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Glutamine-synthesizing activity in lungs of fed, starved, acidotic, diabetic, injured and septic rats

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The maximal catalytic activity of glutamine synthetase was measured in lung homogenates of the rat (being 5.46 ± 0.29 μmol/min per g wet wt. or 31.70 ± 2.62 nmol/min per mg of protein at 37 °C, in fed animals). The activity is similar to that of liver, but 16-fold higher than that in quadriceps muscles. Chronic (NH_4Cl-induced) or acute (HCl-induced) metabolic acidosis had no effects on enzyme activity, but there was a marked increase in the activity of glutamine synthetase in starved (30–40%), streptozotocin-diabetic (17%), dexamethasone-treated (18–22%), laparotomized (25–27%) and septic rats (24–45%).

INTRODUCTION

Glutamine is the most abundant amino acid in the body. It has the highest concentration in plasma, and it accounts for over 50% of the intracellular amino acid content (Felig, 1975). Catabolic disease states such as trauma, sepsis, major surgery, burns and uncontrolled diabetes are characterized by accelerated muscle glutamine and alanine release. The last two amino acids account for more than half of the amino acids released by skeletal muscle during stress states, and hence are the principal nitrogen carriers from the periphery to the visceral organs (for reviews see Newsholme *et al.*, 1988, 1989). Glutamine is consumed by the gastrointestinal tract, where it is used as a fuel (for review see Windmueller, 1984), by the kidney, where it supports renal ammoniagenesis (for review see Welbourne, 1987), and by the immune-system cells, where it provides energy and metabolic intermediates (for reviews see Ardawi & Newsholme, 1985; Newsholme *et al.*, 1989) Alanine is removed mainly by the liver, where it can be converted into glucose via gluconeogenesis.

Skeletal muscle is thought to be the major site of endogenous formation of glutamine and alanine in normal and catabolic disease states, and it has been suggested that it contains the flux-generating step for the pathway of glutamine utilization by various tissues (see Newsholme *et al.*, 1988). Recently, marked net release of glutamine by the lungs of surgically septic patients has been described, and it was suggested that the ability of the lungs to export large amounts of glutamine is most likely secondary to accelerated intracellular synthesis of the amino acid *de novo* (Plumley *et al.*, 1990).

It is known that the only specific enzymic reaction for the formation of glutamine in muscle is catalysed by the non-equilibrium reaction of glutamine synthetase (EC 6.3.1.2), which is known to have a low activity in this tissue and to be affected by various catabolic disease states (King *et al.*, 1983; Ardawi, 1988; Ardawi & Jamal, 1990). The present work was designed to determine the effects of various catabolic conditions (e.g. sepsis, uncontrolled diabetes, glucocorticoid administration and surgical trauma) on the maximal catalytic activity of glutamine synthetase in lung extracts of the rat and to compare with that of skeletal muscle of the same animal.

MATERIALS AND METHODS

Animals

Male Wistar albino rats (200–240 g) and other animals (all adult males) were supplied by King Fahd Medical Research Centre, King Abdulaziz University, Jeddah, Saudi Arabia. Rats were maintained on a standard diet [commercial rat cubes containing (by wt.) approx. 18% protein, 3% fat, 77% carbohydrate and 2% organic salt mixture with a vitamin supplement] (Grain Silos and Flour Mills Organization, Jeddah, Saudi Arabia) and water *ad libitum*, and were kept in a controlled environment (constant temperature 24 °C, and a light cycle of 12 h on/12 h off). Rats were made acidotic by two types of treatments: (i) rats were made chronically acidotic by addition of 1.5% (w/v) NH_4Cl plus 5% (w/v) sucrose in the drinking water for 6–7 days (1.5% NaCl plus 5% sucrose were added to the drinking water of control animals); (ii) short-term acidosis was induced by administration of HCl (10 mmol/kg body wt., in a volume of water equal to 2.5% of the body wt.); controls were given water. Rats were made diabetic by a single intravenous injection of streptozotocin (70 mg/kg body wt. in 50 mM-sodium citrate, pH 4.5) under light ether anaesthesia: rats showing blood glucose greater than 20 mM were used at 7 days after induction of diabetes with appropriate controls. In another treatment group, rats were treated with dexamethasone (30 μg/day per 100 g body wt.) by a daily intramuscular injection (200–300 μl) for 9 days under light ether anaesthesia. The amount of dexamethasone injected daily corresponded approximately to the increased amount secreted by the adrenal steroids during major stress states (see Ardawi *et al.*, 1988). Control rats were injected with 0.9% (w/v) NaCl. Sepsis was induced by caecal ligation and puncture technique (see Ardawi *et al.*, 1989), and septic rats were starved and killed 48 h after the operation. Laparotomy was performed as described previously (Ardawi *et al.*, 1989). Controls for septic and laparotomized rats were sham-operated but fed rats.

