BIOCHEMICAL JOURNAL LETTERS

Glutamine transport and metabolism in mammalian skeletal muscle

We were interested to read the recent paper by Wu *et al.* (1991) on glutamine metabolism in skeletal muscle and the subsequent letter by Parry-Billings & Newsholme (1991). Unfortunately Parry-Billings & Newsholme, who propose a glutamine/glutamate substrate cycle involving glutaminase and glutamine synthetase in combination with a transmembrane glutamine translocation cycle (with supposed benefits for metabolic control), have apparently made only a selective interpretation of the paper by Wu *et al.* (1991). In that paper, the results were consistent with oxidative decarboxylation of glutamine in rat skeletal muscle mainly involving sarcoplasmic glutamine aminotransferase/ω-amidase and *not* mitochondrial glutaminase as Parry-Billings & Newsholme (1991) suggest. Wu *et al.* (1991) clearly showed that in rat muscle: (*a*) the transaminase/ω-amidase enzymes can support a *greater* rate of glutamine catabolism than muscle glutaminase, and (*b*) the glutaminase inhibitor 6-diazo-5-oxo-L-norleucine (DON), which is accumulated in muscle (Hundal & Rennie, 1989), did not inhibit glutamine breakdown.

Wu and colleagues emphasize the potential role of the glutamine aminotransferase/ω-amidase pathway in modulating intramuscular glutamine turnover. This area clearly merits further research, not least because there are some difficulties *a priori* in accepting a physiological function for the pathway. We are not aware of published values for the concentrations of favoured oxo-acid substrates (e.g. phenylpyruvate, 4-methylthio-2-oxobutyrate) in skeletal muscle but those of the branched-chain oxo-acids are in the appropriate range (5–50 μM) for the aminotransferase reaction (e.g. Hutson *et al.*, 1980) and, although these oxo-acids could accept amino groups from glutamine to produce branched-chain amino acids, much of the nitrogen for muscle glutamine synthesis is probably derived already from branched-chain amino acids (Darmaun & Dechelotte, 1991): the net benefit to the organism would therefore be small. A potential for phenylalanine synthesis from phenylpyruvate has been demonstrated for man *in vivo* (Halliday *et al.*, 1981), but only about 30% of an intravenous dose is transaminated (the remaining 70% apparently removed by competing processes such as oxidation and renal excretion).

Thus the results of Wu *et al.* (1991) do not readily fit the theory of a physiologically useful glutamine/glutamate cycle involving glutamine synthetase and glutaminase within mammalian muscle cells, not least because it appears that the two enzymes may be localized within different cell types in whole skeletal muscles (Kelso *et al.*, 1989). Synthesis and degradation of glutamine in liver (and probably kidney) occurs to a large extent separately in distinct types of cell (Häussinger, 1990; Watford, 1991) and the concerted action of the processes owes much to the micro-anatomical arrangement of these within the tissue. Intrahepatic compartmentation of glutamine synthetase and glutaminase and the intercellular substrate cycle developed by their simultaneous activity are well established (Häussinger, 1990), but the additional occurrence of the glutamine aminotransferase/ω-amidase pathway (which is active in liver when suitable substrates are present) could also contribute to glutamine turnover (Häussinger *et al.*, 1985). it is conceivable that in muscle tissue an intercellular glutamine/glutamate cycle involving glutamine synthetase and glutamine aminotransferase/ω-amidase could exist; indeed, metabolic compartmentation of glutamine/ammonia (glutamate) metabolism apparently occurs in the heart (Krivokapich *et al.*, 1984) and an analogous arrangement could occur in skeletal muscle.

On a broader front, we suggest that the whole question of the general applicability and utility of *intracellular* substrate cycles should be challenged: there is evidence that in man the intracellular fatty acid/triacylglycerol cycle proposed by Newsholme & Crabtree (1976) for adipocytes does not operate *in vivo* (Wolfe *et al.*, 1990); furthermore, the proposed fructose 1,6-bisphosphate/fructose 6-phosphate cycle is not only quantitatively insignificant but seems to be a constant fraction of the hepatic glucose production under all circumstances, disqualifying it as a putative amplification mechanism operating to rapidly match metabolic requirements to acutely imposed metabolic needs (Miyoshi *et al.*, 1988). Identification of the components necessary for effecting a substrate or translocation cycle are not clear signs of its operation *in situ* and still less of its operation contributing to an enhancement of physiological control. Thus the concept of an intracellular glutamine/glutamate cycle in muscle might usefully be viewed with caution. Furthermore, the arguments set out by Newsholme & Parry-Billings (1990) for a glutamine translocation cycle across the sarcolemma appear to be flawed. They argue that inward and outward steps of glutamine transport across the muscle membrane are not near-equilibrium and occur by separate processes. However, ΔG for glutamine entry is not $-12.3\ \mathrm{kJ \cdot mol^{-1}}$ as they claim, but actually only $-3.6\ \mathrm{kJ \cdot mol^{-1}}$ (the error appears to have resulted from incorrect attribution of the sign in that part of their calculation dealing with the products:reactants ratio). The corrected value is less than the value of $4.2\ \mathrm{kJ \cdot mol^{-1}}$ that these authors use (for reasons not clarified by the reference they cite) as a threshold for the identification of non-equilibrium reactions. In addition, when calculating ΔG for the process of glutamine release by skeletal muscle, they effectively assume both that the equilibrium constant for the reaction is unity and also that transport is unaffected by ions or membrane potential, without supporting evidence.

