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Expression cloning of a Na^+-independent neutral amino acid transporter from rat kidney

(*Xenopus* oocytes/system L transport)

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ABSTRACT **Uptake of long-chain and aromatic neutral amino acids into cells is known to be catalyzed by the Na^+-independent system L transporter, which is ubiquitous in animal cells and tissues. We have used a *Xenopus* oocyte expression system to clone the cDNA of a system L transporter from a rat kidney cDNA library. The 2.3-kilobase cDNA codes for a protein of 683 amino acids. The transporter has four putative membrane-spanning domains and bears no sequence or structural homology to any known animal or bacterial transporter. When transcribed and expressed in *Xenopus* oocytes, the transporter exhibits many, but not all, of the characteristics of L-system transporters, suggesting that this represents one of several related L-system transporters.**

Nutrients, such as glucose and amino acids, are transported into cells by carrier-mediated systems. Transporters for the uptake of glucose by mammalian cells have recently been cloned and partially characterized (1). However, there is as yet little information available, at the molecular level, concerning mammalian amino acid transporters. Three principal transport systems have been recognized for neutral amino acids (NAAs) (2, 3), but attempts at their purification have met with limited success (4, 5). The system L transporter is Na^+ independent, shows a preference for the transport of branched-chain and aromatic amino acids such as leucine, isoleucine, phenylalanine, and tyrosine, and is largely responsible for the transport of nutritionally essential NAAs. In a previous report we described the expression of system L-type, mammalian, Na^+-independent amino acid transport in *Xenopus laevis* oocytes following injection of mRNA from rat kidney and human lymphoid cells (6). By employing the oocyte expression system to screen a rat kidney cDNA library for the transport of leucine and phenylalanine, we have succeeded in obtaining a cDNA clone that encodes a system L-type amino acid transporter. The nucleotide and deduced amino acid sequences[§] are presented, and a tentative topological model is proposed for the protein. The cloned transporter was expressed in oocytes, and some of its functional properties were examined.

MATERIALS AND METHODS

Materials. ^3H-labeled amino acids (highest specific activity available) and [α-^{32}P]dCTP were from Amersham. 2-Aminobicyclo[2.2.1]heptane-2-carboxylic acid (BCH) was a gift from H. N. Christensen (University of Michigan Medical School). The SuperScript plasmid system for the construction of cDNA libraries was from Bethesda Research Laboratories. The Riboprobe RNA transcription kit and T7 RNA polymerase were from Promega. Restriction endonucleases were from Boehringer Mannheim. Rat kidney poly(A)$^+$ RNA was isolated as described (6). Oocyte-positive *X. laevis* females, from Nasco (Fort Atkinson, WI), were maintained at 19°C.

Construction and Screening of a Rat Kidney cDNA Library. The cDNA library was constructed in the plasmid vector pSPORT 1, using the SuperScript protocol. cDNAs larger than 1.5 kilobases (kb) were selected and directionally cloned into the *Sal* I and *Not* I sites of the vector. The cDNA inserts in this vector are flanked by T7 and SP6 RNA polymerase promoters at their 5′ and 3′ ends, respectively, allowing sense as well as antisense transcription. The library consists of $\approx 7.7 \times 10^5$ independent clones.

For screening, aliquots of the rat kidney cDNA library containing 2000, 4000, 8000, 12,000, 20,000, and 40,000 clones were amplified, and plasmid DNA was purified from each pool using the Qiagen anion-exchange column system (kits purchased from Qiagen, Chatsworth, CA). Plasmid DNA was linearized with *Not* I and transcribed *in vitro* with T7 RNA polymerase in the presence of the GpppG cap, using a protocol supplied with the Riboprobe transcription system (Promega) except that the final concentrations of GTP and the cap analog were 0.3 and 1 mM, respectively. RQ1 DNase digestion was carried out to remove the template, and the RNA transcripts were purified by using an RNaid RNA purification kit (Bio 101, La Jolla, CA). The complementary RNAs (cRNAs) were injected into *Xenopus* oocytes, which were then assayed for expression of L-leucine and L-phenylalanine transport. The pool of 8000 clones that scored positive was subdivided as follows: six aliquots of the amplified pool, about 5000 bacteria each, were plated on 13.7-cm nitrocellulose membrane filters. Replicas were made as described (7) and grown until about 1-mm diameter colonies were obtained. Bacteria were then scraped off the filter, amplified, and screened for expression of NAA transport. The master filter, corresponding to the replica that scored positive, was cut into eight sectors; replicas of each sector were made and screened as described above. This was repeated until a sector containing about 150 colonies remained. The colonies were picked and individually seeded into the wells of a 48-well, flat-bottom, culture plate to produce a grid system that allowed identification of a positive clone in a single screening cycle involving the testing of rows and columns from the positive-scoring plate.

