Differential Recognition of \( \beta \)-Lactam Antibiotics by Intestinal and Renal Peptide Transporters, PEPT 1 and PEPT 2*

(Received for publication, June 5, 1995, and in revised form, August 30, 1995)

Malliga E. Ganapathy‡§, Matthias Brandsch¶, Puttur D. Prasad†, Vadivel Ganapathy†, and Frederick H. Leibach‡

From the Departments of ‡Medicine and §Biochemistry, and Molecular Biology, Medical College of Georgia, Augusta, Georgia 30912

This study was initiated to determine if there are differences in the recognition of \( \beta \)-lactam antibiotics as substrates between intestinal and renal peptide transporters, PEPT 1 and PEPT 2. Reverse transcription-coupled polymerase chain reaction and/or Northern blot analysis have established that the human intestinal cell line Caco-2 expresses PEPT 1 but not PEPT 2, whereas the rat proximal tubule cell line SKPT expresses PEPT 2 but not PEPT 1. Detailed kinetic analysis has provided unequivocal evidence for participation of PEPT 2 in SKPT cells in the transport of the dipeptide glycylsarcosine and the aminopeptidase B from the circulation. Therefore, detailed studies on the interaction of \( \beta \)-lactam antibiotics with the peptide transporters in these cells have shown that the peptide transporters expressed in the small intestine and kidney are structurally different. The human intestinal peptide transporter (PEPT 1) and the human kidney peptide transporter (PEPT 2) exhibit only about 50% homology in amino acid sequence. PEPT 1 is expressed primarily in the small intestine and, to a small extent, in the kidney, whereas PEPT 2 is expressed only in the kidney (12). Nonetheless, both transporters accept small peptides as substrates and are driven by a transmembrane electrochemical \( \text{H}^+ \) gradient. The current investigation was undertaken to study in detail the interaction of \( \beta \)-lactam antibiotics with PEPT 1 and PEPT 2 and to determine whether there are differences between these two transporters in the recognition of these drugs as substrates.

Peptide transporters are primarily expressed in the small intestine and kidney. The endogenous substrates for these transporters are small peptides consisting of two or three amino acids (1). These transporters function in the absorption of peptides arising from digestion of dietary proteins (small intestine) and in the reabsorption of peptides present in the glomerular filtrate (2-4). It became apparent that the peptide transporters can serve as carriers for exogenous compounds which bear structural resemblance to the physiologically occurring peptide substrates, when the transport of cephalixin, a \( \beta \)-lactam antibiotic, was shown to be mediated in the kidney (5) and small intestine (6) by the peptide transport system. The peptide substrates of the peptide transport system and cephalixin share certain structural features such as a peptide bond with an \( \alpha \)-amino group and a terminal carboxylic acid group. This structural similarity is apparently the basis for the molecular mimicry, enabling the peptide transporters to accept cephalixin as a substrate. The pharmacological relevance of the peptide transporters became immediately evident from these studies because of the enormous potential of these transporters to serve as carriers for a variety of peptidomimetic drugs. Subsequent studies have indeed identified a wide spectrum of pharmacologically active compounds that are accepted as substrates by the peptide transporters in the intestine and/or kidney (7-9).

The two organs, the small intestine and the kidney, in which the peptide transporters are primarily expressed play an important role in the therapeutic efficacy of \( \beta \)-lactam antibiotics. The intestinal peptide transport system is responsible for the oral absorption of these drugs. The renal peptide transport system, which functions in the reabsorption of these drugs from the glomerular filtrate, enhances the half-life of these drugs in the circulation. Therefore, detailed studies on the interaction of \( \beta \)-lactam antibiotics with the peptide transporters in these two organs are vital to the understanding of the pharmacodynamics of these drugs. Recent molecular cloning studies (10-13) have shown that the peptide transporters expressed in the small intestine and kidney are structurally different. The human intestinal peptide transporter (PEPT 1) and the human kidney peptide transporter (PEPT 2) exhibit only about 50% homology in amino acid sequence. PEPT 1 is expressed primarily in the small intestine and, to a small extent, in the kidney, whereas PEPT 2 is expressed only in the kidney (12). Nonetheless, both transporters accept small peptides as substrates and are driven by a transmembrane electrochemical \( \text{H}^+ \) gradient. The current investigation was undertaken to study in detail the interaction of \( \beta \)-lactam antibiotics with PEPT 1 and PEPT 2 and to determine whether there are differences between these two transporters in the recognition of these drugs as substrates.

