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CHARACTERISTICS OF ACIDIC, BASIC AND NEUTRAL AMINO ACID TRANSPORT IN THE PERFUSED RAT HINDLIMB

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SUMMARY

1. We have employed a paired-tracer isotope dilution technique in a perfused rat hindlimb preparation to obtain information on the kinetics of transport across the sarcolemmal membranes of acidic, neutral and basic amino acids.

2. We have defined the characteristics of the saturable transport of amino acids normally regarded as paradigm substrates for the A, ASC, L, y^+(basic) and the dicarboxylic amino acid transport systems. Their maximal transport capacities (V_{max}, nmol min^{-1} (g muscle)$^{-1}$ and substrate concentrations for half-maximal transport (K_m, mM) of representative amino acid substrates are as follows: 2-aminoisobutyrate (AIB), $V_{max} = 15 \pm 7$, $K_m = 1{\cdot}26 \pm 0{\cdot}6$; alanine, $V_{max} = 332 \pm 53$, $K_m = 3{\cdot}9 \pm 0{\cdot}9$; serine, $V_{max} = 410 \pm 61$, $K_m 3{\cdot}4 \pm 0{\cdot}5$; leucine, $V_{max} = 2800 \pm 420$, $K_m = 20 \pm 2$; lysine, $V_{max} = 136 \pm 46$, $K_m = 2{\cdot}1 \pm 1{\cdot}3$; glutamate, $V_{max} = 86 \pm 6$, $K_m = 1{\cdot}05 \pm 0{\cdot}05$; proline, $V_{max} = 196 \pm 48$, $K_m = 4{\cdot}1 \pm 0{\cdot}6$.

3. Glycine uptake was faster than expected on the basis of diffusion but was not saturable and showed uptake that could be best described by a first-order rate constant of $0{\cdot}07 \pm 0{\cdot}003$ min^{-1}.

4. We have attempted to discriminate kinetically between possible routes of entry for an amino acid on the basis of competitive and non-competitive interaction between substrates potentially sharing common routes. On this basis, the major routes of alanine entry appear to be via the ASC and L systems with the A system playing a quantitatively minor role. Glutamate and aspartate appear to be transported exclusively by a dicarboxylate amino acid carrier. The branched-chain amino acids (BCAA) and the aromatic amino acid, phenylalanine, are almost equivalent substrates for an L-like system.

5. Insulin had no detectable effect on the uptake of paradigm substrates for ASC, L, y^+, the dicarboxylic amino acid or glycine transport systems.

6. Transport of serine and lysine was Na^+ dependent. Lysine transport apparently occurred with a stoichiometry of 2 Na^+: 1 lysine. With the exception of alanine, whose transport was partially Na^+ dependent, all other amino acids examined in the present study were transported in a Na^+-independent manner.

INTRODUCTION

The existence of a number of parallel systems for transport of amino acids across cell membranes in mammalian tissues is now well recognized (Shotwell, Kilberg & Oxender, 1983). It is therefore somewhat surprising that, given the quantitatively important role of skeletal muscle in intermediary amino acid metabolism (particularly of alanine, glutamine, glutamate and the BCAA) (Snell & Duff, 1984) and the major contribution of muscle to whole-body protein turnover (Waterlow, Garlick & Millward, 1978), so little is known about muscle amino acid transport. Chiefly on the basis of measurements of uptake of non-metabolizable amino acids such as 2-aminoisobutyrate and cycloleucine (Akedo & Christensen, 1962; Kipnis & Parrish, 1965; Riggs & McKirahan, 1973) systems broadly corresponding to the A and L systems of other tissues have been identified in muscle. Furthermore, Riggs & McKirahan (1973) identified the presence of an ASC-like system (transporting alanine, serine and cysteine) in skeletal muscle and attempted to partition the transport of alanine, a substrate for both the A and ASC systems, between them.

There is little information available on the possible existence in muscle of other transport systems specific for acidic and basic amino acids e.g. glutamate and lysine respectively, or for amino acids such as glycine and proline. Even where transport was thought, by analogy with the situation in other tissues, to be saturable, kinetic information regarding the maximal transport capacity (V_{max}) and the external concentration of amino acid that gives half-maximal transport (K_m) was sparse, although information regarding the initial rates of transport at normal rat plasma concentrations of amino acids is available (Baños, Daniel, Moorhouse & Pratt, 1973).

We have therefore turned to the use of the perfused skinned rat hindlimb preparation, which contains mixed-fibre skeletal muscle of fibre-type composition similar to mixed muscles of other mammals (Ariano, Armstrong & Egerton, 1973). The preparation is able, in many circumstances, to mimic the metabolic behaviour of skeletal muscle *in vivo*. This preparation excludes arterial supply to the foot and to the femur. Thus the only tissues apart from skeletal muscle which are perfused are the bones of the lower leg, some intrafascicular fat and the tissues of the vasculature. We estimate the contribution by weight of skeletal muscle to the preparation to be between 92 and 95%. Thus we feel that the transport values overwhelmingly reflect the behaviour of skeletal muscle.

The transport of glutamine has been dealt with in a previous paper (Hundal, Rennie & Watt, 1987). The aims of the present work were to identify and characterize transport of acidic (glutamate), neutral (alanine, serine, BCAA, glycine and proline) and basic (lysine) amino acids. We have also investigated the transport of some other physiologically occurring amino acids such as 3-methylhistidine, the transport characteristics of which are important in assessing its usefulness as an indicator of myofibrillar breakdown (Rennie & Millward, 1983).

METHODS

Animals and perfusion techniques

Overnight-fasted female Wistar rats (200 g, Bantin & Kingman, Hull, UK) were used throughout. Rats anaesthetized with pentobarbitone (Sagatal, May & Baker, Dagenham; 60 mg kg^{-1}, I.P.) were prepared for perfusion of a single hindlimb (about 6 g of muscle in these rats) by methods reported previously (Rennie, Idström, Mann, Scherstén & Bylund-Fellenius, 1983; Hundal *et al.* 1987).

