Transport of amino acids in muscle, gut and liver: relevance to metabolic control

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That the characteristics of tissue amino acid transport might have implications for control of amino acid and protein metabolism in mammals is a notion of considerable vintage, but only recently has good evidence for a controlling role of transport in the disposition of amino acids been obtained (see [1] and [2] for discussion). When we turned our attention to amino acid transport in skeletal muscle, our main interest was in protein synthesis, a relatively slow process which a priori seemed an unlikely process to be controlled by modulation of membrane transport of its precursors. Nevertheless, muscle exhibited some odd phenomena such as a very high sarcolemmal distribution ratio for glutamine [3], higher than for all other metabolically active amino acids, and also its remarkable pathophysiological intramuscular depletion [4]; these seemed worthy of investigation in their own right. We decided to investigate skeletal muscle amino acid transport in general because a literature search revealed only scanty information concerning its nature and to study glutamine transport in particular because of the lack of any explanation for the size of the glutamine distribution ratio and its pathophysiological alterations.

In other tissues glutamine can be transported via carriers for similar amino acids (e.g. system A) or large neutral amino acids (e.g. system L) [2]. Thus it initially seemed likely that system A, a supposedly near ubiquitous Na⁺-dependent transporting system, would be responsible for glutamine accumulation. However, if this were so, it was puzzling why the sarcolemmal glutamine distribution ratio should be much higher than that of, e.g. alanine, which is a good substrate for the concentrative system A and system L (which ought to dissipate any intracellular accumulation of amino acids).

By using the isolated perfused rat hindlimb [5, 6] and, more recently, sarcolemmal vesicles prepared from rat [7] and human skeletal muscle [8], we were able to demonstrate the existence in muscle of many of the known transporter systems; however, their nature has been somewhat unexpected. The contribution of certain systems (e.g. system A) has been less than we presumed and we found an unusual glutamine transporting system resembling system N [9, 10]. When we turned our attention to amino acid transport in skeletal muscle, our main interest was in protein synthesis, a relatively slow process which a priori seemed an unlikely process to be controlled by modulation of membrane transport of its precursors. Nevertheless, muscle exhibited some odd phenomena such as a very high sarcolemmal distribution ratio for glutamine [3], higher than for all other metabolically active amino acids, and also its remarkable pathophysiological intramuscular depletion [4]; these seemed worthy of investigation in their own right. We decided to investigate skeletal muscle amino acid transport in general because a literature search revealed only scanty information concerning its nature and to study glutamine transport in particular because of the lack of any explanation for the size of the glutamine distribution ratio and its pathophysiological alterations.

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Work in sarcolemmal vesicles [7] has shown that system N° is voltage-dependent and quite distinct from systems A, L, ASC, γ⁺ and χ⁻ for which the paradigm substrates are 2-(methylamino)isobutyric acid, leucine or bicycloamo-no-heptane, cysteine, lysine and glutamate.

We currently view glutamine transport across the sarcolemma in each direction as being almost entirely by system N° with asparagine and histidine being capable of transport by other systems such as A, ASC, L and γ⁺. In particular, we perceive glutamine to be a very poor substrate for system L in skeletal muscle, explaining why the distribution ratio is so high. We have hypothesized that the net sarcolemmal movement of glutamine, i.e. the balance of the inward and outward fluxes, is controlled by the membrane potential and by the prevailing concentrations of sodium and glutamine on either side of the membrane [15].

An alternative view has been taken by Eric Newsholme who has suggested instead [16] that concentric inward transport may indeed be by a system-N° like mechanism, but with outward transport by a separate ion-independent transporter. The only mechanism we can imagine fulfilling anything like the required criteria is system L, which does indeed transport glutamine in the liver (see later). System L is normally subject to trans-stimulation, so that if it were important, given a large outward gradient of glutamine, the presence of a system L substrate on the extracellular face of a membrane ought to stimulate glutamine efflux. Preliminary experiments suggest that increasing the presence of system L substrates does not stimulate tracer glutamine efflux from preloaded perfused rat muscle (Fig. 1), although glutamine itself does. Furthermore, our experiments in sarcolemmal vesicles have demonstrated glutamine efflux to be at least partly Na⁺-dependent; it may also be K⁺-dependent (A. Ahmed, P. M. Taylor & M. J. Rennie, unpublished work).

