Discrimination of two amino acid transport activities in 4F2 heavy chain-expressing *Xenopus laevis* oocytes

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Expression of the type II membrane proteins of the rbAT/4F2hc family in *Xenopus laevis* oocytes results in the induction of amino acid transport activity. To elucidate the mechanism of action, amino acid transport was investigated in oocytes expressing the surface antigen 4F2hc. Leucine transport was mediated by a Na^+-independent and a Na^+-dependent transport mechanism. Both systems could be further discriminated by their stereochemical constraints. Isoleucine, with a branch at the β-position, shared only the Na^+-independent transport system with leucine.

Both transport systems were sensitive to inhibition by arginine, but only the Na^+-independent system was sensitive to inhibition by 2-aminobicyclo[2,2,1]heptane-2-carboxylic acid. When compared with known transport systems the two transport activities could be described as similar to, but not identical with, mammalian systems $b^{0,+}$ and y^+L. The Na^+-independent $b^{0,+}$-like transport system was found both in rbAT and 4F2hc expressing oocytes, indicating that both proteins act in a similar way.

INTRODUCTION

Mammalian cells possess different amino acid transport systems with overlapping substrate specificity [1–3]. Besides substrate specificity they are usually categorized by Na^+-dependence and some other criteria [4]. To decide if, in a certain cell type, two amino acids are transported by the same or different transport systems, 'ABC-testing' has been used [4,5]. In the AB-part of the test mutual competition between the two amino acids is investigated. In the C-part the inhibitory effect of a third amino acid on the transport of each of the two other amino acids is quantified.

The classification of amino acid transport systems has largely been confirmed by cDNA cloning for about half of the physiologically characterized transport systems. Most of the cDNAs involved in the transport of amino acids encode polytopic membrane proteins with more than eight membrane-spanning domains [3], which constitute the transmembrane pathway for transport. In contrast to these families stands the rbAT/4F2hc (4F2 heavy chain) family of transport-related proteins [6–11]. The hydrophobicity plot of these proteins indicates only one transmembrane helix. A topology with up to four transmembrane helices has been suggested by Mosckovitz et al. [12], based on experiments using site-specific antibodies. Both proteins are associated with additional smaller proteins, which have not been identified so far [13–15]. Expression of rbAT and 4F2hc cRNA in *Xenopus laevis* oocytes results in manyfold stimulation of amino acid transport activities. Overexpression of the rbAT and 4F2hc cDNA in mammalian cells, in contrast, did not result in increased transport activity [8,16] or increased the transport activity only to a small extent [17]. The rbAT protein is thought to be necessary for expression of amino acid transport system $b^{0,+}$ in mammalian cells [18], the 4F2hc protein has been associated with amino acid transport system L [11,17] and y^+L [19–21]. The mechanism of action of these proteins is still unclear. For the rat rbAT protein (also called NBAT) it has been proposed that it may activate more than one transport activity [22,23] when expressed in *X. laevis* oocytes, although in mam-

malian cells only system $b^{0,+}$ seems to be affected when rbAT expression is diminished or lacking [18].

In this study we have used ABC-testing to dissect amino acid transport activities in 4F2hc expressing *X. laevis* oocytes. Two different activities, one resembling mammalian system $b^{0,+}$, the other resembling system y^+L, were identified. This suggests that 4F2hc does not directly participate in membrane transport but increases the activity of several amino acid transporters.

EXPERIMENTAL

Materials

L-[U-^{14}C]Isoleucine (11.4 Gbq/mmol), L-[U-^{14}C]leucine (11.2 GBq/mmol) and L-[U-^{14}C]alanine (5.66 Gbq/mmol) were purchased from Amersham Buchler (Braunschweig, Germany); L-[U-^{14}C]arginine (12.2 GBq/mmol) was bought from NEN Life Science Products (Frankfurt, Germany). The RNA cap structure analogue 7 mG(5′)ppp(5′)G was purchased from New England Biolabs (Schwalbach, Germany). Restriction enzymes, nucleotides and RNA polymerases were from Life Technologies (Eggenstein, Germany) or Boehringer Mannheim (Germany). Collagenase (EC 3.4.24.3; 0.6–0.8 units/mg from *Clostridium histolyticum*) was from Boehringer-Ingelheim (Germany); lots were tested for their suitability for oocyte expression. All other chemicals were of analytical grade and supplied by E. Merck, (Darmstadt, Germany), Roth (Karlsruhe, Germany) or Boehringer Mannheim (Germany).

