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System y^+L-like Activities Account for High and Low Amino-Acid Transport Phenotypes in Chicken Erythrocytes

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Abstract. The functional properties of the transport of lysine across the chicken erythrocyte membrane were investigated. The animal population studied (male Leghorn chickens, 6–14 weeks old) was found to consist of two groups presenting either low (LT, 19 individuals) or high transport rates (HT, 20 individuals). The rates of influx in the two groups, measured at a concentration of L-lysine of 1 μM, differed by a factor of 34. The transport activities observed in LT and HT erythrocytes were compatible with the general features of system y^+L, but showed some differences in specificity. The transporter in the LT group was found to bind L-lysine, L-leucine, L-methionine and L-glutamine with high affinity, in the presence of sodium, as described for system y^+L in human erythrocytes. The activity present in HT erythrocytes exhibited a much lower affinity for L-leucine, but was able to interact strongly with L-glutamine and L-methionine. The specificity pattern of the HT transporter, has not been described in other cell types. In other respects, the properties of the two systems were similar. Sodium replacement with potassium, drastically reduced the affinity for L-leucine, without affecting lysine transport. Both transporters function as tightly coupled exchangers, are inactivated by *p*-chloromercuribenzene sulfonate and resistant to N-ethylmaleimide. These findings explain previous results obtained in selective breeding experiments of chicken with high and low amino-acid transport activity.

Key Words: Erythrocytes — Transport — Amino acid — Lysine — Kinetic — Chicken.

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Introduction

Important individual differences in the capacity to transport amino acids were noticed in early studies with chicken erythrocytes (Lerner et al., 1978). To investigate the origin of the heterogeneity, animals were selected for either low or high transport rates and the amino-acid transport properties of the progeny were analyzed in three consecutive generations (Somes, Smagula & Lerner, 1981). It was observed that those animals which exhibited high leucine transport rates, also presented a higher ability to transport lysine. This finding was unexpected, because leucine and lysine were then assumed to use separate transporters (Eavenson & Christensen, 1967; Christensen & Antonioli, 1969). Further investigation was conducted with the aim of disclosing a "common metabolic component" that might be responsible for this finding. Intracellular Na^+ and K^+ concentrations, cell fragility and reticulocyte count were found to be similar in the high and low transport lines and the molecular bases for this phenotypic difference remained unexplained (Somes et al., 1981; Lerner et al., 1982).

Broad-spectrum transporters, able to transport neutral and cationic amino acids, have been described in recent years (for reviews *see* Devés & Boyd, 1998, 2000; Palacín et al., 1998; Verrey et al., 1999, 2000). One of these transporters, system y^+L, has been shown to be present in human erythrocytes, where it accounts for approximately one half of the lysine flux at low concentrations (Devés, Chávez & Boyd, 1992). System y^+L has been referred to as the broad-scope, cation-modulated amino-acid transporter, because its specificity varies depending on the ionic composition of the medium (Devés, Angelo & Rojas, 1998).

In light of these findings, it is reasonable to assume that the behavior observed in the selective breeding ex-

periments was indicative of the presence of a transporter able to interact with lysine and leucine. The aim of the present study was to test this hypothesis.

Lysine transport across the cell membrane of chicken erythrocytes was characterized with respect to specificity, ion dependence, transport mechanism and sensitivity towards sulfhydryl reagents. The results show that two different lysine transport phenotypes exist in the population studied, exhibiting either high (HT) or low lysine transport rates (LT). The properties of lysine transport in the two groups correspond to the general definition of system y^+L. The activity observed in LT erythrocytes is very similar to the activity described in human erythrocytes, but the activity present in the HT erythrocytes exhibits a considerably reduced affinity towards leucine. The specificity pattern of the HT transporter has not been described for other experimental systems. It is shown that the kinetic properties of these two activities explain the polymorphism described by Somes et al., (1981).

Materials and Methods

Materials

L-[^{14}C]-Lysine (approximately 300 mCi/mmol) was purchased from ARC (American Radiolabeled Chemicals) and unlabeled amino acids, chloramphenicol, N-ethylmaleimide (NEM), and *p*-chloromercuribenezene sulfonate (pCMBS) from Sigma (St. Louis, MO). All other chemicals were of commercial reagent grade. Healthy male Leghorn chickens, 6 to 14 weeks old, were obtained from a commercial farm and fed on a regular poultry diet.

Preparation of Cells

Fresh blood was obtained by cardiac puncture (under ketamine anesthesia) or from the brachial wing vein using heparin as an anticoagulant. The blood was spun and the plasma, buffy coat and upper layer of cells (including white cells and platelets) were removed by aspiration. Erythrocytes were then washed with "Na^+ medium" containing (in mM): 133 NaCl, 3 KCl, 1.2 $MgSO_4$, 3 NaH_2PO_4, and 6 Na_2HPO_4 (pH 7.4) and packed by centrifugation. To reduce the endogenous amino-acid concentration, cells were incubated as a 2.5% hematocrit suspension in the same buffer plus chloramphenicol (0.02%) during 3 hr at 25°C. Following incubation, cells were washed and packed in an isotonic medium (the composition depending on the particular experiment). Other isotonic solutions used were (in mM): 1.2 $MgSO_4$, 3 KH_2PO_4, 6 K_2HPO_4 plus 133 KCl (K^+ medium) or 133 choline chloride (choline medium). Unless indicated, experiments were performed in Na^+ medium.

Measurement of Entry Rates

Entry was followed as previously described (Devés et al., 1992). Briefly, packed cells were added to a solution containing L-[^{14}C]lysine in isotonic saline (10% hematocrit, 39°C). The composition of the external medium varied depending on the experiment. Three samples of the suspension were withdrawn at intervals (up to approximately 15 min with LT erythrocytes and up to 4 min with HT erythrocytes) and placed in tubes containing dibutylphthalate. After centrifugation, the cells sedimented below the organic layer. The aqueous supernatant was taken off by aspiration and the walls of the tube were thoroughly washed. Following the aspiration of dibutylphthalate, the cells were disrupted by addition of 5% trichloroacetic acid. The suspension was spun (1 min at 11,000 × g) and the radioactivity in the supernatant determined by scintillation counting. All determinations were done in duplicate runs.

