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Amino acid transport System L in muscle cells: biochemical properties and its relation to protein synthesis [1]

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Objectives were to characterize mechanisms and biochemical properties of transport systems responsible for the uptake of branched-chain amino acids (BCAAs) in muscle cells. Rat omega myoblasts (RMo) were grown to confluency and allowed to differentiate prior to conduct of transport assays. Myotubes concentrated cycloleucine (cLeu) in a sodium (Na)-free medium. The Na gradient-independent transporter possessed high affinity ($K_m = 0.12$ mM) and high capacity ($V_{max} = 6.4$ nmol cLeu/mg protein per min). Cycloleucine transport was strongly inhibited by nonpolar neutral amino acids but not by α-aminoisobutyric acid or lysine. Myotubes possessed a Na gradient-independent trans-exchange mechanism. Hence, myotubes possess a System L-like transporter. In the second part of the study we determined that various inhibitors (KCN, oligomycin, iodoacetamide and cycloheximide) increased leucine transport. Their actions were not mediated by reductions in ATP concentration but were instead associated with changes in protein synthesis. Hence, regulation of muscle protein synthesis may also influence transporter activity.

Introduction

Amino acids enter mammalian cells through actions of several transport systems [1–3]. Concentrative activities of some systems are driven by the Na gradient [1–3]. However, Na gradient-independence of other transport systems indicates their reliance upon other mechanisms for energization [1–4]. System L, a ubiquitous Na gradient-independent system which recognizes nonpolar neutral amino acids as substrates, may be energized via coupling to the Na/H exchanger [5,6].

Skeletal muscle protein balance is influenced by branched-chain amino acids (BCAAs) and glutamine. Leucine, which is transported by System L, stimulates muscle protein synthesis [7,8] and its transamination product, α-ketoisocaproic acid, inhibits muscle protein degradation [8,9]. Glutamine, which in skeletal muscle is transported by System N^m [10], stimulates muscle protein synthesis [11,12]. Noncompetitive inhibition of glutamine efflux from muscle by BCAAs is another mechanism by which BCAAs may influence muscle protein balance [11]. Because BCAAs control muscle protein homeostasis via several unique mechanisms, systems by which they enter muscle cells and regulation of these systems may be important determinants of muscle protein balance. Therefore, objectives of this research were to characterize biochemical properties of BCAA transport in cultured muscle cells. During this study we detected unanticipated regulation of transport following exposure of muscle cells to metabolic inhibitors. Hence, objectives of the second part of this research were to investigate the significance and metabolic basis for the regulation of amino acid transport by agents which interfere with ATP formation and protein synthesis.

Methods

Cell culture. RMo myoblasts [13] were obtained from Dr. Gary Merrill (Oregon State University), plated at a density of 20000 cells per cm^2 and were grown to confluency in a humidified water-jacketed CO_2 incubator in 10 cm plastic dishes containing Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% calf serum (CS). Each liter of DMEM contained 10 ml of a 1% penicillin G/streptomycin sulfate mixture (Gibco Laboratories, Grand Island, NY). At confluency the culture medium was replaced with DMEM lacking CS but supplemented with bovine insulin (10^{-6}

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M) and dexamethasone (10^{-7} M). In this medium formation of myotubes occurred over 48 h after which medium was replaced with DMEM containing 10% CS for 24 h. At this time in excess of 90% of nuclei were myotubular.

Modified Earle's balanced salt solution (EBSS; pH 7.4; 37°C) was used for preincubation and transport studies. Na-free EBSS (choline-EBSS) was prepared by replacing Na salts of EBSS with choline salts at equimolar concentrations. In both Na-containing EBSS (Na-EBSS) and choline-EBSS, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (Hepes; free acid) was added to a final concentration of 20 mM. This replaced equimolar NaCl or choline-Cl, respectively. Final buffer pH was adjusted to 7.4 with potassium hydroxide (KOH). All EBSS buffers contained glucose (1 g/l) and Phenol red (10 mg/l).