Expression in *X. laevis* oocytes

For expression studies the rat 4F2 heavy chain (r4F2hc) cDNA, *Not*I–*Sal*I cloned into plasmid pSPORT [11], the human 4F2hc cDNA, cloned into plasmid pSP65 [24] and rabbit rbAT cDNA, cloned into pBluescript SK + [6], were used. For *in vitro* transcription plasmid DNA was linearized with *Not*I (r4F2hc), *Hin*dIII (h4F2hc; human 4F2 heavy chain) or *Kpn*I (rbAT) and

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transcribed *in vitro* with T7 (r4F2hc), T3 (rbAT) or SP6 (h4F2hc) RNA polymerase in the presence of a cap analogue. The protocols supplied with the polymerases were followed, with the exception that all nucleotides and the cap analogue were used at 2-fold concentrations (1 mM) to increase the yield of complementary RNA (cRNA). Template plasmids were removed by digestion with RNase-free DNase. The cRNA was purified by phenol/chloroform extraction followed by precipitation with a half volume of 7.5 M ammonium acetate and two volumes of ethanol to remove unincorporated nucleotides. After determination of the amount of cRNA by measuring absorption at 260 nm the integrity of the transcript was verified by denaturing agarose-gel electrophoresis.

X. laevis females were generously supplied by Dr. P. Hausen (Max-Planck-Institut für Entwicklungsbiologie, Tübingen, Germany). Oocytes (stages V and VI) were isolated as described [25] and allowed to recover overnight. They were micro-injected with either 10 nl of water or 10 nl of cRNA in water at a concentration of 1 μg/μl, by using a microinjection device (Bachofer, Reutlingen, Germany).

Uptake experiments were performed as described before [11]. The concentration of ^{14}C-labelled compounds was taken into account for the adjustment of final substrate concentrations. Uptake of labelled isoleucine is proportional to time up to at least 60 min [11]. No significant metabolism occurs during this timescale [25].

Determination of the ATP content

Oocytes were incubated for 30 min in OR2+ buffer or choline-OR2+, in which NaCl was replaced by choline chloride. Subsequently, the buffer was aspirated and the oocytes were lysed by addition of 100 μl DMSO. The suspension was immediately diluted by addition of 400 μl ice-cold water. These samples could be stored frozen for several days. For the determination of the ATP content, samples were centrifuged for 5 min in a tabletop centrifuge at maximum speed. The supernatant was further diluted 1:10 with water, 100 μl of which were used for the ATP determination. A bioluminescent assay (FL-AA; Sigma, Deisenhofen, Germany) was used to analyse the samples by following the manufacturers' instructions. Accordingly, a 100 μl sample was mixed with 100 μl firefly luciferase extract. The light emission, integrated over a time period of 10 s, was determined after 30, 60 and 90 s by using a BioOrbit 1250 luminometer (Colora, Lorch, Germany). The final DMSO content (1%) did not have any influence on the signal.

Calculations

The transport activity of seven oocytes was used to calculate the mean$\pm$S.D. for each data point in Figures and Tables. All measurements were performed with equal numbers of cRNA and water-injected oocytes. All values represent net uptake rates calculated as: (uptake rate of cRNA-injected oocytes)$-$(uptake rate of water-injected oocytes) = (net uptake rate) using Gauss' law of error propagation for the calculation of the final standard deviations. Each experiment presented was performed at least twice with similar results. Competition experiments were analysed by non-linear regression of data to the equation: $v = V_{max}[S]/\{K_m(1+[I]/K_i)+[S]\}$ where [S] is the substrate concentration, [I] the inhibitor concentration, K_i the inhibition constant and K_m is the Michaelis–Menten constant. For self-inhibition the formula was simplified by setting $K_i = K_m$.

RESULTS

Oocytes expressing the rat 4F2hc cRNA displayed a remarkable difference in their transport activities for leucine and isoleucine (Table 1). Isoleucine uptake, determined at a substrate concentration of 100 μM, was mediated by a Na$^+$-independent transport mechanism, whereas leucine transport, measured at the same concentration, was largely Na$^+$-dependent. At lower substrate concentrations (10 μM) the Na$^+$-dependence of leucine transport was not visible. These results suggested that isoleucine and leucine transport was mediated by different mechanisms. In

