. This error probably accounts for differences in the number of days after confluence at which maximal enzyme specific activities are reached in replicate experiments. Cultures were washed three times with phosphate-buffered saline, scraped into buffer (20 mM imidazole·HCl, pH 7.0/150 mM

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KCl/0.1 mM EDTA), frozen, and stored for 1–2 weeks at −80°. Thawed samples were sonicated (10 sec; 4°; Ultrasonics Sonifier model W140D, setting 3, microtip) and clarified by centrifugation (12,000 × *g*; 4°; 15 min), and aliquots were taken for assay.

Glutamine synthetase was assayed by two methods. For either assay, 1 unit of glutamine synthetase activity is defined as the amount of activity catalyzing the formation of 1 μmol of product (either γ-glutamyl hydroxamate or L-[^{14}C]glutamine) per min under the specified assay conditions. The γ-glutamyl transfer assay (L-glutamine and NH_2OH are reactants, and γ-glutamyl hydroxamate is a product) was performed by using modifications of published procedures (15). Assay mixtures, incubated at 37° for 15–60 min, contained in 1.0 ml: 50 mM imidazole·HCl (pH 6.8), 50 mM NH_2OH, 100 mM L-glutamine, 25 mM potassium arsenate, 0.2 mM ADP, 0.5 mM $MnCl_2$, and 50–250 μl of clarified cell sonicate. Reactions were initiated by the addition of cell sonicate and terminated by the addition of 1.0 ml of 0.37 M $FeCl_3$/0.3 M trichloroacetic acid/0.6 M HCl. The precipitate was removed by centrifugation (1150 × *g*; 10 min; 21°), and γ-glutamyl hydroxamate was determined by comparing the absorbance at 505 nm to that observed when authentic γ-glutamyl hydroxamate was substituted for the cell sonicate. Parallel incubations of cell sonicate in reaction mixtures lacking ADP and arsenate served as controls (16).

The [^{14}C]glutamine biosynthetic assay was performed as described by Prusiner and Milner (17), with minor modifications. Assay mixtures, incubated at 37° for 30–60 min, contained in 0.1 ml: 50 mM imidazole·HCl (pH 7.0), 10 mM L-[^{14}C]-glutamate (0.05–0.20 μCi/μmol), 10 mM NH_4Cl, 10 mM ATP, 15 mM $MgCl_2$, and 10–50 μl of clarified cell sonicate. The rate of product formation (μmol/min) for both purified rat liver glutamine synthetase and for sonicates of 3T3 adipocytes is approximately 20-fold greater in the γ-glutamyl transfer assay than in the [^{14}C]glutamine biosynthetic assay. This difference in reaction rates is expected because the reactions are different (15).

Hexokinase (ATP:D-hexose 6-phosphotransferase, EC 2.7.1.1) (18) and glucose-6-*P* dehydrogenase (D-glucose-6-phosphate: $NADP^+$ 1-oxidoreductase, EC 1.1.1.49) (19) were assayed by minor modifications of published procedures. The activity of each enzyme was determined by measuring the initial rate of NADP reduction at 21°. For both hexokinase and glucose-6-*P* dehydrogenase, 1 unit of activity is the amount of activity catalyzing the reduction of 1 μmol of NADP per min under the specified assay conditions. Hexokinase assay mixtures contained in 1.0 ml: 50 mM potassium *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonate (pH 7.5), 10 mM $MgCl_2$, 5 mM ATP, 50 mM D-glucose, 1.0 mM EDTA, 0.4 mM NADP, 0.4 unit of yeast glucose-6-*P* dehydrogenase (Sigma cat. no. G-6378), and 20–50 μl of clarified cell sonicate. Glucose-6-*P* dehydrogenase assay mixtures contained, in 1.0 ml: 50 mM potassium *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonate (pH 7.5), 10 mM $MgCl_2$, 0.4 mM NADP, 2 mM D-glucose-6-*P*, and 20–50 μl of clarified cell sonicate. Blanks in each assay were reaction mixtures lacking either ATP or D-glucose-6-*P*. For all enzyme assays, rate of product formation was directly proportional to incubation time and amount of cell sonicate assayed. Protein was quantified by the method of Lowry *et al.* (20).

