J. Membrane Biol. 153, 37–44 (1996)

The Journal of
Membrane
Biology

The Binding Specificity of Amino Acid Transport System y^+L in Human Erythrocytes is Altered by Monovalent Cations

S. Angelo, C. Irarrázabal, R. Devés
Department of Physiology and Biophysics, Faculty of Medicine, University of Chile, Santiago, Chile

Received: 22 January 1996/Revised: 26 April 1996

Abstract. System y^+L is a broad-scope amino acid transporter which binds and translocates cationic and neutral amino acids. Na^+ replacement with K^+ does not affect lysine transport, but markedly decreases the affinity of the transporter for L-leucine and L-glutamine. This observation suggests that the specificity of system y^+L varies depending on the ionic composition of the medium. Here we have studied the interaction of the carrier with various amino acids in the presence of Na^+, K^+, Li^+ and guanidinium ion. In agreement with the prediction, the specificity of system y^+L was altered by the monovalent cations. In the presence of Na^+, L-leucine was the neutral amino acid that interacted more powerfully. Elongation of the side chain (glycine - L-norleucine) strengthened binding. In contrast, bulkiness at the level of the β carbon was detrimental. In K^+, the carrier behaved as a cationic amino acid specific carrier, interacting weakly with neutral amino acids. Li^+ was found to potentiate neutral amino acid binding and in general the apparent affinities were higher than in Na^+; elongation of the nonpolar side chain made a more important contribution to binding and the carrier was more tolerant towards β carbon substitution. Guanidinium stimulated the interaction of the carrier with neutral amino acids, but the effect was restricted to certain analogues (e.g., L-leucine, L-glutamine, L-methionine). Thus, in the presence of guanidinium, the carrier discriminates sharply among different neutral amino acids. The results suggest that the monovalent cations stabilize different carrier conformations.

Key words: Lysine — Transport — Amino acids — Carrier — Specificity — System y^+L

Correspondence to: R. Devés

Introduction

Early experimental studies of transport showed that neutral and cationic amino acids exhibited mutual competitive interactions and suggested that these analogues could be recognized by the same transporter (Christensen, 1964). Subsequently, the analysis of these and other observations led to the proposal that cationic amino acids were transported by a single entity (system y^+) which was able to interact with neutral amino acids (Christensen & Antonioli, 1969; Thomas, Shao & Christensen, 1971; White, Gazzola & Christensen 1982; Christensen, 1984; White, 1985). However, the apparent affinity of system y^+ for these two type of amino acids was seen to differ significantly. For example, in fibroblasts the K_i for arginine was 40 μM and the K_i for homoserine about 10 mM (White et al., 1982); in reticulocytes the corresponding values were 70 μM and 30 mM (Christensen & Antonioli, 1969). Thus, system y^+ showed a marked preference for positively charged substrates.

Recently three novel ''broad-scope systems'' have been defined on the basis of their substrate specificities and interactions with monovalent cations (Van Winkle, Christensen & Campione, 1985; Van Winkle, Campione & Gorman, 1988; Devés, Chávez & Boyd, 1992). We define as a ''broad-scope system'' one that interacts with neutral and cationic amino acids with equivalent affinity. System $B^{o,+}$, which was identified in blastocysts, is Na-dependent and transports lysine, alanine and the leucine analogue 3-amino-endo-bicyclo[2,2,1]heptane-2-carboxylic acid (BCO) (Van Winkle et al., 1985). System $b^{o,+}$, also described in blastocytes, is Na-independent and accepts lysine and leucine as substrates. In contrast to system $B^{o,+}$, it interacts weakly with smaller amino acids (valine or alanine) and with bulky amino acids that branch at the β carbon (BCO) (Van Winkle et al., 1988). A $b^{o,+}$-like activity was observed in *Xenopus* oocytes

after injection of cRNA derived from homologous clones from rat (D2) and rabbit kidney (rBAT) (Wells & Hediger, 1992; Bertran et al., 1992*b*, Bertran et al., 1994; Palacín, 1994). The corresponding human gene has also been cloned and found to be mutated in the case of cystinuria (Bertran et al., 1993; Calonge et al. 1994). The precise functional relation between rBAT (or D2) and system $b^{o,+}$ has not been elucidated. It has been suggested that these proteins, which have been proposed to contain either one (Bertran et al., 1992*b;* Wells & Hediger, 1992) or four transmembrane domains (Tate, Yan & Udenfriend, 1992; Mosckovitz et al., 1994) may act as modulators or be components of a more complex transporter.