Tracer uptake and kinetics of unidirectional influx

The rates of unidirectional uptake of ^{3}H-labelled amino acids from the perfusate into hindlimb tissue were determined by the paired-tracer isotope dilution technique (Yudilevich & Mann, 1982). The method was similar to that previously used for the study of glutamine transport in the same preparation (Hundal *et al.* 1987); [^{3}H]- or [^{14}C]mannitol was used as the extracellular reference marker depending on which of these labels was used for the amino acid under study. [^{14}C]Mannitol and all ^{3}H-radiolabelled amino acids were puchased from the Radiochemical Centre, Amersham, UK. [^{3}H]Mannitol and [^{14}C]aminoisobutyrate were purchased from New England Nuclear, Dreiech, FRG. The specific activities of all substances used as tracers were as follows: 2-amino[1-^{14}C]-isobutyric acid, 54 mCi $mmol^{-1}$; L-[2,3-^{3}H]alanine, 54 Ci $mmol^{-1}$; L-[G-^{3}H]glutamic acid, 39 Ci $mmol^{-1}$; L-[2,3-^{3}H]glycine, 11 Ci $mmol^{-1}$; L-[4,5-^{3}H]leucine, 55 Ci $mmol^{-1}$; L-[4,5-^{3}H]lysine, 75 Ci $mmol^{-1}$; [^{14}C]3-methylhistidine, 55 mCi $mmol^{-1}$; L-phenyl[2,3-^{3}H]alanine, 56 Ci $mmol^{-1}$; L-[2,3-^{3}H]-proline, 24 Ci $mmol^{-1}$; L-[2,3-^{3}H]valine, 30 Ci $mmol^{-1}$; D-[1-^{14}C]mannitol, 55 mCi $mmol^{-1}$; D-[1-^{3}H]-mannitol, 19 Ci $mmol^{-1}$. All unlabelled amino acids were obtained from Sigma Chemical Co. Radioisotopes were mixed in the ratio of 5 μCi : 1 μCi (^{3}H :^{14}C) and dried under N_2 gas; tracers were redissolved in appropriate perfusate solutions. Details concerning the injection into the perfused rat hindlimb, processing of samples for radioactive counting and the calculation of unidirectional uptake (taken at plateau values of tracer extraction, see Fig. 1) have been described previously (Rennie *et al.* 1983; Hundal *et al.* 1987). Inhibitor amino acids were co-injected at 50 mM with the labelled tracers as described previously (Hundal *et al.* 1987). This procedure is useful for initially discriminating between possible routes of amino acid entry on the basis of any observed interaction. The carrier tracee concentrations for the tracers injected were in the femtomolar to picomolar range so they were likely to be far below the K_m values for any amino acid transport system (which are usually between 1 and 10 mM, except for system L substrates which have a K_m of $\sim$20 mM, see later). The concentration of the competing amino acid will fall due to dilution, but the ratio of the tracer to native amino acid will of course remain constant, no matter what the absolute concentration due to dilution. The approximate inhibitor concentration at the transport site can, however, be calculated from knowledge of the distribution volume (0·27 ml g^{-1}, Hundal *et al.* 1987) and the injectate volume (100 μl). During the initial mixing the 50 mM bolus concentration will fall to about 4 mM. This is of the order of most K_m values (and of competing K_i values) and ought to cause some depression in the uptake of the tracer amino acid if interaction does take place between the test and inhibitor amino acid. Uptake of tracer amino acids was followed immediately after injection for a period of up to 2 min. In kinetic experiments in which the hindlimb was perfused with steady-state amino acid concentrations, the period of equilibration was routinely in excess of 10 min. The methods used to assess competitive and non-competitive inhibition by the addition of other amino acids to injectates and perfusates were identical to those discussed previously (Hundal *et al.* 1987). Furthermore, that paper contains a discussion of the assumptions underlying the method and arguments of the proposition that the paired-tracer isotope dilution technique measures unidirectional transport of amino acids across the sarcolemmal membrane.

Values for V_{max} and K_m were calculated using the Hanes plot (Price & Stevens, 1982; i.e. $[S]/v_t$ *vs.* [S]) obtained from least-squares regression using commercially available software for the Apple IIe microcomputer. Competitive and non-competitive inhibition were distinguished by carrying out statistical analysis of K_m and V_{max} values obtained in the absence and presence of the putative inhibitor by Students's *t* test.

Flow rate and perfusion pressures

Hindlimbs were perfused at constant flow rates above those for which amino acid transport has been shown to be flow-limited (Hundal *et al.* 1987) i.e. 2 ml min^{-1} $hindlimb^{-1}$. Perfusion pressures were monitored using an anaeroid manometer; they were routinely 40 mmHg. Alterations in perfusate pH or the presence of mersalyl did not affect pressure–flow relationships nor did injection of different amino acids or their presence at high transient or steady-state concentrations in the perfusate.

Inhibitors

In experiments involving alanine or the BCAA, in order to avoid problems of metabolism, aminooxyacetate (Sigma Chemical Co.), an aminotransferase inhibitor, was added to the perfusate at a fixed concentration of 5 mM. In experiments involving serine and lysine the effects of the thiol-group-specific reagent mersalyl acid (Sigma Chemical Co.) were tested at a perfusate concentration of 2 mM.

Na^+ dependence of amino acid transport

The Na^+ dependence of amino acid transport was investigated by partially replacing perfusate Na^+ (normally 145 mM) by choline (choline chloride and bicarbonate replacing sodium chloride and bicarbonate respectively). The extent of replacement was limited to 80% beyond which further replacement of Na^+ resulted in vasoconstriction of the preparation.

In order to check that the possible agonist effects of choline did not interfere with transport measurements we added 0·4 mM-acetylcholine to the perfusate in the presence of 5 μM-neostigmine in two experiments. The uptake of glutamine (transported by a Na^+-dependent carrier) and phenylalanine (Na^+-independent) were within 10 ± 5% of control values indicating that the agonist effects of choline were likely to be negligible.

Effect of insulin on amino acid transport

Porcine insulin (Wellcome Foundation, Beckenham, Kent) was added to the perfusate at a final concentration of 1 mU ml^{-1}, in order to examine its effect on amino acid uptake. Muscle was pre-exposed to insulin for 25 min before measurement of amino acid transport.

RESULTS

Movement of amino acids by passive diffusion

Amino acids studied in the present investigation have previously been shown to be taken up by muscle much faster than expected on the basis of their likely membrane permeability, derived from olive oil–water partition coefficients and the sarcolemmal surface area of rat muscle (Hundal *et al.* 1987).

Alanine transport

[^{3}H]Alanine uptake was inhibited by the presence of 50 mM-L-alanine co-injected with the label, but was not affected by the presence of 50 mM-D-alanine (Fig. 1). [^{3}H]Alanine uptake was progressively inhibited by increasing concentrations of perfusate L-alanine. L-Alanine uptake showed Michaelis–Menten kinetics with a V_{max} of 332 ± 53 mmol min^{-1} (g muscle)$^{-1}$ and K_m of $3{\cdot}9 \pm 0{\cdot}90$ mM (Fig. 2). The kinetic profile shows no evidence of a major diffusive component and calculation of the uptake which might be expected as a result of diffusion suggests that it would be a very small component (Hundal *et al.* 1987) of the total transport at saturating concentrations. Although it seemed likely that the kinetic profile shown in Fig. 2 would eventually turn out to be the result of the contributions of a number of distinct

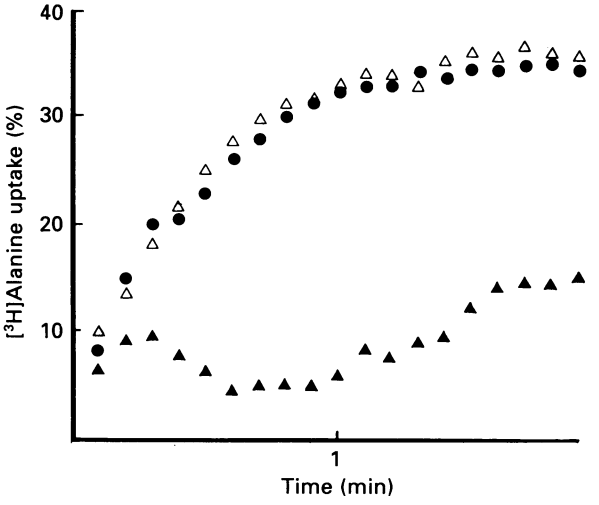

Fig. 1. Uptake profile of [³H]alanine (△) alone and in the presence of 50 mM-D-alanine (●) or L-alanine (▲). Profile obtained from a single perfused preparation when D- or L-isomers of alanine were co-injected with the radiolabelled amino acid.

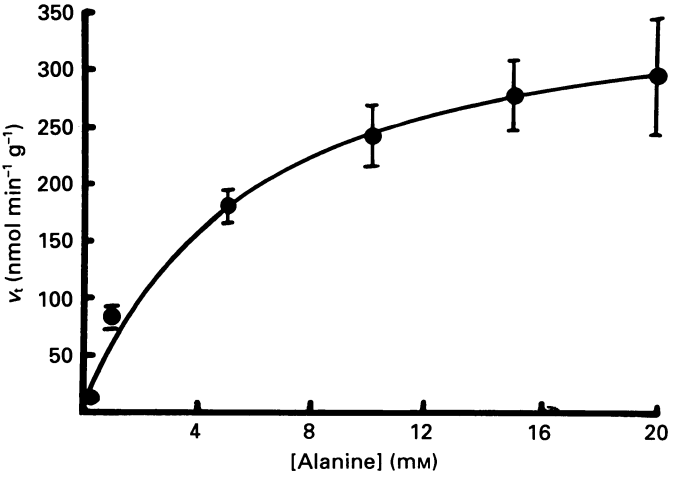

Fig. 2. Kinetics of alanine transport ($V_{max} = 332 \pm 53$, nmol min^{-1} g^{-1}; $K_m = 3{\cdot}9 \pm 0{\cdot}9$ mM). Values are means ± S.D., $n = 4$.

amino acid transport systems all handling alanine, we did not attempt mathematically to strip out individual components because this method becomes unreliable with more than two transporters for a common substrate and we suspected the involvement of, at least, systems A, ASC and L.