Antibody Preparation. Rat liver glutamine synthetase was purified to apparent homogeneity as described by Tate *et al.* (21). The purified enzyme (50 μg) in 1 ml of 150 mM NaCl was mixed with an equal volume of complete Freund's adjuvant and injected intradermally (20 sites) into a 3-kg rabbit on days 0 and 30. On day 40, blood was collected and allowed to clot at 4°, and serum was obtained by centrifugation. Preimmune serum was similarly obtained prior to immunization. The globulin fraction was prepared by two precipitations at 33% $(NH_4)_2SO_4$ saturation followed by dialysis against 250 volumes of buffer (20 mM imidazole·HCl, pH 7.0/150 mM NaCl). The volume of the final preparation was adjusted with the buffer to that of the original serum.

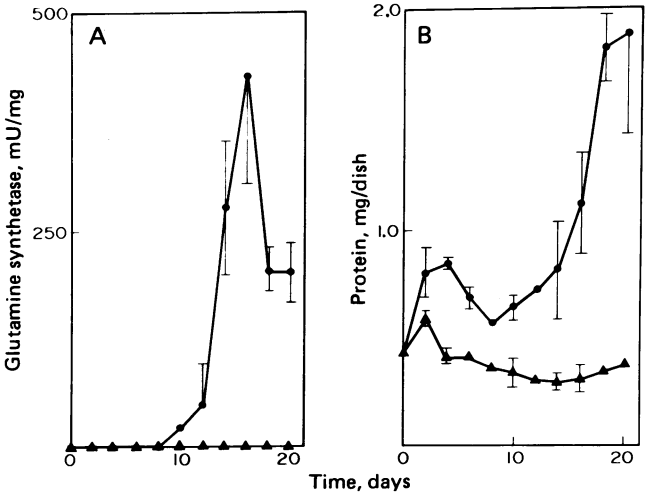

FIG. 1. Glutamine synthetase specific activity and protein content in 3T3-L1 cells maintained after confluence in the absence (▲) or presence (●) of insulin. Cells were plated in 20-cm^2 dishes at a density of 3.2 × 10^4 cells per cm^2 and grown in medium containing 4 mM glutamine but lacking insulin. At confluence, insulin (10 μg/ml) was added to the medium in half the cultures. Every 2 days, cultures were fed with 5 ml of medium either containing added insulin or not containing added insulin. At 2-day intervals from 0 to 20 days after confluence, insulin-treated and control cultures were harvested. Data points are mean ± SD for triplicate cultures. Absence of an error bar indicates SD less than the radius of the data point. (*A*) Glutamine synthetase specific activity (mU, milliunit). Glutamine synthetase was determined by the γ-glutamyl transfer assay. (*B*) Total protein per culture.

RESULTS

Glutamine Synthetase Specific Activity. During the course of the insulin-mediated 3T3-L1 adipocyte conversion there was at least a 50- to 100-fold increase in glutamine synthetase specific activity (Figs. 1*A* and 2*A*; Table 1). In contrast, cultures of 3T3-L1 cells maintained for up to 20 days in the absence of added insulin (control cultures) continued to have a low specific activity of glutamine synthetase. Thus, by the time glutamine synthetase activity reached a maximum (16 days in the experiment illustrated in Fig. 1), the specific activity of this enzyme in insulin-treated cultures was more than 100-fold higher than that in control cultures (Fig. 1*A*). After this peak was reached, glutamine synthetase specific activity declined significantly although total activity in the cultures decreased relatively little. Culture protein content was higher for insulin-treated cells than for control cells (Fig. 1*B*; Table 1). During prolonged insulin treatment, total culture protein declined after an initial rise, then began to rise again. This discontinuous increase in culture protein was reproduced in separate experiments (data not shown). The explanation for it is not evident; however, it may reflect a loss of protein-rich lipid-filled cells by detachment from the dish followed by proliferation and adipocyte conversion in a relatively small population of remaining adipocyte precursors, as suggested by Green and Kehinde (3).