More recently, a third broad-scope transporter (system y^+L) was identified in human erythrocytes (Devés et al., 1992). The specificity of system y^+L closely resembles that of system $b^{o,+}$, but the interaction of the two transporters with monovalent cations differs (Devés, Angelo & Chávez, 1993). System y^+L presents a very striking cation dependence since the strength of substrate binding is differentially affected by monovalent cations, depending on the net charge of the substrate. Thus, whereas the apparent affinity for lysine remains unchanged when Na^+ in the medium is replaced with K^+, the affinity for neutral amino acids (leucine or glutamine) decreases markedly by the same manoeuvre. Interestingly, Li^+ appears to be a good substitute for Na^+. This type of cation dependence was first described by Christensen's group in the early studies with Ehrlich cells (Christensen, Handlogten & Thomas, 1969; Thomas et al., 1971).

The effect of the monovalent ions on the activity of system y^+L, was seen to be restricted to the binding process; the carrier-substrate complexes with leucine or glutamine were shown to translocate at similar rates in the presence of Na^+, K^+, or Li^+ (Angelo & Devés, 1994). System y^+L activity has also been observed in brush border membranes obtained from human placenta (Eleno, Devés, Boyd, 1994; Furesz, Moe & Smith, 1995) and in *Xenopus* oocytes, after expression of a cRNA derived from a clone encoding for the heavy chain of the 4F2 human surface antigen (4F2hc) (Bertran et al., 1992*a;* Wells et al., 1992). This protein shows 30% sequence identity to rBAT or D2. In a recent report, system y^+L was induced in *Xenopus* oocytes after injection of mRNA obtained from human choriocarcinoma cells. Hybrid depletion of 4F2hc blocked induction, in agreement with the proposal that system y^+L and 4F2hc are functionally related (Fei et al., 1995).

The dissimilar effects of monovalent cations on the interaction of system y^+L with lysine and neutral amino acids suggests that when external Na^+ is replaced with K^+, the broad spectrum transporter alters its conformation and converts into a cationic amino acid-selective transporter. With the purpose of testing this hypothesis we have performed a detailed study of the substrate specificity of system y^+L in media of varying ionic compositions. The original observations made for lysine, leucine and glutamine, were extended to a variety of neutral and cationic amino acids, including a series of five amino acids with increasing linear nonpolar side chain (glycine - L-norleucine). Experiments were designed to investigate which are the substrate structural features that contribute to binding in Na^+, K^+, Li^+ and guanidinium medium.

Materials and Methods

Chemicals

Uniformly labeled L-[^{14}C]lysine was purchased from Amersham (approximately 12 GBq $mmol^{-1}$), unlabeled amino acids and NEM from Sigma, and dibutylphthalate from Merck. All other chemicals were of commercial reagent grade.

Preparation of Cells

Human blood was obtained fresh from donors, using heparin as an anticoagulant. The cells were spun and the plasma, buffy coat and upper layer of cells removed by aspiration. The red cells were washed four times with 5 mM sodium phosphate buffer (pH 6.8) containing 150 mM NaCl and 4 mM KCl and incubated to reduce endogenous amino acids (2.5% hematocrit, 0.02% chloramphenicol, 17 hr at 25°C). After this period, the cells were concentrated by centrifugation and packed in the same buffered solution.

Treatment with NEM

Washed erythrocytes (hematocrit 2.5%) were incubated with NEM at 25°C for 15 min in 5 mM sodium phosphate buffer (pH 6.8) plus 150 mM NaCl and 4 mM KCl. The reaction was terminated by addition of 2-mercaptoethanol (10 mM final concentration). The cells were then washed, packed in the corresponding assay buffer as indicated for each experiment, and tested for transport. NEM treated cells were used in all experiments.

Measurement of Entry Rates

Entry was followed by adding packed cells (0.3 ml) at time zero to a solution of L-[^{14}C]lysine (1 μM) in isotonic saline (10% hematocrit, 37°C). The composition of the external medium varied depending on the experiment. All buffers contained 5 mM K^+ phosphate (pH 6.8) and either 154 mM NaCl (Na^+ medium), 154 mM KCl (K^+ medium), 154 mM LiCl (Li^+ medium) or 124 mM KCl plus 30 mM guanidinium chloride (guanidinium medium).

Three samples of the suspension (0.8 ml) were withdrawn at intervals (up to approximately 4 min) and placed in tubes containing dibutylphthalate (0.5 ml). After centrifugation (45 seconds, 11,000 × *g*) the cells sedimented below the organic layer. The aqueous supernatant was taken off by aspiration and the walls of the tubes were thoroughly washed to eliminate contaminating radioactivity. Dibutylphthalate was removed and the cells were precipitated by addition of

5% trichloroacetic acid (0.6 ml). The suspension was centrifuged, and the radioactivity in the supernatant (0.5 ml) determined by scintillation counting.

All determinations were performed in duplicate runs and each rate was estimated from linear regression analysis of six time points.