Table 1   Na$^+$-dependence of isoleucine and leucine transport in r4F2hc expressing oocytes
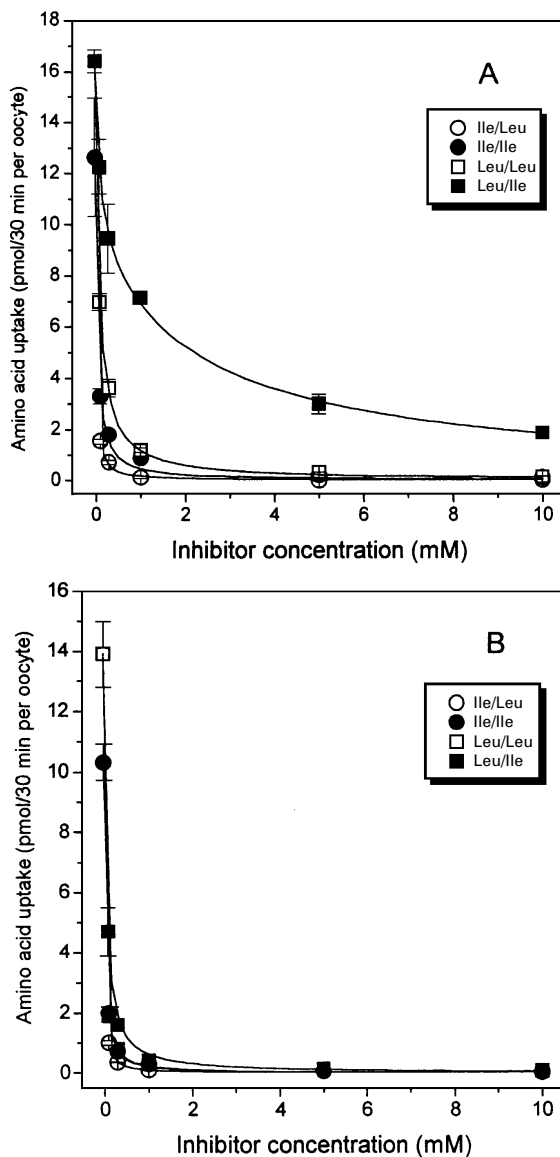
Oocytes were injected with 10 ng r4F2hc cRNA or 10 nl water. After an expression period of 5 days, amino acid transport activity was determined in the presence and absence of Na$^+$ during a 20 min incubation with labelled isoleucine or leucine.

| Substrate | Concentration (μM) | Presence of Na$^+$ | Transport activity in oocytes (pmol/20 min) injected with | | Net uptake (pmol/20 min) |
			r4F2hc cRNA	H$_2$O	
Isoleucine	10	−	8.0 ± 0.6	0.6 ± 0.1	7.4 ± 0.6
	10	+	15.6 ± 1.0	9.1 ± 1.0	6.5 ± 1.4
	100	−	14.6 ± 1.4	1.5 ± 0.2	13.1 ± 1.4
	100	+	35.2 ± 1.0	21.6 ± 1.2	13.6 ± 1.6
Leucine	10	−	9.3 ± 0.5	0.8 ± 0.1	8.5 ± 0.5
	10	+	15.0 ± 1.1	10.3 ± 0.7	4.7 ± 1.3
	100	−	16.1 ± 1.4	1.7 ± 0.2	14.4 ± 1.5
	100	+	63.0 ± 4.3	20.0 ± 1.4	43.0 ± 4.5
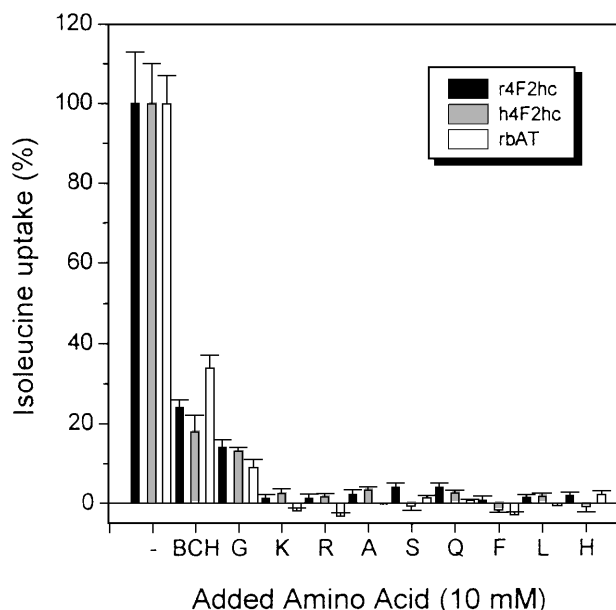
Figure 1   Substrate specificity of Na$^+$-independent neutral amino acid transport in 4F2hc- and rbAT-injected oocytes

