Effects of Truncation of the COOH-terminal Region of a Na⁺-independent Neutral and Basic Amino Acid Transporter on Amino Acid Transport in Xenopus Oocytes*

(Received for publication, January 3, 1996, and in revised form, March 8, 1996)

Ken-ichi Miyamoto, Hiroko Segawa, Sawako Tatsumi, Kanako Katai, Hironori Yamamoto, Yutaka Takekami, Hiromi Haga, Kyoko Morita, and Eiji Takeda

From the Department of Clinical Nutrition, School of Medicine, Tokushima University, Kuramoto-Cho 3, Tokushima 770, Japan

To determine the role of a neutral and basic amino acid transporter (NBAT) in amino acid transport, we microinjected several COOH-terminal deletion mutants of NBAT cRNA into Xenopus oocytes and measured transport activity for arginine, leucine, and cystine in the presence and absence of sodium. Wild-type NBAT significantly stimulated the uptake of all three amino acids 10- to 20-fold compared with controls. On the other hand, no mutant, except a Δ511-685 mutant, stimulated the uptake of these amino acids. The Δ511-685 mutant significantly increased the uptake of arginine. In the presence of sodium, the Δ511-685 mutant also increased the uptake of leucine. The Δ511-685 mutant did not stimulate cystine uptake in the presence or absence of sodium. The stimulation of arginine uptake by the Δ511-685 mutant was inhibited by a 100-fold excess of unlabeled leucine in the presence of sodium. Inhibition of L-arginine uptake by L-homoserine was seen only in the absence of sodium. The Δ511-685 mutant did not stimulate cystine uptake in the presence or absence of sodium. The stimulation of arginine uptake by the Δ511-685 mutant was inhibited by a 100-fold excess of unlabeled leucine in the presence of sodium. Inhibition of L-arginine uptake by L-homoserine was seen only when the extracellular pH was decreased. Furthermore, an inward current in oocytes injected with the Δ511-685 mutant was recorded electrophysiologically when basic amino acids were applied. Homoserine was also taken up, but sodium was necessary for their transport. These properties of the Δ511-685 mutant correspond to those of the y⁺ amino acid transporter. If NBAT is a component of the b0⁺-like amino acid transport system, it is unlikely that a mutant protein (Δ511-685) is able to stimulate an endogenous y⁺-like transport system. These results suggest that NBAT functions as a neutral and basic amino acid transporter (NBAT) functions as a neutral and basic amino acid transporter for system b0⁺(4–8). The specific mutations in the human NBAT gene have been identified in type II cystinuria patients (9–14). These mutations nearly abolish the amino acid transport activity induced by NBAT in Xenopus oocytes (9). However, the predicted structure of the NBAT protein is not particularly hydrophobic and contains only one or four transmembrane-spanning domains, suggesting that it functions as a transport activator and a regulatory subunit (4–6, 8). The predicted NBAT protein is believed to be a type II membrane glycoprotein, as has been shown for 4F2 (15, 16). The 4F2 cell surface antigen is a 125-kDa disulfide-linked heterodimer composed of an 85-kDa glycosylated heavy chain and a 41-kDa nonglycosylated light chain (17, 18). It was identified originally by the production of a mouse monoclonal antibody against the human T-cell tumor clone HSB-2 (18). Both Bertran et al. (15) and Wells et al. (16) have demonstrated that 4F2 cRNA injection into oocytes results in the stimulation of y⁺-like transport activity but not system b0⁺ (15). The murine receptor for the ectotropic murine leukemia virus has been identified as being responsible for system y⁺ activity, a sodium-independent, cation-preferred amino acid carrier (19, 20). The predicted protein for the ectotropic murine leukemia virus contains several membrane-spanning domains and has no homology with 4F2 antigen (15, 16). Thus, the function of the 4F2 proteins remains unclear. Furthermore, recent studies demonstrated that NBAT-mediated transport of neutral and dibasic amino acids is associated with net outward and inward currents, respectively, which may be caused by an exchange of neutral with dibasic amino acids (21–23).