Determination of the Inhibition Constant (K_i)

Inhibition constants were calculated according to the following equation:

$$v_o/v = 1 + [A]/K_i$$

where, v_o and v are the rates of labeled lysine entry in the absence and presence of unlabeled amino acid respectively, [A] is the concentration of unlabeled amino acid and K_i the inhibition constant. This equation assumes that the external lysine concentration is very low ([lysine] << K_m).

K_i values represent means of the constants calculated for at least two different cell samples. Analogues were added at a concentration giving approximately 50% inhibition.

Determination of the $K_{0.5}$ for Guanidinium on the reduction of the Inhibition Constant for L-leucine (K^+ Medium)

The effect of guanidinium on the inhibition constant for leucine was analyzed according to the following equation:

$$K_{\text{iapp}} = K_{\text{iLeu}}[K] \frac{(1 + [G]/K_G}{(1 + [G]/K_{GL})} \quad (2)$$

where $K_{\text{iLeu}}[K]$ is the leucine inhibition constant measured in K^+ medium and K_{iapp} the leucine inhibition constant at varying guanidinium concentrations. K_G and K_{GL} are the apparent dissociation constants for the interaction of guanidinium with the free carrier and the carrier-leucine complex, respectively. The concentration of guanidinium ion that causes 50% of the effect ($K_{0.5}$) corresponds to K_{GL}.

Results

The strength of interaction of amino acid analogues with system y^+L was estimated by measuring the effect of external unlabeled analogues on the initial rate of lysine entry (cis-inhibition) into NEM-treated cells. This sulfhydryl reagent has been found to inactivate system y^+ selectively, without affecting system y^+L (Devés et al., 1993). Consistently, the inhibition kinetics in treated cells follow the simple behavior expected for a single system, leading to complete inhibition at high analogue concentrations. Experiments were carried out a very low lysine concentration (1 μM) for under these conditions the inhibition constant (K_i) coincides with the I_{50}. Inhibition constants were calculated from the effect of a single concentration of analogue, giving approximately 50% inhibition (Eq. 1).

The K_i values determined for a number of amino acids, in Na^+, K^+ and Li^+ medium, are listed in Table 1. The Table also lists the ratio of the inhibition constants measured in Na^+ or Li^+ vs. the value obtained in K^+; this ratio can be considered as an index of the relative potencies. All neutral amino acids tested were found to interact more strongly with the transporter in the presence of Na^+ than in the presence of K^+; nevertheless, the magnitude of the effect varied for the different analogues. In contrast to the observations with zwitterionic amino acids, the apparent affinities for L-ornithine and L-arginine were unaffected by Na^+ replacement. This is consistent with the Na-independence of lysine transport observed previously (Devés et al., 1993; Angelo & Devés, 1994).

Interestingly, Li^+ was able to strengthen the interaction of the transporter with neutral amino acids. Moreover, the apparent affinity measured in Li^+ medium exceeded that observed in Na^+.

With the purpose of testing whether organic monovalent cations could potentiate neutral amino acid binding, as observed with some inorganic cations, the interaction of system y^+L with guanidinium ion was investigated. As shown in Fig. 1 (insert) guanidinium ion was found to inhibit lysine entry with low affinity (K_i = 140 ± 28 mM). This was not unexpected because guanidinium is a structural analogue of arginine. It was further found that guanidinium could potentiate leucine binding, but interestingly this effect occurred at considerably lower concentrations (Fig. 1); the $K_{0.5}$ was 1.7 ± 0.27 mM. In our analysis (Eq. 2) this constant corresponds to the half-saturation constant for the binding of guanidinium to the leucine-carrier complex.

The effect of the organic cation on the apparent affinity of the transporter towards the other neutral amino acids is shown in Table 2. Guanidinium (30 mM) was found to enhance the interaction of the transporter with neutral amino acids (relative to K^+), but the activation was less important than that observed with Na^+ and Li^+. The largest effect was obtained in the case of L-leucine and L-methionine. The same results were obtained when guanidinium was assayed at 40 mM and the medium osmolarity was kept constant with sucrose instead of K^+ (*not shown*).

The data listed in Tables 1 and 2 have been summarized in Fig. 2. This representation clearly reveals the difference in the substrate specificity of the carrier site, as well as the substrate structural features that contribute to (or hinder) binding, in the presence of the various monovalent cations.