EXPERIMENTAL PROCEDURES

Materials

\[ \text{[2-\text{H}]Glycyl-[1-\text{C}]sarcosine (specific radioactivity, 109 mCi/mmol)} \]

was custom synthesized by Cambridge Research Biochemicals (Cleveland, United Kingdom). \[ \text{[2-\text{H}]Cephalixin (specific radioactivity, 10.5 \mu Ci/\mu g)} \]

was a generous gift from SmithKline Beecham Pharmaceuticals.
cals (King of Prussia, PA). [3-35S]cystein (specific radioactivity, 3000 Ci/mmol) was purchased from Amersham. Cell culture media were purchased from Life Technologies, Inc. (Gaithersburg, MD). Fetal bovine serum, dexamethasone, apotransferrin, nigericin, benzylpenicillin, ampicillin, and cephalothin were obtained from Sigma. Cytochalasin, clephalexin, and cefadroxil were generous gifts from Dr. T. Hashi, University of Shizuka, Shizuka, Japan. The rat renal proximal tubule cell line SKPT was provided by Dr. Ulrich Hopfer, Case Western Reserve University, Cleveland, OH. The human colon carcinoma cell line Caco-2 was obtained from the American Type Culture Collection. All other chemicals were of analytical grade.

Methods

Cell Culture and Uptake Measurements—SKPT cells and Caco-2 cells were cultured in Dulbecco's modified Eagle's IF-12 (1:1) medium and in minimal essential medium, respectively, as described previously (14, 15). Uptake of [14C]glycylsarcosine and [3H]cephalexin in cells was measured with the uptake buffer whose composition was 25 mM Hepes/Tris (pH 7.5) or 25 mM Mes/Tris (pH 6.0), 140 mM NaCl, 5.4 mM KCl, 1.8 mM CaCl2, 0.8 mM MgSO4, and 5 mM glucose. The time of incubation for Tris/Hepes (pH 9.0), containing 140 mM NaCl, 5.4 mM KCl, 1.8 mM CaCl2, 0.8 mM MgSO4, and 5 mM glucose, and the other containing 25 mM Tris/Hepes (pH 9.0), 140 mM NaCl, 5.4 mM KCl, 1.8 mM CaCl2, 0.8 mM MgSO4, and 5 mM glucose, and these buffers were mixed to give the desired pH. The culture medium was aspirated from the dish and the cell monolayer was washed once with 1 ml of the uptake buffer. Uptake was initiated by the addition of 1 ml of the uptake buffer containing radiolabeled substrate. Incubation was continued for the desired time, after which uptake was terminated by the removal of the medium followed by three times washing with ice-cold uptake buffer. The cells were then solubilized with 1 ml of 0.2 M NaOH, 1% SDS and the contents were transferred to a counting vial for determination of radioactivity.

mRNA Isolation and Northern Blot—Poly(A)+ RNA was isolated from Caco-2 and SKPT cells using the FastTrack mRNA isolation kit (Invitrogen). The cDNAs encoding PEPT 1 and PEPT 2 were radiolabeled with [32P]dCTP by random priming. Northern blot analysis was carried out under high stringency conditions as described previously (11). The same blot was used for probing with PEPT 1 cDNA and PEPT 2 cDNA by sequential hybridization.