Initial uptake rates were calculated by linear regression from the relationship of the intracellular radioactivity and time. All determinations were performed in duplicate runs, and therefore initial rates were generally estimated from six time points. When deviation from initial rates was evident the measurements performed at longer times were not considered in the analysis. Mediated transport was estimated by subtraction of the transport rates measured in presence of a saturating concentration (2 mM) of unlabeled lysine from the total flux.

Measurement of Exit Rates

Exit was followed as previously described (Angelo & Devés, 1994). Washed cells were incubated in Na^+ medium (50% hematocrit), containing L-[^{14}C]-lysine (10 μM) and chloramphenicol (0.02%) and incubated at 37°C for 2 hours (LT erythrocytes) or 30 min (HT erythrocytes). After this period, the cells were separated from the external medium by centrifugation and repeated washes with ice-cold buffer. Aliquots of loaded cells were added to the assay medium (10% hematocrit, 39°C) and three samples were withdrawn at intervals and rapidly centrifuged in tubes containing dibutylphthalate. The cells sedimented below the organic layer and the radioactivity in the supernatant was determined by scintillation counting. All determinations were done in duplicate runs.

Treatment of the Cells with Sulfhydryl Reagents

Washed cells were resuspended in Na^+ medium with NEM (0.2 mM, 2.5% hematocrit) or pCMBS (0.075 mM, 5% hematocrit) and incubated at 25°C for 15 min or 10 min, respectively. Reaction with NEM was terminated by addition of 2-mercaptoethanol (final concentration, 10 mM). Treatment with pCMBS was stopped by centrifugation in cold buffer.

Determination of the Half-saturation Constants (K_i)

Inhibition by unlabeled amino acids was analyzed according to the following equation:

$$v_0/v = 1 + [A]/K_{iA}$$

where v_0 and v are the rates of entry of labeled lysine in the absence or presence of unlabeled amino acids, respectively. $[A]$ is the concentration of unlabeled amino acid and K_{iA} the half-saturation (or inhibition) constant for analogue *A*. It is assumed that the external lysine concentration is very low ($[\text{lysine}] \ll K_m$). Inhibition constants were calculated by nonlinear regression analysis of the data. This is a valid assumption in the present study because the substrate concentration used was 1 μM.

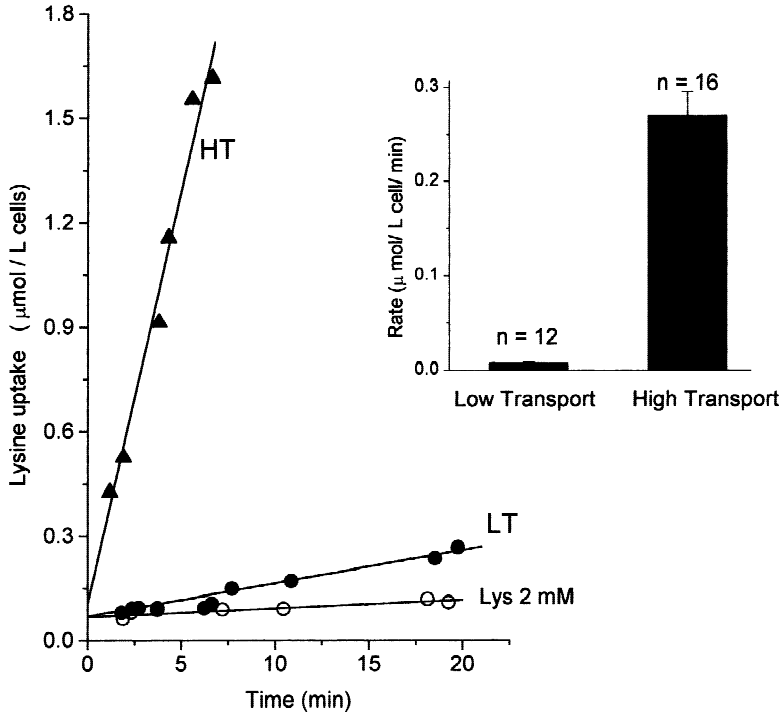

Fig. 1. [^{14}C]-L-lysine uptake in chicken erythrocytes exhibiting low transport (LT) or high transport phenotype (HT). The concentration of lysine was 1 μM. Rates of lysine uptake in cells from two individuals, representative of each one of these groups, were (μmol/l cells/min): 0.0095 ± 0.00048 (LT) and 0.237 ± 0.019 (HT). The effect of unlabeled L-lysine (2 mM) on the uptake in LT erythrocytes is also shown. The insert shows the average rate of mediated uptake in the HT (n = 16) and LT (n = 12) groups.