Maintenance of confluent cells in culture medium lacking added L-glutamine (the glutamine concentration due to 10%

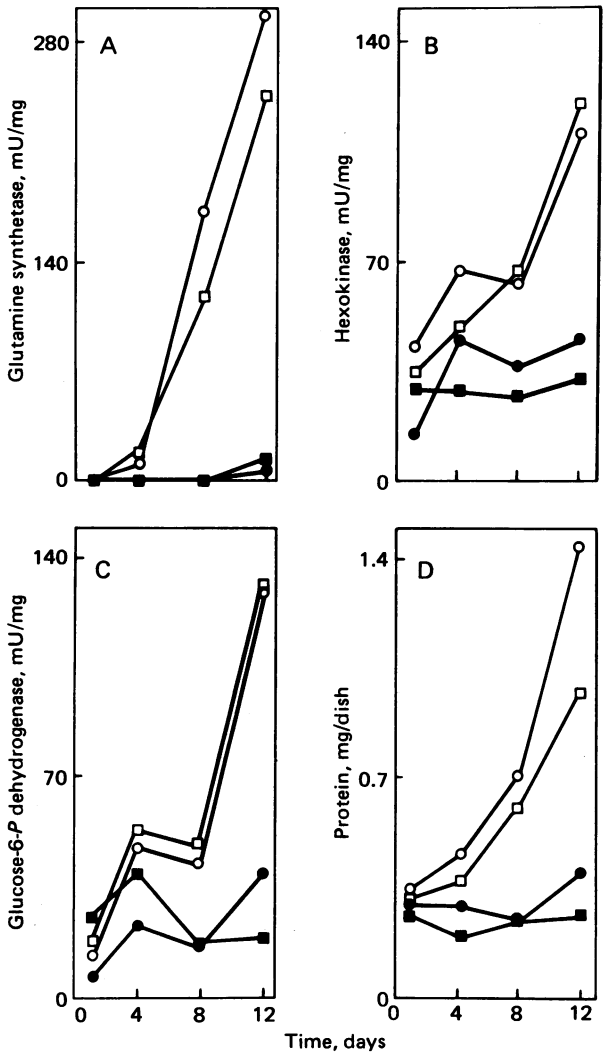

FIG. 2. Glutamine synthetase (*A*), hexokinase (*B*), and glucose-6-*P* dehydrogenase (*C*) specific activities and culture protein content (*D*) in 3T3-L1 cells. Cells were plated in 20-cm^2 dishes at a density of 0.5 × 10^4 cells per cm^2 and grown to confluence (day 0) in medium containing 4 mM glutamine either in the absence (●,■) or in the presence (○,□) of crystalline porcine insulin at 10 μg/ml. Confluence, assessed by phase-contrast microscopy, was achieved 3–4 days after seeding. Beginning 5 days after plating (day 0), cultures were maintained in medium containing: ○, insulin (10 μg/ml) and 4 mM L-glutamine; ●, no added insulin but 4 mM L-glutamine; □, insulin (10 μg/ml) but no added L-glutamine; ■, no added insulin or L-glutamine. Cells were fed with 5 ml of medium every 2 days. At the indicated times, cells were washed twice with 5 ml of phosphate-buffered saline, harvested by scraping into buffer, and stored at −80°. Glutamine synthetase was assayed by using the γ-glutamyl transfer assay.

calf serum is <0.1 mM) had relatively little, if any effect, on glutamine synthetase activity in either control or adipocyte cultures (Fig. 2*A*).

Treatment of confluent 3T3-L1 cultures with hydrocortisone (1 μg/ml, 2.76 μM) for 3 days prior to harvesting resulted in an increase in glutamine synthetase specific activity (Table 1) relative to untreated cultures. This increase was 12-fold for control cultures maintained for 13 days after confluence in the absence of insulin and 1.4-fold for adipocyte cultures maintained for 13 days after confluence in the presence of insulin (10 μg/ml). In contrast, hydrocortisone had little effect on culture protein content. The data from this experiment, shown

Table 1. Effect of insulin, hydrocortisone, and dibutyryl cyclic AMP on 3T3-L1 glutamine synthetase activity

Culture medium additions				Glutamine synthetase specific activity, mU/mg	Protein, μg/dish
Insulin, μg/ml	Hydrocortisone, μg/ml	Dibutyryl cyclic AMP plus theophylline, mM	*n*		
0	0	0	6	10 ± 5	459 ± 56
0	0	1	5	0	410 ± 38
0	1	0	5	119 ± 15	438 ± 40
0	1	1	4	12 ± 7	485 ± 45
10	0	0	6	543 ± 79	1498 ± 100
10	0	1	5	8 ± 7	1008 ± 68
10	1	0	5	758 ± 46	1334 ± 109
10	1	1	4	66 ± 9	1233 ± 104

