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UPTAKE AND METABOLISM OF L-ALANINE BY FRESHLY ISOLATED TROUT (*SALMO TRUTTA*) HEPATOCYTES: THE EFFECT OF FASTING

BY P. CANALS, M. A. GALLARDO, J. BLASCO AND J. SÁNCHEZ*

Departamento de Bioquímica i Fisiologia, Unitat de Fisiologia, Facultat de Biologia, Avenida Diagonal 645, E-08071 Spain

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Summary

Uptake and metabolism of L-alanine by freshly isolated trout hepatocytes has been analyzed. This amino acid is incorporated 'uphill' by different carriers, either Na^+-dependent or Na^+-independent. Na^+-dependent uptake shows the characteristics of an ASC system on the basis of cation dependence and substrate preferences. The Na^+-independent uptake is split between an L-cysteine-sensitive system and a non-saturable component. No uptake through system A has been found, suggesting that this carrier is lacking in trout hepatocytes. On the basis of inhibition by several preferred amino acids, L-alanine is not taken up through either the L or N systems.

Fasting induced changes in both the uptake of L-alanine and its metabolism to CO_2 and glucose. There was an increase in uptake that showed an inverse relationship with L-alanine plasma levels. Glucose production from L-alanine rose during food deprivation, while CO_2 production showed an initial increase, similar to that of glucose. At the end of the fasting time considered, however, there was a drop in CO_2 production, indicating a different kind of regulation for alanine oxidation and gluconeogenesis.

Introduction

Blood-borne amino acids are used by liver cells as a source of energy and to produce a variety of molecules (glucose, peptides, etc.). This feature appears to be especially important in carnivorous species, such as salmonids, which utilise carbohydrate poorly (Walton and Cowey, 1982) and require high-protein diets and mild continuous exercise to achieve maximum growth (Davidson and Goldspink, 1977). However, these species are able to regulate their plasma glucose levels, an essential requirement for the efficient functioning of vital tissues such as the central nervous system.

Of the different amino acids, L-alanine is the most gluconeogenic in mammals

* To whom reprint requests should be sent.

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(Kraus-Friedmann, 1984) but, at equimolar concentrations, L-serine is more gluconeogenic than L-alanine in rainbow trout hepatocytes (French *et al.* 1981). However, the plasma concentration of L-alanine is higher than that of L-serine (Ogata and Arai, 1985; present work) and this difference may partially compensate for the differences in gluconeogenesis.

In any case, transport across the liver cell plasma membrane becomes the rate-limiting step for L-alanine metabolism (Fafournoux *et al.* 1983) and several carriers are able to transport amino acids across the membrane of different cell types. These carriers can be distinguished by their substrate specificity, cation dependence and type of regulation (Guidotti *et al.* 1978; Shotwell *et al.* 1983; Barker and Ellory, 1990). Two main Na^+-dependent carrier systems for short-chain neutral amino acids have been described, the A and ASC systems, as well as one Na^+-independent system, the L system. There is increasing evidence for another Na^+-independent system, similar to the ASC system in substrate preference, which has been called the asc system (Fincham *et al.* 1985, 1990; Vadgama and Christensen, 1985).

'ASC' is the commonly accepted abbreviation for an amino acid carrier which has L-alanine, L-serine and L-cysteine as preferred substrates. The upper case denotes that it is energized by means of the transmembrane Na^+ electrochemical gradient. 'asc' is the abbreviation for a carrier similar to the 'ASC' carrier with respect to preferred substrates; the lower case indicates that it is Na^+-independent. 'A' is the abbreviation for a Na^+-dependent carrier which has L-alanine and other short-chain neutral amino acids as preferred substrates. Its tolerance to *N*-methylated analogues differentiates it from the ASC system. 'L' is the abbreviation for a Na^+-independent carrier which has L-leucine as preferred substrate. Despite its Na^+-independence, the upper case abbreviation is used. 'N' is the abbreviation for a Na^+-dependent amino acid carrier which has L-glutamine, L-asparagine and L-histidine as preferred substrates.

There are abundant data on the presence of these carriers in avian and mammalian cells, but references to their expression in other vertebrates are scarce (Inui and Ishioka, 1983; Ballatori and Boyer, 1988; Fincham *et al.* 1990; Machado *et al.* 1991; Gallardo *et al.* 1992). The study of membrane amino acid transport in ectothermic vertebrates is interesting not only from an evolutionary point of view, but also because the A system seems to be the only amino acid carrier subjected to hormonal (Guidotti *et al.* 1978) or adaptive (Shotwell *et al.* 1983) regulation in mammals. However, Moule *et al.* (1987) have reported that the A, ASC and N systems are regulated by cyclic AMP levels in isolated rat hepatocytes. Because changes in cyclic AMP levels can be related to hormone action, the ASC and N systems could also be regulated in the same way.