Oocytes were injected with 10 ng cRNA derived from r4F2hc (filled bars), h4F2hc (shaded bars) and rbAT (open bars) cDNA clones. After 4 days isoleucine uptake activity was determined at a substrate concentration of 100 μM in the presence of other amino acids. The uninhibited control was set to 100%; the data represent the difference in uptake activity between cRNA- and water-injected oocytes. Amino acids are given in the one-letter code.
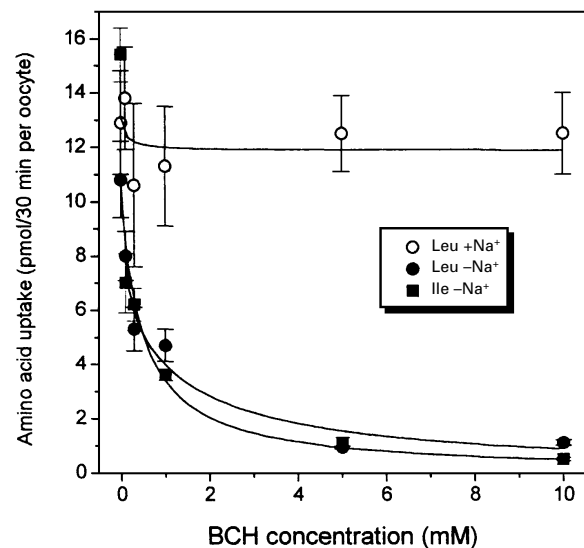
Figure 2   Mutual inhibition of leucine and isoleucine uptake in r4F2hc-expressing oocytes

Oocytes were injected with 10 ng cRNA derived from r4F2hc cDNA. After 4 days, uptake of labelled isoleucine ($\bullet$, $\bigcirc$) and leucine ($\blacksquare$, $\square$) was determined in Na^+-containing buffer (**A**) and Na^+-free transport buffer (**B**) at a substrate concentration of 10 μM in the presence of different concentrations of unlabelled leucine ($\bigcirc$, $\square$) and isoleucine ($\bullet$, $\blacksquare$). Data represent the difference in uptake activity between cRNA- and water-injected oocytes.

oocytes expressing the related rabbit rbAT protein, however, leucine as well as isoleucine transport was mostly Na^+-independent. At a concentration of 100 μM, leucine uptake rates of 154 ± 28 pmol/20 min and 112 ± 13 pmol/20 min were determined in Na^+-containing and Na^+-free transport buffer respectively. For isoleucine uptake, rates of 62 ± 5 pmol/20 min and 40 ± 4 pmol/20 min were determined in the presence and absence of Na^+ respectively. The differences in transport activities observed in 4F2hc-injected oocytes did not result from changes in bioenergetic parameters. For example, the ATP content of oocytes in both the presence and absence of Na^+ was found to be 20 ± 2 nmol ($n = 7$). This corresponded to a concentration of 4 mM, assuming an oocyte volume of 0.5 μl.
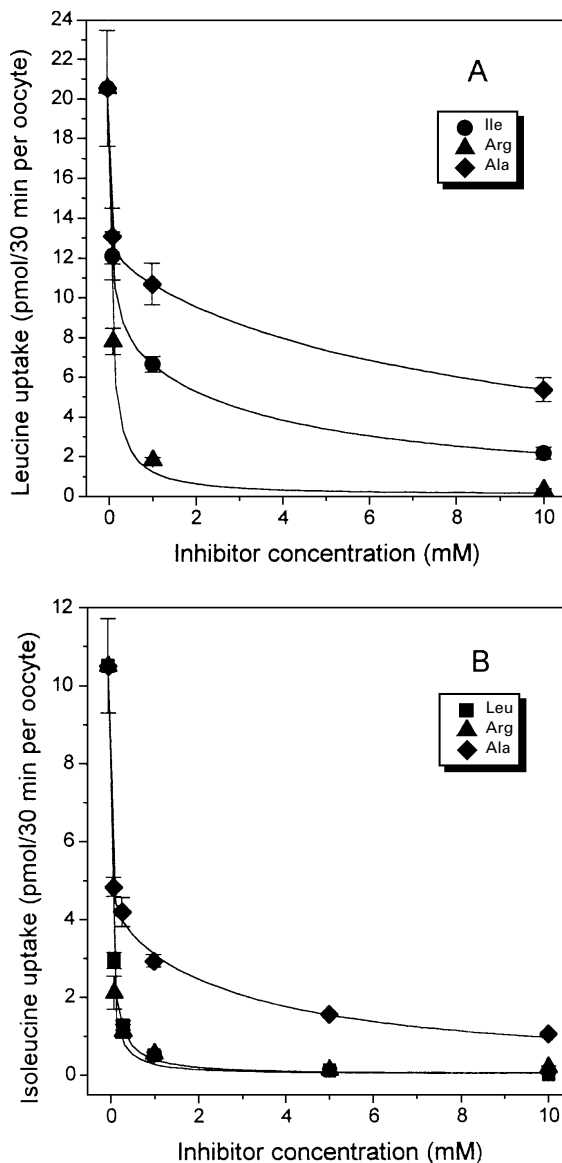
Competition experiments were performed to characterize the