Microinjection of mRNA into *X. laevis* Oocytes and Measurement of the Expressed Amino Acid Transport. *Xenopus* oocytes (stages V and VI) were isolated as described (8) and allowed to recover overnight at 19°C in modified Barth's saline (8) supplemented with 2.5 mM sodium pyruvate, penicillin (100 units/ml), and streptomycin (100 μg/ml). The

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Abbreviations: BCH, 2-aminobicyclo[2.2.1]heptane-2-carboxylic acid; NAA, neutral amino acid; NAA-Tr, neutral amino acid transporter; cRNA, complementary RNA; CAA, cationic amino acid.

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§The sequence reported in this paper has been deposited in the GenBank data base (accession no. M77345).

oocytes were microinjected with either 50 nl of water (controls) or 50 nl of solution containing either rat kidney mRNA (35 ng) or cRNA transcribed *in vitro* from pools of the rat kidney cDNA library. The oocytes were maintained at 19°C. During the initial stages of screening, about 50 ng of transcribed cRNA was injected per oocyte. In the final stages of screening, 10 ng of cRNA was injected per oocyte. For functional characterization of the transporter clone, 5 ng of the transcribed cRNA was injected per oocyte along with 5 ng of the cRNA that codes for a secreted mutant form of human placental alkaline phosphatase, which serves as an internal standard to monitor translation in oocytes (9).

NAA transport into mRNA-injected oocytes was assayed 48 or 72 h after injection as described (6), except that the concentration of the ^{3}H-labeled amino acid (either L-phenylalanine or L-leucine) was 50 μM (in 0.015 M Tris·HCl/0.1 M NaCl, pH 7.6). Uptake was generally determined after a 5- to 20-min incubation at 20°C and is expressed as picomoles of amino acid per oocyte per 5 min, presented as the average of six to eight oocytes.

Sequencing of the cDNA Clone. The nucleotide sequence of the transporter cDNA clone was determined by the dideoxy chain-termination method (10) using the Sequenase DNA sequencing kit (United States Biochemical). Both strands of the clone were sequenced using M13/pUC and T7 sequencing primers as well as synthetic oligonucleotide primers deduced from the partially determined sequence. Subclones containing progressive unidirectional deletions were generated using the Erase-a-Base system (Promega). These were also sequenced, providing additional confirmation of the obtained sequence. Nucleotide and predicted amino acid sequences were assembled and analyzed using the software tools from Genetics Computer Group (Madison, WI). Searches of the National Biomedical Research Foundation protein data base (release 29), GenBank (release 68) data base, and the European Molecular Biology Laboratory (release 26) nucleotide data base were performed by using the FASTA (Genetics Computer Group) search programs.

Northern Blot Analysis. Poly(A)$^+$ RNA (2 μg) prepared from various rat tissues [as described (6)] was fractionated on 1% agarose/formaldehyde gels and blotted onto a Nytran membrane. The entire insert of the cDNA clone was released from the plasmid with the restriction endonucleases *Mlu* I and *Not* I and purified on agarose gels. ^{32}P-labeled probes were prepared by the random oligonucleotide primer labeling method using an oligolabeling kit from Pharmacia. Hybridization and washings of the blot were carried out as described (11). We thank H. Chahine for rat heart and HeLa cell mRNA and also R. Molinar-Rode and P. McKinnon (Roche Institute) for total RNA from PC-12 cells and rat liver. Caco-2 cells, a human colon carcinoma cell line, were from C. Bailey (Hoffmann–La Roche).

RESULTS AND DISCUSSION

Isolation of a Clone Encoding a Rat Kidney NAA Transporter (NAA-Tr). An enriched, directional cDNA library constructed in the plasmid vector pSPORT 1 was screened with the *Xenopus* oocyte expression system and L-phenylalanine and L-leucine as the transported substrates. Initially, only a small increment in transport (3- to 4-fold over controls) was seen in oocytes injected with cRNA synthesized from an aliquot of the library containing ≈8000 individual clones. Each subsequent division of this pool elicited greater expression of L-phenylalanine and L-leucine transport. Eventually, a single clone, pSPORT/NAA-Tr, was isolated from the final positive pool of 48 clones. As little as 1 ng of cRNA transcribed from this clone elicited initial rates of NAA transport in oocytes that were 30- to 40-fold greater than those seen in water-injected oocytes.

