Electrogenic L-Histidine Transport in Neutral and Basic Amino Acid Transporter (NBAT)-expressing Xenopus laevis Oocytes

EVIDENCE FOR TWO FUNCTIONALLY DISTINCT TRANSPORT MECHANISMS INDUCED BY NBAT EXPRESSION

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We have investigated the neutral and basic amino acid transporter (NBAT)-induced transport of L-histidine in Xenopus laevis oocytes. Transport of L-histidine (pH 7.5) was electrogenic and Na⁺-dependent with a 14-fold increase in I_{His} (1 mM) evoked current (I_{His} = 14.7 ± 1.5 nA) in NBAT-expressing oocytes compared with native (water-injected or uninjected) oocytes (−1.0 ± 0.2 nA); the Na⁺-dependent histidine transport showed a stoichiometry of 1:1 (histidine:sodium). I_{His} was stereospecific at pH 7.5 and saturable in both NaCl and tetramethylammonium chloride media. L-Histidine (1 mM) at pH 8.5, at which histidine is uncharged, evoked an Na⁺-independent outward current (11 ± 1.2 nA) in NBAT-expressing oocytes. The total inward 0.1 mM I_{His} increased from −9 ± 0.8 nA at pH 7.5 to −19 ± 2.6 nA at pH 6.5, at which histidine is predominantly cationic. The increase in I_{His}, from pH 7.5 to 6.5 was found to be almost entirely due to the Na⁺-independent component. At pH 7.5, L-histidine weakly inhibited the Na⁺-independent L-arginine uptake; however, this inhibition was much stronger (>90%) at pH 6.5. L-Histidine transport, at pH 7.5, is stimulated by NBAT expression, but unlike L-phenylalanine or L-arginine transport, L-histidine transport is Na⁺-dependent and stereoselective. The induction of Na⁺-dependent L-histidine transport in NBAT-expressing oocytes provides new evidence that NBAT stimulates functionally distinct amino acid transporters including Na⁺-dependent L-histidine and Na⁺-independent L-arginine and L-phenylalanine transporters. The parallel induction of two different mechanisms argues that NBAT is not an amino acid transporter itself but, instead, is a transport-activating protein for a range of amino acid transportocases.

The so called “neutral and basic amino acid transporter” (NBAT) (1) and the homologous rBAT (2) proteins are expressed mainly in renal and intestinal epithelia (3, 4); mutations in rBAT gene have been implicated in cystinuria, characterized by defects in cystine and cationic amino acid transport (5, 6). Although the exact functional identity of NBAT/rBAT proteins is not known, it has been suggested that since characterstics of NBAT/rBAT-stimulated transport in Xenopus laevis oocytes resemble those of a previously described amino acid transporter, System b⁰⁺ (7), the NBAT gene may encode for this transporter (1–4, 8, 9). To assess the substrate specificity of NBAT-induced transport in X. laevis oocytes, we and others previously tested a range of amino acids (10–12), including L- and D-isomers of α-neutral and cationic amino acids and also β-neutral amino acids. Transport of all these amino acids was stimulated in an Na⁺-independent manner, thus supporting the notion that NBAT induces amino acid transport activity resembling System b⁰⁺ (7).

However, due to the atypical structure of NBAT protein, with only one to four membrane-spanning domains (11, 13), a reasonable speculation is that NBAT is not itself a transporter but rather acts as an “activator” (or a regulatory subunit) of other amino acid transporter proteins (5, 11) a property that could be achieved by NBAT associating with another protein to form a functional heterodimer (14). Moreover, we and others have recently described the unique electrogentic properties of NBAT in X. laevis oocytes and shown that transport of neutral amino acids, such as phenylalanine, evoke outward Na⁺- and Cl⁻-independent currents, whereas transport of cationic amino acids, such as arginine, in these oocytes evoke Na⁺-independent inward currents (12). Such apparent disparate common properties could be conceptualized as the result of separate activation of transporters of distinct functional properties.