To evaluate whether NBAT functions as a component of amino acid transporters (amino acid exchanger) or as a transport activator, we measured several mutant forms of NBAT and assayed their ability to stimulate transport activity. The transport and electrophoretic studies suggest that a mutant NBAT (Δ511-685) stimulates an endogenous y⁺-like transport system in Xenopus oocytes distinct from the b0⁺ system activated by wild-type NBAT. These results suggest that NBAT functions as a transport activator.

EXPERIMENTAL PROCEDURES

Materials—L-[³H]Arginine (70 Ci/mmol), L-[¹⁴C]Leucine (300 mCi/mmol), and L-[³⁵S]Cystine (300 Ci/mmol) were purchased from DuPont NEN. Oligonucleotides were synthesized on an Applied Biosystems 380B DNA synthesizer. Klenow enzyme, T7 RNA polymerase, T3 RNA polymerase, DNase I, T4 DNA ligase, restriction endonucleases, and T4 polynucleotide kinase were purchased from Boehringer Mannheim.

Cloning NBAT from a Human Kidney cDNA Library—RNA was

*This work was supported by Grants-in-aid for scientific research from the Ministry of Education and Science. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank/EMBL Data Bank with accession number(s) D82326.

†To whom correspondence should be addressed. Tel.: 81-886-31-3111 (ext. 2327); Fax: 81-886-33-7094.

The abbreviation used is: NBAT, neutral and basic amino acid transporter.
purified from human kidney cortex by the cesium chloride-guanidium isothiocyanate method, and poly(A) RNA was isolated by oligo(dT)-cellulose affinity chromatography as described previously (24). A DNA library was constructed in λgt10 by oligo(dT)-primed cDNA synthesis (Life Technologies, Inc.) (24). A rabbit NBAT cDNA probe was used for library screening (5). Two cDNA clones (pBAT-1 and pBAT-11) were obtained as described in the Materials and Methods. Sequences of the cDNA inserts were determined by automatic DNA sequencing (model 4000L, LI-COR, Lincoln, NE) and the dideoxy chain termination method using a T7 sequencing kit (Pharmacia Biotech Inc.) (24).

Construction of Mutants for NBAT—All deletion mutants were obtained by linearizing pBAT-11 with XbaI using exonuclease III to remove sequences from the 3′-end. Single digestion of the 5′-end overhangs, and DNA repair synthesis with the Klenow fragment of Escherichia coli polymerase. The truncated fragments were gel purified and then ligated to Smal-digested pBA, a vector containing a 3′-noncoding region and the poly(A) site of pBAT-11, in the presence of an oligonucleotide linker, 5′-CTAGACTAGTCTAG-3′, which contains stop codons in all three reading frames. Deletion mutants were sequenced at the junction as described (25).

In Vitro Transcription—pBAT-11 was linearized by digestion with XhoI and transcribed into NBAT cDNA using T3 or T7 RNA polymerase (Promega) (25, 26).

Oocyte Injections, Transport Assays, and Two-electrode Voltage Clamp— Xenopus laevis females were obtained from Hamamatsu (Ibaraki, Japan) (14). Small clumps of oocytes were cultured for 90 min with colcemid at 2 μg/ml, overnight, and then with 50 mM KCl to remove the follicular layer. Following extensive washing, first with ORII solution and then with modified Barth’s solution (14), single oocytes were injected with cRNA solution containing 5 α-amino acids at 20 μM. A resting membrane potential was observed for 4–10 min. Discarded. For electrophysiological studies, oocytes were superfused with 1 M KCl and had resistances ranging from 7 to 14 MΩ. A data acquisition system and commercial software were used to send the voltage pulse protocol and simultaneously to record current and voltage signals.