Functional Characterization of the Clone. cRNA (but not antisense cRNA; data not shown) transcribed *in vitro* from the clone pSPORT/NAA-Tr elicited a large enhancement of L-phenylalanine transport when injected into oocytes (Fig. 1). Similar data were obtained with L-leucine. High levels of transport were maintained in injected oocytes as long as 6 days postinjection. Fig. 1 also shows the level of expression after injection of the original rat kidney mRNA (35 ng per oocyte) for comparison. The rates of uptake of L-phenylalanine and L-leucine in oocytes injected with NAA-Tr cRNA (5 ng per oocyte) were linear up to 10 min. At 20 min, the injected oocytes accumulated L-phenylalanine to a concentration ≈6 times that in the extracellular medium (assuming an oocyte volume of 1 μl). To exclude the possibility that pSPORT/NAA-Tr encodes a transcription factor, oocytes injected with pSPORT/NAA-Tr cRNA were kept for 18 h in modified Barth's saline supplemented with actinomycin D (50 μg/ml) to inhibit endogenous transcription (12). Under these conditions, the injected oocytes exhibited the same levels of L-phenylalanine transport as those incubated in modified Barth's saline in the absence of actinomycin D (data not shown), indicating that injection-induced transcription from the oocyte DNA does not contribute to the observed expression of amino acid transport.

The specificity of Na$^+$-independent uptake into NAA-Tr cRNA-injected oocytes was tested with several amino acids (Fig. 2). The highest rates of uptake were seen with NAAs possessing bulky side chains such as L-phenylalanine, L-leucine, and L-methionine. Next in activity were L-alanine, L-glutamine, and L-histidine (neutral at pH 7.6). α-Aminoisobutyrate, a synthetic substrate for systems A and ASC, was not a substrate for the expressed transporter, and neither was glutamate or lysine. Kinetic studies showed that uptake of L-phenylalanine, L-leucine, L-methionine, L-alanine, and L-histidine is saturable, demonstrating expression of a carrier-mediated transport system. The kinetic parameters calculated from the data are listed in Table 1. The K_m values determined for L-phenylalanine and L-leucine are within the range of values reported for the high-affinity system L-type transporters in a variety of tissues and cells (13–15).

Effects of other amino acids on the transport of Na$^+$-independent L-phenylalanine by NAA-Tr cRNA-injected oocytes are shown in Fig. 3. Similar effects were seen when L-leucine was used as the transported substrate (data not

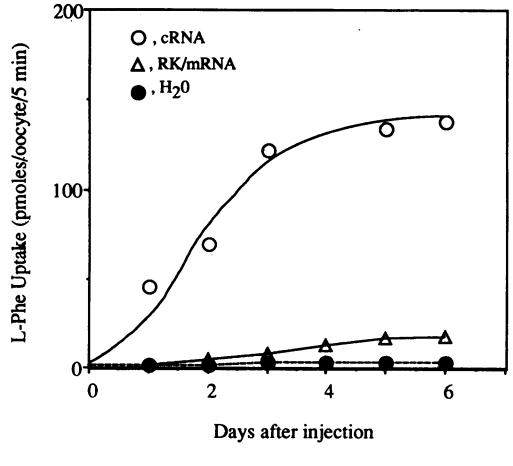

FIG. 1. Expression of the rat kidney Na$^+$-independent NAA-Tr in *Xenopus* oocytes. The oocytes were injected with either water, rat kidney (RK) mRNA (35 ng per oocyte), or NAA-Tr cRNA (5 ng per oocyte). L-Phenylalanine uptake into oocytes at 20°C was determined in 1 ml of Tris/NaCl (0.015 M Tris·HCl/0.1 M NaCl, pH 7.6) containing 50 μM L-[2,6-^{3}H]phenylalanine (final specific activity, 100 μCi/μmol; 1 Ci = 37 GBq). Each point is an average of six to eight oocytes.