The relation between monovalent ion interaction and substrate length was further investigated by measuring the inhibition constants for a series of neutral amino acids with increasing linear nonpolar side chains, extending from glycine to L-norleucine (Fig. 3). As expected from the observations with natural amino acids, the apparent affinity (log $1/K_i$) increased as the chain became longer. The contribution of each methylene was similar

Table 1. Inhibition constants (K_i) for L-amino acids in Na^+, K^+ and Li^+ medium

Amino acid	Inhibition constant Na^+ K_i[Na] (mM)	Inhibition constant K^+ K_i[K] (mM)	Inhibition constant Li^+ K_i[Li] (mM)	Preference Na^+/K^+ K_i[K]/K_i[Na]	Preference Li^+/K^+ K_i[K]/K_i[Li]
Gly	1.6 ± 0.03 (2)	28.8 ± 0.6 (2)	1.0 ± 0.22 (4)	18	29
Ala	0.62 ± 0.11 (3)	8.1 ± 1.1 (3)	0.13 ± 0.04 (2)	13	62
Val	2.5 ± 0.17 (3)	37.6 ± 0.8 (3)	0.15 ± 0.007 (2)	15	251
Ile	0.25 ± 0.02 (3)	10.1 ± 1.9 (4)	0.020 ± 0.002 (2)	40	505
Leu	0.011 ± 0.007[a] (3)	0.77 ± 0.15 (5)	0.0045 ± 0.0003[a] (2)	70	171
Met	0.021 ± 0.003 (4)	0.86 ± 0.07 (2)	0.0087 ± 0.0002 (2)	41	99
Trp	0.049 ± 0.003 (2)	0.48 ± 0.01 (2)	0.038 ± 0.0009 (2)	10	13
Asn	0.098 ± 0.02 (2)	4.5 ± 0.13 (2)	0.20 ± 0.059 (3)	46	23
Gln	0.027 ± 0.003[a] (4)	1.8 ± 0.39[a] (2)	0.033 ± 0.003[a] (2)	67	55
Ser	0.49 ± 0.08 (2)	4.6 ± 0.6 (2)	0.22 ± 0.025 (2)	9.4	21
Homo-ser	0.072 ± 0.013 (3)	2.6 ± 0.19 (4)	0.058 ± 0.008 (2)	36	45
Arg	3.2 ± 0.38[b] (3)	3.4 ± 0.13 (2)		1.1	
Orn	8.3 ± 1.0 (2)	8.2 ± 0.6 (3)		1.0	

Inhibition constants (K_i) for L-amino acids in Na^+, K^+ and Li^+ medium. K_i values are means of determinations performed with at least two different cell samples and the number of samples used is indicated in parenthesis. Errors represent either SDM ($n > 3$) or range ($n = 2$). Experimental details are given in the Methods.

[a] Taken from Devés et al., 1993.

[b] Taken from Forray et al., 1995.

in Na^+ and K^+ medium. However, in Li^+ medium, the apparent affinity rose more steeply with the number of carbon atoms. The ratio $K_{iGly}/K_{inor\text{-}Leu}$ was 25 in Na^+, 38 in K^+ and 163 in Li^+. Elongation of the apolar side chain had a large effect on the affinity when the medium contained guanidinium ion, the ratio $K_{iGly}/K_{inorLeu}$ was 286.

Discussion

The results reported here show that the specificity of system y^+L is altered depending on the ionic composition of the medium. In fact, the most distinctive attribute of this system, namely its inability to discriminate between cationic and neutral amino acids, is lost when external Na^+ is replaced by K^+. The evidence demonstrates that in the presence of K^+, the carrier functions as a cationic amino acid specific carrier.

The new data extend previous observations regarding the specificity of system y^+L in the presence of Na^+. In this medium, the carrier is shown to bind various cationic and neutral amino acids with comparable strengths. Among the neutral amino acids, L-leucine is the substrate that binds most powerfully, whereas smaller amino acids, such as glycine, alanine and serine, behave as weak competitors. Enlargement of the side chain contributes positively to binding, this effect was seen not only for amino acids with apolar residues, but also for those bearing hydroxyl and amide groups. The trend of affinities for a series of five substrates (glycine - L-norleucine), confirmed the above conclusion (Fig. 3).

Although, in general, affinity increases with overall size, the carrier site can accurately discriminate between side chains of comparable hydrophobicity. The different apparent affinities for leucine and isoleucine suggest that a methyl group substituent attached to the β carbon interferes with binding, this is consistent with the finding that the addition of an extra carbon atom (which in general increases binding) is always detrimental when added as a methyl group at this position.

