Glutamine metabolism in isolated incubated adipocytes of the rat

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1. Phosphate-dependent glutaminase activity in the epididymal fat-pad was 15.1 nmol/min per mg of protein. Glutaminase activity demonstrated differences with respect to adipose-tissue sites. Considerable variation was found in different sites of adipose tissue from lean control and Zucker obese animals. 2. Adipocytes incubated in the presence of 2 mM-glutamine utilized glutamine at a rate of 1.8 μ mol/h per g dry wt., and glutamate, ammonia, lactate and alanine were produced. Addition of glucose plus insulin increased the rates of glutamine utilization and glutamate, ammonia, lactate and alanine production. Isoprenaline alone or plus glucose further stimulated the rate of glutamine utilization and formation of end products. 3. The rate of incorporation of 14 C from glutamine into CO_2 was similar to that of glucose, but the rate of incorporation into triacylglycerol was much less. Addition of unlabelled glucose or glucose plus insulin stimulated the rate of incorporation of [14 C]glutamine into triacylglycerol, but had no effect on that of $^{14}\text{CO}_2$ formation. Isoprenaline plus glucose increased the rate of incorporation of [14 C]glutamine into CO_2 , but decreased the rate of incorporation into triacylglycerol. 4. In the absence of insulin, the rate of [14 C]glutamine incorporation into triacylglycerol was related to the glucose concentration (0–10 mM). However, in the presence of insulin, the rate of incorporation of [14 C]glutamine was maximal at 1 mM-glucose.

INTRODUCTION

Adipose tissue has not, in general, been considered to play an important role in amino acid metabolism. It has been shown that 14 C from [14 C]leucine or [14 C]alanine is incorporated into triacylglycerol in isolated adipose tissue (Feller, 1965) and, more recently, that branched-chain amino acids are metabolized by isolated adipose tissue, with the release of alanine and glutamine (Tischler & Goldberg, 1980). The latter finding suggested that adipose tissue metabolized amino acids in a similar manner to skeletal muscle, with the additional capability of converting some of the carbon into triacylglycerol (for review, see Newsholme & Leech, 1983).

Consequently, it was surprising to find a considerable activity of the enzyme that utilizes glutamine, namely phosphate-dependent glutaminase, in adipose tissue (Cooney *et al.*, 1986) and that the activity, per mg of protein, was greater than that in liver and was similar to that in lymphocytes [cf. data of Cooney *et al.* (1986) with those of Newsholme *et al.* (1986)]. Lymphocytes are known to utilize glutamine at a very high rate, a rate which is similar to that in rapidly-dividing cells and tumour cells (Ardawi & Newsholme, 1982, 1983, 1985). The presence of a high activity of this enzyme in adipose tissue raised the question of whether this tissue could utilize glutamine and, if so, what is the fate of glutamine, and does the rate of utilization and its fate change under different conditions?

MATERIALS AND METHODS

Animals, chemicals, biochemicals and enzymes were obtained from sources given previously (Curi *et al.*, 1986; Newsholme *et al.*, 1986, 1987). Obese Zucker rats (male) were obtained as a gift from Beechams

Pharmaceutical Research Division, Great Burgh, Epsom, Surrey, U.K.; they weighed 300–400 g, and were fed *ad libitum* on laboratory chow. U- 14 C-labelled compounds were obtained from the Radiochemical Centre, Amersham, Bucks., U.K.