Immunoprecipitation of [35S]Methionine-labeled NBAT in Oocytes—Oocytes were injected with wild-type or mutant NBAT cRNA in a final volume of 50 nl (50 ng). After 24 h, [35S]Methionine (1 μCi in 50 nl of dialyzed bovine serum-treated water; ICN Biochemical, Inc.) was injected, and the oocytes were incubated for 24 h at 18°C in 0.5 ml of modified Barth’s solution (14). Single oocytes were tritiated in phospho-buffered saline using a Gilson P-200 Pipetman. Oocyte plasma membrane “ghosts” were separated from cytoplasmic contents and collected into a pipette. Plasma membranes then were washed by resuspension in phosphate-buffered saline, followed by centrifugation. An intact oocyte ghost containing 2.5–3.0 μg of plasma membrane protein (approximately 50,000 cpm). The pellets corresponding to membranes obtained from 15–20 oocytes were solubilized in buffer B (10 mM phosphate-buffered saline, pH 7.4, 0.07% Triton X-100, 0.07% SDS, 0.03% deoxycholate, 0.03% bovine serum albumin, and 1 mM phenylmethylsulfonyl fluoride). Samples were mixed with 10 μl of affinity-purified antibody (anti-human NBAT rabbit IgG) and 8 μl of protein A-Sepharose. The samples were then mixed at 4°C overnight. The pellets were then washed twice with 600 μl of buffer B. Aliquots of these samples were used for electrophoresis. After electrophoresis, gels were dried, and autoradiography was performed. For the production of the anti-NBAT antibody, the 15-amino acid sequence (CRPSFKDSKDGGNQD) of the NBAT protein deduced from human NBAT cDNA (amino acids 125–138) was synthesized. The NH2-terminal cysteine residue was introduced for conjugation with keyhole limpet hemocyanin using maleimidobenzoyl-N-hydroxysuccinimide ester. After the peptide (1 mg) was conjugated with keyhole limpet hemocyanin and m-maleimidobenzoyl-N-hydroxysuccinimide ester (1.9 mg) and mixed with a Freund’s complete adjuvant, the emulsion (100 μl of peptide) was injected subcutaneously into rabbits four times at a 2-week interval. The antisera used after the fourth booster was affinity purified using a gel column (Cellu-Cel, Tokyo, Japan) (27).

RESULTS

CDNA Cloning and Functional Expression—Two cDNA clones (pBAT-1 and pBAT-11) were isolated containing 2283 and 3355 base pairs of insert, respectively. Sequencing data indicated that the two clones had the same open reading frame, encoding a 685-amino acid protein, but have different polyadenylation signals (data not shown). pBAT-11 is 3355 base pairs in length as compared with the shorter pBAT-1, which is 2283 base pairs in length. pBAT-11 is approximately 1.1 kilobases longer in 3′-untranslated sequence upstream of its 3′poly(A) signal. pBAT-1 contains an adenine residue at nucleotide 1897, whereas pBAT-11 contains a guanine residue at that position, resulting in substitution of isoleucine for methionine at amino acid 618. The sequence of pBAT-1 is identical to that of human NBAT cDNA reported by Bertran et al. (4). The sizes of two clones (2283 and 3355 base pairs) correspond fairly well to those of the transcripts (2.4 and 3.5 kilobase pairs) seen on Northern blots of kidney mRNA (Fig. 1A). The sizes of two clones (3355 and 3355 base pairs) correspond fairly well to those of the transcripts (2.4 and 3.5 kilobase pairs) seen on Northern blots of kidney mRNA (Fig. 1A). The sizes of two clones (2283 and 3355 base pairs) correspond fairly well to those of the transcripts (2.4 and 3.5 kilobase pairs) seen on Northern blots of kidney mRNA (Fig. 1A).
T7 RNA polymerase. Three days following microinjection, L-[3H]arginine-3 COOH-terminal deletion mutants of NBAT were transcribed with T3 or assayed after 3 days for uptake of the indicated amino acid. cRNAs from 50 nl of water containing 25 ng of each mutant NBAT cRNA were injected with NBAT mutants. Oocytes injected with 50 nl of water or preferring system (bo, oocytes. The induced uptake is different from that seen in wild-type, NBAT-injected oocytes. These observations suggest that the Δ511–685 mutant stimulates another transport system (the cation-specific γ'-like system) but not the neutral and cation-prefering system (b0, oocytes) resultedin the induction of system b0, D 511–685 mutant NBAT-injected oo-