In our attempts to further determine the substrate specificity of NBAT-induced amino acid transport we decided to investigate the transport characteristics of histidine in NBAT-expressing oocytes. L-Histidine, predominantly neutral at physiological pH (pKₐ = 6.04; isoelectric point = 7.6), contains an imidazole ring in its side chain that can act as a cation when the prevailing pH is acidic. We have demonstrated that NBAT expression in oocytes, at neutral pH, stimulates the transport of cationic as well as neutral amino acids in an Na⁺-independent but electrogenic manner (12). We hypothesized that histidine transport in NBAT-expressing oocytes at pH 7.5 should exhibit characteristics of transport of neutral amino acids, the superfusion of which evokes an outward current, but that at pH < 7.0 (when the imidazole nitrogen is partially protonated) it should behave like cationic amino acids such as lysine and arginine, which evoke an inward current (12).

Recent studies of the rabbit protein rBAT (2) have provided some interesting results, which provide clues to the function of rBAT in relation to histidine transport. Busch et al. (15) concluded that rBAT induces an amino acid transport system in oocytes that works via an exchange mechanism involving countertransport of neutral and dibasic amino acids. Whereas these authors (15) showed that histidine transport is pH-dependent with an inward current for cationic histidine that was greater at pH 6.5 than at pH 7.5 and with an outward current for...
uncharged histidine at pH 8.2, crucially, no information was provided on the Na\(^+\) dependence or lack thereof of t-histidine transport (15). The latter is an important distinguishing characteristic of most amino acid transport processes and one with additional significance for histidine transport in NBAT-expressing oocytes, as we describe below.

We have now uncovered direct evidence that suggests that NBAT does not just induce a single broad specificity amino acid transport system (such as b\(_0\)\(^{+}\)) that is Na\(^+\)−independent (12) or one that only induces a neutral-cationic amino acid exchange mechanism as previously thought (15–17), but rather it also induces activity of an Na\(^+\)−dependent amino acid transporter. We report here the characteristics of histidine transport in NBAT-expressing oocytes and, more importantly, provide functional evidence clearly indicating that NBAT is a transport-activating protein for a range of amino acid translocases with disparate transport mechanisms.

**EXPERIMENTAL PROCEDURES**

**Expression of NBAT in Oocytes**—The cDNA clone for NBAT was a kind gift of Dr. S. Udenfriend (Roche Research Center, Nutley, NJ). Synthetic cRNA was transcribed in vitro (transcription kit from Ambion, Austin, TX) from the NBAT cDNA clone (in pSPORT 1 plasmid vector (10)) and 5 ng of cRNA in 50 nl of sterile water injected into each defolliculated stage VI X. laevis oocyte (12). All experiments were performed 3–6 days post-cRNA injection. Native oocytes were injected with 50 nl of sterile water or were uninjected; similar results were obtained in both cases. Electrophysiological experiments (at 21 ± 2 °C), including Cl\(^−\) replacement studies were performed as described elsewhere (12). The superfusion medium for these studies contained 100 mM of either NaCl or tetramethylammonium chloride (TMACl) and including 2 mM KCl, 1 mM CaCl\(_2\), 1 mM MgCl\(_2\), and 10 mM HEPES, pH 7.5, with Tris base (superfusion medium). Amino acids at the indicated concentrations were added to this solution.

Electrophysiological measurements were made in 3–15 individual oocytes (from three or more separate batches) for each experimental maneuver; the data presented here, unless stated, were averaged from all oocytes tested and are expressed as means ± S.E. Amino acid fluxes were measured using \(^{3}H\)-labeled l-histidine (Amersham Corp., UK) and l-arginine (DuPont NEN, UK) by a method described previously (12, 16). Tracer flux data are expressed as means ± S.E. for experiments performed on 10–30 individual oocytes from one to three separate batches. Analysis of variance tests were performed where appropriate.

