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Glutamine synthetase activity of muscle in acidosis

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Metabolic acidosis stimulates the rate of glutamine release from muscle, and this in turn is used by the kidney in acid–base balance. NH_4Cl, HCl or diabetic ketoacidosis increases the maximum activity of glutamine synthetase in skeletal muscle. Starvation and administration of adrenal steroids also increase the activity of the enzyme in muscle.

Glutamine is an important fuel for small intestine (for review see Parsons, 1979) and is also utilized by the kidney during acidosis. Most, if not all, of the glutamine produced from dietary protein in the intestinal lumen is utilized by this tissue, so that the glutamine required by other tissues must arise endogenously. Muscle is thought to be the major site of formation of this glutamine (Felig, 1975; Schrock & Goldstein, 1981). Not only is muscle the major source of endogenous glutamine, but it has been suggested that it contains the flux-generating step for the pathway of glutamine utilization in the kidney (for review see Newsholme & Leech, 1983). Consequently the rate of glutamine production and release by muscle must play a role in the control of glutamine utilization by the kidney. Indeed it has been shown that under some conditions the increased rate of glutamine utilization by the kidney during acidosis is matched almost precisely by its increased rate of release from muscle (Schrock *et al.*, 1980; Schrock & Goldstein, 1981). At present there is no indication of how this metabolic control between kidney and muscle is achieved.

The only specific enzymic reaction for the formation of glutamine in muscle is that catalysed by glutamine synthetase (EC 6.3.1.2), which is known to have a low activity in this tissue (Lund, 1970; Iqbal & Ottaway, 1970). Since acidosis not only increases the rate of glutamine formation and release, but also decreases the concentration of glutamate in the muscle (Goldstein *et al.*, 1983), the activity of glutamine synthetase must be increased by factors other than the concentration of glutamate. One possible factor is an increase in the activity of glutamine synthetase in the muscle. This has been investigated by measuring the maximum catalytic activity of this enzyme in extracts of muscle obtained from normal and acidotic animals.

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Materials and methods

Animals

Male Wistar rats (150–250g) were obtained from Batin and Kingman, Grimston, Yorks. HU11 4QE, U.K. Rats were fed on a standard maintenance diet and water supplied *ad libitum*, except where indicated. Acidosis was induced by three 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 5–6 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 (10mmol/kg body wt., in a volume of water equal to 2.5% of the body weight) by stomach tube under light anaesthesia. Controls were given water. (iii) Rats were made diabetic by streptozotocin injection (150mg/kg) and maintained for several days by insulin injection, and ketoacidosis was induced by withdrawal of insulin treatment (Blackshear & Alberti, 1974). In another treatment group, rats were injected subcutaneously once a day with dexamethasone (0.5mg/100g body wt.) for 3 days. Control rats were untreated.

Chemicals and enzymes

All chemicals and enzymes were obtained from Boehringer Mannheim, Lewes, Sussex, U.K., except for the following: glutamate and imidazole were obtained from Sigma Chemical Co., Poole, Dorset, U.K.; the scintillant and all inorganic reagents were obtained from Fisons Scientific Apparatus, Loughborough, Leics. LE11 0RG, U.K.; and [^{14}C]glutamate was obtained from The Radiochemical Centre, Amersham, Bucks. HP7 9LL, U.K. Dowex (acetate form) was obtained from Bio-Rad (Richmond, CA, U.S.A.).

Preparation of homogenates

The rats were anaesthetized (60mg of pentobarbital sodium/kg, intraperitoneally) on the morn-

ing after the last day of treatment (except for short-term HCl-induced acidosis, for which animals were anaesthetized 5–6h after the last HCl dose). The quadriceps muscle was quickly dissected and the animal killed by cervical dislocation. The tissue was quickly weighed and homogenized in 9vol. of extraction medium (50mM-Tris/2mM-EDTA, pH7.9) with a Polytron set at position 4 for 15s at 0°C. The homogenate was centrifuged at 200*g* for 4min and the supernatant used for enzyme assay.