Chemicals and enzymes

All chemicals and enzymes were obtained from Boehringer Corp., London, U.K., except for the following: streptozotocin and glutamate were obtained from Sigma Chemical Co., Poole, Dorset, U.K.; all inorganic reagents were obtained from Fisons, Loughborough, Leics., U.K.; and [1-^{14}C]glutamate was obtained from The Radiochemical Centre, Amersham, Bucks., U.K. Dowex (acetate form) was obtained from Bio-Rad (Richmond, CA, U.S.A.). Dexamethasone was obtained from MSD Ltd., Hoddesdon, Herts., U.K.

Preparation of homogenates

For the preparation of lung extracts, animals were killed by

cervical dislocation and the thorax was opened, and the lungs were perfused *in situ* with about 30 ml of 0.9% NaCl solution. The lungs were removed and cut into small pieces, weighed and homogenized in 10 vol. of extraction medium (50 mM-Tris/1 mM-EDTA, pH 7.9) by using a Polytron homogenizer (PCU-2, at position 6) for 2 × 20 s at 0–4 °C. The whole homogenate was used for enzyme assay. For the preparation of quadriceps extract, animals were killed by cervical dislocation and the quadriceps muscle was quickly dissected and extracted as described for the lungs. For determination of the apparent K_m of glutamine synthetase for ammonia, lung homogenates were dialysed against extraction medium for 24 h to remove endogenous ammonia.

Assay of enzyme activity

Glutamine synthetase activity was measured radiochemically by an assay method similar to that described by King *et al.* (1983). Briefly, the assay medium (200 μl) contained 50 mM-imidazole/HCl, 25 mM-$MgCl_2$, 20 mM-NH_4Cl, 20 mM-sodium L-glutamate, 0.25 μCi of L-[1-^{14}C]glutamic acid (20 Ci/mol), 15 mM-ATP, 10 mM-phosphocreatine and 1.5 units of creatine kinase, at pH 7.6. The assay was initiated by addition of 20 μl of freshly prepared tissue extract, and the assay mixture was incubated for 10 min at 37 °C. The assay mixture was stopped by addition of 100 μl of 12% (w/v) $HClO_4$, and protein was removed by centrifugation at 13800 *g* for 3 min in a Beckman Microfuge B. The supernatant was neutralized with conc. KOH, followed by 0.1 M-imidazole/HCl, pH 7.0, and the precipitate was removed by centrifugation at 13800 *g* for 3 min. [^{14}C]Glutamine formed in the assay mixture was separated from labelled precursor ([1-^{14}C]glutamate) by ion-exchange chromatography on Dowex (acetate form). A sample of neutralized supernatant (300 μl) was pipetted on top of the ion-exchange column (Pasteur pipette, 5 cm × 0.5 cm) pre-equilibrated with distilled water. The column was washed with 5 × 1 ml of distilled water, and a sample (1 ml) of the effluent was mixed with 10 ml of a scintillation mixture [2 g of 2,5-diphenyloxazole and 0.5 g of 1,4-bis-(5-phenyloxazol-2-yl)benzene in 500 ml of toluene and 250 ml of Triton X-100]. The radioactivity was measured in an LKB-1211 Rack-Beta scintillation counter. The activity of glutamine synthetase was linear with time for up to 35 min, and the activity was proportional to enzyme concentration over a 2–6-fold range. Preliminary experiments established that extraction and assay procedures were such as to provide maximum enzyme activities (see Crabtree *et al.*, 1979) and to exclude interference from glutamine transaminase and phosphate-dependent glutaminase reactions in the assay of glutamine synthetase. Enzyme activities are expressed as μmol/min per g wet wt. and nmol/min per mg of protein. Protein was determined by the bioMerieux protein assay (bioMerieux, Les Bains, France), with bovine serum albumin as standard. Results are presented as means ± S.E.M., and where appropriate comparisons between sets of data were made by Student's *t* test.

RESULTS AND DISCUSSION

The mean value for the maximum activity of glutamine

Table 1. Effects of starvation, acidosis, diabetes, dexamethasone, laparotomy and sepsis on lung and muscle glutamine synthetase activity

Treatment of animals, preparation, extraction of tissues and enzyme-activity measurements were carried out as described in the Materials and methods section. Results are presented as means ± S.E.M. for *n* animals. The numbers in parentheses represent the experimental values expressed as a percentage change over the corresponding control activity. Significance of differences between control and experimental means was tested by Student's *t* test: * $P < 0.05$, ** $P < 0.001$.