With reference to fish, Ballatori and Boyer (1988) indicate that skate hepatocytes do not possess the A system, while the study of Inui and Ishioka (1983) (on eels) is inconclusive about the presence of such a system in teleosts, because the alanine analogue used, aminoisobutyric acid, may be carried by both the A and ASC systems. Changes in the physiological conditions of fish, such as fasting, may

induce alterations in amino acid utilization, increasing their transformation to glucose (French *et al.* 1981), although recent data indicate that these and other changes observed in metabolism during fasting can all be explained by a change in the reference units used (Foster and Moon, 1991). Previous reports of the effect of fasting on amino acid catabolism in fish have given no indications of its effect on the transport process across the membrane.

The aim of the present study is to characterize the transport mechanism mediating L-alanine uptake by isolated trout (*Salmo trutta*) hepatocytes and also to analyze the effect of fasting on its uptake and metabolism.

Materials and methods

Animals and chemicals

Brown trout (*Salmo trutta* L.) (weighing between 250 and 400 g) were obtained from a fish farm (Departament Medi Ambient, Generalitat de Catalunya) in the Pyrenees (Bagà, Spain) and were acclimated to laboratory conditions (a closed water circuit, filled with deionized water and with controlled $[NH_4^+]$ and $[O_2]$, maintained at 15 °C) for at least 1 week before the experiments were performed. Fasting experiments were carried out directly at the fish farm.

All chemicals and L-alanine uniformly labelled with ^{14}C (159.1 mCi mmol^{-1}) were from Sigma Chemical Co. (USA). L-[3-^{3}H]alanine (85 Ci mmol^{-1}) was from New England Nuclear (Germany).

Isolation and incubation of hepatocytes

Trout were anaesthetized in $NaHCO_3$-buffered MS222 and cells were obtained essentially as described by French *et al.* (1981), except that hyaluronidase was omitted and 5 mmol l^{-1} instead of 25 mmol l^{-1} $NaHCO_3$ was used, because of the difference in the gas mixture used to equilibrate all solutions (99.5 % O_2:0.5 % CO_2) before use. Final pH was adjusted to 7.5. Osmolality was determined and adjusted to 305 mosmol kg^{-1} with a micro-osmometer (model 3MO, Advanced Instruments, Inc. USA). Cell viability was routinely assessed by means of Trypan Blue exclusion and more than 95 % viability was obtained. Cell integrity over time was measured by lactate dehydrogenase (LDH) release; less than 5 % of the initial intracellular LDH was found in the medium after 5 h of incubation.

Following isolation, cells were finally suspended in Hanks' solution containing 2 % fatty-acid-free bovine serum albumin. In some instances K^+ was used instead of Na^+ as the main extracellular cation. No loss of cell viability was observed under these conditions.

Uptake and metabolism

For uptake experiments, both cells and solutions were pre-equilibrated at 15 °C before experiments were started by mixing (1:1, v/v) the hepatocyte suspension with the labelled alanine (^{3}H, 0.3 μCi ml^{-1} cells) to obtain the desired concentrations and a final 'hepatocrit' of about 5 %. Incubations were performed

in a shaking bath at 15°C, using the indicated gas mixture as atmosphere. The uptake was stopped by dilution with Hanks' solution. Cells were rinsed with Hanks' solution and centrifuged (1000 *g* for 8 min at 4°C) three times and finally lysed with 0.1% Triton X-100. The resulting solution was counted in a well-type liquid scintillation counter (Packard, Great Britain). 2 mmol l^{-1} amino-oxyacetic acid (AOA; a transaminase inhibitor) was used throughout the uptake experiments.

L-Alanine metabolism experiments were carried out in rubber-sealed vials, previously flushed with the gas mixture. The CO_2 produced from ^{14}C-labelled L-alanine (1 μCi ml^{-1} cells) was trapped in a filter paper containing 2-methoxyethylamine (Merck, Germany). This piece of paper was placed within a plastic well, attached to the rubber seal. Full release of the CO_2 produced by the cells was obtained by acidification of the cell suspension, followed by vigorous shaking for 2 h.

^{14}C-labelled glucose production was measured as described by Walton and Cowey (1979). Briefly, the non-metabolised L-alanine was trapped in a cationic exchange resin, Dowex-50W (200–400 mesh), equilibrated with 1 mol l^{-1} glucose in water. The non-trapped material was eluted with this solution and placed on an anionic exchange resin AG 1-X8 (200–400 mesh), formate form (Bio-Rad, USA). Labelled glucose was eluted with the same 1 mol l^{-1} glucose solution. Incorporation of radioactivity from L-alanine to glycogen was measured as in French *et al.* (1981).