Preparation of adipocytes

Adipocytes were prepared as described by Green & Newsholme (1979). Adipocytes and stromal vascular cells were separated as described by Green & Newsholme (1981). Adipose tissue was obtained from several sites in the rat, and was extracted as described previously (Cooney *et al.*, 1986). Glutaminase activity was assayed as described by Curthoys & Lowry (1973); the assay medium contained 150 mM-phosphate buffer (equimolar mixture of K_2HPO_4 and KH_2PO_4), 0.2 mM-EDTA, 50 mM-Tris/HCl, 20 mM-glutamine and 10–20 μ l of extract [treated immediately before assay with 1% (v/v) Triton X-100] at pH 8.6 in final volume of 1.0 ml. The assay was initiated by addition of freshly prepared glutamine, and the medium was incubated at 37 °C for 10 min (preliminary experiments established that the time course was linear for at least 10 min). The reaction was stopped by addition of 0.2 ml of 25% (w/v) HClO_4 , and protein was removed by centrifugation; the supernatant was neutralized with KHCO_3 , and the concentration of glutamate determined as described by Bernt & Bergmeyer (1974). Preliminary experiments suggested that the conditions of extraction and assay were optimal for glutaminase activity; see Cooney *et al.* (1986). Protein was assayed as described by Bradford (1976).

Adipocytes were incubated as described by Green & Newsholme (1979), and the $^{14}\text{CO}_2$ was collected as described by Leighton *et al.* (1985). To measure the incorporation of 14 C into triacylglycerol from the 14 C-

labelled substrates, the following procedure was used: adipocytes were ruptured by the addition of 5 ml of propan-2-ol/n-heptane/1 M-H₂SO₄ (40:10:1, by vol.), and the triacylglycerol was extracted by addition of 3 ml of n-heptane and 2 ml of water. The aqueous layer was separated by freezing with solid CO₂, and the upper heptane layer was transferred to scintillation vials, evaporated to dryness under compressed air, and the radioactivity incorporated into triacylglycerol was determined after the addition of scintillant.

RESULTS AND DISCUSSION

The activity of glutaminase in extracts of epididymal adipose tissue from normal rats (six) was found to be 0.82 ± 0.08 , 3.3 ± 0.32 and 15.1 ± 2.1 $\mu\text{mol/min per g}$ fresh wt., $\mu\text{mol/min per g}$ dry wt. and nmol/min per mg of protein respectively. These values are similar to those published previously for adipose tissue (Cooney *et al.*, 1986) and, on a mg-of-protein basis, are approximately one-third of those in rat lymphocytes or kidney (40 nmol/min per mg of protein; Ardawi & Newsholme, 1984; Newsholme *et al.*, 1986). The activities of glutaminase were measured in several adipose-tissue sites in

both lean control and Zucker obese rats (Table 1). On the basis of mg of protein, glutaminase activity demonstrated considerable variation between sites, with lower activities found in epididymal and brown adipose tissue. In general, the activities are lower in the obese animal. This finding indicates that the rate of conversion of carbon from glutamine into triacylglycerol is unlikely to play a major role in the cause of obesity in the Zucker rat. It seemed possible that the glutaminase activity could be contained in the stromal-vascular cells rather than in the adipocytes; the two types of cells were separated by centrifugation (see Green & Newsholme, 1981); the major activity (> 90%) was associated with the adipocyte (results not shown).

An important question is whether the presence of glutaminase activity is indicative of an ability of the tissue to utilize glutamine and, if so, what is the fate of the glutamine? Adipocytes incubated with 2 mM-glutamine utilized glutamine at a rate of almost 2 $\mu\text{mol/h per g}$ dry wt. (Table 2). The presence of glutamine increased the rates of formation of ammonia, glutamate, alanine and lactate (Table 2); the rate of production of aspartate was almost zero (results not shown); about 40% of the glutamine utilized was recovered as glutamate. All of the nitrogen from the utilized glutamine could be accounted for as ammonia, glutamate and alanine. However, the rates of production of glutamate, alanine and lactate exceeded that of glutamine used (Table 2), suggesting that metabolism of glutamine may have facilitated an increased rate of utilization of an endogenous non-nitrogen-containing fuel (e.g. glycogen).

When glucose and insulin were added together to the incubation medium, the rate of glutamine utilization and those of ammonia, glutamate, lactate and alanine production were increased (Table 2). However, when added separately they had no effects on the rates of utilization or production (Table 2). β -Adrenoreceptor agonists are known to influence metabolism in adipose tissue (see Smith & Saggerson, 1978), so that it was important to assess their effects on glutamine metabolism. The β -agonist isoprenaline was chosen since it is chemically stable during a 60 min incubation period. It increased the rate of glutamine utilization, and particularly increased the rate of alanine formation (Table 2).