		Glutamine synthetase activity					
		Lung			Skeletal muscle		
Treatment	State of animal	*n*	(μmol/min per g wet wt.)	(nmol/min per mg of protein)	*n*	(μmol/min per g wet wt.)	(nmol/min per mg of protein)
Fed	Control	5	5.46 ± 0.29	31.70 ± 2.62	8	0.33 ± 0.024	1.84 ± 0.12
Starvation (48 h)	Control	6	5.65 ± 0.25	32.17 ± 2.81	6	0.31 ± 0.040	1.80 ± 0.20
	Experimental	5	5.56 ± 0.26* (+34%)	42.50 ± 3.10* (+32%)	6	0.48 ± 0.035** (+55%)	2.68 ± 0.18** (+49%)
Starvation (72 h)	Control	5	5.72 ± 0.38	32.03 ± 2.48	5	0.32 ± 0.036	1.75 ± 0.14
	Experimental	7	7.41 ± 0.24* (+30%)	42.97 ± 3.15* (+34%)	5	0.48 ± 0.018** (+50%)	2.68 ± 0.24** (+53%)
Acidosis (NH_4Cl)	Control	5	5.77 ± 0.06	33.95 ± 3.34	6	0.30 ± 0.016	1.69 ± 0.09
	Experimental	6	4.97 ± 0.15 (−14%)	29.24 ± 0.87 (−14%)	6	0.44 ± 0.028** (+47%)	2.38 ± 0.15** (+41%)
Acidosis (HCl)	Control	4	5.60 ± 0.50	31.81 ± 2.33	4	0.31 ± 0.025**	1.71 ± 0.11
	Experimental	4	5.22 ± 0.68 (−7%)	30.28 ± 3.55 (−5%)	4	0.38 ± 0.032* (+23%)	2.12 ± 0.20* (+24%)
Diabetes	Control	5	5.40 ± 0.34	31.32 ± 2.60	5	0.34 ± 0.04	1.95 ± 0.22
	Experimental	5	6.29 ± 0.10* (+17%)	36.48 ± 2.20* (+17%)	6	0.41 ± 0.028* (+21%)	2.27 ± 0.30* (+18%)
Dexamethasone	Control	5	5.38 ± 0.20	30.94 ± 1.85	6	0.31 ± 0.036	1.75 ± 0.14
	Experimental	6	6.35 ± 0.42* (+18%)	37.60 ± 2.42* (+22%)	6	0.48 ± 0.012** (+55%)	2.64 ± 0.18** (+51%)
Laparotomy	Control	5	5.27 ± 0.46	30.04 ± 2.16	6	0.22 ± 0.0030	1.52 ± 0.12
	Experimental	5	6.56 ± 0.30* (+25%)	38.05 ± 1.88* (+27%)	6	0.30 ± 0.040* (+36%)	1.74 ± 0.15 (+15%)
Sepsis	Control	5	5.39 ± 0.24	31.26 ± 2.22	6	0.29 ± 0.035	1.58 ± 0.22
	Experimental	8	6.66 ± 0.40* (+24%)	45.29 ± 2.50** (+45%)	6	0.50 ± 0.070** (+72%)	2.81 ± 0.25** (+78%)

synthetase in lung homogenates of normally fed rats is approx. 5.46 μol/min per g wet wt. (32 nmol/min per mg of protein) at 37 °C, which is similar to that found in the liver (being 5.92 μmol/min per g wet wt. at 37 °C) but more than 16 times that found in quadriceps muscle of the rat. Similar activities were found in different types of skeletal muscles, and thus enzyme activity in quadriceps should be representative of other skeletal muscles of the rat (see King *et al.*, 1983). Similar enzyme activities were found in lung homogenates of other animals, including man, and were as follows (expressed as μmol/min per g wet wt. at 37 °C and presented as means ± S.E.M.): rabbit [4.20 ± 0.95 ($n = 6$)]; guinea pig [5.11 ± 0.36 ($n = 5$)]; hamster [4.85 ± 0.52 ($n = 5$)]; mouse [5.65 ± 0.45 ($n = 6$)] and man [6.13 ± 2.15 ($n = 3$)]. In the present work, the activity of glutamine synthetase in the lung is much higher, whereas that in skeletal muscle is much lower than that reported previously (Herzfeld & Estes, 1973). These findings could be related to differences in extraction and assay procedures employed.