Figure 3   Inhibition of isoleucine and leucine uptake by BCH

Oocytes were injected with 10 ng cRNA derived from r4F2hc cDNA. After 4 days, uptake of labelled isoleucine ($\blacksquare$) and leucine ($\bullet$, $\bigcirc$) was determined in Na^+-containing (open symbols) or Na^+-free buffer (filled symbols) at a substrate concentration of 10 μM in the presence of different concentrations of unlabelled BCH. Data represent the difference in uptake activity between cRNA- and water-injected oocytes.

transport processes in more detail. In oocytes expressing rat 4F2hc, human 4F2hc or rabbit rbAT protein, Na^+-independent isoleucine transport, determined at a substrate concentration of 100 μM, was inhibited by a 100-fold excess of small neutral, large neutral as well as cationic amino acids (Figure 1). The transport process thus resembled the mammalian transport system $b^{0,+}$. To investigate whether more than one transport system was involved in amino acid transport in 4F2hc cRNA-injected oocytes, ABC-testing was used. In the following the term K_{50} value will be used instead of the term K_i value, because more than one kinetic component was detected under certain conditions. It represents the concentration necessary for a 50 % inhibition of the transport activity. In the AB part of the ABC-test mutual competition between leucine and isoleucine was investigated. For uptake to be mediated by a common transport system for both amino acids, the K_{50} value for competition of amino acid A with amino acid B should be equal to the K_{50} value for amino acid A inhibiting its own uptake. Although the latter value is equal to the K_m value (plus the initial substrate concentration) the term 'K_{50}-value for self-inhibition' will be used throughout because the data were analysed as inhibition of uptake of labelled amino acid by unlabelled amino acid. This type of analysis was chosen to facilitate the comparison of K_{50} values and K_m values. Isoleucine uptake (10 μM), determined in Na^+-containing buffer, was strongly inhibited by leucine and isoleucine itself, resulting in K_{50} values of 0.015 mM and 0.039 mM respectively (Figure 2A). Leucine transport, in contrast, was strongly inhibited by leucine itself ($K_{50} = 0.078$ mM), but only weakly by isoleucine ($K_{50} = 0.72$ mM). Thus inhibition of leucine transport by isoleucine is characterized by a K_{50} value which is almost 20 times higher than the value for self-inhibition of isoleucine transport (Figure 2A). The difference between the K_{50} value of the inhibitory effect of leucine on isoleucine transport (0.015 mM) and the corresponding value for self-inhibition of leucine transport (0.078 mM) also appeared to be significant in this experiment. In further experiments, however, closer-lying K_i values of 0.04 mM and 0.03 mM were determined for inhibition of isoleucine uptake by
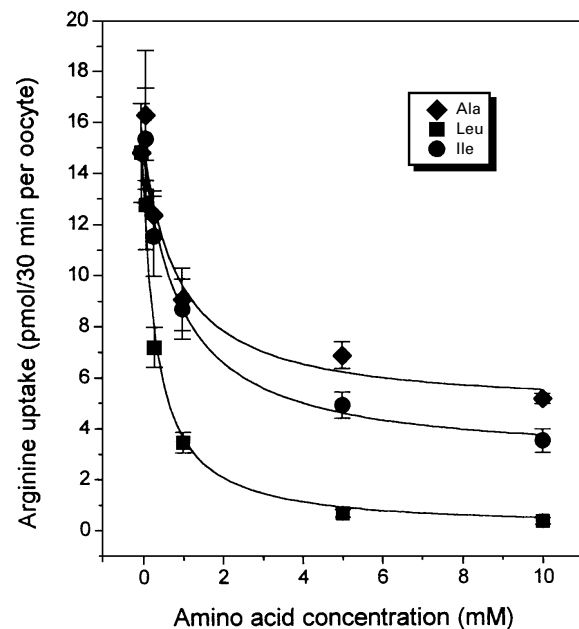
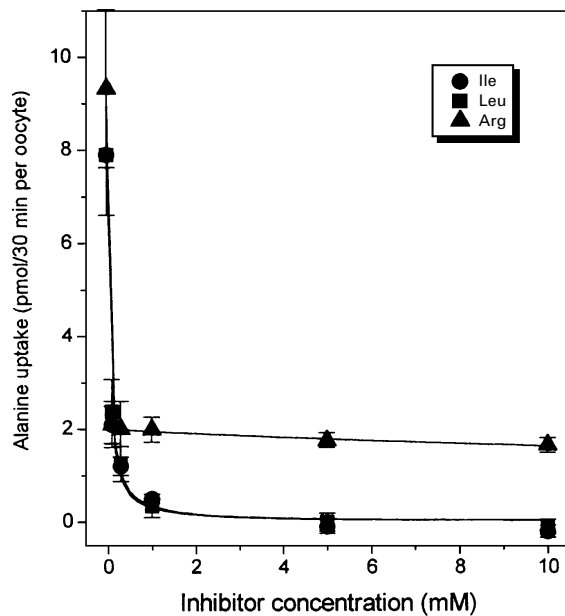**Figure 5   Inhibition of arginine transport by other amino acids**