Cells were plated in 20 cm^2 dishes at a density of 1.23 × 10^4 cells per cm^2 and grown to confluence in medium containing 4 mM glutamine but lacking insulin. Four days after plating, on approximately the day confluence was achieved, insulin was added to the indicated cultures and cells were maintained for an additional 10 days. On the 10th day after confluence, medium was shifted to that indicated. The specific activity of glutamine synthetase on the 10th day after confluence was 5 mU/mg in cultures maintained in the absence of insulin and 200 mU/mg in cultures maintained in the presence of insulin. Cells were fed every 2 days and harvested on day 13 after confluence. *n* is the number of replicate cultures (dishes) assayed. Each assay (enzyme activity and protein) was performed at least twice; variation between assays was <10%. Data are shown as mean ± SD. mU, milliunit.

in Table 1, suggest that increases in glutamine synthetase observed in the presence of hydrocortisone and insulin are at least additive. The maintenance of cultures after confluence in medium containing no added L-glutamine had no effect on the increases in glutamine synthetase activity produced by hydrocortisone or insulin treatment (data not shown).

As shown in Table 1, treatment of confluent 3T3-L1 cultures with dibutyryl cyclic AMP (1 mM) plus theophylline (1 mM) for 3 days prior to harvesting resulted in a decrease in the activity of glutamine synthetase and nearly complete reversal of the increases in the enzyme activity mediated by insulin or hydrocortisone. Incubation of adipocyte cultures with 2 mM butyrate, 1 mM theophylline, or 1 mM AMP resulted in no change in glutamine synthetase activity. Mixing of equal volumes of clarified sonicate from any two of the eight treatments of the experiment described in Table 1 yielded expected average glutamine synthetase activity. This and the direct proportionality between activity measured and volume of clarified sonicate assayed suggest that the presence of activators or inhibitors is an unlikely explanation for activity differences observed due to treatments described in Table 1. Similar relative specific activities of glutamine synthetase were obtained when the clarified sonicates from the experiment described in Table 1 were assayed by the [^{14}C]glutamine biosynthetic assay.

In many studies, γ-glutamyl transfer activity is assumed to accurately reflect glutamine synthetase activity (22–28). Two recent studies (22, 28) have shown that the antibody prepared against purified glutamine synthetase precipitates either crude glutamine synthetase biosynthetic activity (22) or γ-glutamyl