Transport assays. Culture medium was aspirated after which myotubes were preincubated for 10 min in an EBSS-based buffer (10 ml). After this myotubes were washed briefly with EBSS-based buffer (10 ml, 37°C). The washing buffer was aspirated after which EBSS-based buffers (4 ml), which contained the labeled (^{14}C; 0.2 μCi/ml; cLeu or leucine) and unlabeled amino acid under investigation, were added. The plates of myotubes were then covered and incubated at 37°C either in a CO_2 incubator or on a water bath. The incubator was used to maintain buffer temperature and pH when assessing transport for periods of time exceeding 2 min. Transport was stopped by aspiration of the transport buffer followed by three rinses with ice-cold EBSS-based buffers (5 ml each). Application of the first rinse represented termination of the transport assay. Myotubes were scraped from culture dishes then sonicated. Aliquots were taken for determinations of protein, which were completed as outlined by Bradford [14] using bovine serum albumin as a standard, and of ^{14}C activity. Radioactivity in samples was determined using liquid scintillation counting. Na gradient-dependent plus-independent transport was estimated using Na-EBSS-based buffers. Na gradient-independent transport was estimated using choline-EBSS-based buffers. Replicate incubations containing ^{14}C-inulin-carboxyl, [*carboxyl*-^{14}C] (New England Nuclear; Boston, MA) and unlabeled amino acid were used to correct for the labeled amino acid associated with the extracellular buffer. Transport was defined as the inulin-corrected ^{14}C activity associated with myotubular protein. Each treatment, including inulin controls, was repeated three times within a study and studies were replicated with serial passages of myoblasts.

Biochemical characterization of BCAA transport. We evaluated the time-course for cLeu uptake by evaluating distribution ratios (DR) at 0.5, 1, 2, 4, 8 and 16 min of incubation in both Na-EBSS and choline-EBSS buffers. Cycloleucine was provided at 1 mM. Distribution ratios were calculated following determination of intracellular space according to a well-established method [15]. Briefly, total water space associated with myotubes was determined by the equilibration of 3-O-methyl[^{14}C]glucose. Intracellular space was determined by correction of total water space with inulin, which provided an estimate of the extracellular space. The DR was expressed as the concentration of the intracellular amino acid divided by its concentration provided in culture medium. Differences between dose response curves were examined using polynomial regression analysis [16]. For two dose response curves a model was fit with an interaction component after which a model was fit without an interaction component and an extra sum-of-squares F-test was performed [17]. Lack of significance indicated dose–response curves were parallel [16,17].

Non-specific binding of cLeu to muscle cells. Non-specific binding of cLeu to myotubes was examined as outlined by others [18]. Specifically, effects of increasing osmolarity on cLeu transport were examined. Myotubes were pre-incubated for 1 h in Na-free EBSS containing variable concentrations of mannose or mannitol such that variable osmolarities (300–1500 mosM) were obtained. Following pre-incubation, transport of cLeu in Na-free EBSS containing equivalent concentrations of mannose or mannitol and [1-^{14}C]cLeu (aminocyclopentane-1-[^{14}C]carboxylic acid; New England Nuclear, Boston, MA) was examined. Transport was corrected using inulin as described previously.

Kinetics of cLeu transport were examined in both the presence and absence of Na using cLeu concentrations of 0.05, 0.1, 0.25, 0.5 and 1 mM. Kinetic parameters of the Na gradient-independent transport component were derived using least-squares analysis [19] assuming that net entry of cLeu in choline-EBSS resulted from a combination of one Na gradient-independent saturable component and a diffusional component.

Effects of variable concentrations (0, 0.2, 0.5, 2 and 10 mM) of α-aminoisobutyric acid (AIB), leucine, lysine, phenylalanine, tyrosine and 2-amino-2-norbornanecarboxylic acid (BCH; Aldrich Chemical Co.; Milwaukee, WI), which were provided in the transport buffer, on initial rates of saturable Na gradient-independent cLeu transport were evaluated using choline-EBSS-based buffers. Saturable transport was determined by subtracting the nonsaturable component for cLeu entry from total transport. To ascertain the nonsaturable component, concentration-dependence of cLeu transport in choline-EBSS was completed in tandem with these studies. From this, the nonsaturable component was estimated by least-squares analysis [19]. For subsequent studies, the non-saturable component was not determined.

Regulation of amino acid transport. Effects of various inhibitors (KCN, oligomycin, cycloheximide, iodoacet-

amide) on amino acid transport, protein synthesis and ATP concentrations were examined. Rationales and experimental details for these studies are provided in the results section and in legends to figures, respectively.