The DNA content of hepatocytes was measured as described by Buckley and Bulow (1987).

For free plasma amino acid analysis, blood was taken by caudal puncture with heparinized syringes. Plasma was separated by centrifugation. Plasma proteins were precipitated with trifluoroacetic acid and free amino acids were separated by ionic exchange chromatography and analyzed by means of a Chromaspeck-Rank Hilger autoanalyzer fitted with a 300 mm length × 3 mm i.d. column of Dionex DC6A resin. Standard lithium citrate buffers were as recommended by the manufacturer. Post-column derivatization with orthophthaldialdehyde (OPA) yields fluorescent derivatives which were detected with a fluorimeter (λ_{ex}=395 nm; λ_{em}=475 nm). Norleucine was used as the internal standard.

Curve-fitting of experimental data was performed by means of non-linear regression analyses (Graph-Pad 2.0 and Sigma Plot 4.0).

Results

L-Alanine uptake

The time course of uptake of 150 μmol l^{-1} L-alanine by freshly isolated trout hepatocytes is shown in Fig. 1. In the absence of metabolism, due to the presence of 2 mmol l^{-1} AOA, there is a continuous incorporation with a half-equilibration time of 121.4±21.3 min. Cell water content was measured gravimetrically, yielding a mean value of 75%. This gave an intracellular concentration at equilibrium of

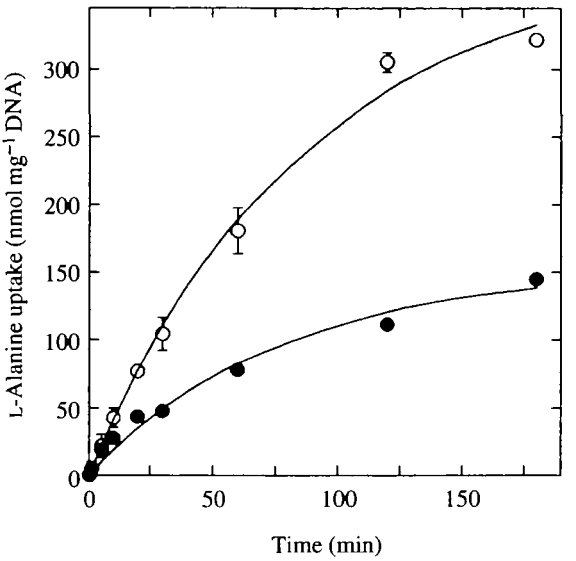

Fig. 1. Time course of L-alanine uptake by trout hepatocytes. Cells were suspended in Hanks' solution and incubated for different times in the presence of 150 μmol l^{-1} L-alanine and 2 mmol l^{-1} amino-oxyacetic acid in either Na^+-containing (○) or K^+-containing (●) medium. Each point is the mean of 3–5 individual experiments. Bars show the standard deviation.

2.4 mmol l^{-1}, i.e. trout hepatocytes can concentrate this amino acid 16-fold over extracellular concentrations. When K^+ was used instead of Na^+ as the main extracellular cation, a fall in the amount of amino acid incorporation was observed. However, L-alanine was still concentrated within hepatocytes (sixfold). Both Na^+-dependent (obtained by subtracting the Na^+-independent uptake from the total uptake) and Na^+-independent uptakes appear to be saturable, indicating the presence of at least two systems capable of transporting L-alanine uphill across the trout hepatocyte membrane.

When the rate of L-alanine incorporation was plotted against the extracellular concentration, the relationship was nonlinear for both Na^+-dependent and Na^+-independent uptakes (Fig. 2). The kinetic constants for the Na^+-dependent uptake were V_{max}=5362.5 nmol mg^{-1} DNA 10 min^{-1} and apparent K_m= 26.7 mmol l^{-1}. Values for Na^+-independent uptake were V_{max}=590.6 nmol mg^{-1} DNA 10 min^{-1} and apparent K_m=9.53 mmol l^{-1}. These values indicate that trout hepatocyte membranes show a high capacity to carry this amino acid, although K_m values are far above the physiological range of L-alanine concentrations (about 500 μmol l^{-1}). A non-saturable component in the Na^+-independent uptake was obtained by measuring the inhibition of uptake of a fixed concentration of radioactive amino acid (10 nmol l^{-1}) by different 'cold' amino acid concentrations (10 μmol l^{-1} to 20 mmol l^{-1}). A maximal inhibition of 90% was obtained. Thus, 10% of the uptake appears to be non-saturable.