685, Δ261–685, Δ382–685, and Δ511–685) in NBAT were made; cRNA was synthesized and injected into Xenopus oocytes; and the uptake of [35S]cystine, [3H]arginine and [14C]leucine was determined. Wild-type NBAT injection into oocytes resulted in the induction of system b0, D 511–685 mutant NBAT was considerably weaker (data not shown). These data suggest that at least part of the reduced activity of several mutants was caused by a reduced plasma membrane content of the mutant protein, whereas the Δ511–685 NBAT was present reported by Bertran et al. (15) and Wells et al. (16). Localization of Δ511–685 NBAT Protein in Xenopus Oocytes—Next we investigated whether wild-type and mutant NBAT are present in the oocyte plasma membrane. A qualitative impression of the relative amount of the various mutant proteins in the oocyte plasma membrane was obtained by immunoprecipitation. These studies were performed using an affinity-purified antibody raised against a 16-residue synthetic peptide corresponding to the conserved region of human and rabbit NBAT proteins. Oocytes expressing wild-type NBAT and the Δ511–685 mutant exhibited strong signals on filter membranes (Fig. 3), whereas signals from oocytes expressing other mutants were considerably weaker (data not shown). These data suggest that at least part of the reduced activity of several mutants was caused by a reduced plasma membrane content of the mutant protein, whereas the Δ511–685 NBAT was present...
at levels in the plasma membrane similar to those of wild-type NBAT.

Characterization of NBAT Mutant D511–685—We next tested whether stimulation of amino acid uptake by the D511–685 mutant (sodium-independent uptake of L-arginine and sodium-dependent uptake of L-leucine) is due to stimulation of a y\(^+\)-like transport system. This transport system has been reported to be a major component of sodium-independent uptake of cationic amino acids in Xenopus oocytes (28). In addition, y\(^+\)-like transport for cationic amino acids is inhibited by neutral amino acids, for which it has a higher affinity in the presence of sodium. To confirm that the amino acid uptake stimulated by D511–685 corresponds to activation of a y\(^+\)-like transport system in Xenopus oocytes, we tested inhibition of uptake by various amino acids. As shown in Fig. 4, sodium-independent L-arginine uptake in the presence of the amino acids tested was identical to the control activity in water-injected oocytes and to the activity stimulated by the D511–685 mutant. This profile is distinct from the y\(^+\)-transport system in mammalian cells but is specific for a cationic transporter (y\(^+\)-like system) in Xenopus oocytes (28). Methylaminoisobutyric acid, an amino acid analogue that is a specific substrate for the sodium-dependent neutral amino acid transport system A, had no effect on the uptake of L-arginine. We also observed that cystine, which is transportable by system b\(^0\)-, and is not taken up by water or mutant NBAT (D511–685)-injected oocytes, caused a 60% decrease in L-arginine uptake but had no effect on L-leucine uptake. The profile of inhibition by cystine agrees with that reported by Wells et al. (16) for 4F2 antigen. In the presence of sodium in the incubation medium, both basal L-arginine uptake (in water-injected oocytes) and uptake stimulation due to D511–685 mRNA were completely inhibited (95%) by a 100-fold excess of unlabeled L-leucine. These results are consistent with stimulation by D511–685 of a component of the transport pathway shared by L-arginine and L-leucine.

We also studied the sodium dependence of inhibition of D511–685 activity by L-homoserine (Fig. 5). It has been demonstrated that the activity of system y\(^+\) is not inhibited by L-homoserine (5 mM), in the absence of sodium, whereas it is largely inhibited by this amino acid in the presence of sodium. In contrast, system b\(^0\)- interacts with L-homoserine in both the presence and absence of sodium. Fig. 5 shows that the basal L-arginine uptake of oocytes and the L-arginine uptake stimulated by water or mutant NBAT (D511–685) RNA were assayed for uptake of 50 \(\mu\)M L-arginine as described for Fig. 3. The uptake assay was performed in the absence (-) and presence (+) of 5 mM L-homoserine. Bars, means \(\pm\) S.E. obtained from seven or eight oocytes from each group in a representative experiment. **, p < 0.01.

Inhibition of L-arginine uptake by L-homoserine (Fig. 5). Oocytes injected with 50 nl of water or 15 ng of mutant NBAT (D511–685) RNA were assayed for uptake of 50 \(\mu\)M L-arginine as described for Fig. 3. The uptake assay was performed in the absence (-) and presence (+) of 5 mM L-homoserine. Bars, means \(\pm\) S.E. obtained from seven or eight oocytes from each group in a representative experiment. **, p < 0.01.

