Transport Properties of a System \( \gamma^+L \) Neutral and Basic Amino Acid Transporter

INSIGHTS INTO THE MECHANISMS OF SUBSTRATE RECOGNITION*

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The properties of system \( \gamma^+L \)-mediated transport were investigated on rat system \( \gamma^+L \) transporter, \( \gamma^+L \)-LAT1, coexpressed with the heavy chain of cell surface antigen \( 4F2c \) in Xenopus oocytes. \( \gamma^+L \)-LAT1-mediated transport of basic amino acids was \( Na^+ \)-independent, whereas that of neutral amino acids, although not completely, was dependent on \( Na^+ \), as is typical of system \( \gamma^+L \)-mediated transport. In the absence of \( Na^+ \), lowering of pH increased leucine transport, without affecting lysine transport. Therefore, it is proposed that \( H^+ \), besides \( Na^+ \) and \( Li^+ \), is capable of supporting neutral amino acid transport. \( Na^+ \) and \( H^+ \) augmented leucine transport by decreasing the apparent \( K_m \) values, without affecting the \( V_{max} \) values. We demonstrate that although \( \gamma^+L \)-LAT1-mediated transport of \( ^{14}C\)-leucine was accompanied by the cotransport of \( ^{22}Na^+ \), that of \( ^{14}C\)-lysine was not. The \( Na^+ \) to leucine coupling ratio was determined to be 1:1 in the presence of high concentrations of \( Na^+ \). \( \gamma^+L \)-LAT1-mediated leucine transport, but not lysine transport, induced intracellular acidification in Chinese hamster ovary cells coexpressing \( \gamma^+L \)-LAT1 and \( 4F2c \) heavy chain in the absence of \( Na^+ \), but not in the presence of physiological concentrations of \( Na^+ \), indicating that cotransport of \( H^+ \) with leucine occurred in the absence of \( Na^+ \). Therefore, for the substrate recognition by \( \gamma^+L \)-LAT1, the positive charge on basic amino acid side chains or that conferred by inorganic monovalent cations such as \( Na^+ \) and \( H^+ \), which are cotransported with neutral amino acids, is presumed to be required. We further demonstrate that \( \gamma^+L \)-LAT1, due to its peculiar cation dependence, mediates a heteroexchange, wherein the influx of substrate amino acids is accompanied by the efflux of basic amino acids.

Molecular cloning approaches have led to the identification of \( Na^+ \)-dependent and \( Na^+ \)-independent amino acid transporters (1). Four amino acid transporter families (three \( Na^+ \)-dependent and one \( Na^+ \)-independent) have been identified so far (2–7). The amino acid permease family SLC7 consists of \( Na^+ \)-independent amino acid transporters composed of two subfamilies: the cationic amino acid transporter (CAT) family, which consists of classical system \( \gamma \) basic amino acid transporters, and the recently identified \( i \)-type amino acid transporter (LAT) family, which includes transporters associated with type II membrane glycoproteins, such as \( 4F2c \) heavy chain (4F2hc) and rBAT (related to \( b_{0,+} \) amino acid transporter) (6, 7). The members of the LAT family exhibit a variety of substrate selectivity and represent the amino acid transport systems L, asp, \( \gamma^+L \), x, \( \gamma^+L \), \( x^-L \), c, and \( b_{0,+} \) (8–17).

Among the members of the LAT family, the system \( \gamma^+L \) transporters, \( \gamma^+L \)-LAT1 and \( \gamma^+L \)-LAT2, are unique because of their peculiar \( Na^+ \) dependence (13, 14). System \( \gamma^+L \) was originally identified as a transport system that transports both neutral and basic amino acids in the erythrocyte plasma membrane (18). The transport of basic amino acids by this system was shown to be \( Na^+ \)-independent, whereas that of neutral amino acids, although not completely, was dependent on \( Na^+ \) (18–21). This characteristic \( Na^+ \) dependence was confirmed for mouse and human \( \gamma^+L \)-LAT1 coexpressed with \( 4F2c \) in Xenopus oocytes (13, 14). In the case of erythrocyte system \( \gamma^+L \)-mediated transport, it was proposed that \( Na^+ \) (and \( Li^+ \)) increases the affinity of neutral but not basic amino acids for the binding sites (19).