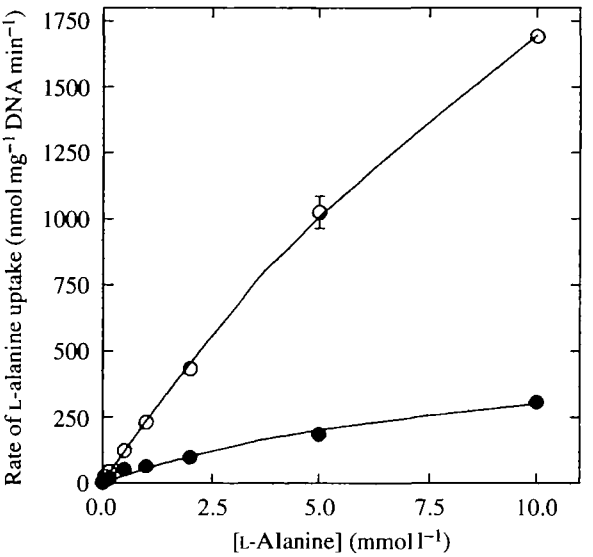

Fig. 2. Concentration dependence of the rate of L-alanine uptake by trout hepatocytes. Initial rates were measured in the presence of buffers containing either sodium or potassium. Cells were incubated for 10 min before the uptake was stopped, as described in Materials and methods. (○) Total uptake. (●) Na^+-independent uptake. Each point is the mean of 3–5 individual experiments. Bars show the standard deviation. Kinetic constants for both Na^+-dependent and Na^+-independent L-alanine uptake are given in the text.

Because there are different Na^+-dependent transport systems for L-alanine, experiments to obtain information about the nature of those present in trout hepatocytes were carried out. Uptake inhibition by α-(methylamino)isobutyric acid (MeAIB) is used to ascertain whether a given amino acid is taken up through system A. Fig. 3 shows that this synthetic amino acid does not inhibit the incorporation of 150 μmol l^{-1} L-alanine into trout hepatocytes, indicating that system A is absent from these cells. In the same way, high concentrations of either L-serine or L-cysteine may be used to inhibit incorporation through the ASC system. Fig. 3A also shows that these amino acids inhibit the incorporation of L-alanine, giving a good indication of the presence of the ASC system in trout hepatocytes.

Kilberg *et al.* (1980) found a Na^+-dependent system in hepatocytes that is relatively specific for glutamine, asparagine and histidine, and which they called system N. Because of the lack of inhibition by high concentrations of these amino acids (except L-asparagine) on the L-alanine uptake, this system (if present in trout hepatocytes) seems to be unable to contribute to the transport of L-alanine across the hepatocyte membrane.

Fig. 3B shows that L-serine and L-cysteine were able to inhibit L-alanine uptake in Na^+-free medium, while the presence of L-leucine in the range 10 μmol l^{-1} to

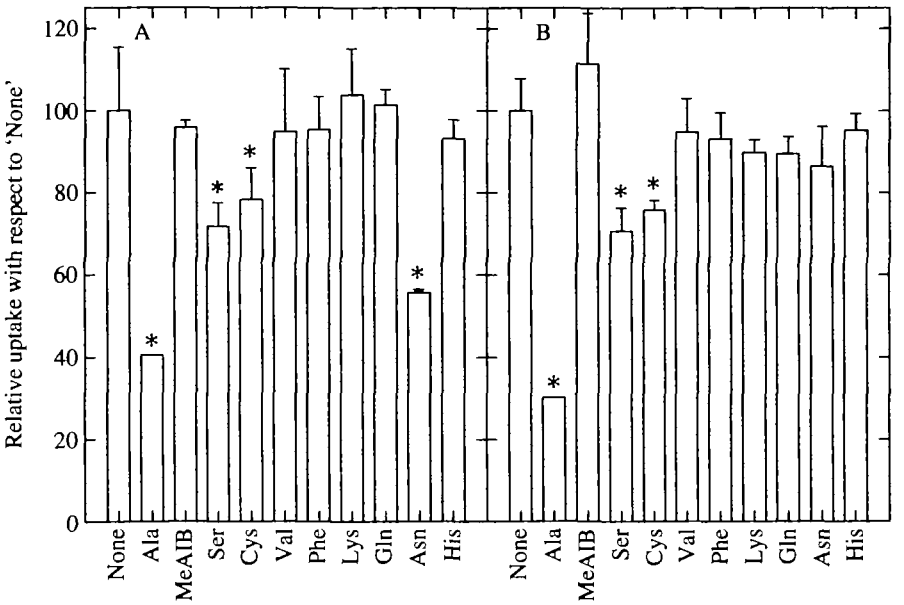

Fig. 3. Effect of different amino acids (1 mmol l^{-1}) on the Na^+-dependent (A) and the Na^+-independent (B) uptake of 150 μmol l^{-1} L-alanine by trout hepatocytes. Cells were incubated for 10 min in either Na^+-containing or K^+-containing medium. Values are the mean of three individual experiments and bars show the standard deviation. Asterisks indicate values significantly different from the control value in the absence of added amino acids ($P<0.05$).