Table 1. Activities of glutaminase in different adipose-tissue sites from lean control and Zucker obese rats

Values are means $\pm$ S.E.M. for six separate male animals in each group. Lean animals weighed 160–200 g and obese animals 300–400 g.

Adipose-tissue site	Glutaminase activity (nmol/min per mg of protein)	
	Lean control	Obese
Interscapular brown	17.1 ± 2.0	14.3 ± 1.1
Perirenal	22.1 ± 1.8	14.7 ± 0.9
Thoracic cavity	30.6 ± 3.2	24.7 ± 2.5
Interscapular white	32.4 ± 2.6	38.3 ± 3.5
Lumbar	45.8 ± 4.2	37.6 ± 5.7

Table 2. Effects of glucose, insulin and isoprenaline on the rates of glutamine utilization and production of ammonia, glutamate, alanine and lactate in isolated rat adipocytes

The concentration of glutamine was 2 mM. Values are corrected for a control incubation containing no glutamine. Minus sign indicates utilization. Values are means $\pm$ S.E.M. Values in parentheses indicate numbers of incubations with separate adipocyte preparations.

Additions to incubation medium	Rates of utilization or production ($\mu\text{mol/h per g}$ dry wt.)				
	Glutamine	Ammonia	Glutamate	Alanine	Lactate
None	-1.80 ± 0.3 (3)	1.30 ± 0.30	0.76 ± 0.2	1.61 ± 0.9	2.40 ± 1.1
1 mM-glucose	-2.30 ± 0.3 (3)	1.63 ± 0.30	0.80 ± 0.1	2.29 ± 1.2	3.01 ± 0.98
Insulin (10 munits/ml)	-1.70 ± 0.20 (3)	0.96 ± 0.10	0.89 ± 0.60	1.0 ± 0.5	2.40 ± 0.22
1 mM-glucose plus insulin (10 munits/ml)	-3.48 ± 0.40 (3)	2.10 ± 0.25	1.29 ± 0.13	3.89 ± 0.13	5.47 ± 1.40
Isoprenaline (100 μM)	-2.91 ± 0.32 (3)	1.80 ± 0.09	1.01 ± 0.12	5.03 ± 1.1	4.02 ± 0.80
1 mM-glucose plus isoprenaline (100 μM)	-4.09 ± 0.17 (3)	2.30 ± 0.18	1.35 ± 0.20	6.81 ± 2.4	8.43 ± 1.94

This could be explained by an increase in the rate of glycogenolysis, and hence in the concentration of pyruvate, which would facilitate transamination from glutamate; the rate of glycolysis was not measured in this study, but isoprenaline is known to stimulate phosphorylase activity in incubated adipocytes (Cheng & Lerner, 1987). The highest rate of glutamine utilization was observed in the presence of isoprenaline and glucose (Table 2). The mechanism(s) by which insulin plus glucose or isoprenaline stimulate glutamine utilization is not known. The rate of glutamine utilization in the absence of any addition is about 100 $\mu\text{mol/h}$ per 10^{10} cells, which is similar to the rate of utilization in lymphocytes, colonocytes, enterocytes and thymocytes (Ardawi & Newsholme, 1985), which are considered to use glutamine at high rates. Furthermore, similarly to lymphocytes, colonocytes and macrophages (Ardawi & Newsholme, 1985; Newsholme *et al.*, 1987), a high proportion of the glutamine that is utilized appears as glutamate. However, in contrast with these other cells, there was almost no production of aspartate (results not shown).