To gain some insight into the interaction between alanine and carriers of other amino acids we co-injected [³H]alanine with solutions (at 50 mM) of other amino acids known to be preferred substrates for a particular transport systems in other tissues. The results (Table 1) show substantial interactions between [³H]alanine and

TABLE 1. Uptake of radiolabelled amino acids (% of control) alone and in the presence of unlabelled amino acids co-injected at 50 mM. Values are means ± S.D. from three to four preparations in each case. Uptake of labelled amino acids was measured relative to mannitol. Each preparation provided a value with a standard error of less than 2%

Inhibitor amino acid	[^{3}H]Alanine	[^{3}H]Serine	[^{3}H]Glutamine	[^{3}H]Lysine	[^{14}C]3-Methylhistidine
None (control)	100 ± 4	100 ± 5	100 ± 8	100 ± 4	100 ± 11
L-Alanine	62 ± 4	74 ± 4	89 ± 8	100 ± 2	—
D-Alanine	100 ± 4	—	—	—	—
L-Serine	59 ± 2	69 ± 3	100 ± 5	100 ± 2	95 ± 1
D-Serine	—	100 ± 3	—	—	—
L-Cysteine	—	72 ± 4	—	—	77 ± 7
L-Glutamine	89 ± 4	69 ± 2	94 ± 5	—	—
L-Histidine	—	—	—	69 ± 2	47 ± 2
2-Aminoisobutyrate	88 ± 2	97 ± 2	100 ± 5	100 ± 2	96 ± 2
L-Glycine	100 ± 2	98 ± 2	100 ± 1	100 ± 4	97 ± 2
L-Leucine	69 ± 4	93 ± 2	100 ± 1	100 ± 3	42 ± 4
L-Proline	98 ± 2	—	—	—	—
L-Lysine	98 ± 2	97 ± 1	100 ± 6	62 ± 2	96 ± 3
D-Lysine	—	—	—	100 ± 3	—
L-Glutamate	100 ± 2	97 ± 3	11 ± 1	100 ± 2	96 ± 2
D-Glutamate	—	—	100 ± 3	—	—
L-Aspartate	—	—	11 ± 1	—	—
L-Arginine	—	—	—	62 ± 2	—
L-Ornithine	—	—	—	60 ± 1	—
3-Methylhistidine	—	—	—	—	47 ± 2

AIB, leucine, serine and glutamine when they were co-injected; however, glutamate, lysine, glycine and proline were without effect on alanine uptake. This apparently ruled out the possibility of alanine travelling via the dicarboxylic or basic amino acid carriers or those responsible for glycine and proline transfer.

The possibility existed, however, that alanine shared carriers for which AIB, leucine, serine and glutamine were substrates (i.e. systems designated A, L, ASC and

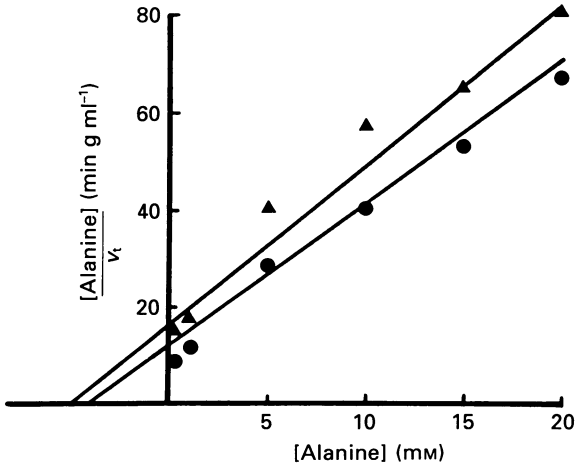

Fig. 3. Hanes plot showing the effect of 3 mM-AIB (▲; $V_{max} = 306 \pm 48$ nmol min^{-1} g^{-1}, not significant from control; $K_m = 4{\cdot}9 \pm 0{\cdot}2$ mM, significantly different from control, $P < 0{\cdot}05$) on L-alanine transport (●; $V_{max} = 332 \pm 53$ nmol min^{-1} g^{-1}; $K_m = 3{\cdot}9 \pm 0{\cdot}9$ mM). Values are means ± S.D. from three to four preparations.

the recently characterized system for glutamine in skeletal muscle designated by us as N^m, Hundal *et al.* 1987). Accordingly, the nature of the interactions between each of these four amino acids and alanine was investigated by constructing plots of the rate of alanine uptake against alanine concentration in the presence of a fixed concentration of the interacting amino acid. The results (Figs 3 and 4) show clearly that in the cases of AIB and leucine the interaction is of a simple competitive nature suggesting that both these amino acids share a route of entry with alanine. The K_i values for AIB and leucine were respectively $1{\cdot}75 \pm 0{\cdot}40$ and $16{\cdot}32 \pm 2{\cdot}3$ mM (values are means ± S.D. of three to four rats) which closely correspond to the K_m values of their own transport through the respective systems for which they are preferred substrates (see later).

The effect of serine on alanine was more complex since it appeared that both the perfusate concentration for half-maximal transport and the maximal rate of alanine uptake were altered by serine (Fig. 5). This suggested inhibition of a 'mixed' type (Price & Stevens, 1982).

Glutamine appeared to exert its inhibitory effect on alanine uptake in a non-competitive manner, the maximal capacity of alanine transport being depressed by the concentration at which half-maximal transport (K_m) occurred being unaltered. We have previously reported (Hundal *et al.* 1987) that alanine exerted no inhibitory

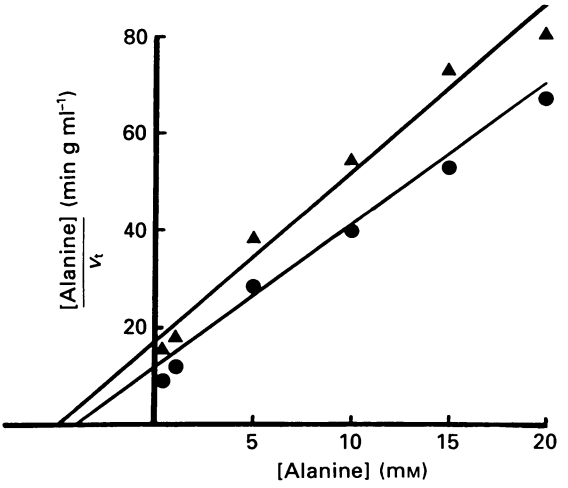

Fig. 4. Hanes plot showing the effect of 1·8 mM-leucine (▲; V_{max} = 298±35 nmol min^{-1} g^{-1}, not significant from control; K_m = 4·9±0·2 mM, significantly different from control, $P < 0·05$) on L-alanine transport (●; V_{max} = 332±53 nmol min^{-1} g^{-1}; K_m = 3·9±0·9 mM). Values are means±S.D. from three to four preparations.