To understand the mechanisms of substrate recognition and transport by system \( \gamma^+L \)-LAT1 transporters, we isolated a \( \gamma^+L \)-LAT1 cDNA from the rat kidney and expressed it in Xenopus oocytes. We report here that not only \( Na^+ \) and \( Li^+ \), but also \( H^+ \), are capable of supporting the neutral amino acid transport and that these monovalent cations are, in fact, cotransported with the neutral amino acids. Because of the peculiar cation dependence, \( \gamma^+L \)-LAT1 mediates the exodus of basic amino acids through an obligatory exchange mechanism.

EXPERIMENTAL PROCEDURES
cDNA Cloning of Rat \( \gamma^+L \)-LAT1—The cDNA for an expressed sequence tag (GenBank™ EBI/DBJ accession number R82979) corresponding to the human \( \gamma^+L \)-LAT1 was obtained from the Integrated and Molecular
Analysis of Genomes and their Expression (IMAGE). A 0.3-kilobase EcoRI fragment was excised from the cDNA (IMAGE clone number 187050) and used as the probe for screening a rat kidney cDNA library (11, 22, 23). cDNAs in positive ZipLex boxes were sequenced into plasmid pZL1 and sequenced as described previously. A cDNA clone was isolated, which contained an open reading frame encoding a 512-amino acid protein, designated as ryLAT1 (rat yLAT1: GenBank™ EBI/DDBJ accession number AB020520) with the amino acid sequence identity to yLAT1 from humans (90%) and mice (94%) (13, 14).

Xenopus Oocyte Expression—cRNAs were obtained by in vitro transcription using SP6 RNA polymerase for ryLAT1 (rat yLAT1) in pZL1 linearized with BstEII and T7 for rat 4F2hc in pT7script II SK linearized with XbaI as described elsewhere (24). The Xenopus oocyte expression studies and uptake measurements were performed as described previously (22, 25). The uptake of [3H]leucine (50 μCi) by control oocytes was isolated, which contained an open reading frame encoding a 512-amino acid protein, designated as ryLAT1 (rat yLAT1: GenBank™ EBI/DDBJ accession number AB020520) with the amino acid sequence identity to yLAT1 from humans (90%) and mice (94%) (13, 14).

**Fig. 1. Functional expression of ryLAT1.** a, functional association of ryLAT1 and 4F2hc. The uptake of [14C]leucine (50 μCi) by Xenopus oocytes injected with water, ryLAT1 cRNA (ry LAT1), 4F2hc cRNA (4F2hc), and both ryLAT1 cRNA and 4F2hc cRNA (ryLAT1 and 4F2hc), was measured in the standard uptake solution 3 days after the injection. b, Na+ dependence of the transport. The uptake of [14C]leucine (Leu) and [14C]lysine (Lys) (50 μCi) by Xenopus oocytes was measured in standard uptake solution (100 mM Na+ (●) or Na+−free uptake solution (−) 3 days after the injection of water (open column) or yLAT1 and 4F2hc cRNAs (filled column).

were used for each data point. Each data point in the figures represents the mean ± S.E. of the uptake by six to eight oocytes. To confirm the reproducibility of the results, experiments were performed in triplicate for each measurement using different batches of oocytes and cRNA transcribed in vitro. Representative results from the experiments are shown in the figures.

Intracellular pH Monitoring—ryLAT1 and rat 4F2hc cDNAs were subcloned into the mammalian expression vector pcDNA3.1 (Invitrogen) and cotransfected into Chinese hamster ovary (CHO) cells by electroporation (16). In brief, the cell suspension (800 μl, 2.4 × 106 cells/ml) was mixed with ryLAT1 plasmid and 4F2hc plasmid (10 μg each). The mixture was electroporated at 250 V, 960 μF in a 0.4-cm cuvette in a Gene Pulsar (Bio-Rad). The cells were cultured in Ham’s F-12 nutrient mixture containing 10% fetal bovine serum for 3 days. They were then examined using an Ultima-z confocal laser scanning microscope (Molecular Dynamics, Sunnyvale, CA) connected to a Zeiss Epifluorescence microscope. The cells were incubated at room temperature for 1 h with Na+−containing medium (containing 137 mM NaCl, 5.4 mM KCl, 1.3 mM CaCl2, 0.5 mM MgCl2, 0.4 mM MgSO4, 0.44 mM KHPO4, 0.34 mM K2HPO4, 4.2 mM NaHCO3, 5.5 mM glucose; pH 7.4) containing 5 μM 3′-O-acetyl-2′,7′-bis(carboxyethyl)-4-(5′-carboxyfluorescein), diace-toxymethyl ester (26, 27). The dye-loaded cells were washed three times with Na+−containing medium or Na+−free medium, which was similar in composition to the Na+−containing medium except that NaCl was replaced by choline chloride, and then further incubated in the Na+−containing medium or Na+−free medium for fluorescence measurements. To examine the behavior of 2′,7′-bis(carboxyethyl)-4(5′)-carboxyfluorescein in the dual-excitation mode, two laser sources were mounted on the confocal laser scanning system (26, 27). The excitation light at 485 nm (pH-dependent wavelength) was provided by an argon ion laser. A He-Cd laser was used to adapt the confocal laser scanning microscope for excitation at 442 nm (pH-independent wavelength). The intensity of the fluorescence signal was measured with a photomultiplier and visualized on a personal computer.