Previous studies have provided good evidence that, in the liver, the inward and outward steps of glutamine transport do occur largely by different pathways: namely, concentrative influx through the Na^+-dependent system N and dissipative efflux through the Na^+-independent system L (Pogson *et al.*, 1990). Nevertheless, the situation in skeletal muscle appears to be different (Rennie *et al.*, 1990): influx is mediated by a variant of system N (system N^m) but efflux does *not* exhibit the characteristic features of system L or its variants {i.e. *trans*-stimulation by amino acids such as leucine, phenylalanine or 2-amino-2-carboxybicyclo[2,2,1]heptane (BCH) and independence of the sodium gradient and membrane potential}. It does, however, show *trans*-stimulation by the system N^m substrates glutamine and asparagine (Willhoft & Rennie, 1992). There is therefore

some evidence for coupling of influx and efflux of glutamine across the muscle membrane, although at present we do not know the extent to which the two processes share the same transporter or reaction mechanism.

In our view, the balance of the evidence at present available does not support the existence of metabolically-important substrate or translocation cycles involving glutamine in skeletal muscle, at least in the form described by Parry-Billings & Newsholme.

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Received 23 December 1991

Some evidence for the existence of substrate cycles and their utility *in vivo*

The concept of substrate cycles for improving sensitivity in control was first proposed by Newsholme & Gevers in 1967, extended by Newsholme & Crabtree in 1970, and the quantitative basis of this suggested improvement, and the potential for heat generation, were established by Newsholme & Crabtree in 1976. A historical account of the development of the concept of cycling up to that time is given by Newsholme (1976). On the basis of publications since then, a sample of which is given below, we consider there is considerable evidence to support the original suggestion of utility rather than futility of such cycles and hence the statement by Rennie *et al.* (1992) that "the whole question of the general applicability and utility of intracellular substrate cycles should be challenged" is somewhat out-of-date.

First, it is important to appreciate that the original concept encompassed *both* intracellular and extracellular cycles. Thus the *extracellular* triacylglycerol/fatty acid cycle between liver and adipose tissue was discussed by Newsholme & Crabtree (1976) and it was *hypothesized* that it could play a role in "maintaining the concentrations of a fuel (fatty acid) despite large variations in its rate of utilisation". Strong support for this hypothesis is provided in the paper cited by Rennie *et al.* (1992), that of Wolfe and co-workers (1990).

Second, in contrast to the statement of Rennie *et al.* (1992), Wolfe and co-workers did report a rate of the *intracellular* triacylglycerol/fatty acid substrate cycle in man: "the rate of intracellular cycling was 1.3 μmol$\cdot$kg$^{-1}\cdot$min^{-1} at rest, which was 20% of the total fatty acid released". Indeed this may represent a minimum rate of cycling since Wolfe *et al.* (1990) assume that adipose tissue contains *no* glycerol kinase: this is not the case; see Robinson & Newsholme (1967). Furthermore, Wolfe *et al.* (1987*a*) demonstrated that the *intracellular* cycle is increased 15-fold in adipose tissue of patients suffering from severe burn injury and that this one cycle could contribute significantly to the increased thermogenesis in these patients. Wolfe *et al.* (1987*b*,*c*) provide evidence that infusion of glucose in man increases the rate of this cycle in adipose tissue and such changes are consistent with changes in the sensitivity of lipolysis. Other workers have also reported significant rates of the *intracellular* triacylglyerol/fatty acid cycle in adipose tissue of man *in vivo* (Coppack *et al.*, 1989, 1990) and *in vitro* (Leibel & Hirsch, 1985; Hammond & Johnson, 1987).

Third, it was proposed by Newsholme (1980) that enhanced activity of substrate cycles after exercise might explain some if not a high proportion of the oxygen debt (now known as recovery oxygen): the work of Bahr *et al.* (1990) in man has shown that, at 3 h after cessation of prolonged exercise, 50% of the recovery oxygen could be accounted for by the increased rate of the tricylglycerol/fatty acid substrate cycle.

Fourth, a direct method for measuring the rate of the intracellular triacylglycerol/fatty acid substrate cycle in adipose tissue *in vivo* in experimental animals by using tritiated water was established by Brooks *et al.* (1983). This technique has been used to show that not only does this cycle exist in adipose tissue of mouse, rat and hamster *in vivo* but that the rate can be increased by catecholamines, by glucose administration and after exercise (Brooks *et al.*, 1983; Dobbin, 1986; Mattacks & Pond, 1988; Tagliaferro *et al.*, 1990); it is known that the rate of this cycle is increased *in vivo* in man by catecholamines (Wolfe *et al.*, 1987*a*), by glucose (Wolfe *et al.*, 1987*b*) and by exercise (Wolfe *et al.*, 1990); it is also increased after exercise in several adipose tissue depots, particularly intermuscular depots in the hamster (Mattacks & Pond, 1988) and it is decreased in the adipose tissue of the rat by lactation (Hansson *et al.*, 1987).

Fifth, although a considerable amount of work has been done on the triacylglycerol/fatty acid cycle, rates of other substrate cycles both *in vitro* and *in vivo* have been demonstrated. For example, flight in the death's head hawk moth (*Acherontia atropos*) increased the rate of glucose/glucose 6-phosphate cycle by 60-fold (Surholt & Newsholme, 1983). In the bumble bee (*Bombus terrestris*), during the warm-up period prior to flight, the rate of the fructose 6-phosphate/fructose 1,6-bisphosphate cycle in the flight muscle *in vivo* is increased by more than 2000-fold and it is shown that this cycle is responsible for most of the heat generation in this period (Surholt *et al.*, 1990). This result was predicted by the work of Newsholme *et al.* as early as 1972, in which the ecological importance of this warming up mechanism for pollination on cold spring days was emphasized. Studies *in vitro* with isolated incubated epitrochlearis muscle of the rat demonstrated that catecholamines could increase the rate of this