10 mmol l^{-1} did not produce a significant inhibition (data not shown). Because L-leucine is considered to be a better substrate than L-serine or L-cysteine for system L, these data suggest that this system has a low ability to transport L-alanine in trout hepatocytes and that there is a Na^+-independent system sensitive to L-serine and L-cisteine inhibition.

Changes in L-alanine uptake and metabolism during 21 days of fasting

DNA content

Because fasting alters tissue composition, the parameter used as a reference for experiments on food deprivation is of great importance. Foster and Moon (1991) proposed that DNA content is the best reference. Fig. 4 shows that the DNA content per gram wet mass of hepatocytes drops slightly during the first 2 weeks of fasting, and only after 3 weeks of food deprivation was a rise observed ($P<0.05$). A more pronounced rise was measured by these authors in the yellow perch, but the fasting period was also longer. Changes in cell size, rather than in cell number, may explain this modification.

L-Alanine uptake

Fig. 5A shows that fasting increases the rate of uptake of 150 μmol l^{-1} L-alanine

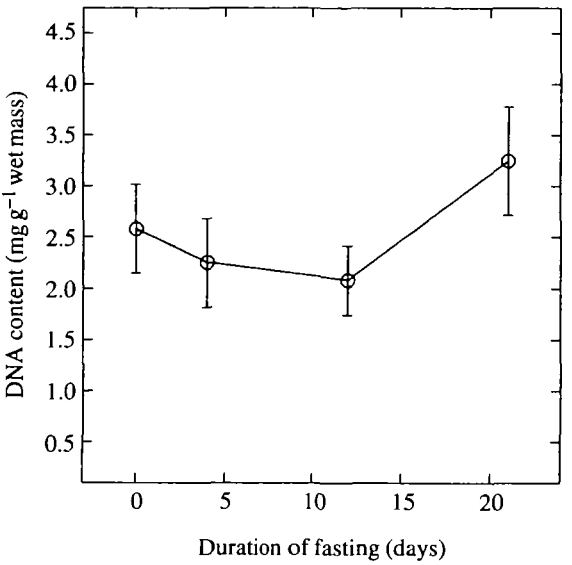

Fig. 4. Variations in DNA content of isolated trout hepatocytes during fasting. Each point is the mean of three individual experiments. Bars show the standard deviation.

and that the Na^+-dependent incorporation accounts for most of the changes observed. Fasting has no clear effect on the L-alanine uptake through Na^+-independent systems (Fig. 5B). Inhibition of uptake caused by addition of 10 mmol l^{-1} L-cysteine reveals that, in a Na^+-free medium, only the rate of uptake through the L-cysteine-sensitive system is modified by fasting, while the rest (the non-saturable component) is not.

Table 1 shows the effect of food deprivation on plasma amino acid levels. A general increase was observed during the first half of fasting, probably due to mobilization from muscle (Johnston and Goldspink, 1973; Mommsen *et al.* 1980; Machado *et al.* 1988; Blasco *et al.* 1991); at the end of fasting, a general drop in plasma levels was observed, indicating a high degree of utilization. When uptake of 150 μmol l^{-1} L-alanine from both control and fasted animals was plotted against their L-alanine plasma concentration, there was an inverse relationship between uptake through either the ASC or the Na^+-independent L-cysteine-sensitive systems and the plasma L-alanine concentration (Fig. 6).

Glucose and CO_2 formation from L-alanine

Besides the fasting-induced rise in L-alanine uptake, there was an increase in glucose production from this amino acid during food deprivation, while its oxidation to CO_2 was depressed (Fig. 7). Glucose formation and L-alanine uptake rate changed almost in parallel, independently of either the nutritional conditions of the cells or the presence/absence of Na^+ in the incubation medium.