RT-PCR—RNA samples from human intestine, human kidney, and Caco-2 cells were subjected to RT-PCR to determine whether these tissues contain PEPT 1 mRNA and/or PEPT 2 mRNA. The primers specific for PEPT 1 corresponded to nucleotide positions 900–917 (sense) and 1542–1560 (antisense) of the cDNA (11). The PEPT 2-specific primers (11, 12) were primers specific for PEPT 2 corresponded to nucleotide positions 1575–1592 (antisense) of the cDNA (11). The cDNAs encoding PEPT 1 and PEPT 2 were radiolabeled with [32P]dCTP by random priming. Northern blot analysis was carried out under high stringency conditions as described previously (11). The same blot was used for probing with PEPT 1 cDNA and PEPT 2 cDNA by sequential hybridization.

To establish unequivocally that Caco-2 cells express PEPT 1 and SKPT cells express PEPT 2, we performed the following experiments. We determined the identity of the peptide transporter present in Caco-2 cells by RT-PCR using PEPT 1- and PEPT 2-specific primers (11, 12). The specificity of each pair of primers was established by PCR using respective cDNAs as templates. RNA samples prepared from human intestine, human kidney, and Caco-2 cells were subjected to RT-PCR using these primers and the products were analyzed by agarose gel electrophoresis. The results of these experiments, given in Fig. 1, show that the PEPT 1-specific PCR product of expected size (~1.2 kb) was generated from all three RNA samples. In contrast, the PEPT 2-specific PCR product (~0.9 kb in size) was generated only from kidney RNA. RNA samples from Caco-2 cells and intestine were negative for this product (Fig. 1). These data demonstrate that Caco-2 cells express PEPT 1 and SKPT cells express PEPT 2.

The nucleotide sequences of the rat homologs of PEPT 1 and PEPT 2 have not yet been determined. Therefore, we investigated the expression of PEPT 1/PEPT 2 in the SKPT cell line by Northern blot hybridization using the human PEPT 1 and PEPT 2 cDNAs as probes (Fig. 2). Poly(A)+ RNA prepared from SKPT cells and Caco-2 cells was size-fractionated and probed constant, Ks, and the maximal velocity, Vmax, were calculated by linear regression of the Eadie-Hofstee plot and confirmed by nonlinear regression methods using the Fig. P, version 6.0 computer program. The calculated parameters are shown as mean ± S.E. Inhibition constants (K) were calculated from IC50 values (i.e. concentration of the unlabeled test compound necessary to inhibit 50% of the uptake of radiolabeled substrate) according to the method of Cheng and Prusoff (18).

RESULTS AND DISCUSSION

Differential Expression of PEPT 1 and PEPT 2 in Caco-2 and SKPT Cells—Mammalian intestine expresses PEPT 1 and not PEPT 2. Mammalian kidney expresses both PEPT 1 and PEPT 2, but the predominant one is PEPT 2. One of the goals of the present study was to investigate the interaction of β-lactam antibiotics, a group of peptidomimetic drugs with pharmacological and clinical importance, with PEPT 1 and PEPT 2, using cultured intestinal and renal cell lines. Caco-2 cells, which are of human intestinal origin, are known to possess a low-affinity H+ /peptide cotransporter, resembling PEPT 1 (15). SKPT cells, which are of rat kidney origin, have been recently demonstrated to express a high-affinity H+ /peptide cotransporter, resembling PEPT 2 (14). Even though the MDCK renal cell line possesses a peptide transport system, available kinetic data suggest that the transport system is most likely PEPT 1 rather than PEPT 2 (19). Therefore, Caco-2 cells and SKPT cells were used in the present study.

The abbreviations used are: Mes, 4-morpholineethanesulfonic acid; RT-PCR, reverse transcriptase-polymerase chain reaction; kb, kilobases.
with these cDNAs. With the PEPT 1 probe, the presence of a major hybridizing band, 3.1 kb in size, was evident in Caco-2 cells, but this band was absent in SKPT cells. With the PEPT 2 probe, there was a primary hybridizing band, 4.2 kb in size, in SKPT cells. This signal was absent in Caco-2 cells. These results indicate that the peptide transporter expressed in SKPT cells is PEPT 2. PEPT 1, which is expressed in Caco-2 cells, is not present in SKPT cells.