Assessment of protein synthesis. Effects of various metabolic inhibitors on myotube protein synthesis were examined by incubating myotubes in choline-EBSS for 1 h in the presence and absence of an inhibitor and [^{3}H]phenylalanine (0.25 μCi/ml; side-chain ^{3}H; ICN, Costa Mesa, CA). Following 1 h, myotubes were washed with ice-cold buffer and scraped from their plates. Cells were sonicated and an aliquot of the sonicated material was taken for protein determination [14] and an aliquot was precipitated by addition of TCA (5%; final concentration). The protein was pelleted by centrifugation, washed twice with 5% TCA and solubilized by heating with Protosol (0.25 ml; New England Nuclear, Boston, MA). Radioactivity associated with the cell pellet was determined by liquid scintillation counting and was corrected for non-specific association of label with cells using zero-time control blanks.

Assessment of myotube ATP. The luminometric method of Hampp [20] was used for ATP determinations. Following exposure of myotubes to metabolic inhibitors for 1 h, myotubes were removed from the CO_2 incubator, medium was aspirated and 1.4 ml of ice-cold perchloric acid (1.66 M) was added. Cells were scraped from their plates and transferred to a microcentrifuge tube. Cell debris was pelleted by centrifugation (2°C) after which the pellet was taken for protein determination [14] and 800 μl of the supernatant was taken for ATP determination. Bicine (1 M; 200 μl) and KOH (4 M; 350 μl) were added. pH of the supernatant was adjusted to 7.6–8.0 by addition of KOH. pH paper (pHydrion Lo-Buff 5-9; Micro Essential Laboratory, Brooklyn, NY) and the residual Phenol red in the sample were used for this purpose. The sample was centrifuged (2°C) to precipitate perchlorate and the supernatant was taken and placed on ice. ATP content of samples was immediately determined with the luciferin-luciferase assay procedure using a Bioluminescent Somatic Cell Assay Kit (Kit FL-ASC; Sigma Chemical Co., St. Louis, MO) and a liquid scintillation counter. A standard curve with freshly-prepared ATP was prepared for each assay. Values obtained for cells were within range of the standard curves. ATP concentration was expressed as a proportion of myotube protein. The study was repeated four times with four different passages of myotubes.

Myotube creatine phosphate (CP) determination. In separate studies, effects of KCN, oligomycin and iodoacetamide on myotube creatine phosphate levels were determined according to the methods of Bergmeyer [21]. Myotubes were treated with inhibitors as outlined earlier prior to CP assay. Myotubes were scraped from their plates in 1.66 M perchloric acid. The pH of the sample was adjusted to 6.0–6.5 using 4 M KOH. The samples were placed on ice for 1 h, then centrifuged (2°C) to precipitate perchlorate. The supernatant (1 ml) was taken and combined with 1.99 ml of triethanolamine buffer (25 mM, pH 7.5) containing 0.23 mM NADP, 3.3 mM $MgCl_2$, 0.15 mM ADP, 16.7 mM glucose, 1.7 μg/ml glucose-6-phosphate dehydrogenase and 470 mU/ml hexokinase, incubated at room temperature for 30 min, then mixed with creatine kinase (10 μl; 5 mg enzyme/ml triethanolamine buffer) and the reaction was allowed to proceed for 20 min. Change in A_{334} was monitored to ensure linearity of reaction conditions and the assay was validated with crystalline CP. The study was repeated four times and triplicates were performed with each study.

Statistical analyses were conducted as outlined by Steel and Torrie [17]. Data were tested for a normal distribution and homogeneity of variance prior to analysis of variance. Differences between treatment means were tested using a Student-Newman-Keul multiple range test. A level of significance of 5% was adopted for all comparisons.

Results

Biochemical characterization of BCAA transport

The time-course for cLeu uptake in the presence and absence of Na is shown in Fig. 1. Irrespective of the presence or absence of Na, myotubes concentrated cLeu 4–5-fold within 16 min of incubation. Differences ($P < 0.05$) in cLeu transport were not detected in the presence or absence of Na.