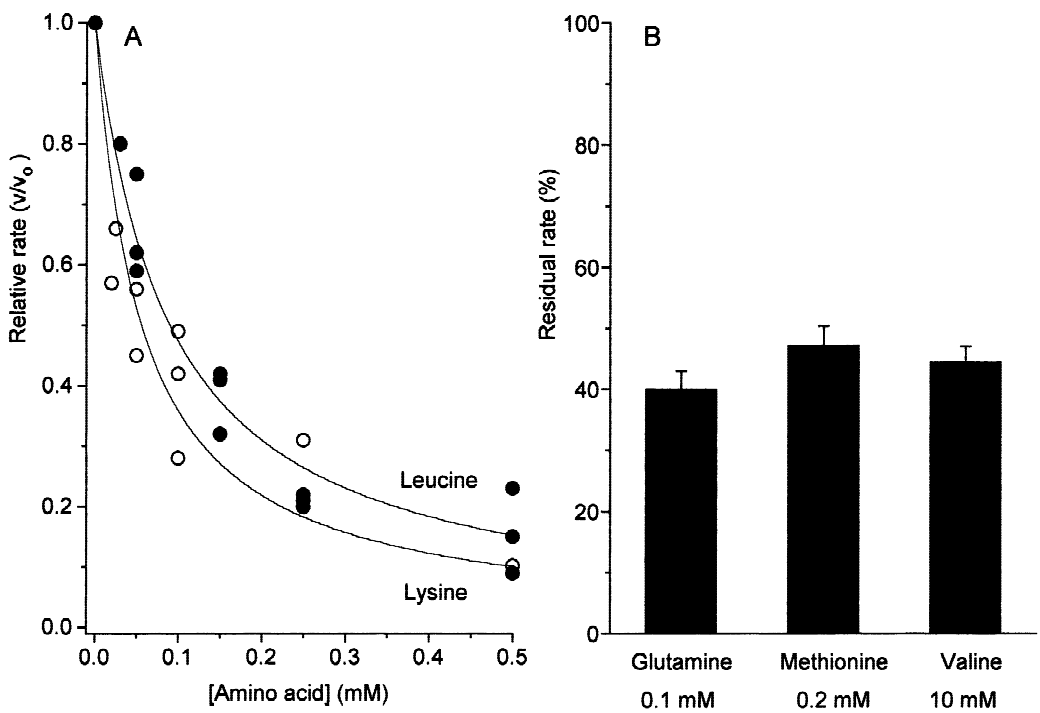

Fig. 2. Specificity of the lysine transporter in LT erythrocytes. (*A*) Relative rates of lysine entry in the presence of varying concentrations of unlabeled lysine (○) or leucine (●). The curves represent the best fit assuming that a single transporter is responsible for the flux (*see* Materials and Methods). (*B*) Relative rates of entry in the presence of L-glutamine, L-methionine and L-valine. L-[^{14}C]lysine concentration was 1 μM. The mean of the control flux (n = 10) was 0.0070 ± 0.0004 μmol/l cell/min. The calculated half-saturation constants are listed in the Table.

Results

The uptake of [^{14}C]-L-lysine (1 μM) into chicken erythrocytes, obtained from two different individuals, is shown in Fig. 1. The rates of transport are seen to differ markedly and have been labeled HT (high transport rate) and LT (low transport rate). These individuals are representative of either one of the two phenotypes described in this study. Entry rates (± SEM) measured in the presence of Na^+ in 28 animals, classified as LT (0.0079 ± 0.0007 μmol/l cell/min, $n = 12$) or HT (0.27 ± 0.025 μmol/l cell/min, $n = 16$) are shown in the insert. The ratio of the average rates of the two populations (HT/LT) was 34. Of all animals used in the study, 19 exhibited low transport rates and 20 presented a high rate of transport.

The specificity of the lysine transporters was inferred from the effect of unlabeled amino acids on the initial influx of radioactive lysine (1 μM). Figure 2 shows the results obtained with the LT group. Relative rates of lysine entry were plotted against the concentration of unlabeled lysine or leucine in the external medium (panel *A*) and the inhibition pattern was analyzed according to the equation in Materials and Methods. The curves represent the best fit assuming that one transporter is responsible for the flux. The half-saturation constants for L-lysine and L-leucine were: K_{mLYS}(LT) = 56.0 ± 7.1 μM and K_{iLEU}(LT) = 90.3 ± 7.1 μM, respectively. The effect of a single concentration of L-glutamine, L-methionine and L-valine is shown in panel *B*. Glutamine and methionine were also found to bind strongly, but the interaction with valine was weak. Saturating concentrations of glutamine and methionine caused complete inhibition (*not shown*). There was no indication of the presence of a transporter showing strict specificity towards cationic amino acids, as it is the case in human erythrocytes, where system y^+ accounts for 50% of the flux at low substrate concentration (Devés, Angelo and Chávez, 1993; Rojas and Devés, 1999).

The specificity of the activity expressed in the HT group differs, the main distinction being its significantly lower affinity for leucine (Fig. 3*A*). The half-saturation constant for leucine is 10 times higher than in the LT group (K_{iLEU}(HT) = 920 ± 100 μM). Since lysine binding is stronger in HT erythrocytes, the ratio of the half-saturation constants (K_{iLEU}/K_{iLYS}) differs markedly in the two groups; 1.6 (LT) versus 52 (HT). Interestingly, the carrier in the HT erythrocytes is still able to bind gluta-

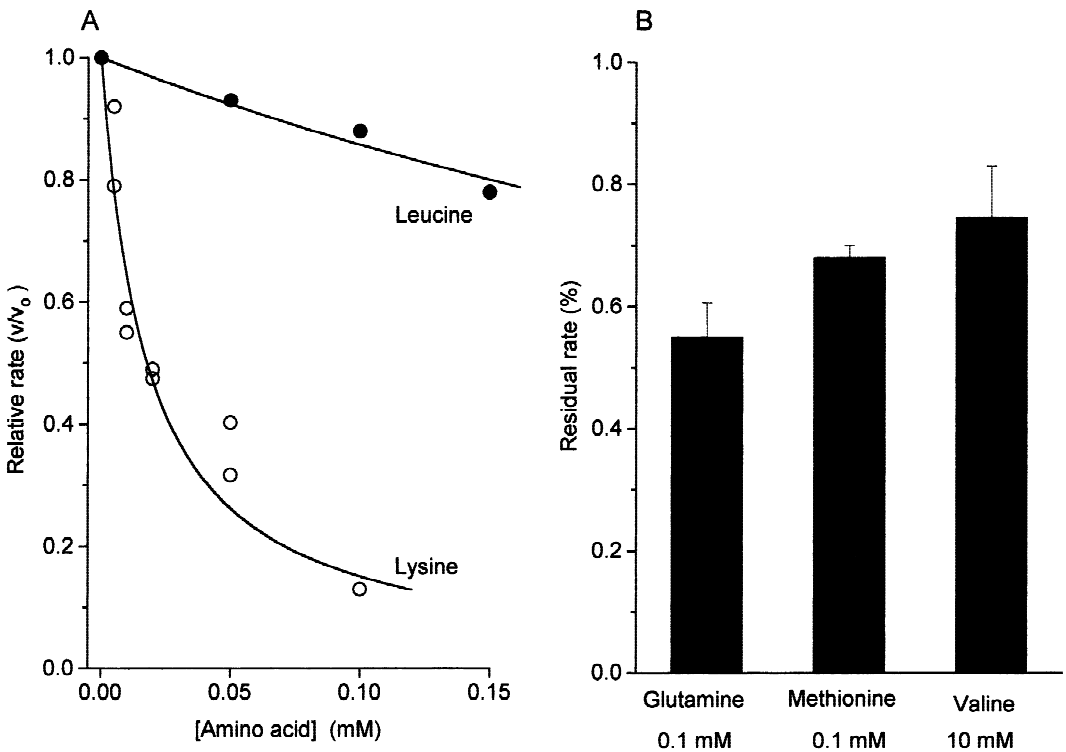

Fig. 3. Specificity of the lysine transporter in HT erythrocytes. (*A*) Relative rates of lysine entry in the presence of varying concentrations of unlabeled lysine (○) or leucine (●). L-[^{14}C]lysine concentration was 1 μM. The curves represent the best fit assuming that a single transporter is responsible for the flux. The inhibition constant for leucine was calculated considering determinations for a wider experimental range (0.05–10 mM), than those shown in this figure. (*B*) Relative rates of entry in the presence of L-glutamine, L-methionine and L-valine. The mean of the control rate ($n = 11$) was 0.336 ± 0.029 μmol/l cell/min. The calculated half-saturation constants are listed in the Table.

mine and methionine (Fig. 3*B*) with relatively high affinity. The values of the half-saturation constants for a series of amino acids are listed in the Table.