The two major fates of glucose used by adipose tissue are formation of CO_2 and triacylglycerol. In the present work, the rates of incorporation of [^{14}C]glutamine, [^{14}C]leucine, [^{14}C]glutamate and, for comparison, [^{14}C]glucose into these end-products were investigated. The rates of incorporation of [^{14}C]glucose into CO_2 and triacylglycerol were similar, and were both markedly increased by insulin, in confirmation of previous work (Table 3). The rate of incorporation of ^{14}C from glutamine or glutamate into CO_2 was similar to that of glucose, but incorporation into triacylglycerol was much less; addition of insulin alone had no effect on rate of either CO_2 or triacylglycerol formation from either amino acid (Table 3). The presence of unlabelled glucose had no effect on the rate of incorporation of ^{14}C from glutamine or glutamate into CO_2 , but increased that into triacylglycerol: a similar effect was seen with leucine. However, addition of insulin plus unlabelled glucose, although having no effect on CO_2 formation, increased (in comparison with glucose alone) the rates of incorporation of ^{14}C from [^{14}C]glutamine or [^{14}C]glutamate into triacylglycerol (Table 3); there was no effect of insulin plus glucose on the rate of ^{14}C incorporation from leucine into triacylglycerol. The presence of a β -agonist increases the rates of incorporation of [^{14}C]glucose into both CO_2 and triacylglycerol, but only increased the rate of CO_2 formation from glutamine or glutamate; for leucine, there was no effect on either process (Table 3). Addition of isoprenaline plus glucose increased the rates of incorporation of ^{14}C from glutamine, glutamate and leucine into CO_2 and decreased the rates of incorporation into triacylglycerol.

The effects of increasing the glucose concentration to 10 mM on the rates of incorporation of [^{14}C]glutamine into CO_2 and triacylglycerol are shown in Table 4. Increasing the concentration of glucose or insulin made no difference to the rate of incorporation into CO_2 ; insulin had no effect on the rate of incorporation into triacylglycerol in the absence of glucose, but in the presence of even 1 mM-glucose the rate was increased 10-fold, which is maximal. However, in the absence of insulin, increasing the concentration of glucose to 10 mM increases markedly the rate of incorporation into triacylglycerol. This suggests that the major effect of

Table 3. Rate of incorporation of [^{14}C]glucose, [^{14}C]glutamine, [^{14}C]glutamate or [^{14}C]leucine into CO_2 and triacylglycerol in incubated rat adipocytes

The final concentration and radioactivity in the incubation medium for the substrates are: glucose (1 mM; 0.25 $\mu\text{Ci/ml}$), glutamine (2 mM; 0.35 $\mu\text{Ci/ml}$), glutamate (2 mM; 0.35 $\mu\text{Ci/ml}$) and leucine (2 mM; 0.35 $\mu\text{Ci/ml}$). Values are presented as means $\pm$ S.E.M. Values in parentheses indicate numbers of incubations with separate adipocyte preparations.

Addition to medium	Rate of incorporation ($\mu\text{mol/h}$ per g dry wt.)					
	[^{14}C]Glucose incorporated into		[^{14}C]Glutamine incorporated into		[^{14}C]Leucine incorporated into	
	CO_2	Triacyl-glycerol	CO_2	Triacyl-glycerol	CO_2	Triacyl-glycerol
None	0.40 $\pm$ 0.026 (4)	0.41 $\pm$ 0.020	0.37 $\pm$ 0.005	0.02 $\pm$ 0.003	0.25 $\pm$ 0.033	0.14 $\pm$ 0.027
Insulin (10 munits/ml)	2.40 $\pm$ 0.280 (4)	3.05 $\pm$ 0.339	0.41 $\pm$ 0.007	0.02 $\pm$ 0.004	0.25 $\pm$ 0.038	0.13 $\pm$ 0.024
Glutamine (2 mM)	0.34 $\pm$ 0.039 (4)	0.44 $\pm$ 0.024	—	—	—	—
Insulin (10 munits/ml) plus glutamine (2 mM)	2.34 $\pm$ 0.209 (4)	3.20 $\pm$ 0.370	—	—	—	—
Glucose (1 mM)	—	—	0.40 $\pm$ 0.08 (4)	0.09 $\pm$ 0.007	0.34 $\pm$ 0.039	0.71 $\pm$ 0.138
Insulin (10 munits/ml) plus glucose (1 mM)	—	—	0.32 $\pm$ 0.007 (4)	0.22 $\pm$ 0.011	0.30 $\pm$ 0.029	0.82 $\pm$ 0.128
Isoprenaline (100 μM)	0.69 $\pm$ 0.013 (4)	0.78 $\pm$ 0.058	0.53 $\pm$ 0.019	0.02 $\pm$ 0.005	0.18 $\pm$ 0.013	0.13 $\pm$ 0.013
Isoprenaline (100 μM) plus glutamine (2 mM)	0.53 $\pm$ 0.096 (4)	0.84 $\pm$ 0.081	—	—	—	—
Isoprenaline (100 μM) plus glucose (1 mM)	—	—	0.70 $\pm$ 0.011 (4)	0.03 $\pm$ 0.004	0.72 $\pm$ 0.078	0.40 $\pm$ 0.100