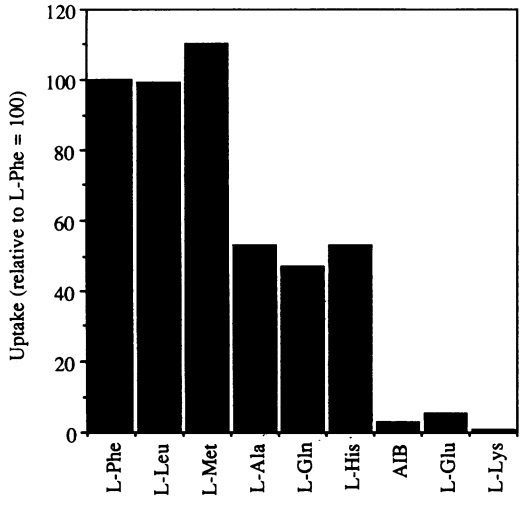

FIG. 2. Substrate specificity of the rat kidney NAA-Tr. The uptake was determined at a radiolabeled amino acid concentration of 50 μM in 1 ml of Tris/NaCl in oocytes injected with NAA-Tr cRNA (5 ng per oocyte). Measurements were made 3 days after the injection, and each value is an average of six to eight oocytes (relative to L-phenylalanine uptake, set at 100). AIB, α-aminoisobutyrate.

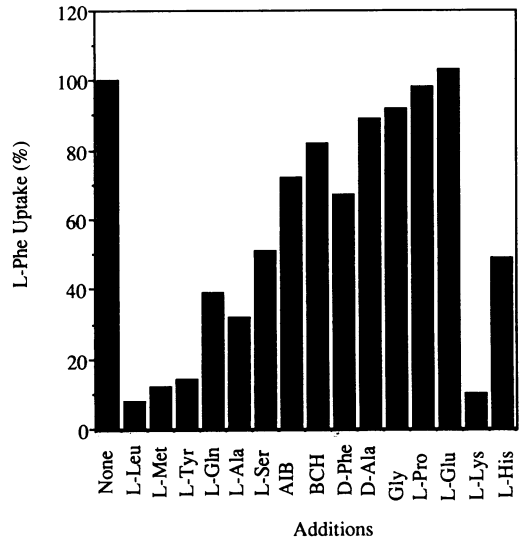

FIG. 3. Effect of various amino acids on L-phenylalanine transport. Oocytes injected with 5 ng of NAA-Tr cRNA were assayed 3 days after injection. Uptake of L-[^{3}H]phenylalanine (50 μM) was determined in Tris/NaCl either without (control = 100) or with added, unlabeled amino acid. The concentration of each added amino acid was 1 mM, except that α-aminoisobutyrate (AIB) and BCH were present at 5 mM.

shown). As expected, other large NAAs were potent inhibitors. Next in inhibitory potency were NAAs such as L-glutamine, L-alanine, L-serine, and L-histidine (the latter is a neutral amino acid at pH 7.6). D-Phenylalanine and D-alanine had little or no effect on L-phenylalanine transport, neither did glycine, L-proline, and L-glutamate. BCH, a specific inhibitor of L-type transport (16), inhibited ≈20% at 5 mM. Unexpectedly, L-lysine, a cationic amino acid that is not a substrate for this transporter (Fig. 2), was a powerful inhibitor of L-phenylalanine and L-leucine uptake (Fig. 3). Although this finding needs further investigation, preliminary experiments suggest that L-lysine most likely interacts with the oocyte membrane. Transport of large NAAs studied in a variety of animal cells is virtually unaffected by L-lysine (13, 17, 18).

Replacing NaCl in the extracellular medium with either KCl or LiCl had no effect on L-phenylalanine transport into NAA-Tr cRNA-injected oocytes (the same was found with L-leucine as substrate), and significant transport (≈60% of that seen in the presence of NaCl) occurred in media containing choline chloride, sucrose, and *N*-methyl-D-glucamine. Thus, pSPORT/NAA-Tr cDNA encodes a rat renal NAA-Tr that exhibits characteristics expected of a system L transporter (i.e., Na^+ independence).

DNA and Deduced Amino Acid Sequences. Sequence analysis of the cDNA insert of plasmid pSPORT/NAA-Tr reveals a 2049-base-pair open reading frame within a 2259-base-pair sequence [excluding the poly(A) tail] (Fig. 4*A*), a 5′ untranslated region of 38 nucleotides, and a 3′ untranslated region of 172 nucleotides. The region immediately surrounding the putative start codon contains purines at positions +4 and −3 and therefore represents a reasonable Kozak consensus sequence (20). The open reading frame predicts a protein containing 683 amino acids with a calculated molecular weight of 78,506 and seven possible N-glycosylation sites. A hydrophobicity plot (19) (Fig. 4*B*) of the deduced amino acid sequence reveals at least four sequences (each 23–28 amino acids) that are candidates for transmembrane domains. The second and fourth putative transmembrane domains (residues 390–413 and 588–610, respectively) include amino acid residues with charged side chains, a feature the NAA-Tr shares with other membrane proteins such as the glucose transporters (21, 22), γ-aminobutyrate (23) and norepinephrine (24) transporters, and several of the ion channels (25). In common with such carriers, the predicted sequence does not contain an amino-terminal signal peptide that conforms to the empirical rules predicted for such sequences (26).