**RESULTS**

In current-clamped NBAT-expressing oocytes (resting membrane potential −62 ± 7 mV) superfusion of l-histidine (0.1 mM in NaCl medium, pH 7.5) caused a depolarization (7 ± 0.4 mV, n = 4 oocytes) of the oocyte cell membrane; there was only a small (<1 mV) depolarization in the membrane potential of native oocytes.

Superfusion of NBAT-expressing oocytes, clamped at a holding potential (V\(_h\)) of −60 mV, with l-histidine (1 mM) in 100 mM NaCl medium at pH 7.5 evoked a reversible inward current (I\(_{\text{His}}\) = −14.7 ± 1.6 nA, n = 10 oocytes; Fig. 1A) which was 14-fold higher than I\(_{\text{His}}\) in native oocytes (−1.0 ± 0.2 nA, n = 5 oocytes; Fig. 1A). Superfusion of NBAT-expressing oocytes with l-histidine (1 mM) in 100 mM TMACl medium also evoked an inward current (−5.7 ± 0.8 nA, n = 5 oocytes; Fig. 1B). In native oocytes, superfusion with l-histidine under similar conditions evoked a small inward current (−0.4 ± 0.3 nA, n = 6 oocytes, Fig. 1B), which was not significantly different from zero. Other neutral amino acids such as l-phenylalanine evoked outward currents at pH 7.5 (24 ± 1.7 nA, n = 5 oocytes; Fig. 1C). Although Na\(^+\)−dependent, the I\(_{\text{Ph}}\) was found to be Cl\(^−\)-independent, since currents of similar magnitudes were observed for 0.1 mM l-histidine in 100 mM NaCl (−7.5 ± 1.2 nA) or equimolar sodium isothionate (−6.1 ± 1.4 nA, n = 11 oocytes) media; these currents were not statistically different (p > 0.5).

Although predominantly neutral at physiological pH, l-histidine (pH = 7.6) is protonated at acidic pH (<7.0) and essentially uncharged at basic pH (>8.0). We therefore tested the hypothesis that at relatively acidic pH values, at which the imidazole ring of the histidine molecule is protonated, histidine would behave like a cationic amino acid with the result that the magnitude of I\(_{\text{His}}\) would increase. Indeed, the magnitude of I\(_{\text{His}}\) did increase progressively with decreasing pH (Table I) in both NaCl and TMACl media; furthermore, subtraction of I\(_{\text{His}}\) measured in TMACl medium from that in NaCl indicates that this increase in I\(_{\text{His}}\) was Na\(^+\)-independent (Table I).

The inward I\(_{\text{His}}\) at pH 7.5 and 6.5 was saturable (Fig. 2 and Table II) with an Na\(^+\)-dependent I\(_{\text{His}}\) in NaCl (I\(_{\text{His}}\) in NaCl − I\(_{\text{His}}\) in TMACl medium) and Na\(^+\)-independent (I\(_{\text{His}}\) in TMACl medium) component (Table II); the I\(_{\text{max}}\) of the Na\(^+\)-dependent component at pH 6.5 and 7.5 was similar (Table II). These findings were corroborated by the tracer flux studies, which showed that the uptake of l-[\(^{3}H\)]histidine was Na\(^+\)- and pH-dependent and that the initial rate of histidine uptake was greater at pH 6.5 than at pH 7.5 (Table III). This increase was largely Na\(^+\)-independent, as found for I\(_{\text{His}}\). The measurement of flux using radiolabeled histidine also allowed us to calculate (by use of Faraday’s constant: F = 9.65 × 10\(^{4}\) C mol\(^{−1}\)) the apparent stoichiometry of the l-histidine: charge movement (Table III). The calculated charge flux ratio for l-histidine:charge movement was found to be similar for the Na\(^+\)-dependent components at pH 6.5 and 7.5 (Table III).