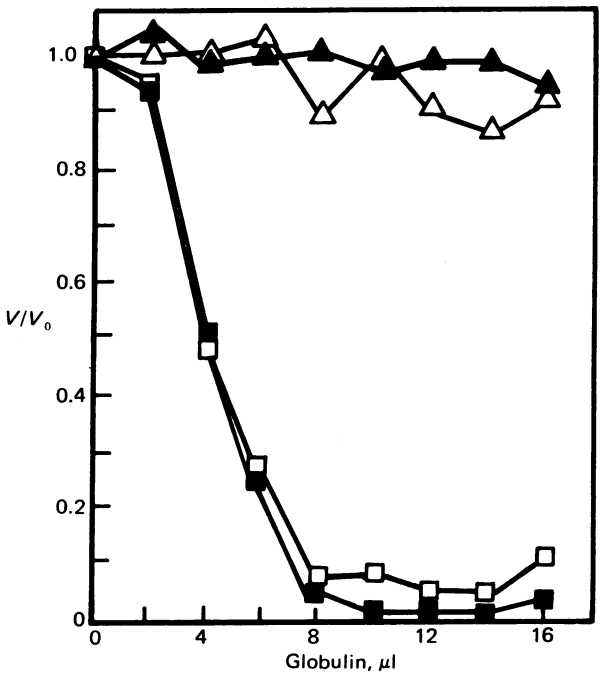

FIG. 3. Immunoprecipitation of glutamine synthetase from 3T3-L1 adipocyte sonicate. Clarified cell sonicate was prepared from 3T3-L1 cells maintained at confluence for 14 days in the presence of insulin at 10 μg/ml. The indicated volume of preimmune (▲,△) or immune (■,□) globulin fraction was added to 0.5 ml of clarified sonicate in 20 mM imidazole·HCl, pH 7.0/150 mM KCl. The mixture was incubated for 18 hr at 4°. An aliquot of each incubated mixture was used to determine the total glutamine synthetase activity in each sample (data not shown). Incubation of cell sonicate with 2, 4, 6, or 8 μl of immune globulin preparation resulted in 0, 13, 18, or 25% inhibition, respectively, of activity measured by the γ-glutamyl transfer (▲,■) and the [^{14}C]glutamine biosynthetic (△,□) assays. The same volumes of preimmune globulin did not inhibit the enzyme activity measured by either assay. Incubated samples were sedimented (15,000 × *g*; 15 min; 4°) and aliquots of the supernatants were assayed to determine the fraction (V/V_0) of glutamine synthetase activity remaining in the supernatant. V_0, activity measured in the supernatant of the sample containing no added globulin fraction; V, activity measured in the supernatant of the sample containing the indicated volume of globulin fraction. The activity in the sample containing no added globulin fraction (V_0) was 476 millunits/ml for the γ-glutamyl transfer assay and 25.2 milliunits/ml for the [^{14}C]glutamine biosynthetic assay.

transfer activity (28). We have also prepared an antibody against purified rat liver glutamine synthetase. This antibody preparation precipitated, in parallel, both the γ-glutamyl transfer activity and the [^{14}C]glutamine biosynthetic activity from clarified 3T3-L1 adipocyte sonicates (Fig. 3). This observation further suggests that the γ-glutamyl transfer activity accurately reflects glutamine synthetase activity, and it supports the conclusion that the γ-glutamyl transfer assay provides a valid measurement of glutamine synthetase activity in extracts of cultured cells.

Hexokinase and Glucose-6-*P* Dehydrogenase Specific Activities. During the course of the insulin-mediated 3T3-L1 adipocyte conversion there was an increase in the specific activity of hexokinase and glucose-6-*P* dehydrogenase (Fig. 2 *B* and *C*). The specific activity of hexokinase was increased about 2-fold and that of glucose-6-*P* dehydrogenase was increased about 3-fold in adipocyte cultures compared to control cultures. These increases in specific activities associated with insulin treatment are far less than those observed for glutamine synthetase in the same cell sonicates. Similarly, treatment of adipocyte or control cultures with hydrocortisone or dibutyryl cyclic AMP plus theophylline had relatively little or no effect on hexokinase and glucose-6-*P* dehydrogenase activities (not shown) or on total protein content of these cultures (Table 1). By the time the insulin-mediated adipocyte conversion was more than 50% complete (13 days after confluence in this experiment), specific activities of hexokinase and glucose-6-*P* dehydrogenase were 1.7- and 2.8-fold higher, respectively, in adipocyte cultures than in control cultures. Hydrocortisone treatment (Table 1) had no effect on hexokinase activity in adipocytes or control cells, whereas it increased glucose-6-*P* dehydrogenase specific activity 1.4-fold in adipocytes and 1.5-fold in control cells. Dibutyryl cyclic AMP (1 mM) plus theophylline (1 mM) treatment (Table 1) had no effect on hexokinase specific activity in control cells; it resulted in a 22% or 53% lower specific activity in adipocytes in the absence or presence of hydrocortisone treatment, respectively. The effect of dibutyryl cyclic AMP plus theophylline treatment on 3T3-L1 glucose-6-*P* dehydrogenase activity was also small: it resulted in a 25% to 30% lower specific activity of this enzyme in control cells and adipocytes both in the absence and presence of hydrocortisone treatment.