Interaction of β-Lactam Antibiotics with PEPT 2 Expressed in SKPT Cells—Having established that the SKPT cells functionally express the kidney-specific PEPT 2, we investigated the interaction of β-lactam antibiotics with PEPT 2 using these cells. Initial studies were done by assessing the ability of several unlabeled β-lactam antibiotics to inhibit the uptake of radiolabeled glycylsarcosine, a dipeptide substrate for peptide transporters. In these experiments, uptake measurements were made at pH 6.0. Under these conditions, there exists a medium-to-cell H\(^+\) gradient (pH of the cell cytoplasm is ~7.4), which provides the driving force for PEPT 2, a H\(^+\)-dependent transporter. We have used three cephalosporins (cephalexin, cephalothin, and cefadroxil) and three penicillins (cyclacillin, ampicillin, and benzylpenicillin) (Fig. 3). These experiments have shown that cefadroxil, cyclacillin, and cephalaxin interact with PEPT 2 with high affinity. The IC\(_{50}\) values (i.e. concentration necessary to cause 50% inhibition of glycylsarcosine uptake) for these antibiotics were 3.0 ± 0.2, 42 ± 2, and 73 ± 6 \(\mu\)M, respectively. The affinity of PEPT 2 for ampicillin, cephalothin, and benzylpenicillin was comparatively low, the IC\(_{50}\) values being 13 ± 0.2, 7.5 ± 2.0, and >10 \(\mu\)M, respectively.

We then investigated the kinetics of inhibition of glycylsarcosine uptake by cefadroxil (a cephalosporin) and cyclacillin (a penicillin). The presence of the antibiotics decreased the affinity of PEPT 2 for glycylsarcosine, without affecting the maximal velocity (Fig. 4). The Michaelis-Menten constant (K\(_m\)) for glycylsarcosine in the absence of the antibiotics was 48 ± 4 \(\mu\)M. This value was increased 3.3-fold to 156 ± 17 \(\mu\)M in the presence of 40 \(\mu\)M cyclacillin. Similarly, the K\(_m\) value was increased 2.3-fold to 112 ± 3 \(\mu\)M in the presence of 3 \(\mu\)M cefadroxil. Therefore, the β-lactam antibiotics and the dipeptide substrates apparently compete for the same binding site on PEPT 2.

We also employed radiolabeled cephalaxin as a substrate to study the interaction of β-lactam antibiotics with PEPT 2 in SKPT cells. Our initial characterization studies have established that the uptake of cephalaxin in these cells was stimulated by an inwardly directed H\(^+\) gradient (Fig. 5). The uptake of the antibiotic (0.1 \(\mu\)M) was stimulated severalfold upon acidification of the extracellular medium. However, this stimulation was abolished when the cells were pH-clamped (i.e. intracellular pH = extracellular pH) with nigericin. Kinetic analysis, done over a cephalaxin concentration of 25–250 \(\mu\)M, showed that the uptake occurred via a single, saturable process (Fig. 6). The Michaelis-Menten constant (K\(_s\)) for the uptake process was 49 ± 8 \(\mu\)M and the maximal velocity (V\(_{\text{max}}\)) was 1.5 ± 0.1 nmol/mg of protein/20 min.