To determine whether the time-dependent increase in myotube radioactivity represented transport or instead represented non-specific binding of cLeu to cells, effects of increasing medium osmolarity on initial rates of cLeu transport were investigated. When osmolarity

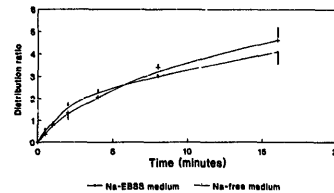

Fig. 1. Time-course for cLeu entry into RMo myotubes in the presence and absence of Na. Myotubes were preincubated at 37°C for 10 min in either Na-EBSS or choline-EBSS prior to assay of transport in either Na- or choline-EBSS buffers. cLeu concentration was 1 mM. Values are expressed as distribution ratios and are means of three replicate studies with three passages of myoblasts. Distribution ratio (DR) is the ratio of intracellular cLeu/extracellular cLeu.

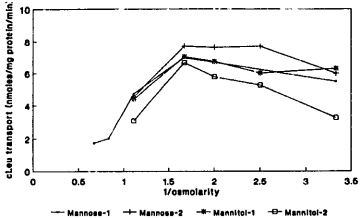

Fig. 2. Effects of osmolarity on cLeu transport. Myotubes were pre-incubated for 60 min in Na-free EBSS (0.3 osM) containing variable concentrations of mannose or mannitol such that final osmolarities of media varied from 0.3 osM (no additional mannose or mannitol) to 1.5 osM. Following this, effects of osmolarity on initial rates of cLeu transport (1 min, 0.1 mM) were examined using Na-free EBSS buffers containing variable mannose or mannitol. The study was conducted twice for mannose and twice for mannitol.

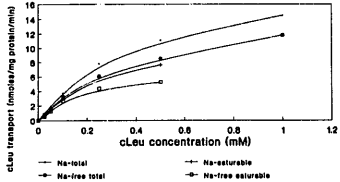

Fig. 3. Concentration dependence of cLeu transport. Myotubes were preincubated (37°C) in Na-EBSS or choline-EBSS for 10 min prior to assessing transport (1 min) of cLeu in either Na- or choline-EBSS buffers, respectively. Values are means of four separate determinations and are expressed as nmol cLeu/mg protein per min ± S.E.. Saturable components for transport in the presence of Na (Na-saturable) and absence of Na (Na-free saturable) are also shown. Estimated V_{max} and K_m for the Na gradient-independent component are 6.4 ± 1.7 nmol cLeu/mg protein per min and 0.12 ± 0.04 mM, respectively. Values are means. S.E. values are not given as their dimensions were smaller than the symbols used.

was increased from 300 to 600 mosM, no changes ($P > 0.05$) in cLeu transport were detected. This is in agreement with results of Hundal et al. [20] who reported that a 50% increase in perfusate osmolarity did not affect glutamine transport in perfused muscle. However, at higher osmolarities we found that cLeu transport was progressively diminished (Fig. 2). We estimate that at infinite osmolarity, transport would approach zero and conclude that the time-dependent increase in cLeu association with myotubes (Fig. 1) represents transport.

Kinetics of cLeu transport in the presence and absence of Na are shown in Fig. 3. Na gradient-independent cLeu transport accounted for approx. 70% of total carrier-mediated cLeu transport. V_{max} and K_m estimates for the Na gradient-independent component were 6.4 ± 1.7 nmol cLeu/mg protein per min and 0.12 ± 0.04 mM, respectively. Diffusion accounted for 5.1 ± 2.3% of initial (1 min) cLeu entry in choline-EBSS.

Effects of other amino acids on initial rates of Na gradient-independent cLeu transport are shown in Fig. 4. Nonpolar amino acids, including BCH, leucine, phenylalanine and tyrosine competed strongly with cLeu for transport whereas a nonpolar neutral amino acid (AIB) and a basic amino acid (lysine) did not compete strongly with cLeu for transport. Nonpolar amino acids, if supplied at sufficiently high concentration (10 mM), completely suppressed saturable Na gradient-independent cLeu transport.