**Fig. 5. Inhibition of L-arginine uptake by L-homoserine in the presence and absence of sodium.** Oocytes injected with 50 nl of water or 15 ng of mutant NBAT (D511–685) RNA were assayed for uptake of 50 \(\mu\)M L-arginine as described for Fig. 3. The uptake assay was performed in the absence (-) and presence (+) of 5 mM L-homoserine. Bars, means \(\pm\) S.E. obtained from seven or eight oocytes from each group in a representative experiment. **, p < 0.01.

**Fig. 6. Inhibition of L-arginine uptake by L-histidine as a function of extracellular pH.** Oocytes injected with 50 nl of water (A) or 50 nl of water containing 25 ng of mutant NBAT (D511–685) (B) were assayed 3 days later for uptake of 50 \(\mu\)M L-arginine in different media at the pH values indicated. The values represent the means \(\pm\) S.E. obtained from seven to eight oocytes from each group. [], without L-histidine; ■, with L-histidine.

| Amino acid | Current | D511–685 mutant | Wild type | Water |
|------------|---------|----------------|-----------|-------|
| L-Arginine (NaCl) | -19 ± 1.6 | -25 ± 1.2 | -0.4 ± 0.2 |
| L-Leucine (NaCl) | 14 ± 1.2 | 30 ± 1.9 | ND* |
| L-Arginine (TMA) | -14.6 ± 1.9 | -26 ± 1.6 | -0.5 ± 0.4 |
| L-Leucine (TMA) | 16.7 ± 1.3 | 0.3 ± 0.2 | ND |
| L-Homoserine (NaCl) | -10.4 ± 1.2 | ND | ND |
| L-Homoserine (TMA) | -0.2 ± 0.1 | ND | ND |

* L-Leucine at 50 \(\mu\)M for 20 s, L-arginine at 50 \(\mu\)M for 20 s, and L-homoserine at 1 mM for 20 s.

* ND, not detected.

lated by D511–685 are both inhibited by L-homoserine only in the presence of sodium. (L-Arginine uptake was inhibited only 11–20% by L-homoserine in the absence of sodium but was inhibited 70–75% by L-homoserine in the presence of sodium.)

We next measured L-histidine uptake inhibition as a function of extracellular pH (Fig. 6). Uptake of histidine by the y\(^+\)-like dibasic amino acid transport system is pH dependent, with increased uptake as the pH is lowered from neutral toward the pK\(_a\) of histidine. The majority of L-histidine molecules are cationic, with a pK\(_a\) of 6.1, and are transported by the y\(^+\)-like system. As shown in Fig. 6, L-histidine inhibition of L-arginine uptake in oocytes injected with the D511–685 mutant in-
The sodium-dependent uptake of 
L-arginine and L-leucine induced 
um-independent uptake of L-arginine was inhibited completely. 
Failed to stimulate L-arginine, L-cystine, or L-leucine transport. 
Amino acid transport in 
eral deletion mutants of NBAT and analyzed their effects on 
transport of basic amino acids described above. In oocytes 
expressing the 
mutant NBAT cRNA in injected oocytes show different currents 
the transport of basic amino acids is inhibited by some neutral 
amino acids, such as homoserine, but only in the presence of 
sodium, as shown in Fig. 5. Homoserine itself induced an inward current in mutant NBAT-injected oocytes, but this was 
completely dependent on the presence of extracellular sodium (Table I). Thus, mutant NBAT transports basic amino acids in 
a sodium-independent manner and not neutral amino acids 
by a sodium-dependent process. These results indicate that 
mutant NBAT functions as the sodium-independent basic 
amino acid uptake system generally known as y’.