Superfusion of NBAT-expressing oocytes with 1 mM l-histidine at pH 8.5 (when the imidazole ring of histidine should be
entirely uncharged) evoked a saturable outward current in TMACl medium ($K_n = 657 \pm 110 \mu M$; $I_{max} = 13 \pm 1.5 \mu A$; $n = 5$ oocytes), which was Na$^+$-independent (outward current for 1 mM L-histidine was $6.7 \pm 1.4 \mu A$ versus $7.7 \pm 1.3 \mu A$, $n = 4$, in 0.1 M NaCl and TMACl media, respectively), as observed for other neutral amino acids in NBAT- and rBAT-expressing oocytes (12, 15) (see also Fig. 1C). L-Histidine (1 mM), in NaCl medium, evoked an inward current ($-1.1 \pm 0.1 \mu A$, $n = 3$) in native oocytes at pH 8.5.

Transport of histidine in NBAT-expressing oocytes was stereospecific, since similar currents were observed, at neutral pH (7.5), in both native and NBAT-expressing oocytes upon superfusion of 1 mM D-histidine ($-1.5 \pm 0.5 \mu A$ versus $-1.8 \pm 0.3 \mu A$, $n = 6$, for native and NBAT-expressing oocytes, respectively, in NaCl medium). This is in contrast with results obtained previously for phenylalanine or arginine, whose transport shows poor stereospecificity in NBAT-expressing oocytes (12); D-isomers of both phenylalanine and arginine evoke outward and inward currents, respectively, of similar magnitude (between 60 and 80%) to those observed with L-isomers in NBAT-expressing oocytes (12).

We next tested the ability of NBAT-induced histidine transporter to accept structurally related analogues of histidine. Superfusion of 1 mM L-3-methyl-histidine (pH 7.5) evoked an inward current ($-8.5 \pm 1.1 \mu A$, $n = 4$) which was $-6$-fold higher than those observed in native oocytes ($-1.3 \pm 0.1 \mu A$, $n = 3$). Similar currents were observed upon superfusion of equimolar L-1-methyl-histidine ($-6 \pm 0.8 \mu A$, $n = 5$) in NBAT-expressing oocytes (a small inward current was observed in native oocytes: $-1.5 \pm 0.4 \mu A$, $n = 3$). Also, NBAT-expressing oocytes show stimulated transport of a- as well as $\beta$-isomers for amino acids such as phenylalanine (12) or alanine.

In order to test the hypothesis that NBAT-induced L-histidine transport shared this characteristic, we investigated the action of histidinol, a $\beta$-analogue of histidine, on NBAT-expressing oocytes. No detectable currents were observed upon superfusion of histidinol, indicating that unlike the NBAT-induced phenylalanine transport, the NBAT-induced histidine transport does not accept the $\beta$-isomers of its common substrate.

In order to determine the substrate specificity of the NBAT-induced histidine transporter we measured the uptake of L-[3H]histidine in the presence of neutral and cationic amino acids known to be transported in NBAT-expressing oocytes (10–12). At pH 7.5, the total uptake (NaCl medium) of L-[3H]histidine (0.05 mM) in current-clamped native oocytes was inhibited by most neutral amino acids (at 1 mM) including the L-isomers of histidine, alanine, phenylalanine and the non-chiral $\beta$-alanine by 40–80% (Table IV); cationic L-arginine, at a 20-fold excess, inhibited L-histidine uptake by 59% (Table IV); b-histidine had a small (20%) inhibitory effect, whereas L-$\beta$-phenylalanine had no inhibitory effect on the uptake of L-[3H]histidine in native oocytes. The pattern of inhibition was similar in current-clamped NBAT-expressing oocytes except that L-$\beta$-phenylalanine, at a 20-fold inhibitor excess, had a strong (80%) inhibitory effect on histidine uptake, whereas $\beta$-alanine, which inhibited the endogenous histidine transporter, caused no inhibition of the NBAT-induced histidine uptake. b-Histidine had a small (20%) inhibitory effect on the transport L-[3H]histidine, indicating that the NBAT-induced histidine transporter was stereospecific, corroborating the results obtained with electrophysiological measurements.