Oocytes were injected with 10 ng cRNA derived from r4F2hc cDNA. After 4 days, uptake of labelled arginine was determined in Na$^+$-containing buffer at a substrate concentration of 10 μM in the presence of different concentrations of unlabelled isoleucine ($\bullet$), leucine ($\blacksquare$) and alanine ($\blacklozenge$). Data represent the difference in uptake activity between cRNA and water-injected oocytes.

Figure 4   Inhibition of isoleucine and leucine transport by other amino acids

Oocytes were injected with 10 ng cRNA derived from r4F2hc cDNA. After 4 days, uptake of labelled leucine (**A**) and isoleucine (**B**) was determined in Na$^+$-containing buffer at a substrate concentration of 10 μM in the presence of different concentrations of unlabelled leucine ($\blacksquare$), isoleucine ($\bullet$), arginine ($\blacktriangle$) and alanine ($\blacklozenge$). Data represent the difference in uptake activity between cRNA- and water-injected oocytes.

leucine in r4F2hc- and h4F2hc-injected oocytes respectively. The differences between isoleucine and leucine disappeared when the same experiment was repeated in Na$^+$-free transport buffer (Figure 2B). In this case the four inhibition curves were almost superimpossible, resulting in the following K_{50} values: K_{50}(Leu/Leu) = 0.015 mM; K_{50}(Leu/Ile) = 0.021 mM; K_{50}(Ile/Ile) = 0.020 mM; K_{50}(Ile/Leu) = 0.008 mM.

The difference between leucine and isoleucine transport also became apparent when the amino acid analogue 2-amino-bicyclo[2,2,1]heptane-2-carboxylic acid (BCH) was used as inhibitor (Figure 3). In the absence of Na$^+$, leucine as well as isoleucine uptake was sensitive to inhibition by BCH, with K_{50} of about 0.2 mM. In the presence of Na$^+$, only a small part of leucine uptake could be inhibited by BCH (Figure 3). Since

isoleucine transport was completely Na$^+$-independent, no inhibition curve was determined for isoleucine in the presence of Na$^+$. Nevertheless, at a fixed concentration of 10 mM, BCH was able to inhibit the uptake of 0.1 mM isoleucine by 72 ± 12 and 76 ± 2 % in the presence and absence of Na$^+$ respectively.

Despite the strong discrimination between isoleucine and leucine in r4F2hc-expressing oocytes, uptake of both amino acids was sensitive to inhibition by arginine. Arginine completely inhibited isoleucine transport (10 μM) with a K_{50} value of 0.022 mM, and leucine transport (10 μM) with a K_{50} value of 0.056 mM (Figures 4A and 4B). Both isoleucine and leucine uptake were also inhibited by alanine, but the data could not be satisfactorily fitted by one kinetic component. A considerable portion of isoleucine and leucine uptake was inhibited by alanine, with K_{50} values of 0.015 mM and 0.017 mM respectively. The remaining activity was only suppressed by this amino acid at millimolar concentrations (Figure 4A and 4B).

Arginine transport was completely inhibited by leucine, with a K_{50} value of 0.2 mM (Figure 5). All data points could be well fitted by assuming only one kinetic component. Although isoleucine transport could be completely blocked by low concentrations of arginine, the reverse situation revealed only a partial inhibition of arginine transport by isoleucine. The data points could not be fitted by an equation using a single K_{50} value. The best fit was obtained by assuming two components with K_{50} values of 0.54 and 18.4 mM. The extent of inhibition of arginine transport by isoleucine did not depend on the presence or absence of Na$^+$ (see [11]).

Alanine transport was completely inhibited by leucine and isoleucine at sub-millimolar concentrations with K_{50} values similar to the K_{50} values for self-inhibition (Figure 6). The inhibition of alanine transport by arginine was also characterized by a low K_{50} value but was incomplete (Figure 6). The K_{50} values of all experiments are summarized in Table 2.