Table 1. Kinetic parameters for the transport of amino acids by the rat kidney NAA-Tr

Amino acid	K_m, μM	Relative V_{max}
L-Phenylalanine	29	100
L-Leucine	22	97
L-Alanine	50	56
L-Methionine	71	151
L-Histidine	167	126

Xenopus oocytes were injected with 5 ng of NAA-Tr cRNA per oocyte. Three days after injection, amino acid uptake was determined in Tris/NaCl (pH 7.6) at 20°C. The concentration of each amino acid was varied from 10 to 100 μM. Preliminary experiments showed that uptake increased linearly with time up to 10 min. V_{max} was expressed as pmol of amino acid taken up per oocyte per 5 min.

Tissue Distribution of the NAA-Tr mRNA. Northern transfer and hybridization analysis with the full-length NAA-Tr cDNA probe revealed the presence of a 2.4-kb RNA in poly(A)$^+$ RNA isolated from rat kidney (Fig. 5). From the size of the cRNA and its ability to be functionally expressed in oocytes, we conclude that the cloned cDNA is probably full length. Northern transfer and hybridization signals (although much weaker) were also obtained with rat heart (about 5 kb), lung (2.5 kb), and tongue, and with Caco-2 cells (2.4 kb) (data not shown). Unexpectedly, poly(A)$^+$ RNA obtained from RPMI-8226 cells (a lymphoblastic cell line derived from a patient with multiple myeloma) did not yield a positive Northern signal with the NAA-Tr cDNA. This is significant (see below) since we had previously shown expression of system L-type amino acid transport in oocytes injected with mRNA from these cells (6). The NAA-Tr cDNA probe did not react with mRNAs from rat liver, brain, or muscle (data not shown).

The function of transporters is to serve as portals for regulating the entry of compounds that would otherwise not penetrate the plasma membrane. They must, therefore, be transmembrane proteins. Indeed, four transmembrane do-

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                                                                                                    CTACTGGAAAGCACTGGGAAGAGCTGCACCGGATAGAC     38

ATGAATGAGGACAAAGACAAGAGAGACTCTATCCAGATGAGTATGAAGGGATGCCGGACGAATAACGGGTTTGTCCAAAATGAAGACATCCAGGAGCAGGACCCAGACTCCAGGGACACT    158
MetAsnGluAspLysAspLysArgAspSerIleGlnMetSerMetLysGlyCysArgThrAsnAsnGlyPheValGlnAsnGluAspIleGlnGluGlnAspProAspSerArgAspThr     40

CCACAGTCCAACGCTGTTAGTATCCCTGCTCCAGAGGAGCCTCAACTAAAGGTGGTGCGGCCCTATGCAGGGATGCCCAAGGAGGTGCTGTTCCAGTTCTCTGGCCAGGCTCGCTACCGG    278
ProGlnSerAsnAlaValSerIleProAlaProGluGluProGlnLeuLysValValArgProTyrAlaGlyMetProLysGluValLeuPheGlnPheSerGlyGlnAlaArgTyrArg     80

GTGCCTCGGGAGATCCTCTTCTGGCTCACCGTGGTCTCCGTGTTCCTGCTCATCGGAGCCACCATAGCCATCATCATCATCTCTCCAAAATGCCTTGACTGGTGGCAGGCAGGTCCCATG    398
ValProArgGluIleLeuPheTrpLeuThrValValSerValPheLeuLeuIleGlyAlaThrIleAlaIleIleIleIleSerProLysCysLeuAspTrpTrpGlnAlaGlyProMet    120

TACCAGATCTACCCGAGGTCTTTTAAGGACAGTGACAAGGATGGGAATGGAGACCTGAAAGGTATCCAAGAGAAGCTGGACTACATCACTGCTTTAAATATAAAGACCATTTGGATCACT    518
TyrGlnIleTyrProArgSerPheLysAspSerAspLysAspGlyAsnGlyAspLeuLysGlyIleGlnGluLysLeuAspTyrIleThrAlaLeuAsnIleLysThrIleTrpIleThr    160