The effect of the ionic composition of the medium on the specificity was explored by comparing the inhibition produced by leucine in media containing Na^+, K^+, Li^+ or choline, as the principal cation. Figure 4 illustrates the behavior of the LT transporter. Leucine was found to bind with high affinity in the presence of Na^+ and even more strongly in the presence of Li^+, but the interaction was weak in the presence of K^+ or choline. The same ion dependence was observed for the HT group (Fig. 5), but the differences were more dramatic in this case. The K_i measured in the presence of Li^+ was one twentieth of the K_i measured in Na^+, and binding in the presence of K^+ or choline was negligible, even at a concentration of 2 mM. The transport of lysine was of similar magnitude in the presence of Na^+, Li^+ or K^+, but lower in the presence of choline (24% in LT erythrocytes and 50% in HT erythrocytes).

The transporters were found to function as tightly coupled exchangers. Figure 6 illustrates the exit of L-[^{14}C]lysine from preloaded cells in LT erythrocytes in the absence or presence of unlabeled amino acids (lysine, leucine and glutamine) in the *trans*-compartment. The rate of exit was found to increase approximately 10-fold by the addition of saturating concentrations of these amino acids. Trans-acceleration was also observed in

Table. Half-saturation constants (K_i) for L-amino acids

L-Amino acid	Half-saturation constant or K_i (μM)	
	LT erythrocytes	HT erythrocytes
Lysine	56.0 ± 7.1 (Na^+)	17.8 ± 1.3 (Na^+)
	57.7 ± 7.0 (K^+)	
Arginine	26.5 ± 2.6 (Na^+)	
Leucine	90.3 ± 7.1 (Na^+)	920 ± 100 (Na^+)
	2450 ± 146 (K^+)	>10000 *(K^+)
	21.5 ± 1.1 (Li^+)	40.0 ± 4.8 (Li^+)
	1070 ± 97 (Choline)	>10000 *(Choline)
Glutamine*	91.5 ± 12.7 (Na^+)	123 ± 27 (Na^+)
Methionine*	184 ± 20 (Na^+)	198 ± 4 (Na^+)
Valine*	8050 ± 810 (Na^+)	>20000 (Na^+)

Half-saturation constants (K_i) for L-amino acids in Na^+, K^+, Li^+ and choline medium. K_i values were calculated by nonlinear regression analysis of the effect of varying concentration of unlabeled amino acids, according to the equation or are (*) means of determinations performed with 2 different cell samples at a single amino-acid concentration. The number of different concentrations used in the linear regression analysis were L-lysine (Na^+): (LT, 9; HT, 9); L-lysine (K^+): (LT, 5); L-leucine (Na^+): (LT, 12; HT, 10); L-leucine (K^+): (LT, 8); L-leucine (Li^+): (LT, 7; HT, 10); L-leucine (choline) (LT, 4); L-arginine (LT, 7). Errors represent either SEM or range. Experimental details are given in Materials and Methods.

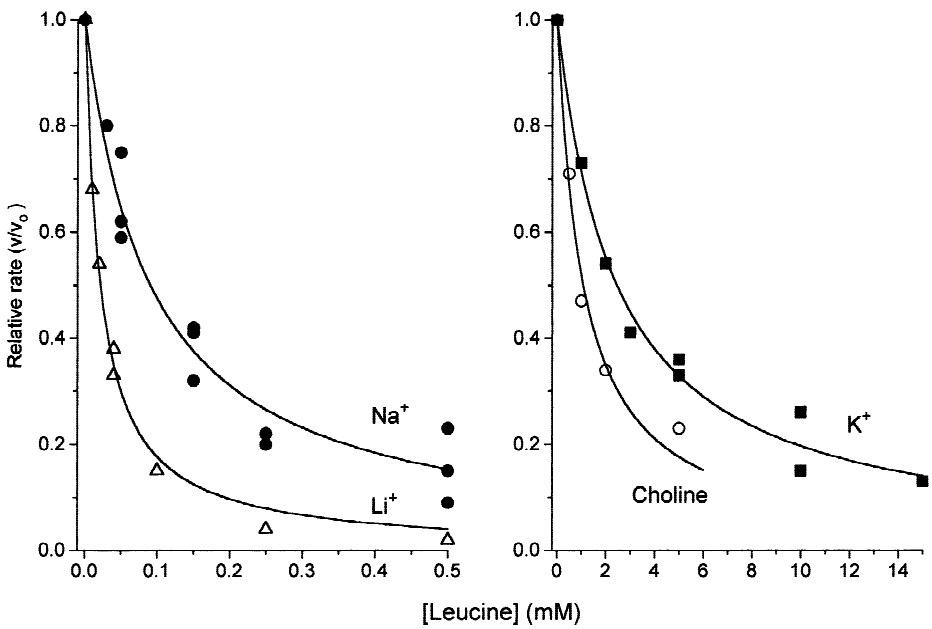

Fig. 4. Effect of unlabeled leucine on the relative rates of L-[^{14}C]lysine entry in LT erythrocytes in media of different ionic compositions. The lysine concentration was 1 μM and the media contained Na^+ (●), Li^+ (△), K^+ (■) or choline (○), as the principal cation (*see* Materials and Methods). The measured inhibition constants are listed in the Table. The data in the presence of Na^+ are the same as those shown in Fig. 2. The means of the control rates were (μmol/l cell/min): 0.0073 ± 0.009 (Na^+) ($n = 4$), 0.0079 ± 0.001 (Li^+) ($n = 2$), 0.0065 ± 0.002 (K^+) ($n = 2$), 0.0056 ± 0.0006 (choline) ($n = 2$).