Figure 6 Inhibition of alanine transport by other amino acids

Oocytes were injected with 10 ng cRNA derived from r4F2hc cDNA. After 4 days, uptake of labelled alanine was determined in Na^+-containing buffer at a substrate concentration of 10 μM in the presence of different concentrations of unlabelled isoleucine (●), leucine (■) and arginine (▲). Data represent the difference in uptake activity between cRNA- and water-injected oocytes.

Table 2 Summary of inhibition constants determined with r4F2hc-expressing oocytes

Inhibition constants were derived by non-linear regression from the data presented in Figures 2–6. All values are given in millimolar. Numbers in parentheses were added when more than one kinetic component was detected.

	Leu	Ile	Arg	Ala
Leu + Na	0.078	0.64(1) 0.08(2)	0.056	4.1(1) 0.017(2)
— Na	0.015	0.021		
Ile + Na	0.03	0.039	0.022	2.2(1) 0.015(2)
— Na	0.008	0.020		
Arg + Na	0.2	18.4(1) 0.54(2)		50.8(1) 0.51(2)
— Na				
Ala + Na	0.028	0.025	37.6(1) 0.001(2)	
— Na				

The 4F2hc cRNA from rat or human tissues displayed identical characteristics when expressed in oocytes. A plot of the relative inhibition of amino acid transport activities determined in r4F2hc-expressing oocytes against the relative inhibition determined in the identical experiments in h4F2hc-expressing oocytes yielded a straight line with a slope of one (Figure 7).

DISCUSSION

The amino acid transport related proteins 4F2hc and rbAT have no sequence similarity to other transport proteins. Topology analysis of these proteins indicates a very unusual structure, suggesting only one transmembrane helix. Despite the information content of the sequence it has nevertheless been suggested that rbAT might constitute a transmembrane channel [8,15].
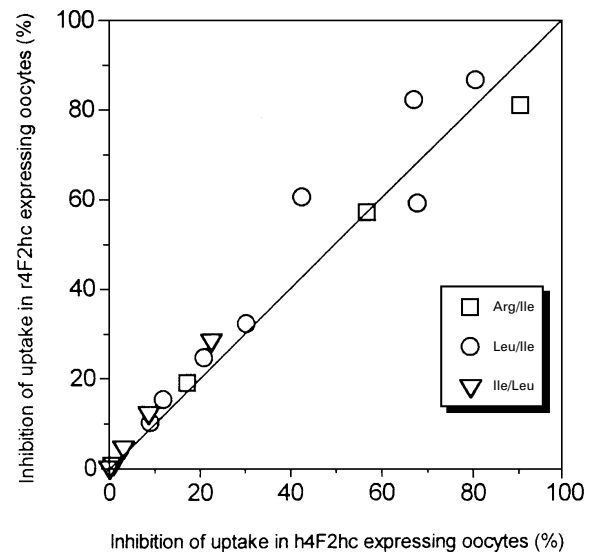
Figure 7 Comparison of the characteristics of r4F2hc and h4F2hc

Oocytes were injected with 10 ng cRNA derived from r4F2hc or h4F2hc cDNA. After 4 days, uptake activity of different amino acids was determined at a substrate concentration of 10 μM in the presence of other amino acids. The uninhibited controls were set to 100%. The relative inhibition of uptake determined in r4F2hc expressing oocytes was plotted against the corresponding data from h4F2hc expressing oocytes. Identical behaviour of both clones is expected to yield data points lying close to a line with a slope of one through the origin. Data were taken from experiments with the following substrate/inhibitor combinations: arginine/isoleucine (□), leucine/isoleucine (○), isoleucine/leucine (▽). Data represent the difference in uptake activity between cRNA and water-injected oocytes.

In this work we have used competition experiments to dissect the transport properties of 4F2hc- and rbAT-injected oocytes. Several observations strongly suggest that two different amino acid transport systems are involved in the uptake of isoleucine and leucine in 4F2hc-injected oocytes. (i) At a substrate concentration of 100 μM, isoleucine transport was always Na^+-independent, whereas leucine transport was predominantly Na^+-dependent. (ii) The inhibition constant of isoleucine for leucine transport was about twenty times higher than the K_{50} value for self-inhibition. When the same parameters were determined in the absence of Na^+, matching values of K_{50} and K_m were obtained. The inhibition constant for leucine on isoleucine transport, in contrast, was nearly identical with the K_{50} value for self-inhibition, regardless of the presence or absence of Na^+. These data showed that the AB-part of the ABC-test was heterogeneous for isoleucine and leucine. The C-part of the ABC-test, in contrast, was relatively homogeneous for both substrates. Arginine strongly inhibited both isoleucine and leucine transport. In the reverse situation, arginine uptake was completely inhibited by leucine at low concentrations, but only partially inhibited by isoleucine, again demonstrating that a second transport system with a high K_{50} value for isoleucine is present in 4F2hc-expressing oocytes. The K_{50} values for both, isoleucine and leucine are, however, considerably higher than the K_m values of both substances. This suggests that a large part of arginine transport in r4F2hc-expressing oocytes may be independent of the characterized leucine and isoleucine transport. It must be pointed out that this indication should be confirmed by a complete ABC-test similar to the one presented here for leucine and isoleucine.