When the cells were incubated in K^+ medium the

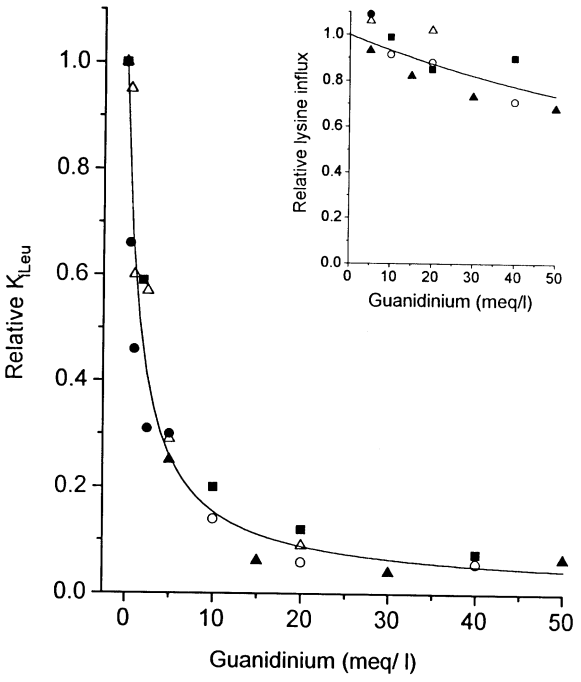

Fig. 1. Effect of varying concentrations of guanidinium ion on the inhibition constant for leucine (K_{Leu}). The isotonicity of the external medium was kept constant with KCl and the solution was buffered with 5 mM K^+ phosphate (pH 6.8); guanidinium was added as the chloride salt. The lysine concentration was 1 μM. The symbols identify five different cell samples. Control rates ranged from 0.02 ± 0.001 to 0.034 ± 0.001. The curve was fitted with Eq. 2 (*see* Materials and Methods). K_{iLeu} in the absence of guanidinium was 0.77 ± 0.15 mM and the concentration of guanidinium producing half the effect was 1.7 ± 0.27 mM. The insert shows the effect of guanidinium ion on lysine entry (in the absence of leucine). The curve was fitted with Eq. 1 and K_i for guanidinium was 140 ± 28 mM.

Table 2. Inhibition constants (K_i) for L-amino acids in guanidinium-medium

Amino acid	Inhibition constant K_i[G] (mM)	Preference Guan/K^+ K_i[K]/K_i[G]
Gly	11.2 ± 0.61 (2)	2.6
Ala	4.7 ± 0.44 (2)	1.7
Val	18.5 ± 0.15 (2)	2.0
Ile	1.7 ± 0.12 (2)	5.9
Leu	0.044 ± 0.008 (5)	18
Met	0.11 ± 0.009 (2)	7.8
Trp	0.20 ± 0.023 (2)	2.4
Asn	6.1 ± 0.31 (2)	0.7
Gln	0.37 ± 0.020 (2)	4.9
Ser	3.5 ± 0.48 (2)	1.3
Homo-ser	1.0 ± 0.04 (2)	2.6

Inhibition constants (K_i) for neutral amino acids in guanidinium medium. K_i values are means of determinations performed with at least two different cell samples and the number of samples used is indicated in parenthesis. Errors represent either SDM ($n > 3$) or range ($n = 2$). Experimental details are given in Materials and Methods.

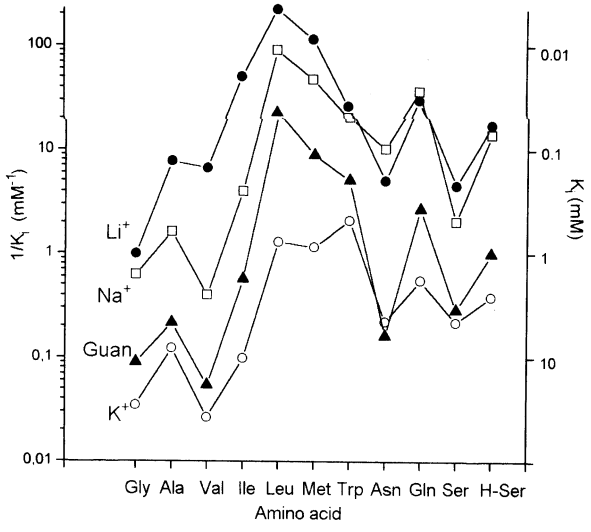

Fig. 2. Structure-affinity relations for the interaction of neutral amino acids with system y^+L in Na^+ (□), K^+ (○), Li^+ (●) and guanidinium (▲) medium. The figure summarizes the data listed in Tables 1 and 2.

apparent affinity for cationic amino acids did not change significantly. In contrast, the strength of interaction for neutral amino acids was found to decrease markedly. As illustrated in Fig. 2, the affinity for neutral amino acids was found to be lower in all cases, but the reduction was more important for those amino acids that are the best substrates in Na^+. Thus, in K^+ medium the transporter binds neutral amino acids with lower affinity and is less able to discriminate among them. Elongation of the side chain increased binding, as had been observed for Na^+, and further, the magnitude of the contribution made by successive additions of methylenes was similar in the two media (Fig. 3). The most important consequence of Na^+ substitution with K^+ is the decrease in the affinity for substrates lacking a net positive charge.