**RESULTS**

**Transport Activity and the Effect of Na+**—As reported for human and mouse yLAT1 (13, 14), ryLAT1 required 4F2hc for its functional expression in Xenopus oocytes (Fig. 1a). All the following experiments were therefore performed in coexpression of ryLAT1 and 4F2hc. The uptake of 50 μM [14C]leucine or [3H]lysine was measured in the presence or absence of Na+. As shown in Fig. 1b, leucine transport was dependent on the extracellular Na+, whereas lysine transport was not affected by the removal of Na+ from the extracellular medium. It is noticeable that the low level of leucine transport...
The uptake of 22Na was measured in standard uptake solution (Na 100), Li+ uptake solution (Li 100), high K+ uptake solution (K 102), and Na+-free uptake solution (Choline 100) at pH 7.5 and 5.5. See “Experimental Procedures” for the uptake solutions.

The Effects of Monovalent Cations on Leucine Transport—To determine which monovalent cations can take the place of Na+ to support leucine transport, the uptake of [14C]-leucine (200 μM) was measured in Li+ uptake solution, high K+ uptake solution, and low pH uptake solution (Na+-free uptake solution with a pH of 5.5). The values were compared with that in Na+-free uptake solution, pH 7.5. As shown in Fig. 2, Li+ was able to take the place of Na+ to support leucine transport. Leucine transport was increased by lowering the pH, whereas it was not affected by increased K+ concentration of the uptake solution (Fig. 2).

Dependence of Substrate Transport on Na+ and pH—To determine the role of Na+ in ry/LAT1-mediated transport, the concentration dependence of l-leucine transport and l-lysine transport was determined by measuring it in standard uptake solution (100 mM Na+) and in Na+-free uptake solution (0 mM Na+). The Km value for leucine transport into oocytes coexpressing ry/LAT1 and 4F2hc was dramatically increased in Na+-free uptake solution, the Vmax value was less affected (Km = 44 μM, Vmax = 6.7 pmol/oocyte/min in standard uptake solution containing Na+ 100 mM, and Km = 423 μM, Vmax = 4.8 pmol/oocyte/min in Na+-free uptake solution, both determined in the same batch of oocytes) (Fig. 3a). On the other hand, neither the Km nor the Vmax value for lysine transport was affected by changing Na+ concentration (Km = 68 μM, Vmax = 9.0 pmol/oocyte/min in standard uptake solution containing Na+ 100 mM, and Km = 50 μM, Vmax = 7.5 pmol/oocyte/min in Na+-free uptake solution, both determined in the same batch of oocytes) (data not shown).

The effect of pH on ry/LAT1-mediated transport was further examined by comparing the uptakes of [14C]-labeled leucine and lysine at pH 7.5 and 5.5. Although the Km value for leucine transport was markedly altered with a change of pH of the uptake solution, the Vmax value was less affected (Km = 383 μM, Vmax = 5.7 pmol/oocyte/min in Na+-free uptake solution, pH 7.5, and Km = 119 μM, Vmax = 6.5 pmol/oocyte/min in Na+-free uptake solution, pH 5.5, both determined in the same batch of oocytes) (Fig. 3b). On the other hand, neither the Km nor the Vmax value for lysine transport was affected with a change of pH (Km = 74 μM, Vmax = 7.6 pmol/oocyte/min in Na+-free uptake solution, pH 7.5, and Km = 81 μM, Vmax = 6.7 pmol/oocyte/min in Na+-free uptake solution, pH 5.5, both determined in the same batch of oocytes) (data not shown). The concentration dependence profiles of neither leucine nor lysine uptake measured in the standard uptake solution (100 mM Na+) were affected by the pH of the solution (data not shown).