Assay of enzyme activity

Glutamine synthetase activity was measured by a radiochemical assay modified from that of Tiemeier & Milman (1972). The reaction was started by the addition of 20μl of the supernatant to 180μl of reaction medium. The latter consisted of 50mM-imidazole/HCl, 20mM-$MgCl_2$, 20mM-NH_4Cl, 20mM-sodium L-glutamate, 0.25μCi of L-[1-^{14}C]-glutamic acid (20Ci/mol), 15mM-ATP, 10mM-phosphocreatine and 1.2 units of creatine kinase, at pH7.6. The mixture was incubated for 15min at 37°C, and then the reaction was stopped by adding 100μl of 12% (v/v) $HClO_4$. Perchlorate was removed by addition of concentrated KOH, followed by 0.1M-imidazole/HCl, pH7.0, and the precipitate was removed by centrifugation at 10000*g* for 2min. [^{14}C]Glutamine formed in the assay was separated from the labelled precursor ([^{14}C]glutamate) by ion-exchange chromotography on Dowex (acetate form). A sample of supernatant (350μl) was pipetted on top of the ion-exchange column (Pasteur pipette, 5cm × 0.5cm) previously equilibrated with distilled water. The column was washed with 5ml of distilled water, and a sample (1ml) of the effluent added to 10ml of a scintillation mixture [2g of 2,5-diphenyloxazole and 0.5g of 1,4-bis-(5-phenyloxazol-2-yl)-benzene in 500ml of toluene plus 250ml of Triton X-100]. The radioactivity was measured in a Beckman scintillation counter model LS 800. This separation procedure resulted in recovery of over 90% of the glutamine formed, whereas less than 0.1% of the glutamate came through the columns. Glutamine synthetase activity was linear with time for up to 30min, and the activity was proportional to enzyme concentration over at least a 2-fold range. Enzyme activity is expressed as nmol/min per g wet wt. and nmol/min per mg of protein. Protein was measured by the Bio-Rad protein assay. Statistical analysis was performed using Student's *t* test for group data.

Results and discussion

The K_m of muscle glutamine synthetase for glutamate was 5.0mM, which may be compared with the values of 13 and 15mM found by Lund (1970) for the brain and liver enzyme, by a similar assay procedure. The tissue homogenate contained sufficient ammonia nearly to saturate the enzyme and to preclude the possibility of determining a K_m for ammonia.

The mean value for the activity of glutamine synthetase in muscle of normal rats is approx. 233nmol/min per g, which is similar to activities reported by Iqbal & Ottaway (1970) and Lund (1970) for rat skeletal muscle. The glutamine synthetase activities in quadriceps, soleus and epitrochlearis muscles are 303, 240 and 290nmol/min per g respectively. Since similar activities were found in these different muscles, only quadriceps muscle was used in the following studies. The enzyme activity in muscle is low compared with that in liver (4917nmol/min per g). A similar difference between the activities in liver and skeletal muscle of rat was noted previously by Lund (1970).

Administration of NH_4Cl in the drinking water for 5–6 days produced about a 50% increase in muscle glutamine synthetase activity when expressed on a fresh-weight or protein basis (Table 1). To rule out the possibility that the NH_4^+ moiety of NH_4Cl was the factor causing the increase in glutamine synthetase, acidosis was induced by administration of HCl by stomach tube. As shown in Table 1, administration of HCl for only 1.5 days raised muscle glutamine synthetase activity by 40%. This result indicates that acidosis and not the NH_4^+ load is responsible for the rise in glutamine synthetase activity during NH_4Cl treatment. Diabetic ketoacidosis leads to a large increase in muscle glutamine release (Schrock & Goldstein, 1981). Therefore we tested the effects of diabetic ketoacidosis on muscle glutamine synthetase activity. As shown in Table 1, diabetic ketoacidosis raised enzyme activity by about 70%.

Starvation increases glutamine release from skeletal muscle (Adibi, 1971), and adrenal steroids increase glutamine synthetase activities in a variety of cells (Harmon & Thompson, 1982). As shown in Table 1, both starvation and administration of the glucocorticoid dexamethasone produced significant increases in the activity of glutamine synthetase in muscle.