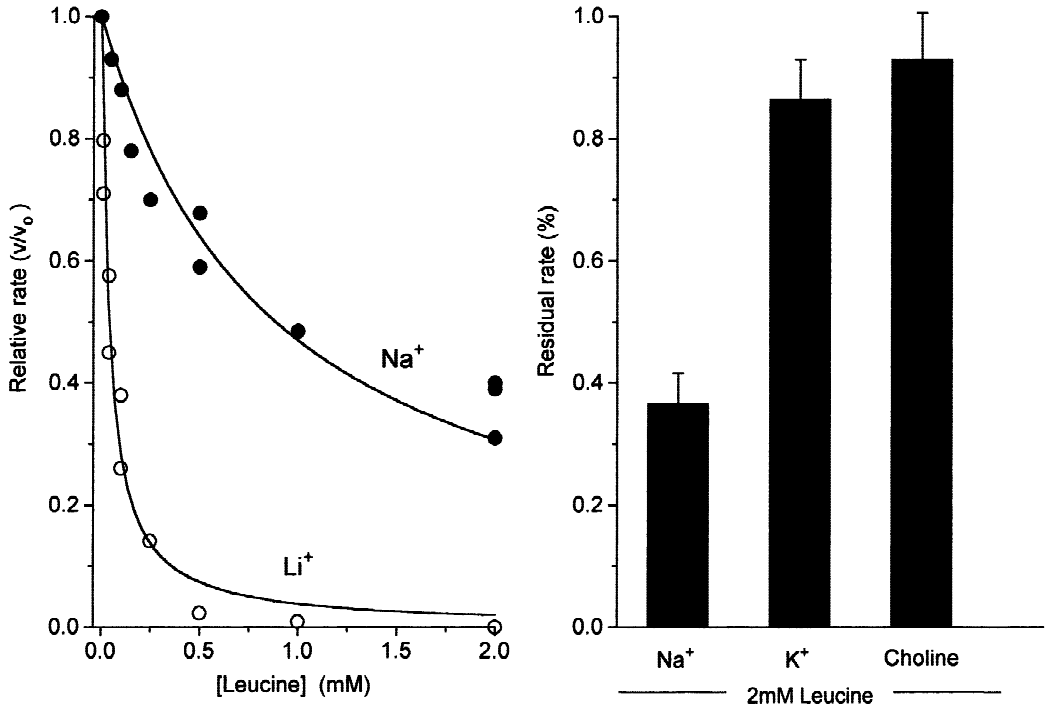

Fig. 5. Effect of unlabeled leucine on the relative rates of L-[^{14}C]lysine entry in HT erythrocytes in media of different ionic compositions. The lysine concentration was 1 μM and the media contained Na^+ (●), or Li^+ (○) (panel *A*) and Na^+, K^+ or choline (panel *B*) as the principal cation (*see* Materials and Methods). Some of the determinations in the presence of Na^+ are also shown in Fig. 3. The measured inhibition constants are listed in the Table. The means of the control rates were (μmol/l cell/min): 0.304 ± 0.038 (Na^+) (n = 6), 0.326 ± 0.075 (Li^+) (n = 3), 0.327 ± 0.038 (K^+) (n = 2), 0.152 ± 0.034 (choline) (n = 2).

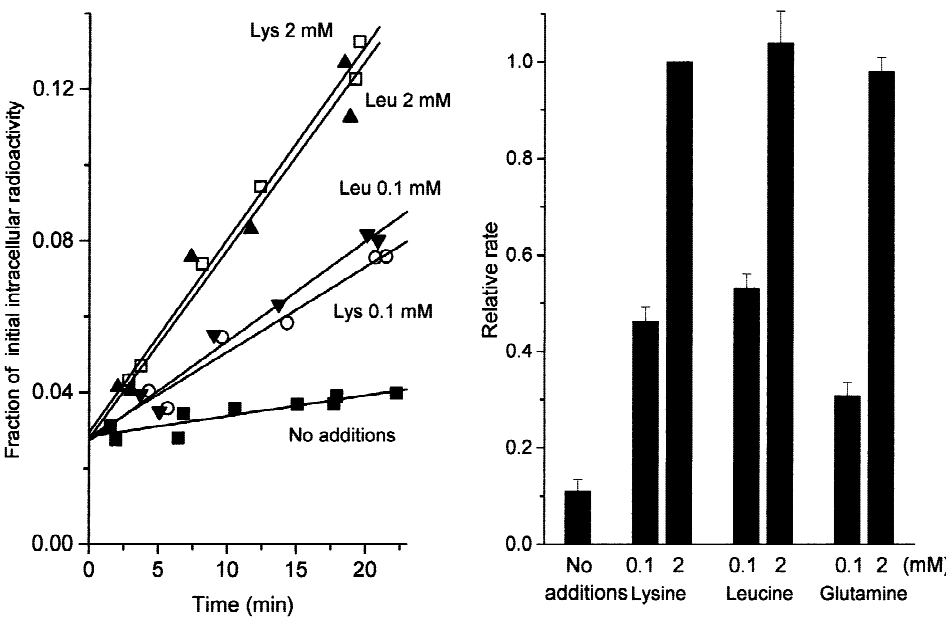

Fig. 6. Effect of unlabeled amino acids, present in the external medium, on the rate of exit of L-[^{14}C]lysine from LT erythrocytes. The initial concentration of lysine in the intracellular medium was 5 μM. (*A*) Representative experiment showing the efflux into media without added substrate (■) or with 0.1 mM lysine (○), 2 mM lysine (□), 0.1 mM leucine (▼), 2 mM leucine (▲). The rate in the presence of 2 mM lysine was 0.026 ± 0.005 μM/min. (*B*) Relative efflux in the absence or presence of L-leucine (n = 4), L-lysine (n = 3) and L-glutamine (n = 2). The rates have been normalized to the rates measured in the presence of 2 mM L-lysine.