Transport of Na+ and H+ Accompanies Amino Acid Transport—The uptake of 22Na was measured in the presence or absence of amino acids (200 μM l-leucine or l-lysine) in the presence of 40 mM Na+ in the uptake medium containing ouabain, amiloride, and bumetanide to inhibit endogenous Na+ transport in the oocytes. In the oocytes coexpressing ry/LAT1 and 4F2hc, marked 22Na uptake was detected in the presence of leucine in the medium, whereas no significant 22Na uptake was detected in the absence of leucine (Fig. 4a). Lysine, however, did not induce 22Na uptake (Fig. 4a). In the same batch of oocytes, the uptakes of 200 μM [14C]-l-leucine and [14C]-l-lysine were measured under the same conditions as for the 22Na uptake measurements to verify that the uptakes of [14C]-l-leucine and [14C]-l-lysine were almost identical (Fig. 4b). Leucine-induced 22Na uptake was calculated as the difference between the 22Na uptakes measured in the presence and absence of leucine, and was compared with ry/LAT1-mediated [14C]-l-leucine uptake. The ratio of the fluxes of 22Na and [14C]-l-leucine was 1.088 (13.9 ± 2.6 pmol/oocyte/min for 22Na flux versus 12.3 ± 1.0 pmol/oocyte/min for [14C]-l-leucine flux, both determined in the same batch of oocytes, Fig. 4, a and b).

To confirm the Na+:leucine stoichiometry, the dependence of leucine-induced 22Na uptake on the leucine concentration was compared with the concentration dependence of [14C]-l-leucine uptake measured under the same conditions in the same batch of oocytes. 22Na uptake and the [14C]-l-leucine exhibited almost identical dependence on the leucine concentration (Km = 71.8 μM and Vmax = 12.7 pmol/oocyte/min for 22Na uptake; Km = 105.9 μM and Vmax = 14.8 pmol/oocyte/min for [14C]-l-leucine uptake), indicating that the Na+ flux and leucine flux were well correlated (data not shown). The ratio of the Vmax values for 22Na uptake and [14C]-l-leucine uptake was
calculated to be 1:1.17.

\[ \text{Na}^+ \] uptake was further measured in a medium containing only trace levels of \( \text{Na}^+ \) (0.28 \( \mu \text{M} \)). Addition of leucine (200 \( \mu \text{M} \)) to the medium increased the \( \text{Na}^+ \) uptake, however, the level of uptake was still much lower than that of \( [^{14}\text{C}]\text{-leucine} \) uptake measured under the same conditions as in \( a \).

Cotransport of \( \text{H}^+ \) with amino acids was examined by measuring the intracellular pH changes induced by extracellularly applied amino acids in CHO cells cotransfected with \( \text{ry}^{1}\text{LAT1} \) and 4F2hc. As shown in Fig. 5, extracellularly applied leucine (1 mM) induced intracellular acidification in the Na\(^+\)-free medium, whereas in a Na\(^+\)-containing medium (141 mM Na\(^+\)) extracellularly applied leucine had no effect on the intracellular pH. In contrast, extracellularly applied lysine (1 mM) did not induce intracellular pH changes regardless of the presence or absence of Na\(^+\) in the medium. No pH change was detected in the control mock-transfected CHO cells with the addition of amino acids to the medium (data not shown).

**Amino Acid Exchange via \( \text{ry}^{1}\text{LAT1} \)—Trans-stimulation of \( [^{14}\text{C}]\text{-leucine} \) uptake by intracellularly loaded amino acids** was examined by microinjecting high concentrations of nonlabeled amino acids into the oocytes. As shown in Fig. 6a, the uptake of \( [^{14}\text{C}]\)-leucine measured in the standard uptake solution was stimulated by the intracellular injection of L-lysine, whereas no such stimulatory effects on the uptake were observed following intracellular injection of neutral amino acids such as leucine, L-arginine, L-ornithine, and L-histidine also trans-stimulated \( [^{14}\text{C}]\)-leucine uptake (data not shown).