Previous experiments had shown that Li^+ could replace Na^+ in the interaction of the carrier with glutamine and leucine (Angelo & Devés, 1994). In agreement with these observations, Li^+ was found to potentiate neutral amino acid binding in all cases and, moreover, the apparent affinity was seen to be higher than that estimated in Na^+. The difference was most important for those analogues bearing a methyl group substituent at the β carbon position which are less discriminated by the carrier in Li^+. It can be concluded that in the presence of Li^+ the binding strength is higher, but the selectivity is less marked than in the presence of Na^+. Once more, lengthening of the side chain potentiated binding (Fig. 3); the addition of each methylene group made a nearly constant contribution to the binding energy. The standard free energy of binding was approximately −600 cal/mol. In contrast to the results obtained in Na^+ and K^+

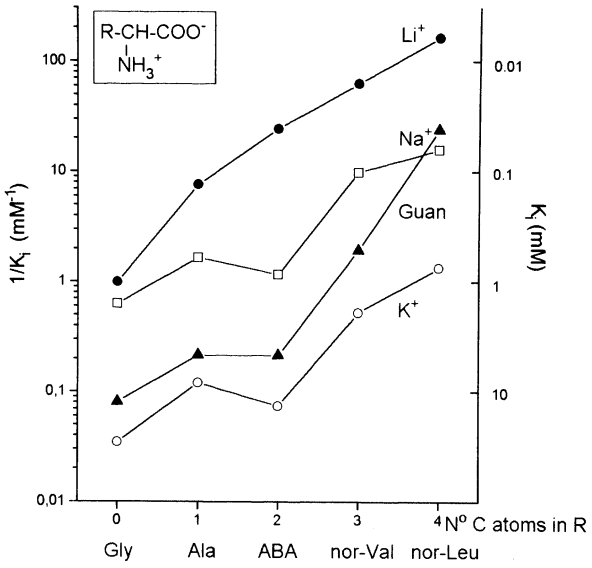

Fig. 3. Apparent affinity (log $1/K_i$) for a series of amino acids with increasing nonpolar linear side chain in Na^+ (□), K^+ (○), Li^+ (●) and guanidinium (▲) medium. Data represent mean values obtained with 2 to 6 different cell samples.

medium, the apparent affinity for aminobutyric acid exceeded that for alanine. It is interesting to notice that the alanine-aminobutyric acid transition involves structural changes at the β carbon position which, as pointed out earlier, appears to interact differently with the carrier in Li^+ medium.

With the aim of investigating whether organic monovalent cations could strengthen neutral amino acid binding, the effect of guanidinium ion was tested. This ion was chosen because of its structural similarity to arginine. Guanidinium ion was found to potentiate the binding of some neutral amino acids (relative to K^+) and moreover, very low guanidinium ion concentrations (which do not interfere with lysine binding) were sufficient to produce the effect (Fig. 1). In contrast to the observations with Na^+ or Li^+, the stimulatory effect was substantial only for leucine, methionine, glutamine, isoleucine and norleucine. Thus, in the presence of guanidinium, system y^+L is very selective towards some neutral amino acids. The ability of guanidinium ion to affect the binding of some neutral amino acids, but not others, appears to be related to substrate size (Fig. 3). In the presence of guanidinium, the carrier was also able to discriminate against β carbon substitution.

The observations described above show that the substrate specificity of system y^+L changes significantly in the different ionic environments and suggest that the distinct monovalent cations are able to stabilize different carrier conformations.

The problem of the structural selectivity of cationic amino acid transporters and its modulation by monovalent cations was addressed years ago in an investigation of Ehrlich ascites tumor cells and rabbit reticulocytes (Thomas et al., 1971). The selectivity patterns in these two cell types were found to differ, suggesting that distinct carriers participate in the transport of neutral amino acids across their membranes (although this statement was not explicitly made). The influx of arginine (50 μM) into reticulocytes was inhibited weakly by neutral amino acids and the inhibition did not vary substantially in the presence of various inorganic cations. For example, norvaline (added at a concentration of 50 mM) reduced arginine influx by 40% in the presence of Na^+ and 55% in the presence of K^+ or Li^+. Hydroxy-norvaline inhibited transport by 45% in the presence of Na^+, 55% in Li^+ and 38% in K^+. Monovalent inorganic cations did not affect arginine influx in the absence of neutral amino acids.