We have examined changes in net efflux from the skeletal muscle of rat hindlimb in vivo under circumstances associated with muscle protein loss, such as chronic corticosteroid treatment, diabetes and muscle denervation [15, 17-19], and in all these there is good evidence that the sarcolemma has a controlling role in accelerating the net loss, since efflux increases as the glutamine distribution ratio falls. We have shown that muscle glutamine synthetase and glutaminase activities do not change sufficiently rapidly to explain the results and, furthermore, in the conditions studied there appears to be no shortage of substrates such as aspartate, glutamate or ammonia for glutamine synthesis.
suggesting that control of the size of the intramuscular pool of glutamine is not dependent on substrate availability for the synthetase.

What might be the relevance of glutamine to the metabolism of muscle in general, and to the pathophysiological changes in disease and injury? The steady-state concentration of glutamine seems to influence the extent of net anabolism of protein and carbohydrate in muscle, by stimulation of protein accretion (by increasing protein synthesis and decreasing protein breakdown [20, 21]) and stimulation of glycogen synthesis [22, 23]. Although some other workers have been unable to see any relationship between glutamine availability and muscle protein synthesis in vivo [24, 25], we have also observed an effect of glutamine in increasing protein synthesis in vivo in normal and denervated rat muscle (P. W. Watt & M. J. Rennie, unpublished work), as well as in normal healthy men in whom glutamine availability was increased by infusion of the dipeptide alanyl-glutamine [26].

In thinking through the consequences of injury-induced loss of muscle glutamine, we became interested in the fate of glutamine in the small intestine and liver. We investigated glutamine uptake by the blood-facing membranes of the small intestine [27] and discovered that its glutamine utilization was restricted by a transport mechanism which was mostly Na+-independent with a $K_m$ in the physiological range ($= 0.9$ mM). The output of ammonia, alanine, proline, glutamate and citrulline derived from glutamine [28] therefore depends upon the rate of supply to the gut of glutamine, which is largely derived from muscle under normal circumstances and which would likely increase after any insult to muscle glutamine production. Ureagenesis depends crucially upon ammonia concentration within liver cells [29] and this is determined to some extent by its availability in the portal blood [30]. Ammonia stimulates liver glutaminase which itself supplies ammonia and an $\alpha$-amino group (in aspartate via glutamate) for ureagenesis. Since glutamine availability appeared to be a factor modulating ureagenesis, we investigated the extent to which modulation of inward and outward glutamine transport across the hepatocyte membrane could be involved in the control of hepatic glutamine catabolism. Work carried out in collaboration with Chris Pogson, Richard Knowles and Mark Salter showed that both inward and outward transport steps (largely mediated by systems N and L, respectively), as well as mitochondrial glutaminase, each had importance in the control of glutamine catabolism in the liver [31]; in isolated hepatocytes the control strengths of the transport steps (Na+-dependent system N, Na+-independent system L) were roughly equal but opposite in sign, with mitochondrial oxidation of glutamine having a control strength of about 1.0. We have also studied glutamate transport in rat liver and showed the presence of a basolateral (sinusoidal) membrane Na+-dependent transporter in perivenous cells [32]. This transporter has also been identified in cultured perivenous hepatocytes, where it is induced by counter-regulatory hormones [33] and it may be important for perivenous-cell scavenging of ammonia which has escaped perportal cell ureagenesis [34]. In liver sinusoidal vesicles from normal rats glutamate transport is predominantly Na+-independent, but in rats which have undergone procedures causing elevation of blood ammonia (such as streptozotocin-induced diabetes and corticosteroid treatment), we observe [35] a large (≈5-fold) induction of Na+-dependent glutamate transport in isolated vesicles, presumably of perivenous origin. Induction of glutamate transport could be very important in ketoacidosis for ammonia detoxification and acid-base balance, since glutamine synthesized in the liver is an important source of renal ammonium.

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