We had now established, first, that the cationic form of L-histidine was mainly transported by the Na$^+$-independent component and that L-arginine strongly inhibited the total uptake of L-[3H]histidine and, secondly, that histidine transport at pH 7.5, unlike that of the Na$^+$-independent arginine or
phenylalanine transport, was stereoselective in NBAT-expressing oocytes. It is known that cationic amino acids (e.g. arginine and lysine) cause a depolarization of the resting membrane potential in NBAT-expressing oocytes (12, 15). In fact, at 1 mM (the inhibitor concentration at which arginine strongly inhibits histidine uptake in current-clamped oocytes; see Table IV) arginine causes a depolarization of 29 mV (n = 3) of the oocyte cell membrane in NBAT-expressing oocytes. Thus, we went on to test the hypothesis that cationic histidine was a substrate for the Na+-independent, nonstereoselective NBAT-induced arginine transporter. We therefore determined whether or not (i) the strong inhibition of histidine transport by arginine in current-clamped oocytes (Table IV) was also observed under voltage-clamped conditions; (ii) there was a differential inhibition of L-arginine uptake by L-histidine at pH 7.5 and 6.5, in NaCl and TMACl medium; and (iii) there was an increase in the magnitude of L-histidine-evoked current, in an Na+-independent manner, at pH 6.5 (at which D-histidine is predominantly cationic).

First, under voltage-clamped (V_h = −60 mV) conditions, 40% of NBAT-induced L-[3H]histidine (50 μM) uptake was not inhibited by 1 mM L-arginine (Fig. 3). Conversely, under current-clamped conditions, 1 mM L-arginine caused >95% inhibition of L-[3H]histidine (50 μM) uptake (Fig. 3). These results indicate that a significant portion of arginine inhibition of histidine uptake is a secondary event of membrane depolarization that would reduce electrogenic histidine transport. Second, unlike for histidine uptake, there was no significant difference in L-[3H]arginine (50 μM) uptake at either pH 7.5 or 6.5 (Fig. 4), as would have been expected if the change in the rate of substrate transport were a function of the charge on the molecule, since the ionizable guanidinium group of arginine is fully protonated at neutral pH. Similar results were obtained for L-arginine-evoked currents at pH 7.5 and 6.5 with electrophysiological measurements (Fig. 5). L-[3H]Arginine (0.05 mM) uptake at pH 7.5 was inhibited by ~34% in the presence of 1 mM L-arginine in NaCl medium (Fig. 4), and a similar decrease in L-arginine uptake in the presence of L-histidine was observed in TMACl medium (Fig. 4), indicating that this inhibition was Na+-independent. These results also show that transport of

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### TABLE II

| pH        | NaCl | TMACl | Na+-independent |
|-----------|------|-------|-----------------|
| 7.5       | 56 ± 7 | 61 ± 9 | 116 ± 26 |
| 6.5       | 61 ± 2 | 116 ± 26 | 69.7 ± 10 |

### TABLE III

| Amino acid | Control | L-Arginine | D-Histidine | +L-Arginine |
|------------|---------|------------|-------------|-------------|
| Control histidine | 100 ± 8 | 100 ± 5 |
| +L-Arginine | 11 ± 1 | 50 ± 9 |
| +L-Arginine | 6 ± 0.3 | 36 ± 14 |
| +L-Arginine | 8 ± 2 | 36 ± 5 |
| +L-Arginine | 20 ± 5 | 150 ± 20 |
| +L-Arginine | 3 ± 0.7 | 41 ± 9 |
| +L-Arginine | 77 ± 5 | 80 ± 13 |
| +L-Arginine | 120 ± 30 | 63 ± 5 |