A different behavior was observed with Ehrlich ascites cells and, although a direct comparison with the present results is not possible (because the earlier data are expressed in qualitative terms), the observations resemble the results reported here for system y^+L. First, hydroxynorvaline (concentration not stated) inhibited homoarginine influx by 75%–80%, both in Na^+ and Li^+ medium and only by 10% in the presence of K^+. Second, the apparent affinities for a series of substrates with increasing chain length (alanine–norleucine) were found to increase in the presence of Li^+. Third, the corresponding apparent affinities in the presence of Na^+ were lower. It is likely therefore that, an important fraction of the cationic amino acid uptake in Ehrlich cells occurs through a transporter analogous to system y^+L. Reticulocytes, on the other hand, do not show any evidence of a y^+L-like activity and the observations in this case reflect the activity of a cationic amino acid specific system interacting with low affinity with neutral amino acids. It is interesting to notice that cationic amino acid transporter mCAT-1, which was expressed in *Xenopus* oocytes by injecting the corresponding cRNA, was able to transport lysine, arginine and ornithine stereospecifically (with affinities corresponding to system y^+), but exhibited either weak (Wang et al., 1991) or no interaction (Kim et al., 1991) with neutral amino acids. This is consistent with the observation in human erythrocytes and placenta where two cationic amino acids transporters were discriminated on the basis of their interaction with neutral amino acids (Devés et al., 1992, 1993; Eleno et al., 1994).

In the framework of their pioneer studies, Christensen and coworkers proposed an explanation for the differential effects of monovalent cations on the binding of neutral and cationic amino acids in Ehrlich cells, in particular the loss of binding strength of neutral amino acids when Na^+ was replaced by K^+ (Christensen, Handlogten & Thomas, 1969; Christensen, 1984). Their hypothesis stated that ''certain neutral amino acids and so-

dium react cooperatively with the transport system for cationic amino acids in various cells, the sodium ion hypothetically occupying the position otherwise taken by the distal cationic group of the basic amino acid (Thomas et al., 1971).'' They further proposed that the amino acid and the cation interacted directly to form a species which was highly complementary to the receptor site.

Although in the present study we do not deal with the mechanism of activation directly, we believe that our results are more compatible with a model in which the cations affect the binding of neutral amino acids by altering the conformation of the substrate site, and not by direct interaction with the amino acid. Large stimulation by Na^+ and Li^+ was seen for amino acids with nonpolar chains, such as isoleucine and leucine, where bridging between the cation and amino acid side-chain cannot be conceived.

Whether the cations affect the carrier conformation by binding inside or outside the substrate site cannot be decided on the basis of the present results. The very weak inhibition of lysine influx by guanidinium (in the absence of neutral amino acid; K_i, 140 mM), and the similarity of the rates of lysine transport measured in the presence of Na^+, Li^+ and K^+, appear to argue against the model that places the cation within the substrate site; according to this model the ''activators'' should compete with lysine. However, since the interaction of the cation and the neutral amino acid is cooperative, this possibility cannot be rule out until a detailed study of the cation concentration dependence of the stimulation of neutral amino acid binding is carried out.

We are grateful to FONDECYT-Chile (1940561) for financial assistance.

References

Angelo, S., Devés, R. 1994. Amino acid transport system y^+L of human erythrocytes: specificity and cation dependence of the translocation step. *J. Membrane Biol.* **141:**183–192

Bertran, J., Magagnin, S., Werner, A., Markovich, D., Biber, J., Testar, X., Zorzano, A., Kühn, L., Palacin, M., Murer, H. 1992a. Stimulation of system y^+-like amino acid transport by the heavy chain of human 4F2 surface antigen in *Xenopus laevis* oocytes. *Proc. Natl. Acad. Sci.* **89:**5606–5610

Bertran J., Testar, X., Zorzano, A., Palacín, M. 1994. A new age for mammalian plasma membrane amino acid transporters. *Cell Physiol. Biochem.* **4:**217–241

Bertran, J., Werner, A., Chillaron, J., Nunes, V., Biber, J., Testar, X., Zorzano, A., Estivill, X., Murer, H., Palacín, M. 1993. Expression cloning of a human renal cDNA that induces high affinity transport of L-cystine shared with dibasic amino acids in *Xenopus* oocytes. *J. Biol. Chem.* **268:**14842–14849

Bertran, J., Werner, A., Moore, M., Stange, G., Markovich, D., Biber J., Testar, X., Zorzano, A., Palacin, M., Murer, H. 1992*b*. Expression cloning of a cDNA from rabbit kidney cortex that induces a single transport system for cystine and dibasic and neutral amino acids. *Proc. Natl. Acad. Sci. USA* **89:**5601–5605

Calonge, M.J., Gasparini, P., Chillaron, J., Galluci, M., Rousaud, F., Zelante, L., Testar, J., Dallapiccola, B., Di Silverio, F., Barcelo, P., Estivill, X., Zorzano, A., Nunes, V. and Palacin, M. 1994. Cystinuria caused by mutations in rBAT, a gene involved in the transport of cystine. *Nature Genetics* **6:**420–425