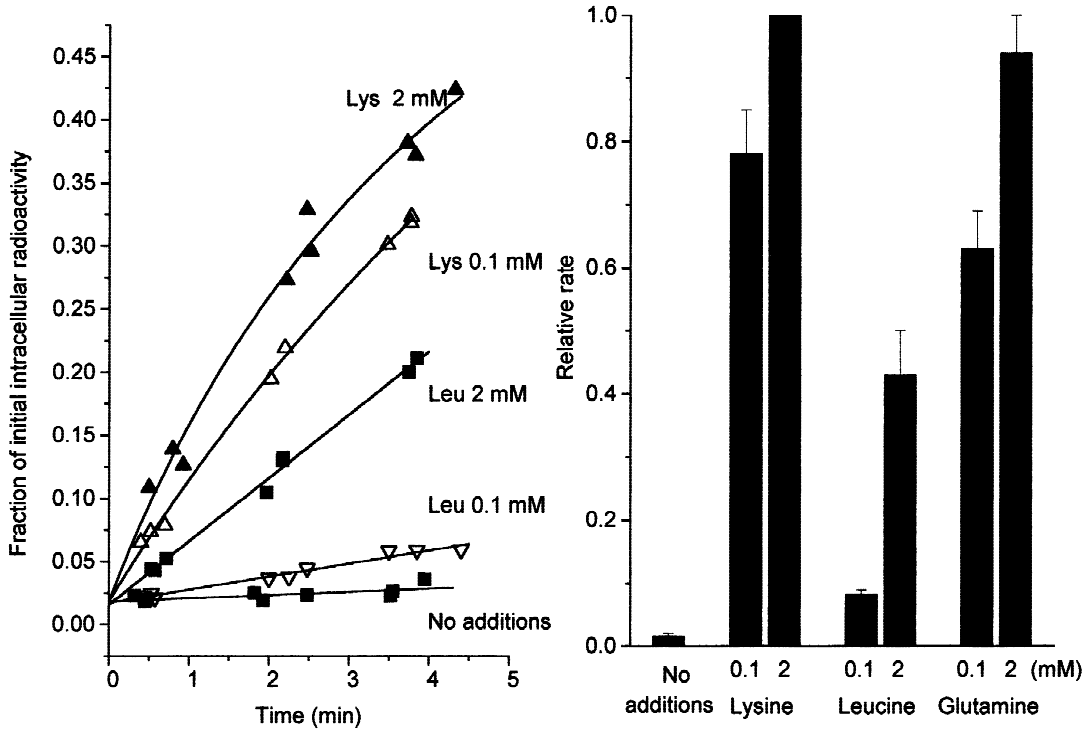

Fig. 7. Effect of unlabeled amino acids present in the external medium on the rate of exit of L-[^{14}C]lysine from HT erythrocytes. The initial concentration of labeled lysine in the intracellular medium was 10 μM. (*A*) Representative experiment showing the efflux into media without added substrate (■) or with 0.1 mM lysine (△), 2 mM lysine (▲), 0.1 mM leucine (▽), 2 mM leucine (■). The rate in the presence of 2 mM lysine was 1.38 ± 0.17 μM/min. (*B*) Relative efflux in the absence ($n = 3$) or presence of L-leucine ($n = 3$), L-lysine ($n = 3$) and L-glutamine ($n = 2$). The rates have been normalized to the rates measured in the presence of 2 mM L-lysine.

HT erythrocytes (Fig. 7) and activation was even more pronounced; the ratio of the rate of exit in the presence of saturating L-lysine relative to the rate of the control was 48, 55 and 89, in three experiments, respectively. Glutamine was as effective as lysine, but stimulation by leucine (0.1 and 2 mM), in the presence of Na^+, was less important, as expected from its lower affinity. In Li^+ medium, leucine produced the same stimulation as lysine (*result not shown*).

Lysine flux in LT and HT erythrocytes was found to be insensitive to N-ethylmaleimide (0.2 mM, 15 min), but inactivated by pCMBS (0.075 mM, 10 min) as shown in Fig. 8.

Discussion

The results reported in this study confirm the existence of two different phenotypes in chicken erythrocytes, exhibiting high or low lysine-transport rates. The magnitude of lysine influx in the high-transport phenotype (HT), measured at a concentration of 1 μM, exceeded by 34-fold that of the low-transport phenotype (LT). It is proposed that the basis of this polymorphism resides in the differential expression of two y^+L-type transport activities.

The functional properties of the transporter present in LT erythrocytes closely resemble those of system y^+L in human erythrocytes (Angelo, Irarrázabal & Devés, 1996). In the presence of Na^+ (or Li^+), the transporter is able to recognize and translocate cationic and neutral amino acids with comparable rates, but Na^+ replacement with K^+ or choline selectively reduces the affinity towards neutral amino acids. The transporter functions as a tightly coupled exchanger, like most members of the heterodimeric family of transporters (Chillarón et al., 1996; Verrey et al., 1999). The pattern of inactivation by sulfhydryl reagents is also consistent with that of system y^+L since it is resistant to NEM (an inhibitor of system y^+) and sensitive to pCMBS (Estévez et al, 1998).

In spite of some evident differences with the LT transporter, the general features of the activity expressed in HT erythrocytes are also compatible with a y^+L-type system. The most singular attribute of this activity is its low affinity for leucine (K_{iLEU} is 10 times larger than that of the LT group). Nevertheless, the selectivity towards