**DISCUSSION**

To investigate whether NBAT functions as a component of 
transporter systems or as a transport activator, we made 
several deletion mutants of NBAT and analyzed their effects on 
amino acid transport in Xenopus oocytes. Most of the mutants 
fail to stimulate L-arginine, L-cystine, or L-leucine transport. 
One COOH-terminal deletion mutant (Δ511–685), with a deletion in a leucine zipper motif, stimulated L-leucine transport in 
the presence of sodium and the sodium-independent uptake of 
L-arginine. Mutant NBAT (Δ511–685) cRNA-stimulated, sodium-independent uptake of L-arginine was inhibited completely 
by L-leucine in the presence of sodium. The stimulation both of 
sodium-dependent uptake of L-arginine and L-leucine induced by mutant NBAT (Δ511–685) cRNA was inhibited completely 
by basic L-amino acids. In voltage clamp experiments, dibasic amino acids induced inward currents in Xenopus oocytes 
injected with the Δ511–685 mutant, but neutral amino acids did not induce outward currents. These results demonstrate that 
mutant NBAT (Δ511–685) may stimulate a cation-prefering amino acid transport system identical to the y’-like transport 
already present in oocytes. If NBAT is a component of the 
amino acid transport system, it is unlikely that mutant NBAT 
(Δ511–685) stimulates other amino acid transport systems. 
These results strongly suggest that NBAT functions as a transport activator.

As shown in Fig. 8A, Mosckovitz et al. (29) have demonstrated that NBAT has four transmembrane domains using site-directed polyclonal antibodies directed against NBAT. Two mutations (M467T and M467K), which so far are the most common cystinuric mutations in the Spanish and Italian populations, are located in the third transmembrane domain (9, 12, 13). These mutations abolish the amino acid transport activity induced by NBAT in oocytes (9), suggesting that the transmembrane domain of NBAT is important to stimulate the amino acid transport activity. However, the Δ511–685 mutant, which is truncated at the fourth transmembrane domain, can activate the y’-like system in oocytes. If wild-type NBAT has four membrane-spanning domains, the truncation, which at most deletes one of the four transmembrane segments, causes a different topology of the rest of the protein, which is highly unlikely. In addition, NBAT protein and 4F2 have a very similar localization of the putative transmembrane domains within their sequences (15, 16). Both Bertran et al. (15) and Wells et al. (16) have found significant amino acid sequence homology between 4F2 and NBAT, with 26% identity and 45%
similarity overall between the two amino acid sequences. As shown in Fig. 7, four regions in the amino acid sequences are highly conserved, with homologies of 67–80% between the two proteins (15). The A511-685 mutant has a deletion in one of these highly conserved regions the leucine zipper motif of NBAT. However, the structure of mutant NBAT (Δ511–685) cRNA still may be similar to that seen with the 4F2 antigen. We therefore suggest that NBAT has one transmembrane domain, as shown in Fig. 8B. Furthermore, the COOH-terminal (mutant 511–685) of NBAT is important for determining its specificity as a transport activator. Further work is needed to clarify the transport systems associated with members of the type II membrane glycoprotein family.