Regulation of amino acid transport

Following completion of the preceding studies we examined regulation of System L. Leucine replaced cLeu as a model amino acid at this stage in our studies because its susceptibility to trans-exchange with other non-polar amino acids was greater than trans-exchange noted for cLeu (data not shown). Hence, it was surmised that more physiologically-meaningful data could be obtained with use of a naturally-occurring amino acid. Effects of KCN on leucine transport were examined because this has been used as a criterion for reliance upon cellular energy reserves by transport systems [4,22]. Preloading of myotubes with leucine or phenylalanine prior to assessing transport was used to

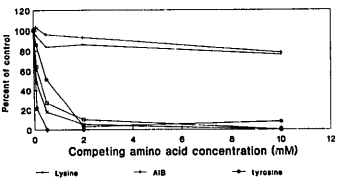

Fig. 4. Inhibition of saturable Na gradient-independent cLeu transport by AIB, BCH, leucine, lysine, phenylalanine and tyrosine. Myotubes were preincubated for 10 min in choline-EBSS after which a choline-EBSS-based buffer containing cLeu (0.1 mM) and ^{14}C-cLeu with either no additions or additions of other amino acids (0.2, 0.5, 2 or 10 mM) was added. Transport assays were conducted for 1 min. The nonsaturable (diffusion) component was subtracted from total transport to allow determination of effects of competing amino acids on Na gradient-independent cLeu transport. Values are expressed as a percent of control and are means of three separate determinations. Transport of cLeu in absence of other amino acids was 3.1 nmol/mg protein per min. For clarity of presentation, S.E. values are not shown.

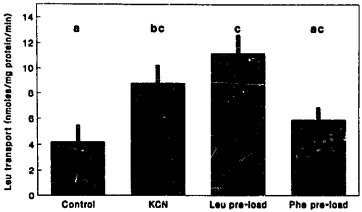

Fig. 5. Effects of KCN and of preloading on leucine transport by RMo myotubes. Myotubes were preincubated for 60 min in choline-EBSS containing no additions (control) or additions of KCN (5 mM), leucine (20 mM) or phenylalanine (20 mM) after which they were washed with ice-cold choline-EBSS then leucine transport (0.1 mM; 1 min) was assayed. Values are means ± S.E. of three replications. Differences ($P < 0.05$) between treatments are indicated by lack of a common superscript above bars.

examine trans-exchange of leucine for leucine and leucine for phenylalanine, respectively.

Preincubation of myotubes with KCN increased leucine transport 2-fold ($P < 0.05$) and preloading myotubes with leucine enhanced leucine transport (Fig. 5). Preloading myotubes with phenylalanine did not affect ($P > 0.05$) leucine transport.

To determine whether effects of KCN on leucine transport were due to added potassium or due, instead, to the presence of cyanide, we evaluated effects of KCN, KCl and oligomycin on leucine transport (Fig. 6). Trypan blue exclusion was also assessed to provide an index of viability. KCN and oligomycin increased leucine transport ($P < 0.05$) but KCl was without effect ($P > 0.05$). KCl, KCN and oligomycin did not affect

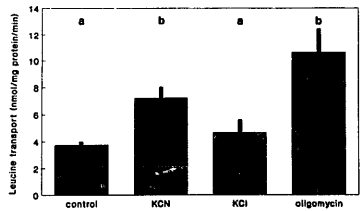

Fig. 6. Effects of KCN, KCl and oligomycin on Na gradient-independent leucine transport by RMo myotubes. Myotubes were preincubated as outlined in the legend to Fig. 5 in the presence of KCN (5 mM), KCl (5 mM) or oligomycin A (5 μg/ml) or in the absence of additions after which leucine transport (1 min) in choline-EBSS was determined. Leucine concentration in the transport medium was 0.1 mM. Values are means of three replications ± S.E.. Differences ($P < 0.05$) between treatments are indicated by lack of a common superscript above bars.

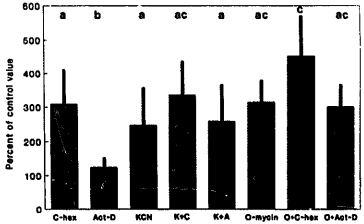

Fig. 7. Effects of cycloheximide (C-hex; 10 μg/ml), actinomycin D (A; 1 μg/ml), KCN (K; 5 mM), oligomycin A (O; 5 μg/ml) and combinations of these treatments on Na gradient-independent leucine transport by RMo myotubes. Myotubes were exposed to these treatments for a 60 min preincubation period in choline-EBSS. Conditions for assessing leucine transport are given in the legend to Fig. 5. Values are expressed as a percent of control and are means of three replicate studies ± S.E.. Differences ($P < 0.05$) between treatment means are indicated by lack of a common superscript above bars. All treatments means except actinomycin D differed ($P < 0.05$) from control (4.16 ± 0.84 nmol leucine/mg protein per min).