The apparent K_m of lung glutamine synthetase for glutamate was 13.35 ± 1.20 mM (mean ± S.E.M. for five separate determinations), which may be compared with values of 5.0, 13.0 and 15.0 mM found, by similar assay procedures, for skeletal muscle, brain and liver enzymes respectively (see Lund, 1970; King *et al.*, 1983). The apparent K_m of lung glutamine synthetase for ammonia was 10.63 ± 1.70 mM (mean ± S.E.M. for four separate determinations). Starvation of rats for 48 or 72 h increased significantly the activity of glutamine synthetase in both lung and quadriceps homogenates (see Table 1). It is known that starvation increases glutamine release from skeletal muscle (Adibi, 1971) and, according to the changes in lung glutamine synthetase activity, it is expected that starvation may enhance glutamine release from the lungs. The latter was confirmed in incubated rat lung slices (M. S. M. Ardawi, unpublished work).

Unlike glutamine synthetase of skeletal muscle, which was found to be responsive to acidosis (see Table 1), that of the lung was not affected by acidosis induced via the administration of NH_4Cl or HCl. Conversely, streptozotocin-induced diabetes (for 7 days) resulted in a moderate increase in the activity of lung glutamine synthetase (17%).

It is known that administration of adrenal steroids enhances the activity of glutamine synthetase in a variety of cells (Harmon & Thompson, 1982). As shown in Table 1, administration of the glucocorticoid dexamethasone resulted in a marked increase in the activity of glutamine synthetase in both lung and quadriceps preparations. The effect on muscle is consistent with previous observations (e.g. King *et al.*, 1983; Ardawi & Jamal, 1990).

Both sepsis and laparotomy increased significantly the activity of glutamine synthetase in lung and quadriceps preparations. These findings are consistent with the observations that there is an enhanced rate of release of glutamine across the lungs of surgically septic patients (Plumley *et al.*, 1990) and across the leg of surgically ill patients (Stjernstrom *et al.*, 1986).

The increase in the activity of glutamine synthetase in various catabolic disease states is consistent with the enhanced rate of release of glutamine by skeletal muscle under these conditions (see Newsholme *et al.*, 1988). This is consistent with the view that this enzyme plays a role in the production of glutamine for release by muscle and lungs (based on the findings of the present work) and that the increase may be part of a mechanism that enables both muscle and lungs to respond to the increase in demand for glutamine by other tissues (e.g. gut, cells of the immune system). However, the basis for this increase in activity is unknown, but it could be either related to an increase in the concentration of the enzyme protein or due to covalent modification of the enzyme. Furthermore, the physiological stimulus for the increase in the activity of glutamine synthetase in lungs as well as in muscle is not known, but it is possible that it is hormonally mediated. The latter is supported by the changes in enzyme activity in response to starvation, diabetes, administration of glucocorticoid, trauma and sepsis.

It is possible that the activity of the enzyme *in vivo* is regulated allosterically by lung metabolites (see also King *et al.*, 1983). For instance, the addition of leucine, isoleucine and valine (added to the incubation medium at a final concentration of 10 mM) increased the enzyme activity by 95.7, 45.5 and 84.7% respectively. Whether the concentrations of branched-chain amino acids and/or other metabolites in the lungs change in response to catabolic disease states and contribute to the increased rate of glutamine synthesis and release is not known, and further work is needed in this respect.

The lung is a heterogeneous tissue comprised of approx. 40 different cell types (Sorokin, 1970), including type II pneumocytes, macrophages and lymphocytes, and the specific cell type(s) that contains the activity of glutamine synthetase and has the capacity to release glutamine into blood is unknown. Therefore the activity of glutamine synthetase in the lung will only reflect the mean enzyme activity of all the enzyme-specific constitutive cell types present in the tissue.

Although the ability of the lungs to release large amounts of glutamine [being about 85 μmol/h for a 200 g rat (M. S. M. Ardawi, unpublished work)] is related to the large flow of blood through the pulmonary vasculature, its glutamine synthetase activity, being high, further supports a greater capacity of the lung to release glutamine per g wt. of tissue compared with that of muscle. However, the weight of the lungs ($< 1\%$ of total body wt.) is markedly less than that of skeletal muscle (30–40% of total body wt.); this is consistent with the view that skeletal muscle is the principal site of free intracellular glutamine. The latter suggests that the large amounts of glutamine released by lungs are therefore must likely secondary to an accelerated intracellular synthesis *de novo* rather than the release of already existing intracellular stores, which is consistent with the fact that the lungs contain less free glutamine per g of tissue than does muscle (Herbert *et al.*, 1966).

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