The Na^+-independent transport system has a high affinity for branched-chain amino acids, ranging between 15 and 20 μM. In the presence of Na^+, leucine was transported with a similar K_m value (78 μM). This explains why not all K_i values could be

resolved in Na$^+$-containing buffer, especially when using 100 μM as the lowest inhibitor concentration. The observed variations of K_{50} values from one experiment to another could have been caused by (i) the limited number of concentrations which could be handled in one experiment, (ii) variations in the relative expression levels of Na$^+$-dependent and -independent components, and (iii) incomplete aspiration of the washing buffer. To be on the safe side, 2- or 3-fold differences in K_{50} values were not considered as significant.

The data are most easily interpreted by proposing two transport systems of broad substrate specificity, both recognizing neutral as well as cationic amino acids. One system transports neutral amino acids only in the presence of Na$^+$, the other in the absence of Na$^+$. Leucine is a substrate of both transporters, whereas isoleucine transport is mediated only by the Na$^+$-independent system. The apparent contradiction that a transport system can discriminate between isoleucine and leucine but otherwise has a broad substrate specificity, including arginine and alanine, can be solved by closer examination of the structures of these amino acids. The carbon skeleton of isoleucine branches in the β-position, whereas all other amino acids, apart from valine, do not have branches before the γ-position. This is in good agreement with the strong inhibition of Na$^+$-independent isoleucine and leucine transport by the amino acid analogue BCH, which even has a branch in the α-position. The Na$^+$-dependent leucine transport, in contrast, is not sensitive to inhibition by BCH at all. It has to be pointed out that the mammalian system b$^{0,+}$, as characterized in mouse blastocysts, cannot be inhibited by BCH [26]. It is widely accepted that BCH is a specific inhibitor of system L. Including the transport activity described in the present work, three transport systems are now known that are sensitive to inhibition by BCH, namely system L [27], system B$^{0,+}$ [28] and 4F2hc-activated b$^{0,+}$. The same type of transport activity was also observed in h4F2hc- and rbAT-expressing oocytes. The Na$^+$-dependent transport system detectable in 4F2hc-expressing oocytes does not accept branching before the γ-position. Despite not being transported by the Na$^+$-dependent system, isoleucine is able to inhibit Na$^+$-dependent leucine transport with low affinity. This resembles monocarboxylate transport, where cinnamic acid derivatives are competitive inhibitors of monocarboxylate transport although not being transported [29]. Since arginine transport in 4F2hc-expressing oocytes is Na$^+$-independent [11], the second transport system is similar to the mammalian transport system y$^+$L [30,31], which also does not accept branching in α- or β-positions. These results also explain the differences observed between studies from our laboratory [11,17] and those from other laboratories [7,10,19,20], which were not caused by the differences between r4F2hc and h4F2hc, but by the choice of substrate, e.g. isoleucine or leucine.

In addition, for the related NBAT protein, evidence has been presented for the activation of several transport systems when expressed in oocytes [22,23]. In contrast with 4F2hc, however, NBAT is thought to be necessary for only one transport activity in mammalian cells [18]. The surface antigen 4F2hc has been correlated with the mammalian system y$^+$L in a number of studies [19–21]. In view of the results presented here and in earlier studies [11,17] it seems hasty to associate expression of 4F2hc firmly with system y$^+$L. Nevertheless, we also have to concede that an exclusive association with system L is similarly problematic.

It is tempting to speculate that the 4F2hc protein may have a more general role in the activation of amino acid transport such as the yeast shr3 protein [32]. This protein is a component of the secretory pathway and is required for the localization of amino acid permeases in yeast [32]. The behaviour of proteins of the rbat/4F2hc family also resembles data gained by expression of the IsK protein in oocytes. The IsK protein, having only one transmembrane helix, induces potassium currents in oocytes [33]. Recent studies have shown that the association of this protein with the K(V)LQT1 protein only gives rise to the functional potassium channel characterized in mammalian cells [34].

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