# FIG. 3

**Inhibition of L-[3H]histidine (50 μM) uptake by 1 mM L-arginine at pH 7.5.** Oocytes (voltage-clamped at −60 mV) were superfused with 50 μM L-[3H]histidine (1.4 kBq/ml) in NaCl transport medium with or without 1 mM L-arginine for 300 s at room temperature. After superfusion, oocytes were washed and counted as described previously (12, 21). For current-clamped oocytes, transport was measured as described under "Experimental Procedures." Data are normalized for the uptake observed in native oocytes in the presence and absence of arginine. Values are means ± S.E. for 5–10 oocytes from three different batches for voltage-clamped experiments and for 30 oocytes from three different batches for current-clamped experiments. Open bars, control L-[3H]histidine uptake; solid bars, histidine uptake in the presence of 1 mM L-arginine.
these two amino acids does not show strong mutual inhibition. When arginine uptake was measured at pH 6.5, a 20-fold excess of histidine (predominantly cationic at pH 6.5) had a strong inhibitory effect (>90%) on arginine uptake in NBAT-expressing oocytes (Fig. 4). As before, this inhibition was found to be the same in both NaCl and TMACI media. Third, there was a 4-fold increase in the n-histidine-evoked current at pH 6.5 compared with those observed at pH 7.5 in NBAT-expressing oocytes, which was similar in both NaCl and TMACI media (Fig. 5). These results indicate that cationic histidine transport, like that of cationic arginine transport (12), is Na\(^+-\)independent and nonstereoselective in NBAT-expressing oocytes.

**DISCUSSION**

We have demonstrated that in NBAT-expressing oocytes L-histidine transport is electrogenic and also that at physiological pH (7.5) influx of L-histidine evokes an inward current that is predominantly Na\(^+-\)dependent. The charge carried could be of a cation, moving in, or an anion, moving out. Since \(I_{\text{His}}\) was similar in the presence and absence of chloride, the first possibility seems most likely, indicating that a small fraction of histidine may be protonated at pH 7.5. The results, nonetheless, clearly demonstrate the dependence of \(I_{\text{His}}\) upon the prevailing Na\(^+\) gradient. Dependence of metabolite transport, particularly amino acid transport processes, on the prevailing Na\(^+\) gradient is a robust criterion by which transporter proteins can be classified and which has been utilized in both the traditional functional classification (based upon the characterization of cloned transporters (4, 19)) and the more recent structural classification (based upon the characterization of cloned transporters (4, 19)). By this criterion, it appears from the present results that, at physiological pH (7.5), histidine transport occurs via a mechanism different from that observed for the neutral phenylalanine or the cationic arginine in NBAT-expressing oocytes.

Another criterion that indicates whether or not two or more metabolites are substrates for the same protein is mutually competitive transport inhibition. At pH 7.5, histidine (1 mM) only weakly inhibited L\(^{3}H\)[H]arginine (0.05 mM) uptake, whereas ~40% of L\(^{3}H\)[H]histidine (0.05 mM) uptake was arginine-insensitive (at 20-fold excess inhibitor) under voltage-clamped conditions, indicating lack of strong mutual inhibition of the uptake of these amino acids. Only partial inhibition of 50 \(\mu\)M histidine uptake by a 20-fold excess of arginine, at pH 7.5, would be expected if the \(K_i\) for this inhibition were in the mM range, unlike the \(K_m\) for NBAT-induced Na\(^+-\)dependent histidine transport, which is 65 \(\mu\)M. These results suggest a separate pathway for histidine transport that may not be shared by arginine. Upon progressive protonation of the imidazole ring of histidine (from pH 7.5 to 7.0 to 6.0) we found almost all of the increase in \(I_{\text{His}}\) for cationic histidine to be attributable to the Na\(^+-\)independent component of \(I_{\text{His}}\). Only cationic histidine (at pH 6.5) reciprocated the strong arginine inhibition of its uptake, and this occurred in an Na\(^+-\)independent manner, indicating that the cationic arginine and cationic histidine transport may be mediated via the same carrier in NBAT-expressing oocytes.