TCCTTTTATAAATCACCTTTGAAAGATTTTAGATATGCTGTTGAAGATTTCAAAGAAATTGACCCTATTTTCGGAACAATGAAAGATTTTGAGAATTTGGTTGCTGCGGTCCATGACAAA    638
SerPheTyrLysSerProLeuLysAspPheArgTyrAlaValGluAspPheLysGluIleAspProIlePheGlyThrMetLysAspPheGluAsnLeuValAlaAlaValHisAspLys    200

GGTTTAAAATTAATAATTGACTTCATACCAAACCACACCAGTGACAAACATCCTTGGTTCCAATCGAGTAGGACACGGAGCGGGAAATACACTGATTATTACATCTGGCACAACTGTACC    758
GlyLeuLysLeuIleIleAspPheIleProAsnHisThrSerAspLysHisProTrpPheGlnSerSerArgThrArgSerGlyLysTyrThrAspTyrTyrIleTrpHisAsnCysThr    240

CACGCCAACGGTGTAACCACCCCTCCCAACAACTGGCTGAGCGTGTATGGAAACTCCAGCTGGCAGTTTGATGAAGAACGAAAGCAATGTTATTTTCACCAGTTTTTGAAAGAGCAGCCG    878
HisAlaAsnGlyValThrThrProProAsnAsnTrpLeuSerValTyrGlyAsnSerSerTrpGlnPheAspGluGluArgLysGlnCysTyrPheHisGlnPheLeuLysGluGlnPro    280

GATCTTAATTTCCGAAATCCTGCTGTTCAAGAAGAAATAAAGGAAATAATAAAGTTCTGGCTCTCAAAGGGTGTTGATGGGTTTAGTTTCGATGCAGTTAAATTTCTTCTGGAAGCAAAG    998
AspLeuAsnPheArgAsnProAlaValGlnGluGluIleLysGluIleIleLysPheTrpLeuSerLysGlyValAspGlyPheSerPheAspAlaValLysPheLeuLeuGluAlaLys    320

GACCTGAGAAATGAAATCCAAGTAAATACATCCCAAATTCCGGACACAGTGACCCGCTACTCAGAACTGTACCACGACTTCACCACTACCCAGGTGGGAATGCATGACCTTGTCCGGGAC    1118
AspLeuArgAsnGluIleGlnValAsnThrSerGlnIleProAspThrValThrArgTyrSerGluLeuTyrHisAspPheThrThrThrGlnValGlyMetHisAspLeuValArgAsp    360

TTCCGGCAGACCATGAACCAGTTCAGCCGGGAGCCTGGCAGATACCGGTTCATGGGGACGGAGGTGTCAGCTGAGAGCACCGAGAGGACCATGGTGTACTATGGCCTGTCATTTATCCAG    1238
PheArgGlnThrMetAsnGlnPheSerArgGluProGlyArgTyrArgPheMetGlyThrGluValSerAlaGluSerThrGluArgThrMetValTyrTyrGlyLeuSerPheIleGln    400

GAAGCTGACTTCCCTTTCAACAAGTACTTAGCCACACTAGACACTCTTTCCGGGCATACTGTGTACGAAGCTATCACATCCTGGATGGAAAACATGCCGGAAGGAAAATGGCCCAACTGG    1358
GluAlaAspPheProPheAsnLysTyrLeuAlaThrLeuAspThrLeuSerGlyHisThrValTyrGluAlaIleThrSerTrpMetGluAsnMetProGluGlyLysTrpProAsnTrp    440

ATGATTGGCGGACCAGAGACTTCTCGGCTGACTTCTCGAGTAGGGAGCGAGTATGTCAATGCCATGAACATGCTTCTGTTCACACTCCCAGGAACCCCCATAACTTACTATGGGGAAGAA    1478
MetIleGlyGlyProGluThrSerArgLeuThrSerArgValGlySerGluTyrValAsnAlaMetAsnMetLeuLeuPheThrLeuProGlyThrProIleThrTyrTyrGlyGluGlu    480

ATAGGGATGGGAGATATTTCCATTACAAACCTCAACGAGCGCTATGACACTAACGCCCTTCTCTCTCCAAGTCACCGATGCAGTGGGACAATAGTTCAAATGCGGGGTTTACTGAGGCCAAC    1598
IleGlyMetGlyAspIleSerIleThrAsnLeuAsnGluArgTyrAspThrAsnAlaLeuLeuSerLysSerProMetGlnTrpAspAsnSerSerAsnAlaGlyPheThrGluAlaAsn    520