We then performed detailed kinetic studies to establish that the uptake of the dipeptide glycylsarcosine and the uptake of the β-lactam antibiotic cephalaxin occur via a common transport system (i.e. PEPT 2) in SKPT cells. The experimental approach employed here for this purpose is the so-called "A-B-C test" which is widely used in the transport field (20–22). We systematically investigated the interaction between glycylsarcosine and cephalaxin during uptake in SKPT cells with the
Kinetics of cephalexin uptake in SKPT cells. Uptake of cephalexin in SKPT cells was measured at pH 6.0 with a 20-min incubation period. Concentration of cephalexin was varied between 25 and 250 μM, keeping the concentration of [14C]glycylsarcosine constant at 200 nM and adding unlabeled cephalexin to desired concentrations. Non-mediated component was determined from the uptake of radiolabel measured in the presence of 10 mM unlabeled cephalexin. This component was subtracted from total uptake to calculate mediated uptake which was used in kinetic analysis. Inset: Eadie-Hofstee plot (V versus V/S). V, cephalexin uptake in nmol/mg of protein/20 min; S, cephalexin concentration in μM.

Differential Recognition of β-Lactam Antibiotics by PEPT 1 and PEPT 2—Cephalosporins as well as penicillins are β-lactam antibiotics, possessing peptide-like chemical structures. The basic structure of these compounds resembles the backbone of a tripeptide in which the C-terminal peptide bond is located in the β-lactam ring. The free carboxylic acid group which constitutes the C terminus is present in the dihydrothiazine ring in the case of cephalosporins and in the thiazolidine ring in the case of penicillins. Our results show that some penicillins such as cyclacillin interact with the renal peptide transporter with greater affinity than some cephalosporins such as cephalexin (Fig. 3). At the same time, there are also examples where certain cephalosporins (e.g., cefadroxil) are better substrates for this transporter than cyclacillin (Fig. 3). This indicates that the dihydrothiazine ring in the cephalosporins and the thiazolidine ring in the penicillins are not differentiated to any significant extent by the renal peptide transporter.

This conclusion is in contrast to the previously held notion that penicillins in general have much lower affinity than cephalosporins for the transporter (23).

Since it has now become clear from molecular biological studies that the intestinal and renal peptide transporters are distinct proteins with significant differences in their primary structure (10–13), we initiated studies to see if there are differences in the substrate recognition pattern between these two transporters. In our studies to compare the substrate recognition pattern of PEPT 1 and PEPT 2, we selected cefadroxil (a cephalosporin) and cyclacillin (a penicillin) as model substrates. Initially, we carried out the experiments with Caco-2 cells (PEPT 1) and SKPT cells (PEPT 2) by determining the relative potency of these two peptidomimetic drugs for the inhibition of the uptake of the dipeptide glycylsarcosine. In Caco-2 cells, the dipeptide uptake was inhibited by cefadroxil and by cyclacillin in a dose-dependent manner (Fig. 7A). The respective IC50 values for the inhibition were 5.4 ± 0.2 μM and 2.5 ± 0.1 μM. Thus, cyclacillin is severalfold more potent than cefadroxil in competing with glycylsarcosine for uptake via

### Table I

Inhibition constants for different compounds to compete with the uptake of radiolabeled glycylsarcosine and cephalexin

| Unlabeled compound | [14C]Glycylsarcosine | [3H]Cephalexin |
|--------------------|----------------------|---------------|
| Glycylsarcosine     | 47.8 ± 4.2a          | 64.0 ± 4.4    |
| Cephalexin          | 68.2 ± 5.2           | 48.5 ± 7.9a   |
| Cyclacillin         | 39.1 ± 1.4           | 36.8 ± 4.9    |
| Cefadroxil          | 2.8 ± 0.2            | 2.5 ± 0.1     |

a Values represent Kᵢ, as well as Kᵢ.
Interestingly, even though the uptake of glycylsarcosine in SKPT cells was inhibited by both cyclacillin and cefadroxil as in Caco-2 cells, there were important differences (Fig. 7B). The potency with which these drugs inhibited the uptake in SKPT cells was much greater than in Caco-2 cells. The IC₅₀ values were in the micromolar range rather than in millimolar range. In addition, there was a significant difference in the relative inhibitory potency between the two drugs. The IC₅₀ values for cefadroxil and cyclacillin in SKPT cells were 3.0 ± 0.2 and 41.6 ± 1.5 μM, respectively. In other words, cefadroxil is manyfold more potent than cyclacillin in competing with the dipeptide for uptake via PEPT 2. Thus, the relative affinities of PEPT 1 and PEPT 2 for cyclacillin and cefadroxil are reversed. These results show that the substrate recognition pattern is significantly different between PEPT 1 and PEPT 2.