Another distinguishing property of NBAT-induced histidine transport is its stereoselectivity. At pH 7.5, NBAT-induced transport of neither the cationic arginine nor neutral phenylalanine is stereospecific (12), whereas histidine transport in these oocytes is highly stereospecific. D-histidine transport in NBAT-expressing oocytes is stimulated only when D-histidine is cationic (i.e. at acidic pH), and it occurs in an Na\(^+-\)independent manner like that of arginine (12). Furthermore, a characteristic feature of NBAT-induced transport is its tolerance of the \(\beta\)-isoforms for neutral amino acids, although we found this did not extend to NBAT-induced histidine transport.

By these important functional criteria for categorizing different transporter proteins (i.e. Na\(^+-\)dependence, stereoselectivity, mutual inhibition, and lack of tolerance for \(\beta\)-analogaes), the modes of transport for histidine, arginine, and phenylalanine show disparate characteristics in NBAT-expressing oocytes. They are so disparate, in fact, that they strongly indicate that in NBAT-expressing oocytes more than one transport activity for amino acids is stimulated, clearly differentiating the histidine transporter from that or those carrying other neutral and cationic amino acids in NBAT-expressing oocytes (11, 12).

Our results indicate that transport of histidine at physiological pH (7.5), when histidine is predominantly neutral, is via an Na\(^+-\)dependent mechanism but that the predominantly cationic form of histidine is transported (like that of cationic arginine) via an Na\(^+-\)independent mechanism. This concept, that NBAT-induced transport of neutral histidine occurs via an Na\(^+-\)dependent carrier different from the arginine transporter, is strengthened because (i) there is lack of strong mutual inhibition at neutral pH of histidine and arginine transport, (ii) the mutual inhibition is observed only for the Na\(^+-\)independent modes of transport of cationic histidine and arginine, and (iii) only cationic and not neutral D-histidine transport is stimulated in NBAT-expressing oocytes. Our results also show that in NBAT-expressing oocytes, the mode of transport of entirely uncharged histidine (at pH 8.5) is distinct from that observed at physiological or acidic pH, since histidine evokes an outward current at pH 8.5, a normal characteristic of the transport of
neutral α- and β-amino acids in these oocytes (12).

Several reports (1–4, 8, 9), based primarily on functional studies, have suggested that electrogenic transport of neutral and cationic amino acids stimulated upon injection of NBAT or rBAT cRNA in X. laevis oocytes resembles the activity of the previously described System b0,−1 translocase; very recently, a model of double gated pore has been proposed as a mechanism for rBAT-induced amino acid transport (17). Conversely, the atypical structure of NBAT (one to four membrane-spanning domains (11, 13), which is unlike the structure of any known metabolite transporters, which have 10–12 membrane-spanning domains (4, 19)) has led to suggestions that NBAT may be an amino acid transport activating protein (4, 11, 14), but no direct functional evidence supporting this hypothesis had so far been provided. However, using NBAT-specific antibodies, Wang and Tate (14) have demonstrated the oligomeric organization of NBAT with other proteins and have postulated that the minimal functional unit of NBAT-mediated transport in kidney and intestine could be a heterodimer containing NBAT (85 kDa) and another, as yet unknown, protein of ~50 kDa. Using the same NBAT-specific antibodies, these authors have also reported that NBAT associates with a protein (or proteins) native to Xenopus oocytes, possibly to form a functional unit. Our results provide clear functional evidence that one of the NBAT-associating proteins transports histidine in a manner distinct from that previously described for other neutral or cationic amino acids in NBAT-expressing oocytes, and the simplest explanation for this is an amino acid transport-activating role for NBAT protein.

Addendum—Since the submission of this manuscript, Miyamoto et al. (20) have reported evidence, by expressing COOH-terminal deletion mutants of NBAT in Xenopus oocytes, which is consistent with the idea that NBAT is an activator rather than a System b0,−1-like transporter.

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