The glucose formed was not incorporated into glycogen since no radioactivity

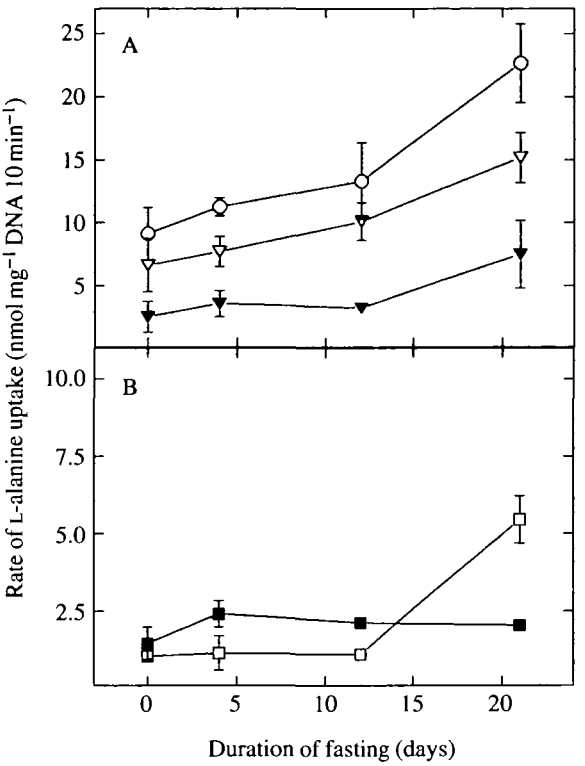

Fig. 5. Effect of fasting time on the rate of uptake of 150 μmol l^{-1} L-alanine by trout hepatocytes. Cells were incubated for 10 min in either Na^+-containing or K^+-containing medium. (A) Total (○), Na^+-dependent (▽) and Na^+-independent (▼) uptake. (B) Na^+-independent uptake split between asc (□) and non-asc (■) components. Each point is the mean of three individual experiments. Bars show the standard deviation.

was found after the extraction procedure, perhaps because of the loss of glycogen during hepatocyte isolation (Moon *et al.* 1988) or because there is no incorporation of synthesized glucose into this molecule. Neither French *et al.* (1981) nor Foster and Moon (1991) give a clear indication whether this process takes place.

Discussion

Isolated trout hepatocytes take up L-alanine by means of specific carriers, one of them belonging to the ASC type, on the basis of its preference for short-chain neutral amino acids and its Na^+-dependence. The lack of inhibition of uptake by MeAIB allows us to discount the presence of system A, which is the main membrane carrier for short-chain neutral amino acids in mammalian hepatocytes (McGivan *et al.* 1981) and for which MeAIB is considered a selective substrate (Barker and Ellory, 1990). The other uptake system is Na^+-independent.

Table 1. *Amino acid levels in plasma after different times of fasting*

Amino acid (μmol l^{-1})	Fasting (days) 0	4	12	21	Significance
Tau	527±103	1377±215	997±132	451±27	0.001
Urea	446±47	523±73	478±119	795±497	NS
Asp	24±4	28±2	33±2	13±1	0.01
Thr	239±33	287±27	296±45	185±28	NS
Ser	139±31	145±19	195±11	101±7	0.05
Asn	123±11	132±5	123±13	73±8	0.01
Glu	73±8	112±15	112±6	69±8	0.05
Gln	710±99	598±53	760±127	388±146	NS
Pro	141±34	220±11	239±8	113±0	0.01
Gly	257±43	367±30	349±4	191±53	0.05
Ala	549±70	946±108	1051±70	290±57	0.001
Val	784±116	959±54	899±51	317±42	0.001
Met	66±2	93±3	96±6	27±10	0.001
Ile	308±27	403±26	362±28	185±26	0.01
Leu	381±36	486±5	432±18	207±26	0.001
Tyr	34±4	76±8	71±2	46±11	0.01
Phe	85±9	162±26	126±6	118±39	NS
GABA	11±7	80±21	137±20	29±16	0.05
Orn	32±6	36±2	39±4	21±3	NS
Lys	238±30	318±16	326±14	160±50	0.05
His	89±13	85±3	135±10	53±8	0.01
Ans	65±5	90±3	89±6	47±25	NS
Arg	116±13	151±5	152±7	74±22	0.05
Essential	2308±226	2948±56	2829±79	1330±196	0.001
Non-essential	2254±239	2838±109	3203±92	1312±257	0.001
Total	5090±514	7164±289	7030±204	3094±437	0.001

Values are expressed as the mean±standard deviation; N=3.
One-way ANOVA significances during the fasting process are indicated; NS, not significant.

Although there is an important gap in our knowledge of amino acid transport systems in fish, a similar picture has been found for the uptake of L-alanine by skate hepatocytes (Ballatori and Boyer, 1988) although, in this case, most of the Na^+-independent uptake was attributed to diffusion. In contrast to both rat and skate hepatocytes, trout hepatocytes show a relatively small incorporation of L-alanine through Na^+-independent systems (or a low diffusion component).