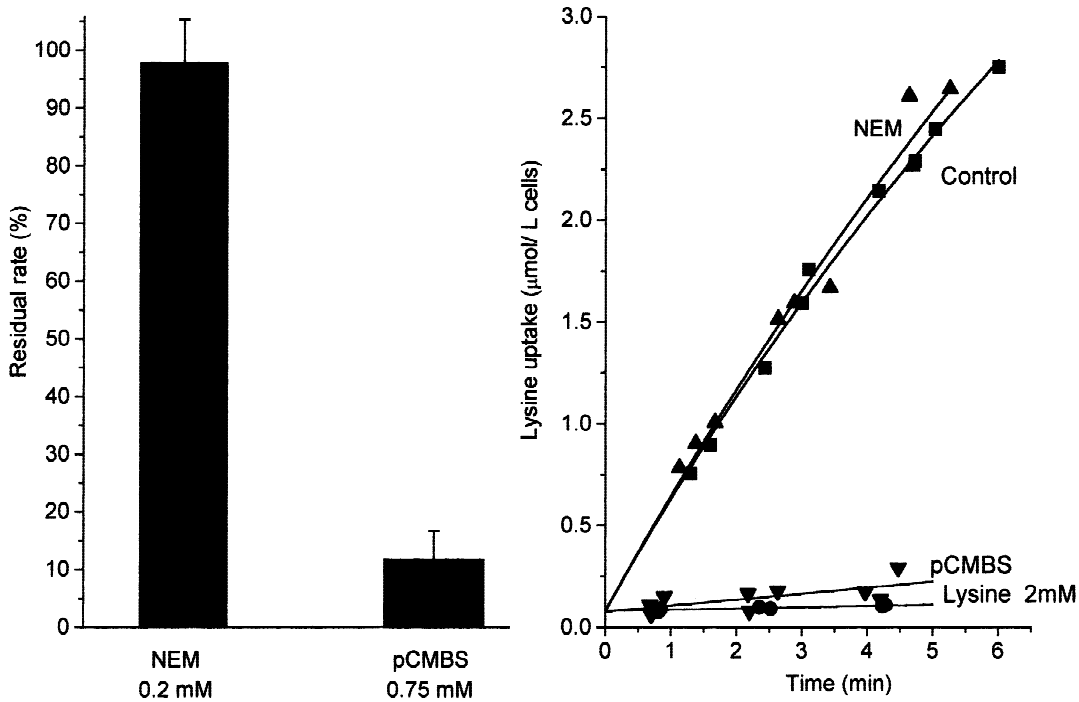

Fig. 8. Effect of NEM and pCMBS on the influx of L-[^{14}C]lysine in chicken erythrocytes. Treatment was as described in Materials and Methods. (*A*) LT erythrocytes; relative rates of influx following treatment with NEM or pCMBS. (*B*) HT erythrocytes; uptake in cells without (■) and with pretreatment with pCMBS (▼), or NEM (▲). The effect of unlabeled 2 mM lysine (●) is also shown. The rates of entry in untreated cells were (μmoles/l cell/min): 0.008 ± 0.0007 (LT, $n = 3$) and 0.48 ± 0.02 (HT).

cationic amino acids is not as large as in the case of system y^+ (present in human erythrocytes and many other cell types) (Christensen & Antonioli, 1969; MacLeod, Finley & Kakuda, 1994; Rojas & Devés, 1999), because the HT transporter interacts strongly with other neutral amino acids (glutamine, methionine). The specificity pattern of the HT transporter has not been described either in functional studies with other cell types or in studies with the two cloned y^+L transporters, y^+LAT-1 or y^+LAT-2 (Broer et al., 2000; Kanai et al., 2000, Pfeiffer et al., 1999; Torrents et al., 1998).

In other respects, the two activities exhibit similar properties. The ionic dependence is qualitatively the same (although the stimulatory effect of Li^+ on the binding of leucine was found to be more marked in HT erythrocytes) and exit was found in both cases to depend strictly on the presence of a substrate in the extracellular medium. The relatively poor ability of leucine to *trans*-accelerate the efflux of lysine in the presence of Na^+ in HT erythrocytes, is consistent with the low affinity exhibited by this amino acid in *cis*-inhibition entry experiments. Furthermore, the efficient *trans*-acceleration by leucine observed in the presence of Li^+ supports the notion that lysine and leucine are transported with equivalent maximum transport rates in HT erythrocytes. The effects of the sulfhydryl reagents were the same as those in the LT group.

Given that system y^+L is an obligatory exchanger, the question arises as to whether the differences observed might be the result of differences in intracellular amino-acid availability. A comparative analysis of entry and exit rates shows that the differences of lysine transport observed in LT and HT erythrocytes are not compatible with a potential effect of different intracellular amino acid concentrations. If the greater magnitude of the influx in HT erythrocytes compared to LT erythrocytes were due to a higher concentration of amino acids in the intracellular medium, the opposite relationship would be expected in efflux; that is, the rate of exit in HT erythrocytes should be lower than that of LT erythrocytes, because the intracellular amino acids would compete for exit with labeled lysine. This was not found to be the case. LT and HT erythrocytes were loaded with 5 and 10 μM labeled lysine, respectively, and the rates of exit of the intracellular labeled lysine (in the presence of saturating unlabeled lysine outside) were : 0.026 ± 0.005 μM/min (LT, Fig. 6) and 1.38 ± 0.17 μM/min (HT, Fig. 7). Thus, the rates of exit in HT erythrocytes exceeds by 53-fold that in LT erythrocytes. Since the initial substrate concentration in HT erythrocytes is twice that in LT cells, the factor is in good agreement with the ratio of 34 fold measured in entry. It can be concluded without ambiguity that the differences in the rates are not due to different intracellular amino acid concentrations.