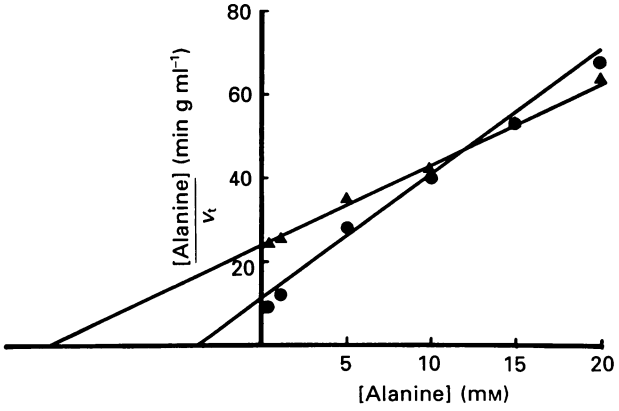

Fig. 5. Hanes plot showing the effect of 3 mM-serine (▲; V_{max} = 516±34 nmol min^{-1} g^{-1}, significantly different from control, $P < 0·01$; K_m = 12·6±2·2 mM, significantly different from control, $P < 0·001$) on L-alanine transport (●; V_{max} = 332±53 nmol min^{-1} g^{-1}; K_m = 3·9±0·9 mM). Values are means±S.D. from three to four preparations.

effect on the uptake of glutamine providing further evidence that these amino acids are transported in perfused rat skeletal muscle by distinct routes.

Examination of alanine transport during alteration of perfusate pH and insulin availability

When perfusate pH was altered between 6·8 and 8·0 (Table 2), in each of four preparations no variation was observed in alanine transport. Furthermore, when alanine transport was examined in four preparations at high (20 mM) and low (0·1 mM) concentrations of alanine in the presence of insulin at a dose of 1 mU ml^{-1}

(sufficient to cause a 30% stimulation of glutamine transport, Hundal *et al.* 1987) no effect was observed. It appears, therefore, that in the perfused rat hindlimb preparation alanine transport is effectively insulin insensitive.

Na^+ dependence of alanine transport

When perfusate Na^+ concentration was varied between 29 and 145 mM at a fixed alanine concentration, alanine transport showed little alteration until external Na^+ concentration fell below 70 mM (Table 3). This complicated relationship was in

TABLE 2. Lack of effect of varying perfusate pH on leucine (5 mM), serine (0·5 mM) and alanine (1 mM) transport

	v_t (nmol min^1 g^{-1})		
Perfusate pH	Leucine	Serine	Alanine
6·8	496 ± 19	56·52 ± 5·96	47·86 ± 12·65
7·4	497 ± 47	59·35 ± 8·73	57·21 ± 5·85
8·0	523 ± 23	57·57 ± 9·63	52·62 ± 9·71

Values are means ± S.D. from three to four preparations.

TABLE 3. Effect of changing extracellular Na^+ on alanine (1 mM), serine (2 mM), leucine (5 mM) and glutamate (0·02 mM) transport. Perfusate Na^+ was replaced with choline

	v_t (nmol min^{-1} g^{-1})			
$[Na^+]_o$ (mM)	Alanine	Serine	Leucine	Glutamate
145	85·69 ± 14·2	166·5 ± 2·5	558 ± 39	3·32 ± 1·8
116	92·70 ± 15·5	140·0 ± 6·0	530 ± 40	3·76 ± 0·8
87	84·38 ± 15·8	129·5 ± 3·5	553 ± 57	3·85 ± 0·7
72·5	80·20 ± 16·7	—	—	—
58	61·28 ± 16·2	98·0 ± 8·4	473 ± 91	3·62 ± 0·3
44	48·21 ± 6·3	76·3 ± 9·9	486 ± 65	3·85 ± 0·4

Values are means ± S.D. for between three and four preparations.

contrast to the simple linear one observed by us for glutamine transport (Hundal *et al.* 1987). It suggested the possible involvement of separate Na^+-dependent and Na^+-independent systems. In other tissues, systems A and ASC are Na^+ dependent and system L is Na^+ independent (Akedo & Christensen, 1962; Christensen, Liang & Archer, 1967; Kilberg, Handlogten & Christensen, 1981). It became apparent that to get more information about the routes of entry of alanine we would need to investigate the kinetics of uptake for substrates for the A, ASC and L systems (see below).

2-Aminoisobutyrate (AIB) transport

The use of AIB to probe transporter specificity depends on its high affinity for the A system and the fact that it is non-metabolizable. In the perfused rat hindlimb, the uptakes of AIB and Me-AIB (methyl AIB) were very much less than those of native amino acids such as alanine, serine etc. We attempted to construct kinetic profiles for both AIB and Me-AIB. This proved impractical for Me-AIB because of cost, but it was

possible for AIB (Fig. 6). Maximal transport capacity was only 15 ± 7 nmol min^{-1} g^{-1}, i.e. approximately 3–4% of maximal alanine transport; the concentration of AIB at which transport was half-maximal was $1{\cdot}26 \pm 0{\cdot}6$ mM. Thus the A system in muscle appears to have a fairly high 'affinity' and a very low capacity. If alanine is a substrate for this system, then the proportion of total alanine transport carried physiologically by this route must be extremely small. Support for this interpretation comes from the observation that in dog skeletal muscle [^{11}C]2-aminoisobutyrate transport occurs at rates much lower than those for naturally occurring amino acids,

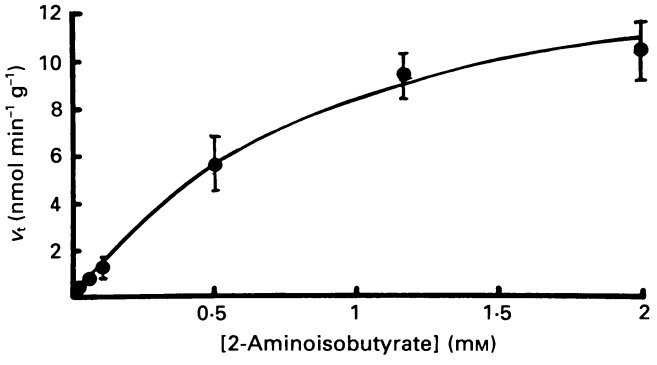

Fig. 6. 2-Aminoisobutyrate (AIB) transport kinetics ($V_{max} = 15 \pm 7$ nmol min^{-1} g^{-1}; $K_m = 1{\cdot}26 \pm 0{\cdot}6$ mM). Values are means ± S.D. from three preparations.

and is not altered by the presentation or deprivation of nutrient substrate or the availability of insulin (Bading, Corbally, Fissekis, DiResta & Brennan, 1987).

We have not investigated the insulin dependence in our preparation of AIB transport via system A, a classically insulin-dependent system (Akedo & Christensen, 1962). However, since the A system appears to play such a minor role in the perfused rat skeletal muscle preparation, even if it is insulin sensitive the lack of measurable insulin stimulation of alanine transport is consistent.

Serine transport

Michaelis–Menten analysis of serine transport suggested that serine uptake was saturable with a V_{max} of 410 ± 61 nmol min^{-1} g^{-1} and K_m of $3{\cdot}4 \pm 0{\cdot}5$ mM.

[^{3}H]Serine uptake was depressed (Table 1) by the presence at 50 mM of unlabelled L-serine, L-alanine, L-cysteine and L-glutamine in the injectate, although no effect was observed as a result of the inclusion in the injectate of D-serine, L-glutamate, L-lysine, L-glycine or AIB (Table 1). The apparent lack of inhibition by D-serine suggests that the serine transporter accepts only amino acids in the L-configuration.