The effect of extracellularly applied L-leucine was examined on oocytes loaded with \( [^{14}\text{C}]\)-lysine. As shown in Fig. 6b, L-leucine applied extracellularly to the oocytes induced the marked efflux of preloaded \( [^{14}\text{C}]\)-lysine from the oocytes coexpressing \( \text{ry}^{1}\text{LAT1} \) and 4F2hc, indicating that \( \text{ry}^{1}\text{LAT1} \) is an amino acid exchanger.

\( \text{Na}^+ \) dependence of extracellularly applied amino acids to induce efflux of preloaded \( [^{14}\text{C}]\)-lysine was examined. As shown in Fig. 7, the efflux induced by L-leucine was dependent on extracellular Na\(^+\), whereas that induced by L-lysine was not affected by the removal of Na\(^+\) from the extracellular medium. In addition, the efflux induced by other neutral amino acids such as L-isoleucine, L-glutamine, and L-methionine was also reduced by the removal of Na\(^+\) (data not shown). The efflux induced by L-arginine and L-ornithine was not affected by the removal of Na\(^+\) (data not shown).

To determine which intracellular amino acids were effectively exchanged via \( \text{ry}^{1}\text{LAT1} \), the efflux of preloaded \( [^{14}\text{C}]\)-amino acids was measured in the standard uptake solution (Na\(^+\) 100 mM) containing 100 \( \mu \text{M} \) L-leucine. A high level of efflux of intracellularly injected \( [^{14}\text{C}]\)-labeled basic amino acids such as L-lysine, L-arginine, and L-ornithine was induced by extracellularly applied L-leucine (Fig. 8). A lower level induction of the efflux of \( [^{14}\text{C}]\)-labeled L-leucine, L-methionine, and L-histidine was detected (Fig. 8).

**DISCUSSION**

**Requirement of Inorganic Monovalent Cations for Substrate Recognition—\( \text{ry}^{1}\text{LAT1} \)-mediated L-lysine transport was not Na\(^+\)-dependent**, whereas the L-leucine transport was, although not completely, dependent on Na\(^+\) (Fig. 1b), consistent with previous observations on human and mouse \( \text{y}^{1}\text{LAT1s} \) (13, 14). As reported for the erythrocyte system \( \text{y}^{1} \)L (20, 21, 28), Na\(^+\) decreased the \( K_m \) value for L-leucine uptake while not affecting that of L-lysine uptake (Fig. 3a), suggesting that Na\(^+\) increases the apparent affinity of neutral amino acid substrates for the substrate-binding site of \( \text{ry}^{1}\text{LAT1} \).

Based on the observation that \( \text{ry}^{1}\text{LAT1} \) can still accept neutral amino acid substrates in the absence of Na\(^+\), it is suggested that some other monovalent cations may also support the binding of neutral amino acids in the absence of Na\(^+\) (Fig. 1b). As shown in Fig. 3b, the \( K_m \) value for L-leucine transport at pH 5.5 was lower than that at pH 7.5. Apparently, this is due to the conformational changes of the transporter protein with the change of pH of the uptake solution, because L-lysine transport was not altered by the change of pH. It is proposed that H\(^+\) takes the place of Na\(^+\) in the absence of Na\(^+\) to support leucine transport. Besides Na\(^+\) and Li\(^+\), H\(^+\) is also capable of supporting the binding of neutral amino acid substrates to the substrate-binding site of \( \text{ry}^{1}\text{LAT1} \) (Fig. 9b).

**Transport of Inorganic Monovalent Cations with Amino Acids—\( \text{ry}^{1}\text{LAT1} \) not only requires Na\(^+\) for the binding of neutral amino acids to its substrate-binding site, but it also translocates the Na\(^+\) along with neutral amino acids.** As shown in Fig. 4, although L-leucine transport was accompanied by \( \text{Na}^+ \) uptake, the transport of L-lysine was not coupled with \( \text{Na}^+ \) transport, consistent with the observation that L-leucine transport was Na\(^+\)-dependent, whereas that of L-lysine was not dependent on Na\(^+\) (Fig. 1b). The Na\(^+\)-L-leucine coupling ratio was 1:1 when measured in the presence of 40 mM Na\(^+\).