Table 4. Effect of glucose and insulin (10 munits/ml) on the rates of incorporation of [¹⁴C]glutamine into CO₂ and triacylglycerol in isolated incubated adipocytes

The concentration of glutamine was 2 mM. Values are means $\pm$ S.E.M. Values in parentheses indicate numbers of incubations with separate adipocyte preparations.

Concn. of glucose (mM)	Rate of incorporation into (μ mol/h per g dry wt.)			
	CO ₂		Triacylglycerol	
	No insulin	+ insulin	No insulin	+ insulin
0	0.39 $\pm$ 0.004 (4)	0.45 $\pm$ 0.011 (4)	0.03 $\pm$ 0.001 (4)	0.03 $\pm$ 0.003 (4)
1	0.42 $\pm$ 0.006 (3)	0.38 $\pm$ 0.010 (4)	0.08 $\pm$ 0.002 (3)	0.27 $\pm$ 0.031 (4)
5	0.38 $\pm$ 0.018 (3)	0.37 $\pm$ 0.009 (4)	0.12 $\pm$ 0.004 (4)	0.28 $\pm$ 0.007 (4)
10	0.35 $\pm$ 0.011 (4)	0.35 $\pm$ 0.008 (4)	0.16 $\pm$ 0.015 (4)	0.26 $\pm$ 0.015 (4)

insulin on this process is achieved via an increase in the rate of glucose transport into the cell. The effect of insulin plus glucose or glucose alone is likely to be due to the increase of the concentration of glycerol 3-phosphate, the necessary cofactor for triacylglycerol synthesis.

The results in Tables 2 and 3 suggest that, if conditions for lipogenesis and triacylglycerol formation are not favourable, much of the glutamine removed by adipose tissue is converted into glutamate and is lost to the tissue; if, however, conditions are favourable, the glutamate is further metabolized to acetyl-CoA to provide the precursor for lipogenesis. If this explanation is correct, it is of interest that the presence of unlabelled glutamine (plus insulin) does not decrease the rate of incorporation of [¹⁴C]glucose into triacylglycerol (Table 3): it is tempting to speculate that, in the presence of insulin, glutamine can further activate the process of lipogenesis, so that it or a metabolite derived from glutamine might act as a regulator of lipogenesis.

The rate of glutamine utilization by adipocytes was approx. 2 μ mol/h per g dry wt. For a 200 g rat, which possesses 10% white adipose tissue, the total rate of utilization of glutamine by adipose tissue (assuming that all sites behave similarly) would be approx. 10 μ mol/h. The total lymphocyte population of a rat may utilize glutamine at a rate of approx. 20 μ mol/h (Ardawi & Newsholme, 1985), and that used by the kidneys of an acidotic rat is approx. 42 μ mol/h (Schröck *et al.*, 1980). Hence the rate of utilization by adipose tissue is not insignificant in comparison with these other tissues.

J. M. K. was the recipient of a Fellowship from the Medical Research Council of Canada, and R. C. was the recipient of a Fellowship from CNPq-Brazil.

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Received 13 July 1987/10 September 1987; accepted 29 September 1987