Of the Na^+-independent systems able to carry L-alanine across the hepatocyte membrane, system L appears to be the most widely distributed among higher vertebrate cells (Barker and Ellory, 1990). From the present results and those of Ballatori and Boyer (1988), who reported that 20 mmol l^{-1} leucine inhibited only a small fraction of L-alanine uptake by skate hepatocytes, this system does not seem to play an important role in L-alanine uptake by fish (or at least certain fish) hepatocytes. These authors also provide evidence that, in a Na^+-free medium,

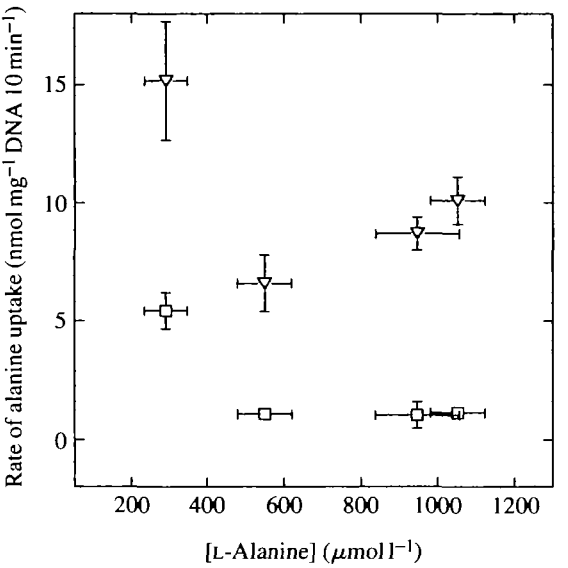

Fig. 6. Relationship between rate of uptake of 150 μmol l^{-1} L-alanine uptake through either the ASC (▽) or the Na^+-independent L-cysteine-sensitive (□) system by trout hepatocytes and plasma L-alanine concentration. Cells from control or fasted animals were incubated for 10 min in Na^+-containing or Na^+-free medium before the uptake was stopped as described in Materials and methods. Each point is the mean of three individual experiments. Bars show the standard deviation.

only L-serine and L-cysteine were able to inhibit L-alanine uptake, but no further comments were made. Such an inhibition pattern, as well as the Na^+-independence, which is similar to that found in the present study, indicates that in the hepatocyte membrane of some fish there is a Na^+-independent cysteine-sensitive system, resembling the asc system found in other cell types and able to carry L-alanine (Fincham *et al.* 1985, 1990; Vadgama and Christensen, 1985). This system has mainly been found in erythroid cells, but it has also been described in rat exocrine pancreatic cells (Norman and Mann, 1987). Although an exact characterisation is lacking, the agreement between the present results and those of Ballatori and Boyer (1988) strongly suggests its presence in fish liver cells.

Previous studies have shown that some amino acids are good gluconeogenic substrates for the liver of starved trout (French *et al.* 1981). There may be an increase in the activity of those enzymes related to this metabolic pathway (Morata *et al.* 1982; Foster and Moon, 1991), but there have been no studies on changes in the amino acid uptake ability of fasted hepatocytes. Our data demonstrate that the increase in gluconeogenesis is accompanied by a rise in the alanine uptake ability of hepatocytes during fasting and that most of the changes observed are related to the Na^+-dependent system.

Two main explanations may account for the changes observed during fasting: hormonal regulation and/or adaptive regulation. Most of the known hormonal

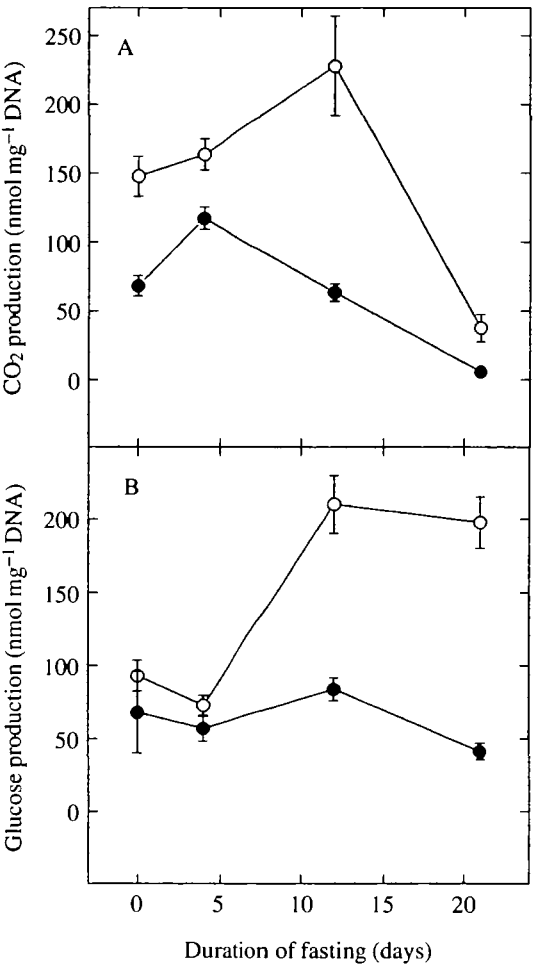

Fig. 7. Effect of fasting time on the CO_2 (A) and glucose (B) production from 150 μmol l^{-1} L-[U-^{14}C]alanine. Cells were incubated for 2 h in either Na^+-containing (○) or K^+-containing (●) medium. Each point is the mean of three individual experiments. Bars show the standard deviation.