In the presence of trace concentrations of Na\(^+\), however, the number of Na\(^+\) coupled with L-leucine was markedly reduced. Considering the pH dependence of \( [^{14}\text{C}] \)-leucine uptake in the absence of Na\(^+\), it is supposed that H\(^+\) instead of Na\(^+\) is cotransported with L-leucine in the presence of trace concentrations of Na\(^+\). In fact, as shown in Fig. 5, L-leucine induced intracellular acidification in CHO cells cotransfected with \( \text{ry}^{1}\text{LAT1} \) and 4F2hc cDNAs in the absence of extracellular Na\(^+\), lending support to the assumption of H\(^+\) cotransport. H\(^+\) was cotransported with L-leucine in the absence or presence of a low concentration of Na\(^+\) but not with L-lysine. Considering the low Na\(^+\)-L-leucine coupling ratio (0.0015:1; see “Results”) in the presence of 0.28 mM Na\(^+\) at pH 7.4 (0.04 mM H\(^+\)), the affinity for H\(^+\) seems to be higher than that for Na\(^+\), provided that most of the leucine is cotransported with H\(^+\) under this condition. In agreement with this observation, the affinity of the Na\(^+\)/glucose cotransporter, which also accepts H\(^+\) in the absence of Na\(^+\), is also much higher for H\(^+\) than that for Na\(^+\) (29).

**Proposed Mechanisms of Substrate Recognition—**As indicated above, Na\(^+\), H\(^+\), or Li\(^+\) is required for neutral amino...
acids to be accepted by ry^1 LAT1. Therefore, the positive charge on the side chains of basic amino acids or that conferred by inorganic monovalent cations is presumed to be required for the recognition of amino acid side chains by the substrate-binding site of ry^1 LAT1 (Fig. 9, a and b). It should be noted that ry^1 LAT1 is structurally related to the system y^+ L amino acid transporters CAT1, CAT2, CAT3, and CAT4 (~30% identity at the amino acid level) (1, 6, 21). The substrate-binding sites of CATs bind specifically with basic amino acids. There is, however, an interesting exception: neutral amino acids, in particular L-homoserine, are accepted with low affinity by CAT1 in the presence of Na^+ (30), indicating that CAT1 behaves like a system y^+ L transporter, particularly for L-homoserine. It is, therefore, suggested that the binding sites of ry^1 LAT1 and CAT1 recognize the positive charges on the side chains of substrate amino acids by a similar mechanism, consistent with the structural similarity between the two proteins.

Considering the requirement of positive charges for substrate binding, it is reasonable to assume that the binding sites
of ry’LAT1 and possibly those of the other system y’L transporters (KIAA0245/ry’LAT2 from humans (13) and SPRM1 from Schistosoma mansoni (9)) contain negatively charged amino acid residues to accept the positive charges of the basic amino acid side chains or of the inorganic cations with neutral amino acid residues to accept the positive charges of the basic amino acids to be present at the substrate-binding sites.

The positive charge recognition site is probably located at a certain distance from the α-carbon recognition site. Therefore, for neutral amino acids to be the substrates of ry’LAT1, the length of the side chains must be within a limited range, so that the side chains accompanied with inorganic cations can fit well into the binding site of ry’LAT1. L-Leucine, L-isoleucine, L-methionine, and L-glutamine, which are accepted by ry’LAT1, are predicted to meet this requirement.

The positive charge recognition site of ry’LAT1 accepts inorganic cations with the following proposed order of affinity: $H^+ \gg Li^+ > Na^+ > K^+$. Some of the Na’-binding sites of Na’-dependent transporters accept not only Na’ but also other inorganic monovalent cations such as $H^+$ and $Li^+$. As already mentioned, the Na’/glucose cotransporter accepts $H^+$ in the absence of Na’. (29). In glumatic transporters, it is proposed that one of the three Na’-binding sites is specific for Na’, whereas the other two are less specific and also accept $Li^+$. (31). The broad cation selectivity of the positive charge recognition site of ry’LAT1 is reminiscent of the less specific Na’-binding sites of Na’-dependent transporters. The positive charge recognition site of the system y’L transporters may be a primitive form of Na’-binding sites; hence, system y’L transporters might be the evolutionary link between Na’-independent transporters and Na’-dependent transporters.

**Amino Acid Exchange Property—**Consistent with the Xenopus oocyte endogenous system y’L activity stimulated by the injection of 4F2hc cRNA and the mouse ry’LAT1 coexpressed with 4F2hc in Xenopus oocytes (14, 32), ry’LAT1 mediated amino acid exchange (Fig. 6b). ry’LAT1-mediated uptake was trans-stimulated by substrate amino acids (Fig. 6a). In addition, the extracellular substrate selectivity and Na’ dependence for the efflux of preloaded [14C]1-lysine was basically identical to those of the uptakes of [14C]amino acids (Fig. 7).