REFERENCES
1. Segal, S., and Thier, S. O. (1989) in The Metabolic Basis of Inherited Disease (Scriver, C. R., Beaudet, A. L., Sly, W. S., and Valle, D., eds) pp. 2479–2496, McGraw-Hill, Inc., New York
2. McKusick, V. A. (1994) in Mendelian Inheritance in Man. McGraw-Hill, Inc., New York (Scriver, C. R., Beaudet, A. L., Sly, W. S., and Valle, D., eds) pp. 2479–2496, 11th Ed., pp. 1750–1751, Johns Hopkins University Press, Baltimore
3. Silbernagl, S. (1988) Physiol. Rev. 68, 911–1007
4. Bertran, J., Werner, A., Chilaron, J., Nunes, V., Biber, J., Testar, X., Zorzano, A., Estivill, X., Murer, H., and Palacin, M. (1993) J. Biol. Chem. 268, 14842–14849
5. Bertran, J., Werner, A., Moore, M. L., Stange, G., Markovich, D., Biber, J., Testar, X., Zorzano, A., Palacin, M., and Murer, H. (1992) Proc. Natl. Acad. Sci. U.S.A. 89, 5601–5605
6. Rebecca, W.-S., Sabha, R. V., Mohandas, T. K., and Hediger, M. A. (1993) J. Clin. Invest. 91, 1959–1963
7. Tate, S. S., Yan, N., and Udenfriend, S. (1992) Proc. Natl. Acad. Sci. U.S.A. 89, 1–5
8. Wells, R. G., and Hediger, M. A. (1992) Proc. Natl. Acad. Sci. U.S.A. 89, 5596–5600
9. Calonge, M. J., Gasparini, P., Chilaron, J., Chillón, M., Gallucc, M., Rousaud, F., Zelante, L., Testar, X., Dallapiccola, B., Silverio, F. D., Barcelo, P., Estivill, X., Zorzano, A., Nunes, V., and Palacin, M. (1994) Nat. Genet. 6, 420–425
10. Pras, E., Arber, N., Aksentijerich, I., Katz, G., Shariro, J. M., Prosen, L., Gruberg, L., Harel, D., Liberman, U., Weissenbach, J., Pras, E., and Kastner, D. L. (1994) Nat. Genet. 6, 415–419
11. Pras, E., Raben, N., Golomb, E., Arber, N., Aksentijerich, I., Shapiro, J. M., Harel, D., Katz, G., Liberman, U., Pras, M., and Kastner, D. L. (1995) Am. J. Hum. Genet. 56, 1297–1330
12. Calonge, M. J., Vopini, V., Biscoeglia, L., Rousaud, F., DeSanctis, L., Beccia, E., Zelante, L., Testar, X., Zorzano, A., Estivill, X., Gasparini, P., Nunes, V., and Palacin, M. (1995) Proc. Natl. Acad. Sci. U.S.A. 92, 9667–9671
13. Miyamoto, K., Katai, K., Tsutsumi, S., Sone, K., Segawa, H., Yamamoto, H., Taketani, Y., Takada, K., Morita, K., Kanayama, H., Kagawa, S., and Takeda, E. (1995) Biochem. J. 310, 951–955
14. Bertran, J., Magagnin, S., Werner, A., Markovich, D., Biber, J., Testar, X., Zorzano, A., Kuhn, L., Palacin, M., and Murer, H. (1992) Proc. Natl. Acad. Sci. U.S.A. 89, 5606–5610
15. Wells, R. G., Lee, W.-S., Kanayama, H., Yang, K., and Hediger, M. A. (1992) J. Biol. Chem. 267, 15285–15288
16. Haynes, B. F., Herrlinger, M. E., Mann, D. L., Eisenbarth, G. S., Shelhamer, J. H., Mostowski, H. S., Thomas, C. A., Strominger, J. L., and Fauci, A. S. (1981) J. Immunol. 126, 1409–1414
17. Herrlinger, M. E., and Strominger, J. L. (1982) J. Immunol. 129, 623–628
18. Hermler, M. E., and Strominger, J. L. (1982) FEBS Lett. 148, 174–178
19. Kim, J. W., Closs, I. E., Albritton, L. M., and Yoon, H. (1991) Biochemistry 30, 7842–7851
20. Ahmed, A., Peter, G. J., Tayor, P. M., Harper, A. A., and Rennie, M. J. (1995) Biochim. Biophys. Acta 1263, 255–261
21. Bush, A. E., Herzer, T., Waldegger, S., Schmidt, F., Palacin, M., Biber, J., Markovich, D., Murer, H., and Lang, F. (1994) J. Biol. Chem. 269, 25581–25586
22. Ahmed, A., Peter, G. J., Taylor, P. M., Harper, A. A., and Rennie, M. J. (1995) J. Biol. Chem. 270, 8482–8486
23. Coady, M. J., J alai, F., Chen, X., Lemay, G., Berteloot, A., and Lapointe, J. Y. (1994) FEMS Lett. 136, 174–178
24. Miyamoto, K., Tsutsumi, S., Morimoto, A., Minami, H., Yamamoto, H., Sone, K., Taketani, Y., Nakabay, Y., Oka, T., and Takeda, E. (1994) Biochem. J. 303, 877–883
25. Agarwal, K., Baek, K. H., Jeon, C. J., Miyamoto, K., Ueno, A., and Yoon, H. (1991) Biochemistry 30, 7942–7951
26. Miyamoto, K., Tsutsumi, S., Sonoda, T., Yamamoto, H., Minami, H., Taketani, Y., and Takeda, E. (1995) Biochem. J. 305, 81–85
27. Hisano, S., Haga, H., Miyamoto, K., Takeda, E., and Fukui, Y. (1996) Brain Res. 710, 299–302
28. Van Winkle, L. J. (1993) Biochim. Biophys. Acta 1154, 157–172
29. Moschkowitz, R., Udenfriend, S., Felix, A., Heimer, E., and Tate, S. S. (1994) FASEB J. 8, 1069–1074