DISCUSSION

The studies reported here demonstrate substantial amounts of glutamine synthetase activity in 3T3-L1 cells incubated in the presence of added insulin. It is noteworthy that the maximal glutamine synthetase specific activity we observed in 3T3-L1 adipocyte sonicates is considerably greater (at least 10-fold) than that observed in other cultured cell lines (22–28). The glutamine synthetase activity (as measured by the γ-glutamyl transfer assay) in clarified 3T3-L1 adipocyte sonicates (up to 1.50 units/mg) is comparable to that which we measured in clarified extracts of rat (body weight, 200–250 g) epididymal adipose tissue (0.35–0.70 unit/mg) and rat liver (0.5–1.0 unit/mg). This high 3T3-L1 adipocyte glutamine synthetase activity may reflect the requirement for glutamine in the biosynthesis of the proteins, nucleic acids, and complex polysaccharides (9) that are needed for adipocyte differentiation. Although the insulin concentration used in this study exceeds physiological levels, the concentration of insulin required for a half-maximal increase in glutamine synthetase specific activity (approximately 0.01 μg/ml) is much closer to normal serum levels.¶

When glutamine synthetase activity has been studied in cultured cells as a function of the glutamine content of the culture medium, an inverse relationship between activity and glutamine content has been observed (22–28). In contrast, in the 3T3-L1 cells studied here, exclusion of glutamine from the culture medium had little, if any, effect upon the enzyme activity. Similarly, omission of glutamine did not significantly alter the effects of insulin or hydrocortisone on enzyme activity.

The effects of insulin and glucocorticoid on glutamine synthetase activity are not unique to 3T3-L1 cells. Insulin prevents at least part of the glutamine-mediated decrease in Chinese hamster cell glutamine synthetase activity (22) and glucocorticoids (including hydrocortisone) have also been shown to increase the enzyme activity in several cell lines (22–28). In contrast, the large insulin-mediated increase in glutamine synthetase activity in 3T3-L1 cells maintained either in the absence or in the presence of glutamine has not been reported for other cell lines.

The effects of dibutyryl cyclic AMP plus theophylline on

¶ R. E. Miller and J. F. Jorkasky, unpublished data.

3T3-L1 adipocyte and control cell glutamine synthetase activity contrasts with the results obtained in cultured Chinese hamster cells (23, 24) and in cultured chick embryonic neural retinas (29). In Chinese hamster cells and chick neural retinas, incubation with dibutyryl cyclic AMP alone (1 mM) resulted in 6- and 7.5-fold increases in glutamine synthetase activity, respectively. The observed effect of dibutyryl cyclic AMP plus theophylline on 3T3 adipocytes appears to be relatively specific for glutamine synthetase because there are relatively minor effects of this treatment on hexokinase and glucose-6-*P* dehydrogenase specific activities and on total culture protein. Alternatively, the effect may be nonspecific if dibutyryl cyclic AMP plus theophylline results in decreased synthesis of many proteins. In this case, the rapid decrease in glutamine synthetase activity (time for 50% decrease, 9 hr¶) may reflect a rapid degradation rate for this enzyme as compared with the degradation rate for hexokinase, glucose-6-*P* dehydrogenase, or total culture protein.

Insulin-mediated increases in hexokinase (30) and glucose-6-*P* dehydrogenase (19) activities have been observed in incubated rat epididymal adipose tissue. The maximal specific activities of these enzymes (mean ± SD) in clarified sonicates of 3T3-L1 adipocytes (hexokinase, 126 ± 19 milliunits/mg; glucose-6-*P* dehydrogenase 165 ± 16 milliunits/mg) are greater than those that we have measured in clarified extracts of rat epididymal adipose tissue (hexokinase, 60 milliunits/mg; glucose-6-*P* dehydrogenase, 90 milliunits/mg¶).

L-Glutamine is required in numerous biosynthetic processes directed ultimately toward the synthesis of proteins, nucleic acids, and complex polysaccharides (9). Therefore, glutamine synthetase may be required for insulin-mediated differentiation of 3T3-L1 cells into adipocytes. In support of this hypothesis, we find that the increase in glutamine synthetase activity in insulin-treated 3T3-L1 cells precedes the morphological changes and the increases in culture protein (Fig. 1), hexokinase activity, and glucose-6-*P* dehydrogenase activity.¶

We (31) and others (32, 33) have observed an increase in specific insulin binding in 3T3-L1 adipocytes compared with 3T3-L1 preadipocytes. Because L-glutamine is required for amino sugar biosynthesis and therefore glycoprotein biosynthesis (9), glutamine synthetase can potentially play a regulatory role in the synthesis of cell surface glycoproteins, including insulin receptors (34). Further study should clarify the role of glutamine synthetase in differentiation and the mechanism for the regulation of glutamine synthetase activity by hormones and cyclic nucleotides.

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