These results indicate that the amino acid efflux is tightly coupled to amino acid uptake, suggesting that ry’LAT1 mediates an obligatory exchange of substrate amino acids.

The efflux of intracellularly injected 14C-labeled basic amino acids such as L-lysine, L-arginine, and L-ornithine was efficiently induced by extracellularly applied amino acid substrates (Fig. 8). In contrast, the efflux of 14C-labeled neutral amino acids such as glutamine, leucine, isoleucine, and methionine was low, although they are also substrates of y’LAT1 in the standard uptake solution. This is understandable when considering the low intracellular concentration of Na’. It is assumed that a sufficient concentration of Na’ is required for neutral amino acid substrates to bind not only to the extracellular binding sites but also to the intracellular binding sites.

**Proposed Functional Roles—**It has been proposed that, in the renal proximal tubules and small intestine, basic amino acids are absorbed from the luminal fluid via system b0,+ transporter situated on the apical membrane of the epithelial cells and pass through the basolateral membrane via system y’L transporter into the extracellular fluid and blood stream (32). The results of our present investigation indicate that ry’LAT1 can fulfill this task once it exists on the basolateral membrane of the epithelial cells along with 4F2hc, which has already been shown to be localized on the basolateral membrane (33). As shown in Fig. 8, intracellularly loaded basic amino acids, but not neutral amino acids, in fact, efficiently moved out of the cells via ry’LAT1 through the amino acid exchange mechanism, probably because the intracellular substrate-binding site prefers basic amino acids to neutral amino acids due to the low intracellular concentrations of Na’.

Thus, ry’LAT1 appears to be well suited as an exit path for basic amino acids.

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**REFERENCES**

1. Palacín, M., Estevez, R., Bertran, J., and Zorzano, A. (1998) Physiol. Rev. 78, 969–1054.
2. Amara, S. G., and Kuhar, M. J. (1993) Annu. Rev. Neurosci. 16, 73–93.
3. Kanai, Y. (1997) Curr. Opin. Cell Biol. 9, 565–572.
4. Chaudhry, P. A., Reimer, R. J., Krizaj, D., Barber, D., Storm-Mathisen, J., Copenhagen, D. R., and Edwards, R. H. (1999) Cell 99, 769–780.
5. Varougi, H., Zhu, H., Yao, D., Ming, H., and Erickson, J. D. (2000) J. Biol. Chem. 275, 4049–4054.
6. MacLeod, C. L., Finley, K. D., and Kakuda, D. K. (1994) J. Exp. Biol. 166, 109–121.
7. Verrey, F., Jack, D. L., Paulsen, I. T., Saier, J. M., and Pfeiffer, B. (1999) J. Membr. Biol. 172, 181–192.
8. Kanai, Y., Segawa, H., Miyamoto, K., Uchino, H., Takeda, E., and Endou, H. (1998) J. Biol. Chem. 273, 23629–23632.
9. Mastroberardino, L., Spindler, B., Pfeiffer, B., Skelly, P. J., Laffing, J., Shoemaker, C. B., and Verrey, F. (1998) Nature 395, 258–291.
10. Pineda, M., Fernandez, E., Torrens, D., Estevez, R., Lopez, C., Camps, M., Lloberas, J., Zorzano, A., and Palacin, M. (1999) J. Biol. Chem. 274, 19738–19744.
11. Segawa, H., Fukasawa, Y., Miyamoto, K., Takeda, E., Endou, H., and Kanai, Y. (1999) J. Biol. Chem. 274, 9745–9751.
12. Fukasawa, Y., Segawa, H., Kim, J. Y., Chaisangduang, A., Kim, D. K., Matsus, H., Cha, S. H., Endou, H., and Kanai, Y. (2000) J. Biol. Chem. 275, 9690–9696.
13. Torrens, D., Estevez, R., Pineda, M., Fernandez, E., Lloberas, J., Shi, Y.-B., Zorzano, A., and Palacin, M. (1998) J. Biol. Chem. 273, 32437–32445.
14. Pfeiffer, B., Rossier, G., Spindler, B., Meier, C., Luh, L., and Verrey, F. (1999)
Transport Properties of a System \(y^+L\) Amino Acid Transporter