Christensen, H.N. 1964. A transport system serving for mono- and diamino acids. *Proc. Natl. Acad. Sci. USA* **51:**337–344

Christensen, H.N. 1984. Organic ion transport during seven decades. The amino acids. *Biochimica et Biophysica Acta* **779:**255–269

Christensen, H.N., Antonioli, J.A. 1969. Cationic amino acid transport in rabbit reticulocytes. *J. Biol. Chem.* **244:**1497–1504

Christensen, H.N., Handlogten, M., Thomas, E. 1969. Na+-facilitated reactions of neutral amino acids with a cationic amino acid transport system. *Proc. Natl. Acad. Sci. USA* **63:**948–955

Devés, R., Angelo, S., Chávez, P. 1993. N-ethylmaleimide discriminates between two lysine transport systems in human erythrocytes. *J. Physiol.* **468:**753–766

Devés, R., Chávez, P., Boyd, C.A.R. 1992. Identification of a new transport system (y^+L) in human erythrocytes that recognize lysine and leucine with high affinity. *J. Physiol.* **454:**491–501

Eleno, N., Devés, R., Boyd, C.A.R. 1994. Membrane potential dependence of the kinetics of cationic amino acid transport systems in human placenta. *J. Physiol.* **479:**291–300

Fei, Y., Prasad, P.P., Leibach, F.H., Ganapathy, V. 1995. The amino acid transport system y^+L induced in *Xenopus laevis* oocytes by human choriocarcinoma cell (JAR) mRNA is functionally related to the heavy chain of the 4F2 cell surface antigen. *Biochemistry* **34:**8744–8751

Forray, M.I., Angelo, S., Devés, R. 1995. Transport of nitric oxide synthase inhibitors through cationic amino acid carriers in human erythrocytes. *Biochem. Pharmacol.* **50:**1963–1968

Furesz, T.C., Moe, A.J., Smith, C.H. 1995. Lysine uptake by human placental microvillus membrane: comparison of system y^+ with basal membrane. *Am. J. Physiol.* **268:**C755–C761

Kim, J.W., Closs, E.I., Albritton, L.M. and Cunningham, J.M. 1991. Transport of cationic amino acids by the mouse ecotropic retorvirus receptor. *Nature* **352:**725–728

Mosckovitz, R., Udenfriend, S., Felix, A., Heimer and Tate, S.S. 1994. Membrane topology of the rat kidney neutral and basic amino acid transporter. *FASEB Journal* **8:**1069–1074

Palacin, M. 1994. A new family of proteins (rBAT and 4F2hc) involved in cationic and zwitterionic amino acid transport: a tale of two proteins in search of a transport function. *J. Exp. Biol.* **196:**123–137

Tate, S.S., Yan, N., Udenfriend, S. 1992. Expression cloning of a Na^+-independent neutral amino acid transporter from rat kidney. *Proc. Natl. Acad. Sci. USA* **89:**1–5

Thomas, E.L., Shao, T.C., Christensen, H.N. 1971. Structural selectivity in interaction of neutral amino acids and alkali metal ions with a cationic amino acid transport system. *J. Biol. Chem.* **246:**1677–1681

Van Winkle, L.J., Campione, A.L., Gorman, J.M. 1988. Na^+-independent transport of basic and zwitterionic amino acids in mouse blastocytes by a shared system and by processes which distinguish between these substances. *J. Biol. Chem.* **263:**3150–3163

Van Winkle, L.J., Christensen, H.N. and Campione, A.L. 1985. Na^+-dependent transport of basic, zwitterionic, and bicyclic amino acids by a broad-scope system in mouse blastocysts. *J. Biol. Chem.* **260:**12118–12123

Wang, H., Kavanaugh, M.P., North, R.A. and Kabat, D. 1991. Cell surface receptor for ecotropic murine retroviruses is a basic amino acid transporter. *Nature* **352:**729–731

Wells, R.G., Hediger, M. 1992. Cloning of a rat kidney cDNA that stimulates dibasic and neutral amino acid transport and has sequence similarity to glucosidases. *Proc. Natl. Acad. Sci. USA* **89:**5596–5600

Wells, R.G., Lee, W., Kanai, Y., Leiden, J.M., Hediger, M. 1992. The F42 antigen heavy chain induces uptake of neutral and dibasic amino acids in *Xenopus* oocytes. *J. Biol. Chem.* **267:**15285–15288

White, M.F. 1985. The transport of cationic amino acids across the plasma membrane of mammalian cells. *Biochem. Biophys. Acta* **822:**355–374

White, M.F., Gazzola, G.C., Christensen, H.N. 1982. Cationic amino acid transport into cultured animal cells. Influx into cultured fibroblasts. *J. Biol. Chem.* **257:**4443–4457