Administration of NH_4Cl or HCl and diabetic ketoacidosis are all conditions of sufficiently severe acidosis that demand a high rate of renal ammonia production from glutamine to facilitate proton excretion. The rate of glutamine utilization may increase by up to 10-fold in some of these conditions, and most of this glutamine is provided by the muscle. Indeed, it is known that glutamine release by rat skeletal muscle doubles (Schrock & Goldstein, 1981). The increase in activity of glutamine synthetase in these conditions also almost doubles (Table 1), which is consistent with the view that this enzyme plays a role in the production of glutamine

Table 1. *Effects of acidoses, dexamethasone and starvation on muscle glutamine synthetase activity*

Results are expressed as means ± S.E.M. Glutamine synthase activities in terms of nmol/min per mg of protein for the NH_4Cl-treated rats and their controls were obtained by dividing the mean enzyme activity per g fresh wt. by the mean value for muscle protein per g fresh wt., the latter values being obtained from separate groups of similarly treated control and experimental animals. The number of animals per group is indicated by *n*. The numbers in parentheses represent the experimental values expressed as a percentage increase over the control activity. Significance of differences between control and experimental means was tested by Student's *t* test: * $P<0.05$, ** $P<0.01$ and *** $P<0.001$.

		Glutamine synthetase activity			
		(nmol/min per g wet wt.)		(nmol/min per mg of protein)	
Treatment	*n*	Control	Experimental	Control	Experimental
NH_4Cl (0.28 M, in drinking water for 5–6 days)	10	280 ± 17	432 ± 28** (+54%)	2.30	3.62 (+57%)
HCl (20 mmol/kg per day for 1.5 days)	8	267 ± 27	373 ± 33* (+40%)	2.35 ± 0.22	3.10 ± 0.27* (+32%)
Diabetic ketoacidosis (3 days)	12	232 ± 17	392 ± 25*** (+69%)	1.73 ± 0.13	2.80 ± 0.18*** (+62%)
Dexamethasone (5 mg/kg per day for 3 days)	6	157 ± 17	380 ± 27*** (+142%)	1.15 ± 0.12	2.53 ± 0.20*** (+120%)
Starved (2 days)	6	228 ± 35	480 ± 38*** (+110%)	2.00 ± 0.35	3.85 ± 0.35** (+92%)

for release by muscle and that the increase in activity may be part of the mechanism that enables the muscle to respond to the increase in demand for glutamine by the kidney. The basis for this increase in activity is not known, but it could be either an increase in concentration of enzyme protein or a covalent modification (e.g. phosphorylation) of the enzyme.

The physiological stimulus for the increase in activity of the enzyme in muscle is also not known, but it is possible it is hormonally mediated. This view is supported by the fact that a similar increase in activity to that produced by acidosis was also caused by administration of the glucocorticoid dexamethasone and by starvation, which lowers insulin concentrations (Table 1). It is possible that the activity of the enzyme *in vivo* is also regulated allosterically by muscle metabolites. For example, we found that leucine, isoleucine or valine (added to the incubation medium at a final concentration of 10 mM) increased the enzyme activity by 37, 15 and 47% respectively. Since it is known (Felig *et al.*, 1970; Goldstein *et al.*, 1983) that the concentration of these branched-chain amino acids rises in muscle of animals with diabetic ketoacidosis, this effect may contribute to the increased rate of glutamine release seen *in vivo*.

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References

Adibi, S. A. (1971) *Am. J. Physiol.* **221**, 829–838
Blackshear, P. J. & Alberti, K. G. M. M. (1974) *Biochem. J.* **138**, 107–117
Felig, P. (1975) *Annu. Rev. Biochem.* **44**, 933–955
Felig, P., Marliss, E., Ohman, J. L. & Cahill, G. F., Jr. (1970) *Diabetes* **19**, 727–729
Goldstein, L., Perlman, D. F., McLaughlin, P. M., King, P. A. & Cha, C.-J. (1983) *Biochem. J.* **214**, 757–767
Harmon, J. M. & Thompson, E. B. (1982) *J. Cell. Physiol.* **110**, 155–160
Iqbal, K. & Ottaway, J. H. (1970) *Biochem. J.* **119**, 145–156
Lund, P. (1970) *Biochem. J.* **118**, 35–39
Newsholme, E. A. & Leech, A. R. (1983) *Biochemistry for the Medical Sciences*, pp. 509–535, John Wiley and Sons, London
Parsons, D. S. (1979) *Top. Gastroenterol.* **7**, 253–271
Schrock, H. & Goldstein, L. (1981) *Am. J. Physiol.* **240**, E519–E525
Schrock, H., Cha, C.-J. & Goldstein, L. (1980) *Biochem. J.* **188**, 557–560
Tiemeier, D. C. & Milman, G. (1972) *J. Biol. Chem.* **247**, 2272–2277