To rule out the possibility that the observed differences in substrate recognition of the peptide transporters between these two cell lines may be due to species differences rather than real differences between PEPT 1 and PEPT 2, we performed similar experiments with the cloned human PEPT 1 and human PEPT 2. These two transporters were functionally expressed in HeLa cells using the vaccinia virus expression system and the uptake of cephalixin was determined using 0.5 μM [³H]cephalexin. As shown in Fig. 8A, cephalixin uptake measured at pH 6.0 in HeLa cells expressing PEPT 1 was 1.89 ± 0.05 pmol/10⁶ cells/10 min which was 11-fold greater than the uptake in control cells (i.e. HeLa cells transfected with empty vector) under similar conditions. The H⁺ gradient-dependent nature of the uptake process was evident from the findings that the uptake decreased drastically in PEPT 1-expressing cells when measured at pH 9.0 instead of pH 6.0. Similar results were obtained with PEPT 2 (Fig. 8B). These data demonstrate that PEPT 1 and PEPT 2 catalyze the transport of the β-lactam antibiotic cephalixin. We then used this experimental system to compare the substrate recognition pattern of PEPT 1 and PEPT 2. These transporters were individually expressed in HeLa cells and the uptake of glycylsarcosine was determined in the presence of increasing concentrations of cyclacillin and cefadroxil. As was the case in Caco-2 cells and SKPT cells, the dipeptide uptake via PEPT 1 and PEPT 2 was inhibited by both cyclacillin and cefadroxil. In the case of PEPT 1, the IC₅₀ values for cefadroxil and cyclacillin were 0.87 ± 0.12 and 0.35 ± 0.09 μM, respectively (Fig. 9A). In contrast, the corresponding IC₅₀ values were 66 ± 4 and 610 ± 100 μM in the case of PEPT 2 (Fig. 9B). These data with the cloned human PEPT 1 and PEPT 2 show that there are significant differences between the two peptide transporters in substrate selectivity.

Significant differences were noted in relative potency of cefadroxil and cyclacillin as inhibitors of glycylsarcosine uptake in Caco-2 and SKPT cells which express PEPT 1 and PEPT 2 natively and in HeLa cells which express the cloned human PEPT 1 and PEPT 2. It is possible that post-translational
modifications (e.g. N-glycosylation) of PEPT 1 and PEPT 2 expressed in HeLa cells are not identical to those of the native transporters in Caco-2 and SKPT cells. This may contribute to the observed differences. With respect to PEPT 2, species differences may also be a factor because SKPT cells were derived from rat kidney whereas the PEPT 2 cDNA was cloned from human kidney.

In addition to PEPT 1 and PEPT 2, mammalian tissues may express other peptide transporters. A cDNA clone (HPT-1) has been recently isolated from a Caco-2 cell cDNA library and expression of this cDNA in mammalian cells leads to increased uptake of the peptidomimetic drugs cephalexin and bestatin. Interestingly, there is no sequence homology between the HPT-1 protein and the peptidetransporters PEPT1 and PEPT2. It has also been shown that Caco-2 cells express two functionally distinct peptide transporters, one in the apical membrane and the other in the basolateral membrane. Among these multiple peptide transporters, PEPT 1 and PEPT 2 have been characterized in detail, both at the functional level and at the molecular level. The present study, which focuses on the handling of peptidomimetic drugs by PEPT 1 and PEPT 2, documents a major functional difference between these two transporters in terms of recognition of β-lactam antibiotics as substrates.

Acknowledgment—We thank Bonnie Arms for excellent secretarial assistance.