modifications of the amino acid transport ability of cells are related to the effects of insulin and glucagon in mammalian cells, both of which increase their expression of system A (Fehlmann *et al.* 1979). However, levels of both hormones (especially insulin) fall during the food deprivation period (Moon *et al.* 1989; Navarro, 1990) and it does not seem likely that either of them alone can act as a regulator of amino acid uptake during fasting in fish. There are no data about the influence of variations in the glucagon (glucagon-like-peptides)/insulin ratio on amino acid uptake by liver cells, but Moon *et al.* (1989) have proposed that small changes in the glucagon-like-peptides/insulin ratio occurring during fasting could explain the

rise in gluconeogenic fluxes, despite the drop in the plasma concentration of both hormones. These authors found a rise in this ratio, but not in the glucagon/insulin ratio. Because there is a positive relationship between L-alanine uptake and glucose production in these cells, which is independent of the duration of fasting, a rise in the glucagon-like-peptides/insulin ratio could be responsible for the increase in the incorporation of some amino acids into the hepatocytes.

Other hormones that could exercise some kind of regulation on L-alanine uptake by trout hepatocytes are the catecholamines. However, P. Canals, M. A. Gallardo and J. Sánchez (unpublished data) have shown that both adrenaline and noradrenaline effect only a small increase in the uptake of L-alanine by isolated trout hepatocytes and that this increase is not clearly related to α-receptors. This may be because of the proposed lack of these receptors in trout hepatocytes (Moon and Mommsen, 1990) or because of a seasonal variation in the expression of these catecholamine receptors, as has been proposed for β-receptors of rainbow trout erythrocytes (Cossins and Kilbey, 1989). Glucocorticoids have also been suggested to increase the amino acid uptake by mammalian liver cells (Shotwell *et al.* 1983). Because of the stressor effect of fasting, corticoid levels may be raised in fasting animals, contributing to a rise in amino acid uptake.

A completely different, but not exclusive, explanation of the results obtained is the possible existence of adaptive regulation in trout liver cells, similar to that found in some cell types of higher vertebrates (Guidotti *et al.* 1978). Cultured human fibroblasts showed an enhancement of system A activity when the cells were incubated under conditions of amino acid shortage (derepression phase) and a decrease in activity when they were exposed to a medium supplied with site-A-reactive amino acids (repression phase) (Gazzola *et al.* 1981). Adaptive regulation has been related to the expression of system A and Gazzola *et al.* (1981) indicate that neither the ASC nor the L system is controlled *via* adaptive regulation. However, although human cells express system A, this system does not seem to be present in trout hepatocytes. For this reason, such an adaptation for the ASC system cannot be excluded in the present case, although no attempts to obtain evidence for other requirements (variations in mRNA, protein synthesis, etc.) of such regulation have been made.

Most of studies on the metabolic changes provoked by fasting consider only the experimental 'end point' and the present results agree with these. However, during the first half of the period considered, CO_2 production rose (with a direct relationship to L-alanine uptake; data not shown). This was, perhaps, due to an increase in L-alanine uptake before oxidative activity was depressed, indicating that the L-alanine oxidation rate is not related exclusively to the availability of the amino acid. When L-alanine incorporation is studied in Na^+-free media, CO_2 production changes in the same way as it does in Na^+-containing media. This indicates that fasting induces changes in hepatocyte oxidative ability, but the actual nature of these changes remains unclear. Foster and Moon (1991) found similar results for enzyme activities and CO_2 production from L-alanine in yellow perch fasted for 7 weeks, although the results obtained by French *et al.* (1981)

differ somewhat. Changes in citrate synthase activity were apparent in the yellow perch, but not in the rainbow trout.

This study has demonstrated that trout hepatocytes take up L-alanine (and probably some other short-chain neutral amino acids) mostly by means of an ASC system and that system A is absent from these cells. These data give rise to some questions about the evolution of changes in the transport ability of cells and, at the same time, further support the ranking of the ASC system as more important than the A system, as stated by Christensen (1990). Moreover, fasting induces an increase in glucose formation from L-alanine through an increase in its uptake. The possibility that this raised uptake could be due to an expression of system A was tested, giving negative results (data not shown). Thus, changes in the uptake could be due to adaptive regulation and/or hormone control. In any case, these data add a new perspective to the control of amino acid transport, because the ASC system has been excluded from these types of regulations.

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