CACACCTGGCTCCCCACAAACTCTGACTACCACACAGTGAATGTGGATGTCCAAAAAACCCAGCCGAGCTCAGCACTGAGGCTATATCAGGATCTGAGTCTACTCCATGCCAGAGAGCTG    1718
HisThrTrpLeuProThrAsnSerAspTyrHisThrValAsnValAspValGlnLysThrGlnProSerSerAlaLeuArgLeuTyrGlnAspLeuSerLeuLeuHisAlaArgGluLeu    560

CTTCTCAGCAGAGGCTGGTTTTGCCTTTTGAGGGACGACAATCACTCTGTTGTGTACACCAGGGAGCTGGATGGCATAGATAAAGTCTTCCTTGTGGTTCTGAATTTTGGAGAATCATCA    1838
LeuLeuSerArgGlyTrpPheCysLeuLeuArgAspAspAsnHisSerValValTyrThrArgGluLeuAspGlyIleAspLysValPheLeuValValLeuAsnPheGlyGluSerSer    600

ACTGTGCTAAATCTACAGGAAACTATTTCAGATGTTCCTACAAAACTGAGAATAAGATTAAGTACCAATCCAGCCTCCAAAGGCAGTGATGTTGATACCCATGCCGTTTCTCTGGAGAAG    1958
ThrValLeuAsnLeuGlnGluThrIleSerAspValProThrLysLeuArgIleArgLeuSerThrAsnProAlaSerLysGlySerAspValAspThrHisAlaValSerLeuGluLys    640

GGAGAGGGGCTCATCTTGGAACACAGCATGAAGACTCTCCTCCATCACCAGAAAGCTTTCAGAGACAAATGTTTTATTTCCAACCGTGCATGCTACTCCAGCGTGCTGGACCTTCTGTAT    2078
GlyGluGlyLeuIleLeuGluHisSerMetLysThrLeuLeuHisHisGlnLysAlaPheArgAspLysCysPheIleSerAsnArgAlaCysTyrSerSerValLeuAspLeuLeuTyr    680

AGCTCGTGCTAGGCAGCTCTGTAAGAGGTGGCCACCCTGCGTCTGGTATGCTTGCCATCACACATGCAAGGGCCTCAGGAATGGCATCAGTTCTTAGATATTTCTGTAGCACGAATGCAT    2198
SerSerCys***

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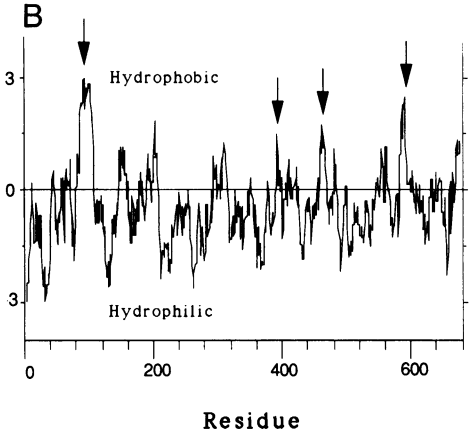

FIG. 4. (*A*) Nucleotide and deduced amino acid sequence of the rat kidney NAA-Tr cDNA. The amino acid sequence is numbered from the presumed initiator methionine, and the termination codon is marked with asterisks. Putative transmembrane domains (four) predicted by the Kyte–Doolittle algorithm are underlined, and the potential N-glycosylation sites are boxed. The polyadenylylation signal is marked by a wavy line. (*B*) Hydropathy plot of the NAA-Tr deduced amino acid sequence. Hydrophobicity values were calculated as described (19) with a window of nine residues. The arrows indicate the four putative membrane-spanning domains.

mains are readily apparent in the cDNA deduced sequence of the cloned renal NAA-Tr (Fig. 4*A*). Furthermore, the absence of a signal peptide implies that the amino terminus is located on the cytoplasmic side of the plasma membrane. A tentative topological model showing the proposed orientation of the renal NAA-Tr is depicted in Fig. 6. Each of the four putative membrane-spanning domains is assigned a transmembrane orientation. The amino acids at the carboxyl terminus are most likely in the cytoplasm. This orientation places all potential N-glycosylation sites extracellularly.