The inhibition exerted by alanine and glutamine upon the uptake of tracer serine was further investigated by constructing kinetic profiles of serine transport in the presence of fixed concentrations of alanine and glutamine. The results (Fig. 7) suggest a complicated interaction between alanine and serine with mixed effects on both of the kinetic parameters V_{max} and K_m such are not explicable by simple competitive interaction. The effect of glutamine on serine uptake (Fig. 8), however, was non-competitive. Serine and glutamine therefore do not appear to share a

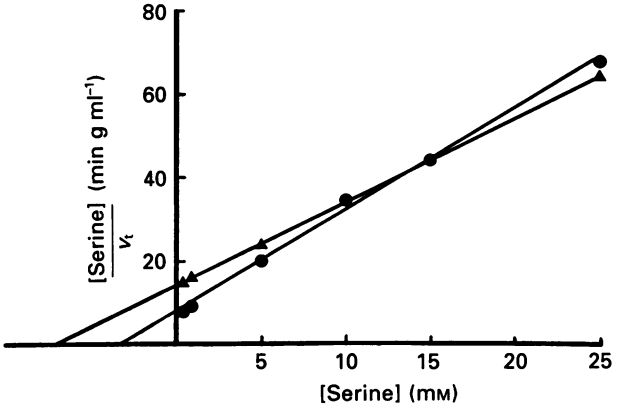

Fig. 7. Hanes plot showing the effect of 3 mM-alanine (▲; $V_{max} = 208 \pm 25$ nmol min^{-1} g^{-1}, significantly different from control, $P < 0{\cdot}01$; $K_m = 7{\cdot}2 \pm 2$ mM, significantly different from control, $P < 0{\cdot}02$) on L-serine transport (●; $V_{max} = 410 \pm 61$ nmol min^{-1} g^{-1}; $K_m = 3{\cdot}4 \pm 0{\cdot}5$ mM). Values are means ± S.D. from three to four preparations.

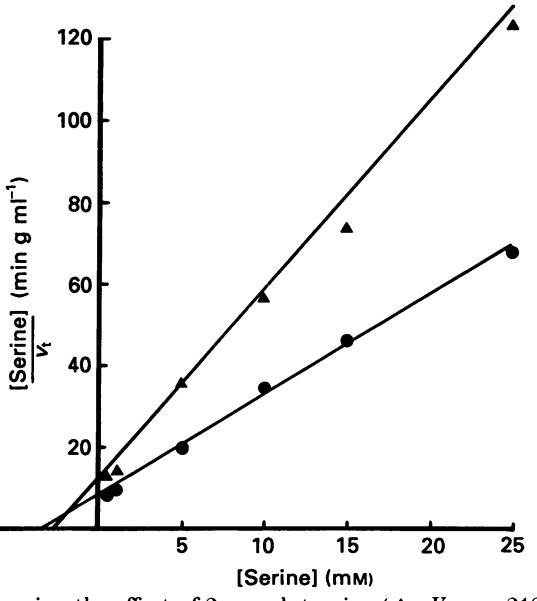

Fig. 8. Hanes plot showing the effect of 2 mM-glutamine (▲; $V_{max} = 218 \pm 45$ nmol min^{-1} g^{-1}, significantly different from control, $P < 0{\cdot}01$; $K_m = 3{\cdot}4 \pm 0{\cdot}5$ mM, not significantly different from control) on L-serine transport (●; $V_{max} = 410 \pm 61$ nmol min^{-1} g^{-1}; $K_m = 3{\cdot}4 \pm 0{\cdot}5$ mM). Values are means ± S.D. from three to four preparations.

common route of entry into muscle, a proposition consistent with our previous observation that serine non-competitively inhibits glutamine transport in the same preparation (Hundal *et al.* 1987).

Serine transport appeared to be dependent upon the presence of Na^+ as unidirectional serine influx fell as perfusate Na^+ was progressively replaced with choline (Table 3).

Effect of variation in perfusate pH and insulin availability on serine transport

As for alanine, there was no apparent alteration in the transport rate of serine over the range pH 6·8–8·0 (Table 2) and no effect on the rate of serine uptake across the entire kinetic profile when this was examined in the presence of insulin at 1 mU ml^{-1}.

Effect of mersalyl acid on [^3H]serine uptake

When [^{3}H]serine uptake was compared to that obtained in the presence of 2 mM-mersalyl acid the maximal uptake (U_{max}) of the amino acid was found to be depressed by approximately 40% (not shown). The inhibition caused by mersalyl acid suggested the presence of reactive -SH groups on the carrier(s), but since the effect of mersalyl acid was only tested at 2 mM, one cannot exclude the possibility of a concentration-dependent effect or the presence of more than one transporter.

Transport of system L substrates

A kinetic profile of the transport of leucine against perfusate leucine concentration showed the characteristics of saturability expected of carrier-mediated transport (not shown). The kinetic parameters turned out to be those of a high-capacity, low-'affinity' system (with a K_m of 20 ± 2 mM, and V_{max} of 2800 ± 420 nmol min^{-1} g^{-1}). Almost identical characteristics were observed for the other BCAA, valine and isoleucine, and for the aromatic amino acid phenylalanine: V_{max} (nmol min^{-1} g^{-1}) and K_m (mM) values were, respectively, as follows: valine, 2575 ± 50, $20{\cdot}1 \pm 0{\cdot}1$; isoleucine, 2550 ± 50, $18{\cdot}3 \pm 1{\cdot}7$; phenylalanine, 3019 ± 384, $19{\cdot}32 \pm 1{\cdot}5$ (values are means ± S.D. of four to six rats).

Although competition could be shown between the above-mentioned amino acids in terms of reciprocal K_m and K_i for pairs of amino acids (results not shown), because of the low 'affinity' of the L-like system (K_m of 20 mM) which transports these neutral hydrophobic amino acids, it was impossible to raise the concentration of other neutral amino acids to a sufficiently high level in the injectate to see any interaction with transport of the L system tracer substrates.

Lack of ion, pH dependence and insulin insensitivity of the L system

The uptake of leucine (Table 3) and phenylalanine were not affected by alteration in perfusate Na^+ concentration. Furthermore, transport of leucine was unaltered by pH as judged by variation in perfusate pH between 6·8 and 8·0 (Table 2). Insulin added to the perfusate at 1 mU ml^{-1} was unable to modulate the transport of these amino acids (results not shown). It appears that, as in most other tissues, the transport of leucine and phenylalanine (not shown) in perfused muscle is Na^+ independent, and insensitive to changes in pH or increased availability of insulin.

Dicarboxylic amino acid transport

Glutamate transport was saturable and obeyed Michaelis–Menten type kinetics, the V_{max} being 86 ± 6 nmol min^{-1} g^{-1} and half-maximal saturation occurring at $1{\cdot}05 \pm 0{\cdot}05$ mM (Fig. 9). The pulse co-injection technique revealed that glutamate uptake was stereospecific, the presence of D-glutamate being without effect on the

uptake of the labelled L-isomer (Table 1). Furthermore, the only other amino acid capable of depressing the uptake of [^{3}H]glutamate was L-aspartate. On closer examination, this interaction was found to be competitive (Fig. 10, K_i of aspartate being 0·96±0·06 mM) suggesting that in perfused rat skeletal muscle a specific carrier system existed for the dicarboxylic amino acids.

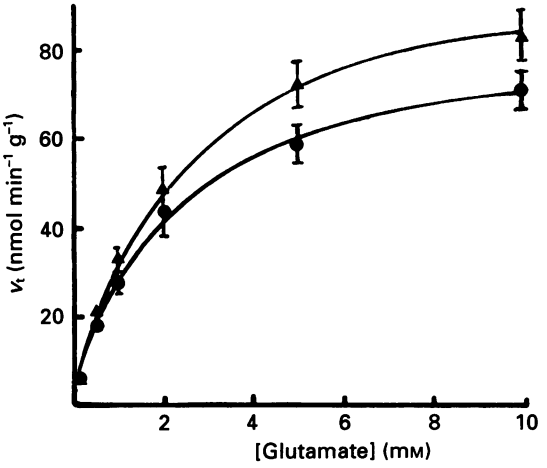

Fig. 9. Effect of changing perfusate pH on glutamate transport kinetics. pH was altered to 7·1 (▲; V_{max} = 112±11 nmol min^{-1} g^{-1}, significantly different from control, $P < 0·02$; K_m = 1·3±0·2 mM, not significantly different from control. Control values at pH 7·4 (●; V_{max} = 86±6 nmol min^{-1} g^{-1}; K_m = 1·05±0·05 mM). Values are means±S.D. from three to four preparations.