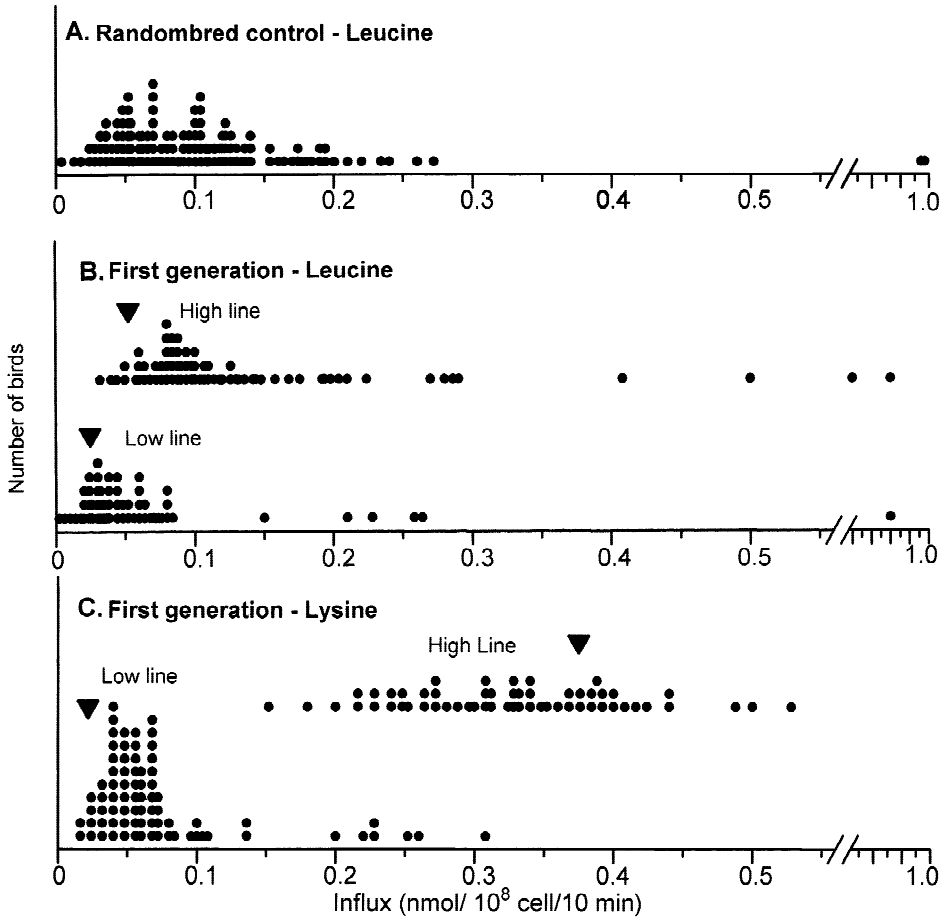

Fig. 9. Distribution of rates of amino-acid entry measured in chicken erythrocytes from random-bred and selectively-bred animals. Data taken from Somes et al., 1981. (*A*) L-Leucine influx in erythrocytes from random-bred chickens. (*B*) L-Leucine influx in erythrocytes from first-generation high- and low-transport lines. (*C*) L-Lysine influx in erythrocytes from first-generation high- and low-transport lines. Average values of the measured lencine-transport rates were (nmol/10^8 cells/10 min): 0.0968 (random-bred), 0.0686 (first generation, low line), 0.121 (first generation, high line). The initial external concentrations of L-leucine and L-lysine were 100 μM and 50 μM, respectively. The average values of the influx of L-leucine of the selected parents for the first generation were (nmoles/10^8 cells × 10 min): 0.0403 ($n = 9$) and 0.175 ($n = 9$) for the low and high lines respectively. The predicted values for the influx of L-leucine (at 100 μM) and L-lysine (at 50 μM), calculated on the basis of the kinetic parameters determined in the present study for the HT and LT transport lines, are indicated (▼) in panels *B* and *C*, respectively (*see* Discussion).

Heterogeneity of amino-acid transport in chicken erythrocytes was first described by Lerner et al. (1978) and Somes et al. (1981) who demonstrated that there were important individual differences in the ability to transport leucine and lysine and, furthermore, that these differences could be emphasized by genetic selection. The ratio of the magnitude of leucine influx (100 μM) in the high and low transport lines, for the first, second and third generations of chickens, was 1.58, 4.88, and 7.08, respectively. Lysine influx (50 μM) was determined for the first generation only, and the equivalent ratio found to be 4.50 (i.e., significantly larger than the ratio obtained for leucine in this generation). The molecular bases for these phenotypic differences have remained unexplained.

Figure 9 reproduces the results obtained by Somes et al. (1981) for the first-generation high and low transport lines. The distributions of mediated transport for leucine and lysine are plotted for the first generation of the high transport line, the first-generation of the low transport line and the random-bred population (leucine transport only). It is evident that the differences in the magnitudes of the fluxes for the high and low transport lines are larger for lysine than for leucine influx. The question that arises is whether this observation is consistent with the hypothesis that both substrates are transported by a common entity.

As the analysis that follows will show, the kinetic parameters determined for the HT and LT transporters can be used to calculate the rates of lysine and leucine transport expected at the concentrations used by Somes et al. (1981). Consistency between the two studies

would provide strong evidence in favor of the hypothesis that the phenotypic differences are due to differences in system y^+L activity.

Rates of transport can be calculated for any given concentration, on the basis of the Michaelis-Menten equation ($v = V_{max}\,[S]/(K_m + [S])$), provided the V_{max} and the K_m of the system are known. Since, in this study, rates were measured at very low concentration, the first step of the analysis was to estimate the maximum velocities.

Replacement in the Michaelis-Menten equation of the average rates measured at 1 μM lysine for each group and of the corresponding half-saturation constants, gives the following maximum velocities or V_{max} values (μmol/l cells/min): 4.8 (HT) and 0.44 (LT). It is of interest that the very low concentration (1 μM) used in the present study made more explicit the differences between the two phenotypes ($v_{HT}/v_{LT} = 34$ at 1 μM, versus $V_{max\,HT}/V_{max\,LT} = 10.9$). Since the *trans*-acceleration experiments indicate that the lysine and leucine translocation rates are essentially the same, the V_{max} values, calculated above, can also be applied to predict the rates of leucine transport at intermediate concentrations.

Rates for lysine and leucine entry at the concentrations used by Somes et al. (1981) (100 μM for leucine and 50 μM for lysine) can be calculated using the corresponding V_{max} and K_i values. The calculated rates are (μmol/l cells/min): 0.21 (LT lysine influx), 3.54 (HT lysine influx), 0.23 (LT leucine influx) and 0.48 (HT leucine influx). That is, as found in the experimental study, the predicted ratios of lysine and leucine influx in HT and LT erythrocytes differ. This behavior, which at first sight may seem peculiar, is due to the opposite affinity shift for these two amino acids in going from LT phenotype to the HT phenotype (K_{iLEU}(HT)/K_{iLEU}(LT) = 10.2 and K_{iLIS}(HT)/K_{iLIS}(LT) = 0.32). For the same reason, in the case of lysine, the difference between the two groups is more noticeable at lower substrate concentrations, whereas the reverse is true in the case of leucine.

The rates calculated above (expressed in nmoles/10^8 cells/10 min) were matched in Fig. 9 to the experimental lysine and leucine fluxes measured in the low and high transport lines. Consistency between the calculated rates and the results of Somes et al. (1981) provides strong evidence in favor of the hypothesis that system y^+L transporters are responsible for the observed phenotypic differences.

No differences in terms of growth rate, size, disease-resistance was observed in the animals with HT or LT and, therefore, whether the two different phenotypes also affect other cell types, is not known. The study of the molecular basis of these phenotypic variations should give, in the future, valuable information regarding the role of the different molecular variants of system y^+L. The two phenotypes could result either from the different expression of two y^+L transporter isoforms or from an amino acid residue substitution of the erythrocyte y^+L transporter. The second mechanism, however, would require that the mutation affects both the maximum velocity (presumably the rate of translocation) and the specificity (binding site conformation).

Although the molecular species responsible for system y^+L in human erythrocytes has not been identified, it appears to be distinct from that present in epithelia (y^+LAT-1). Transport through system y^+L has been found to be normal in the erythrocytes of patients presenting lysine protein intolerance (LPI) (Boyd et al., 2000), a genetic defect characterized by impaired cationic amino-acid transport across the basolateral membrane in renal tubule and small intestine (Desjeux et al., 1980). LPI has been shown to result from mutations in the gene that encodes the light chain protein (y^+LAT-1) of the heteromeric y^+L transporter (Borsani et al., 1999; Torrents et al., 1999).

The study of the molecular bases of the HT and LT phenotype should offer light in the future on the structure-function relationship of the y^+L transporters and its relation to pathophysiology.

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