($P > 0.05$) Trypan blue exclusion. For each treatment percent of myotubes excluding trypan blue was 93%–95%.

To determine whether effects of KCN and oligomycin on leucine transport were transcription- or translation-dependent, we investigated effects of preincubation with and without KCN or oligomycin in the presence and absence of actinomycin D and cycloheximide (Fig. 7). Actinomycin D, added to the preincubation buffer alone, did not affect ($P > 0.05$) leucine transport nor did it affect ($P > 0.05$) abilities of KCN or oligomycin to enhance leucine transport. In more recent studies (data not shown) we have determined that longer exposure (2 h) of myotubes to actinomycin D increases ($P < 0.05$) leucine transport. Cycloheximide, added alone to the preincubation buffer, mimicked actions of KCN and oligomycin ($P < 0.05$). Although effects of KCN on leucine transport were not additive to effects of cycloheximide, the combination of oligomycin and cycloheximide caused a greater induction of transport than did cycloheximide alone ($P < 0.05$; Fig. 7). Because we did not examine concentrations of cycloheximide and oligomycin which maximally increased leucine transport, we cannot infer from the latter observation that oligomycin and cycloheximide regulate leucine transport via distinct mechanisms.

To determine whether the observations were unique to the RMo cell line we examined effects of KCN and oligomycin on leucine transport in L6 myotubes (American Tissue Type Collection; Rockville, MD). This study was repeated three times identically to studies with RMo myotubes and we determined that

KCN and oligomycin effected the same response ($P < 0.05$) for leucine transport in L6 myotubes. Transport of leucine in control-, KCN- and oligomycin-treated L6 myotubes was 3.15 ± 0.74, 7.46 ± 0.34 and 6.84 ± 0.46 nmol/mg protein, respectively.

Based on the preceding observations we believed that the stimulation of transport was due either to a reduction in ATP concentration or to a reduction in protein synthesis. We challenged the former hypothesis by examining effects of iodoacetamide on leucine transport and by examining effects of the inhibitors on myotube ATP concentrations. Iodoacetamide is a potent inhibitor of glycolysis [23] and we expected that it would effect a greater reduction in myotube ATP than KCN or oligomycin. In preliminary studies (data not shown) we determined that 100 μM iodoacetamide was the highest concentration which could be added to culture medium which would not cause myotube detachment. Although pre-incubation of myotubes for 1 h with iodoacetamide (100 μM) increased leucine transport by the same order of magnitude as KCN and oligomycin (data not shown), iodoacetamide and the other inhibitors did not significantly ($P > 0.05$) reduce myotube ATP concentrations. ATP concentrations in control-, KCN-, oligomycin- and iodoacetamide-treated myotubes were 42.9 ± 8.8, 46.5 ± 8.6, 39.9 ± 7.0 and 41.3 ± 10.9 nmol/mg protein, respectively. However, each of the inhibitors including iodoacetamide, cycloheximide and actinomycin D reduced ($P < 0.05$) protein synthesis to varying degrees (Fig. 8). Cycloheximide effected near-complete inhibition of protein synthesis (Fig. 8).

We believed that myotubes may maintain ATP concentrations when treated with metabolic inhibitors by utilizing their high-energy phosphate stores in the form of creatine phosphate (CP). Myotubes were treated with KCN, oligomycin and iodoacetimide for 1 h, after which they were scraped from their plates for assay of CP. Creatine phosphate concentrations were 45.6 ± 5.4, 42.3 ± 2.8, 45.3 ± 8.0 and 67.6 ± 7.6 μmol CP/g protein in control-, KCN-, oligomycin- and iodoacetamide-treated muscle cells, respectively. Compared to control, iodoacetamide increased ($P < 0.05$) CP concentrations. Effects of the other inhibitors on CP concentrations were not significant ($P > 0.05$).