Alteration in perfusate Na^+ had no effect on the transport of glutamate (Table 3) at a perfusate glutamate concentration of 20 μM. Increased availability of insulin also proved to have no effect over the concentration range examined (i.e. 0·1–10 mM, results not shown).

[^{3}H]Glutamate transport was, however, sensitive to changes in perfusate pH. Transport was stimulated 30% (Fig. 9) by a drop in perfusate pH from 7·4 to 7·1. This appeared to be due to an increase of the number of available sites for glutamate uptake as the maximal transport capacity was increased (i.e. it was a V_{max} effect), no significant change in the K_m being observed during alteration of perfusate pH.

Lysine transport

Lysine uptake was stereospecific and saturable as judged from pulse-injection experiments with D-lysine and L-lysine at 50 mM. Co-injection of [^{3}H]lysine with other amino acids revealed that L-lysine uptake was inhibited by unlabelled L-lysine, L-arginine, L-ornithine and L-histidine, all at 50 mM in the injectate, but not by L-glutamate, L-alanine, L-glycine, L-serine or AIB (Table 1).

The kinetic profile for lysine shows its transport obeys Michaelis–Menten kinetics with no evidence for a major diffusive component (not shown). The maximal transport capacity for lysine transport was 136±46 nmol min^{-1} g^{-1} and half-maximal transport occurred at 2·1±1·3 mM.

From Na^+ replacement experiments (using choline), lysine uptake was Na^+ dependent. A linear relationship was obtained using the double-reciprocal plot when the unidirectional influx (v_t) was plotted against the inverse of the square of the extracellular Na^+ concentration (Fig. 11). This suggested that lysine was co-transported along with Na^+ with a stoichiometry of 2 Na^+ : 1 lysine. Insulin appeared to have no effect on lysine transport which was also insensitive to alterations in perfusate pH between 6·5 and 8·0 (not shown).

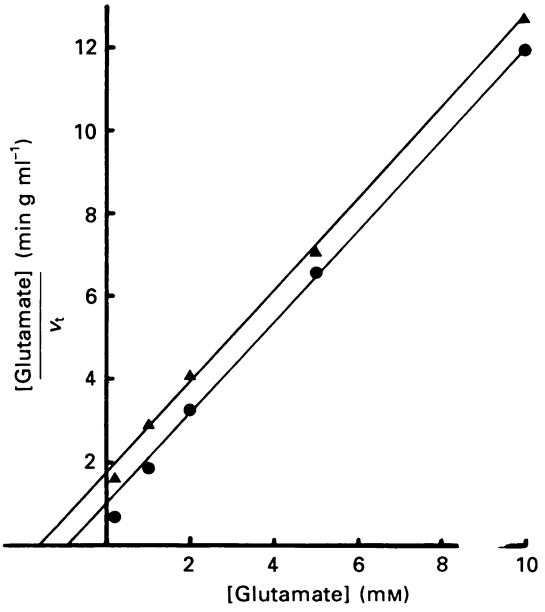

Fig. 10. Hanes plot showing the effect of 0·5 mM-aspartate (▲; V_{max} = 102 ± 14 nmol min^{-1} g^{-1}, not significantly different from control; K_m = 1·6 ± 0·3 mM, significantly different from control, $P < 0·01$) on L-glutamate transport (●; V_{max} = 86 ± 6 nmol min^{-1} g^{-1}; K_m = 1·05 ± 0·05 mM). Values are means ± S.D. from three to four preparations.

The thiol group reagent mersal[illegible]l at 2 mM caused partial inhibition of [^{3}H]-lysine uptake by approximately 40% [illegible]t shown), indicating the presence of some reactive -SH groups on the carrier. The incomplete inhibition indicates, however, that either more than one carrier for lysine is present in skeletal muscle or that inhibition may be concentration dependent, as suggested earlier in the case of serine.

Skeletal muscle 3-methylhistidine transport

The *N*-methyl derivative of histidine at the imidazole N-3 position (3-methylhistidine, methylated post-translationally *in vivo*) is of interest physiologically and clinically because it is an end-product, and thus an index, of myofibrillar protein breakdown; it is not reutilized for protein synthesis, nor otherwise metabolized within muscle (Rennie & Millward, 1983). A full kinetic study of 3-methylhistidine transport was not possible because of the cost of the unlabelled

amino acid needed to construct a kinetic profile. Nevertheless, using the pulse injection technique, it was possible to ascertain that transport was likely to be saturable since unlabelled 3-methylhistidine when co-injected at 50 mM was capable of causing substantial inhibition of tracer [^{14}C]3-methylhistidine uptake (Table 1). The route of transport was difficult to ascertain, but [^{14}C]3-methylhistidine appears not to share routes of entry with glutamate, serine, lysine, AIB and glycine as judged by the inability of these amino acids, when co-injected at 50 mM, to cause any

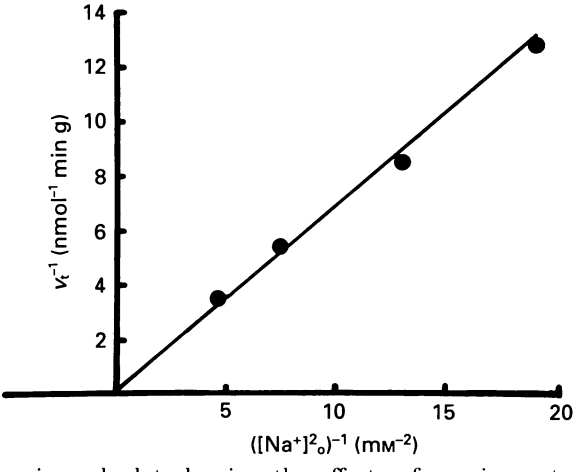

Fig. 11. Double-reciprocal plot showing the effects of varying extracellular Na^+ on unidirectional lysine (0·55 mM) influx (each point is the mean value from at least four preparations).

depression in the uptake of 3-methylhistidine. Amino acids which did interact with the uptake of [^{14}C]3-methylhistidine were histidine, leucine and cysteine which depressed uptake by 55, 48 and 25% respectively. If these effects were truly competitive then 3-methylhistidine may be transferred across cell membranes by carrier(s) responsible for glutamine, asparagine and histidine transfer (system N^m) and/or systems L and ASC. If transport is via the glutamine carrier then the interference by leucine (a system L substrate) and cysteine (a typical ASC substrate) could be non-competitive as occurs in the case of the uptake of glutamine itself (Hundal *et al.* 1987).

Glycine transport

The uptake of [^{3}H]glycine was much higher than anticipated on the basis of diffusion alone. Uptake of glycine appeared not to be saturable up to 50 mM (not shown) and could be described by a single first-order rate constant of $0{\cdot}070 \pm 0{\cdot}003$ min^{-1} ($n = 3$). Since uptake was much faster than could be accounted for by diffusion (Hundal *et al.* 1987), these observations suggest that glycine was carried by a low-'affinity' and very high-capacity transporter or channel. No interaction could be observed between [^{3}H]glycine and AIB, leucine, histidine, serine or glutamate when they were co-injected at 50 mM with the labelled amino acid (results not shown).

Only unlabelled glycine and proline appeared to affect [^{3}H]glycine uptake, but owing to the apparently very low affinity and high capacity of the system transporting glycine, the presence of these amino acids in the injectate at 50 mM caused only a small depression (20%) in the uptake of [^{3}H]glycine.