REFERENCES
1. Matthews, D. M. (1991) Protein Absorption: Development and Present State of the Subject, Wiley-Liss, New York
2. Ganapathy, V., and Leibach, F. H. (1986) Am. J. Physiol. 251, F945–F953
3. Ganapathy, V., Brandsch, M., and Leibach, F. H. (1994) in Physiology of the Gastrointestinal Tract (Johnson, L. R., ed) 3rd Ed., pp. 1773–1794, Raven Press, New York
4. Silbernagl, S. (1988) Physiol. Rev. 68, 911–1007
5. Inui, K., Okano, T., Takano, M., Saito, H., and Hori, R. (1984) Biochim. Biophys. Acta 769, 449–454
6. Okano, T., Inui, K., Maegawa, H., Takano, M., and Hori, R. (1988) J. Biol. Chem. 263, 14130–14134
7. Tsuji, A. (1987) Adv. Biosci. 65, 125–131
8. Kramer, W., Girbig, F., Gutjahr, U., Kleemann, H. W., Leipe, I., Urbach, H., and Wagner, A. (1990) Biochim. Biophys. Acta 1027, 25–30
9. Amidon, G. L., and Lee, H. J. (1994) Annu. Rev. Pharmacol. Toxicol. 34, 321–341
10. Fei, Y. J., Kanai, Y., Nussberger, S., Ganapathy, V., Leibach, F. H., Romero, M. F., Singh, S. K., Boron, W. F., and Hediger, M. A. (1994) Nature 368, 563–566
11. Liang, R., Fei, Y. J., Prasad, P. D., Ramamoorthy, S., Han, H., Yang-Feng, T. L., Hediger, M. A., Ganapathy, V., and Leibach, F. H. (1995) Biochem. Biophys. Acta 270, 6456–6463
12. Liu, W., Liang, R., Ramamoorthy, S., Fei, Y. J., Ganapathy, M. E., Hediger, M. A., Ganapathy, V., and Leibach, F. H. (1995) Biochim. Biophys. Acta 1235, 461–466
13. Ramamoorthy, S., Liu, W., Ma, Y. Y., Yang-Feng, T. L., Ganapathy, V., and Leibach, F. H. (1995) Biochim. Biophys. Acta, in press
14. Brandsch, M., Brandsch, C., Prasad, P. D., Ganapathy, V., Hopfer, U., and Leibach, F. H. (1995) FASEB J., in press
15. Brandsch, M., Miyamoto, Y., Ganapathy, V., and Leibach, F. H. (1994) Biochim. Biophys. Acta, in press
16. Kulanthaivel, P., Cod, D. R., Ramamoorthy, S., Mahesh, V. B., Leibach, F. H., and Ganapathy, V. (1991) Biochem. J. 277, 53–58
17. Felgner, P. L., Gadek, T. R., Holm, M., Roman, R., Chan, H. W., Wenig, M., Northrop, J. P., Ringold, G. M., and Danielsen, M. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 7413–7417
18. Cheng, Y., and Prusoff, W. H. (1973) Biochim. Biophys. Acta 22, 3099–3108
19. Brandsch, M., Ganapathy, V., and Leibach, F. H. (1995) Am. J. Physiol. 268, F391–F397
20. Ahmed, K., and Schoeffield, P. G. (1962) Can. J. Biochem. Physiol. 40, 1101–1110
21. Sorvillo, C. R., and Wilson, O. H. (1964) Nature 202, 92–93
22. Christensen, H. N. (1989) Methods Enzymol. 173, 576–616
23. Daniel, H., and Adibi, S. A. (1993) J. Clin. Invest. 92, 2215–2223
24. Dantzig, A. H., Hoskins, J., Tabas, L. B., Bright, S., Shepard, R. L., enkins, I. L., Duckworth, D. C., Sportsman, J. R., Mackensen, D., Rosteck, P. R., Jr., and Skatrud, P. L. (1994) Science 264, 430–433
25. Saito, H., and Inui, K. I. (1993) Am. J. Physiol. 265, G289–G294