The amino acid sequence of the renal NAA-Tr bears no homology to known mammalian (or bacterial) transporters nor to other membrane proteins. The topological model with four putative transmembrane domains differs strikingly from transporters for glucose (1). The Na^+-glucose cotransporters contain 11 membrane-spanning domains and the facilitative glucose transporters contain 12. The renal NAA-Tr also bears no similarity to transporters for γ-aminobutyric acid (23) and norepinephrine (24), each of which has 12 transmembrane segments. Recently, two reports appeared demonstrating that cRNA transcripts from cDNA encoding a murine eco-

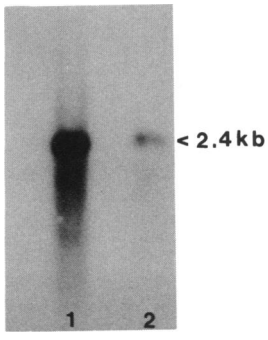

FIG. 5. Northern blot analysis of rat mRNA. A blot prepared from cRNA transcribed *in vitro* from NAA-Tr cDNA (1 ng) (lane 1) and rat kidney poly(A)$^+$ mRNA (2 μg) (lane 2) was hybridized to a ^{32}P-labeled NAA-Tr cDNA probe. Prehybridization and hybridization were at 42°C in 50% formamide, 5× Denhardt's reagent, 5× standard saline phosphate/EDTA, 0.1% SDS, and salmon sperm DNA at 100 μg/ml. The blot was washed with 2× standard saline citrate and 0.1% SDS at 55°C for 1 h and exposed to the film for 6 h.

tropic retrovirus receptor (eco R) expressed transport of the cationic amino acids (CAAs) L-arginine, L-lysine, and L-ornithine (27, 28). The properties of the expressed transporter resemble those ascribed to system y^+, the principal Na^+-independent transporter of CAAs. The rat renal NAA-Tr exhibits no sequence homology to the CAA transporter and is strikingly different with respect to the number of membrane-spanning domains. The four membrane-spanning domain motif is a feature shared by the ligand-gated receptor–ion channel family of proteins that include the nicotinic acetylcholine, γ-aminobutyrate, glycine, and glutamate (kainate subtkype) receptors (31). However, these receptors are synthesized with cleavable NH_2-terminal signal peptides and, in the mature proteins, the NH_2 and COOH terminals are located extracellularly.

System L-type transport of nutritionally essential amino acids has been demonstrated in a large number of tissues and cells (including kidney, intestine, liver, brain, lymphoid cells, etc.). However, Northern blot analysis indicates that the NAA-Tr described here has a limited tissue distribution. While conforming to a typical L-type transporter in many ways (i.e., Na^+ independence and kinetic parameters for uptake of leucine, phenylalanine, and other NAAs), it exhibits some striking differences. The first was the failure of the specific inhibitor of system L transport, BCH, to produce significant inhibition. The second is that mRNA from lymphoblastoid cells, which are known to possess an L-type amino acid transporter, did not yield a Northern transfer and

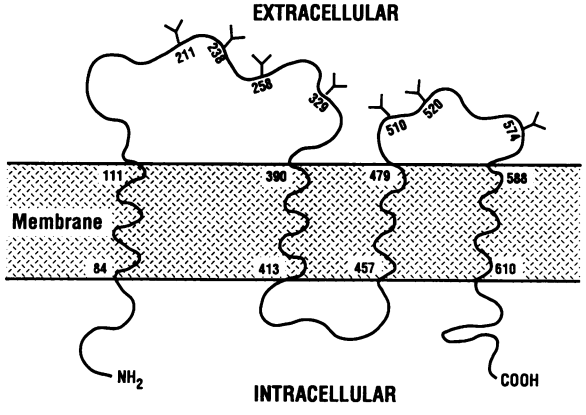

FIG. 6. Schematic representation of the rat kidney NAA-Tr showing the proposed orientation in the plasma membrane. Each membrane-spanning domain is assigned a transmembrane orientation. The amino terminus is placed inside the cell because of the absence of an identifiable leader signal sequence. This orientation places all potential N-glycosylation sites, each marked by a "Y" and numbered, extracellularly. The end points of the membrane-spanning segments are numbered. Note that the various segments are not drawn to scale.

hybridization signal with the full-length NAA-Tr probe. Most striking, however, was the finding that L-tryptophan was not transported into oocytes injected with the NAA-Tr cRNA and that tryptophan did not inhibit transport of leucine or phenylalanine into such oocytes (data not shown). This was so unexpected that, to rule out artifacts, studies were carried out with a variety of intact cells, which did indeed transport tryptophan (data not shown). Tryptophan is an essential amino acid and is known to be transported by intact tissues and cells in a manner that is competitive with leucine and phenylalanine (13–15, 29, 30). The NAA-Tr cloned from rat kidney may be one of, perhaps, several system L-type amino acid transporters with overlapping substrate specificities that are expressed in different tissues or at different stages of development.

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