Proline transport

Transport of this amino acid was suspected to be mediated by the same carrier as glycine since it was the only amino acid that interacted with the uptake of [^{3}H]-glycine. The presence of a specific glycine–proline carrier in the intestine (Stevens, Ross & Wright, 1982) supports this possibility. However, when a kinetic profile of proline transport was constructed it became clear that this was unlikely to be the case. Unlike glycine transport, proline uptake was saturable (not shown) with a V_{max} of 190 ± 48 nmol min^{-1} g^{-1}, and K_m of $4{\cdot}1 \pm 0{\cdot}6$ mM (values are means $\pm$ S.D. from four preparations), with no strong evidence of a major diffusive component.

DISCUSSION

The present results provide some details of the kinetic characteristics (V_{max} and K_m) of L-amino acid transport in a mixed skeletal muscle preparation. These have not been available previously for most of the systems studied. We recognize that the metabolic characteristics of specific types of muscle fibre comprising skeletal muscle differ and that this may also be true for amino acid transport. Nevertheless, since the muscle mass as a whole appears to fulfil a metabolic role which is presumably the integrated sum of its individual components, information on the overall behaviour of the tissue in transporting amino acids should prove useful in understanding the whole-body nitrogen economy.

An important factor to be considered during amino acid transport measurements is the degree to which intracellular metabolism of amino acids is likely to affect the results obtained. The processes most likely to interfere are transamination, oxidation, protein synthesis and protein breakdown.

Of the amino acids studied serine, lysine, glycine and proline do not undergo significant intermediary metabolism in skeletal muscle, protein synthesis and breakdown being the major reactions in which they participate. On the other hand, alanine, the branched-chain amino acids (BCAA) and glutamate are involved in intermediary metabolism (Snell & Duff, 1984). Alanine aminotransferase (EC 2.6.1.2) and the BCAA aminotransferase (EC 2.6.1.42) catalyse the trans-amination of alanine and the BCAA respectively. Thus efflux of radiolabelled pyruvate (from alanine) or of the branched-chain keto-acids (from the BCAA) may make interpretation of results concerning these amino acids difficult. Although the metabolism of alanine and the BCAA was not followed an attempt was made to overcome this problem by the inclusion in the perfusate of a transaminase inhibitor, aminooxyacetate, at 5 mM. The inhibitor did not appear to have any adverse effects on the metabolic integrity of the perfused preparation. That it was effective was demonstrated by the total inhibition of [^{13}C-α]ketoisocaporate production from ^{13}C-labelled leucine added to the perfusate (results not shown).

Glutamate, besides being a substrate for glutamate dehydrogenase, may also be

converted to glutamine by glutamine synthetase. To what extent could these enzymes metabolize exogenous tracer glutamate? Given the size (6 nmol min^{-1} g^{-1}) of the unidirectional glutamate influx we obtained at physiological glutamate concentrations in the present study, and the size of the intramuscular glutamate pool (1·5 μmol (g muscle)$^{-1}$, we calculate that during the period of transport measurement less than 0·5% of the intracellular glutamate pool ought to become labelled. For extracellular glutamate to participate in intermediary metabolism to any significant extent the glutamate pool would need to turn over extremely rapidly. That the fractional efflux of tritiated material we observed was very small suggests that the pool does not turn over sufficiently rapidly. Inhibitors of glutamate dehydrogenase, glutamine synthetase and glutamate–oxaloacetate transaminase were therefore not used; we felt that with the insignificant labelling of the intracellular glutamate pool observed they were unnecessary.

The extent to which transport may affect any metabolic reaction involving amino acids such as alanine, glutamate and the BCAA in skeletal muscle remains unknown. Unfortunately, it has become apparent that no simple answer can be given by comparing the rates of transport and other process and deciding which is smaller. Such an approach has been shown to be useful as a special case in certain specific instances, but a general description of the interactions between steps at which transfer of metabolites occurs and their control coefficients remains incomplete (Kacser & Burns, 1979).

In liver it has been shown that transport can influence tissue amino acid metabolism. The flux through the tryptophan-preferring transporter (system T), which also transports phenylalanine and tyrosine, may effectively control hepatic catabolism of the aromatic amino acids (Salter, Knowles & Pogson, 1986). The extent of the control may be perturbed by corticosteroid treatment when the capacity of aromatic amino acid metabolism is increased. Furthermore, the rate of supply of amino acids to the liver, including their membrane transport, does limit the extent of ureagenesis (Meijer, Lof, Ramos & Verhoeven, 1985). The characteristics of particular skeletal muscle amino acid transporters may allow them to be modulated in such a manner that they influence the control of amino acid metabolism as a whole, e.g. by alteration of Na^+ ion concentration as in the case of glutamine (Rennie, Hundal, Babij, MacLennan, Taylor, Watt, Jepson & Millward, 1986; Hundal *et al.* 1987).

We can be more certain in the case of glutamate. The rate of glutamate transport across the sarcolemma is low enough that it would almost certainly limit glutamine synthesis from extracellular glutamate. Thus glutamate must be synthesized within cells in order to fulfil the requirements of glutamine synthesis. Normally, the supply of anapleurotic intermediates for the Krebs cycle and the activity of alanine aminotransferase appear to be sufficiently high to accomplish this. Nevertheless, there appears to be an effective compartmentation between intra- and extracellular glutamate in muscle principally due to the low activity of the glutamate transporter. This confirms the conclusions of Darmaun, Matthews & Bier (1986) who studied rates of appearance of [^{15}N]glutamate into glutamine in man. They found that the free-glutamate pool available to the ^{15}N-labelled tracer infused into the blood was rapidly labelled and possibly of the same size as the extracellular space, thus excluding the

possibility of significant tracer glutamate entry into major tissues such as skeletal muscle; furthermore, plasma glutamine appeared not to be significantly labelled from the plasma glutamate pool.

How do the characteristics of glutamate transport observed in other tissues compare with those described here? Early studies (reviewed by Lerner, 1978) demonstrated that acidic amino acids were transported across the lumenal membrane of the rat intestine at rates comparable with those of neutral amino acids. Furthermore in the same preparation there was no absolute discrimination between L- and D-stereoisomers, although L-enantiomers were absorbed faster. Schultz, Yu-Tu, Alveraz & Curran (1970) showed that anionic amino acid transport was a saturable, carrier-mediated process in the apical membranes of cells of the rabbit intestine, relying on Na^+ for transport with a coupling stoichiometry of 1 Na^+:1 glutamate. Na^+ dependence also appears to be a feature of certain anionic carriers on liver cell membranes where a system resembling X_{ag}^- has been identified (Taylor & Rennie, 1987).

The results presented here point to the existence in rat skeletal muscle of a low-capacity carrier system which is Na^+ independent and insulin insensitive, but is sensitive to changes in pH.

The pH dependence of the glutamate transporter suggests that an increase in H^+ concentration promotes the uptake of glutamate into skeletal muscle. If the protonated form of the amino acid is the major permeating species an increase in transport may be as a result of recognition by a neutral amino acid carrier as suggested by Christensen (1984) i.e. carrier number would increase. If glutamic acid is the predominant species carried then a fall of 0·3 pH units would cause less than 1% glutamic acid to become protonated. It seems unlikely that such small change could account for the observed stimulation in transport (30%) via a neutral amino acid carrier. It is plausible that conformational changes in the transporter may result from increased H^+ concentration. A change in transporter conformation might affect its mobility in the membrane resulting in the observed increased glutamate influx. Alternatively, changes in pH may allow for the recruitment of a much larger proportion of the inactive carrier population already present in the membrane. Since the effect was acute, it was most unlikely that stimulation of glutamate transport during a fall in pH was as a result of up-regulation by *de novo* carrier synthesis. The extent to which such a mechanism could facilitate the production of glutamine from glutamate during acidosis is not known, but since the rate of the stimulated transport ($\sim$26 nmol min^{-1} g^{-1}) is 5-fold larger than the efflux of glutamine from muscle determined *in vivo* under normal circumstances (Babij, Hundal, Rennie, Ward & Watt, 1986), it is likely that during acidosis net glutamine production may be controlled by glutamate uptake.