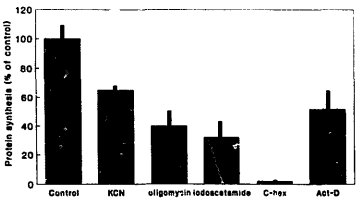

Fig. 8. Effects of KCN, oligomycin, iodoacetamide, cycloheximide (C-hex) and actinomycin D (Act-D) on phenylalanine incorporation into myotube protein. Values are means ± S.E. of two separate studies. Values are expressed as a percent of control. Compared to control, all inhibitors effected a significant ($P < 0.05$) reduction in protein synthesis.

Discussion

Biochemical characterization of BCAA transport

Cultured myotubes contain a transport system which possesses properties of System L noted in other cell types. These properties include the ability to concentrate nonpolar neutral amino acids in a Na-free buffer, inhibition of cLeu transport by nonpolar neutral amino acids but not by polar neutral or basic amino acids and trans-exchange. Myotube System L is an active Na gradient-independent transport mechanism which possesses approximately one-third of the capacity and 120-fold the affinity of the System L described in perfused rat muscle [24]. Reasons for the large differences in affinities between these tissue sources are uncertain. Differences could be due to existence of different System L variants in RMo cells compared to intact hind-limb muscle. Several variants of System L(L1, L2, L3), which differ in their K_ms and capacities, have been identified in other cell types [25]. Alternatively, differences may be due to the different methods used in ours and others' studies. In the hind-limb perfusion studies [10,24] transport was assessed as the disappearance of labeled amino acid from a perfusate. However, the same laboratory group has reported a low K_m for glutamine transport in sarcolemmal vesicles (90 μM [26]). In our studies the K_m for AIB in isolated sheep intercostal muscle was high (11.4 mM [19]). Therefore, it appears that transport studies which use intact muscle yield higher estimates of K_ms than do studies which employ cell culture or vesicle preparations. These differences could be due to unstirred layer effects associated with intact tissues.

Using cLeu as a transport substrate, LeCam and Freychet [22] reported that the K_m of System L in rat hepatocytes was 2.5 mM. The large difference in affinity of System L in hepatocytes versus myotubes may be related to differences in experimental techniques. Alternatively, it is possible that kinetics of System L differ between tissues. Tissue-dependent expression of transporter variants and/or tissue-specific regulation of transporters may underlie prioritization of tissues for utilization of amino acids. A low-K_m System L in skeletal muscle cells would facilitate uptake and utilization of BCAAs by skeletal muscle prior to the uptake of BCAAs by the liver.

Regulation of amino acid transport

In Ehrlich ascites tumor cells [4] and hepatocytes [21] KCN and oligomycin reduce System A and L activities. Interpretation of this has been that transport systems rely upon energy reserves for their concentrative activities. We examined effects of inhibitors of ATP formation on leucine transport and were surprised to detect a stimulation of transport. At first we believed that KCN and oligomycin may effect changes in transport by reducing ATP concentration. However, we later determined that none of the inhibitors had an effect on myotube ATP concentrations. Furthermore, none of the inhibitors reduced CP levels and, in fact, iodoacetamide increased CP levels. Hence, the means by which muscle cells maintained ATP is not clear. It is possible that 1 hour of exposure to inhibitors was insufficient to deplete CP stores detectably. Therefore, our studies did not provide insight into the energetic dependency of myotubular System L. However, we later determined that all inhibitors reduced protein synthesis. Effects of cycloheximide on protein synthesis and of actinomycin D on RNA synthesis are well-established; however, mechanisms by which the other inhibitors reduced protein synthesis are unknown. Fuller et al. [27] have reported a relationship between cardiac protein synthesis and CP concentrations. However, the lack of effect of inhibitors on CP in this study rules this out as a possible means by which the inhibitors altered protein synthesis. Nevertheless, changes in protein synthesis are in common to exposure of myotubes to cycloheximide, KCN, oligomycin and iodoacetamide and it is possible that there exists a relationship between protein synthesis and amino acid transport.

Observations that KCN and oligomycin reduce System A and L activities in hepatocytes and Ehrlich ascites tumor cells [4,22] indicate that the above-noted responses could be unique to skeletal muscle. Differences between liver and muscle transporters exist [22,24] and may include responses to factors which regulate protein synthesis. Christensen [28] has commented on the importance of transport to the determination of interorgan amino acid flows. Perhaps changes in muscle amino acid transport, which may occur upon regulation of muscle protein synthesis, contribute to changes in amino acid flows between muscle and other tissues.

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