The present results concerning routes of alanine uptake were surprising. Muscle alanine transport has, since the early work of Akedo & Christensen (1962) and later work by Riggs & McKirahan (1973) and Narahara & Holloszy (1974), been commonly considered to be largely mediated by a muscle variant of system A, which is normally found to be insulin sensitive. Since many studies of amino acid transport in muscle have used AIB as the system A paradigm substrate, the belief has grown up that muscle amino acid transport is generally insulin sensitive (Aoki, Brennan, Muller,

Moore & Cahill, 1972). The present results cast some doubt on these interpretations because the A system appears to be of very low capacity in the perfused skeletal muscle preparation. This conclusion is supported by the results of studies with [^{11}C]2-aminoisobutyrate uptake in dog skeletal muscle observed in the presence of insulin (Bading *et al.* 1987). In fact alanine uptake appears to be mediated via a mixture of Na^+-dependent and independent but, in any case, insulin-sensitive systems, probably akin to the ASC and L systems found in other tissues. In general we find amino acid transport in perfused skeletal muscle to be poorly insulin sensitive, with a few notable exceptions such as the transport of glutamine (Hundal *et al.* 1987). This conclusion has also been arrived at by a consideration of factors affecting net disappearance of amino acids into human limbs studied by arterio-venous differences during amino acid infusion studies (Lundholm, Bennegård, Zachrisson, Lundgren, Edén & Moller-Loswick, 1987).

The characteristics of alanine transport may help explain some aspects of its physiological behaviour in muscle. The distribution ratio (intracellular:extracellular concentration) for alanine (5:1 in rat skeletal muscle) is not as high as for other amino acids (e.g. glutamine: 12:1; which appears to travel exclusively via a single Na^+-dependent carrier (Hundal *et al.* 1987). It may be that the Na^+ dependence of the A and ASC systems is responsible for the concentrative nature of alanine transport but that alanine accumulation is limited by the exchange properties of system L, for which alanine may also be a substrate. Thus accumulation of alanine via the Na^+-dependent systems (secondary active transport) could result in an increased availability of alanine for outward transport via the L system (tertiary active transport). Such an arrangement would of course be conducive to a generalized increase in L system transport and might explain the ability of L system substrates such as the BCAA and aromatic amino acids to become concentrated within the muscle cell water by about 10–20%, as commonly observed (e.g. Bergström, Fürst, Norée & Vinnars, 1974). Similar arguments can be used to explain the distribution ratios of greater than unity of other amino acids wholly or partially transported by Na^+-dependent and independent systems. For certain amino acids such as aspartate and glutamate which are not transported by a secondary active transport mechanism, yet display large distribution ratios, intracellular production and the barrier properties of the cell membrane play a somewhat greater role in determining their distribution.

There appears to have been no previous information available regarding the transport characteristics of serine and lysine by skeletal muscle, although measurements of initial rates of amino acid entry into skeletal muscle have been made (Baños, Daniel, Moorhouse & Pratt, 1973) and the net uptake of these amino acids determined by arterio-venous blood sampling is also available (Aoki, Muller, Brennan & Cahill, 1973). However, features of the transport of these amino acids in skeletal muscle appear to be similar to those described for other cell types.

Christensen *et al.* (1967) first described a carrier system preferring alanine, serine and cysteine in the Ehrlich ascites cell and designated it system ASC. The transport of serine and these other amino acids is Na^+ dependent and insensitive to changes in pH and availability of insulin. In the hepatocyte Joseph, Bradford & McGivan (1978) reported serine and alanine transport to be mutually competitive and Na^+ dependent.

Similar properties have been observed by us for serine uptake in skeletal muscle; furthermore, the concurrence of K_m values between the liver and muscle systems appears to suggest that they may be structurally related. In the perfused rat pancreas (Mann, Norman, Habara, Munoz & Peran, 1985) the tissue ASC carrier, unlike the muscle serine transporter, is responsive to physiological doses of insulin which affects both the V_{max} and the K_m of the transport process. This may in part be due to structural variation between the ASC transporters of the two tissues since there also appears to be a large difference between their K_m values (Mann *et al.* 1985).

The transport of cationic or basic amino acids like lysine is mediated by system y^+, which reportedly has similar properties in all cells studied in detail at present (reviewed by White, 1985). System y^+ is normally pH insensitive with substrate specificity confined to lysine, arginine and ornithine, characteristics consistent with the present results. System y^+ has generally been found to be Na^+ independent, but partial dependence has been demonstrated in renal and intestinal brush-border membranes (White, 1985). Our results show that lysine uptake into skeletal muscle was mediated by a Na^+-dependent process with a stoichiometry of 2 Na^+:1 lysine, suggesting a deviation from properties of the y^+ carrier described for other cell types (White, 1985).

The differences between the properties of the lysine transporter described here and those of other tissues (see White, 1985) provides another example of pairs of related transport systems like ASC and asc (Vadgama & Christensen, 1985) which have identical substrate selectivity, but differ only in their Na^+ dependence, asc being Na^+ independent.

All twenty common amino acids (other than those that have previously undergone post-translational modification) may participate in protein synthesis. In the general model proposed by Kacser & Burns (1979), all components (e.g. transport of amino acids) in a 'whole' metabolic system must have some influence, however small. The elucidation of the modes of such possible interactions is beyond the scope of the present work. Nevertheless, it is possible to compare the rate of amino acid transfer across the muscle membrane and the rate of protein synthesis. The protein synthetic rate in gastrocnemius muscle of the 200 g female rats we routinely use is, in our hands, found to be $12{\cdot}2 \pm 3{\cdot}1$ % day^{-1}. Data concerning the relative amounts of each amino acid in muscle protein is also available (Waterlow *et al.* 1978). Using these values the incorporation rate for individual amino acids into muscle protein may be calculated to vary between 2 (e.g. histidine) and 10 (e.g. alanine) nmol min^{-1} (g protein)$^{-1}$. For most amino acids entry into skeletal muscle is nearly 2–3-fold greater than their incorporation rate into protein suggesting that under normal conditions amino acid transport is unlikely to limit protein synthesis, a conclusion also arrived at on the basis of similar calculations by Waterlow & Fern (1981) and by Lundholm *et al.* (1987) from arterio-venous flux measurements. Where transport is of the same order as the incorporation rate into protein (e.g. glutamate) proteolysis and intracellular synthesis of the amino acid ensure that the free intracellular pool is large enough to meet the demand of protein synthesis.

One of the major findings of the present work was that, with the exception of substrates of system N^m (Hundal *et al.* 1987), amino acid transport in skeletal muscle

was insulin insensitive. What explanation can therefore be given concerning the common observation that the addition of exogenous insulin or stimulation of endogenous insulin secretion, e.g. following a carbohydrate meal, result in a fall in the concentration of many amino acids in the plasma? The exact mechanism of how intracellular responses to insulin binding are evoked still remains elusive, but it is well documented that net protein synthesis in normal animal muscle is stimulated by insulin both *in vitro* and *in vivo* (discussed extensively by Waterlow *et al.* 1978), in which case, the size of the amino acid precursor pool for protein synthesis should fall. Under physiological conditions the transporter molecules would not be saturated so that a fall in plasma amino acid concentration in the presence of insulin may easily be explained if intracellular events increase amino acid demand.

The present results provide a basis upon which to investigate further the hormonal, nutritional, nervous and ionic modulation of amino acid transport in mammalian skeletal muscle.

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