β-Lactam Antibiotics as Substrates for OCTN2, an Organic Cation/Carnitine Transporter*

Malliga E. Ganapathy‡§, Wei Huang‡, D. Prasanna Rajan‡, A. Lee Carter¶, Mitsuru Sugawara‡, Ken Iseki‖, Frederick H. Leibach‖, and Vadivel Ganapathy¶

From the Departments of ‡Medicine and ¶Biochemistry and Molecular Biology, Medical College of Georgia, Augusta, Georgia 30912 and †Department of Pharmacy, Hokkaido University Hospital, Sapporo, Hokkaido, 060-8648 Japan

Therapeutic use of cephaloridine, a β-lactam antibiotic, in humans is associated with carnitine deficiency. A potential mechanism for the development of carnitine deficiency is competition between cephaloridine and carnitine for the renal reabsorptive process. OCTN2 is an organic cation/carnitine transporter that is responsible for Na⁺-coupled transport of carnitine in the kidney and other tissues. We investigated the interaction of several β-lactam antibiotics with OCTN2 using human cell lines that express the transporter constitutively as well as using cloned human and rat OCTN2s expressed heterologously in human cell lines. The β-lactam antibiotics cephaloridine, cefoselis, cepime, and cellupreneam were found to inhibit OCTN2-mediated carnitine transport. These antibiotics possess a quaternary nitrogen as does carnitine. Several other β-lactam antibiotics that do not possess this structural feature did not interact with OCTN2. The interaction of cephaloridine with OCTN2 is competitive with respect to carnitine. Interestingly, many of the β-lactam antibiotics that were not recognized by OCTN2 were good substrates for the H⁺-coupled peptide transporters PEPT1 and PEPT2. In contrast, cephaloridine, cefoselis, cepime, and cellupreneam, which were recognized by OCTN2, did not interact with PEPT1 and PEPT2. The interaction of cephaloridine with OCTN2 was Na⁺-dependent, whereas the interaction of cefoselis and cepime with OCTN2 was largely Na⁺-independent. Furthermore, the Na⁺-dependent, OCTN2-mediated cellular uptake of cephaloridine could be demonstrated by direct uptake measurements. These studies show that OCTN2 plays a crucial role in the pharmacokinetics and therapeutic efficacy of certain β-lactam antibiotics such as cephaloridine and that cephaloridine-induced carnitine deficiency is likely to be due to inhibition of carnitine reabsorption in the kidney.

OCTN2 is a member of the family of organic cation transporters (1). The ability to transport a wide variety of structurally diverse organic cations is a common characteristic of the transporters belonging to this family. However, OCTN2 is unique because it can mediate the transport of not only organic cations but also the zwitterions carnitine and acylcarnitines (2, 3). Interestingly, the transport of organic cations by OCTN2 is Na⁺-independent, whereas the transport of carnitine and acylcarnitine by the same transporter is Na⁺-dependent (4). Thus, OCTN2 is an organic cation/carnitine transporter. This characteristic is seen with OCTN2 from different animal species (4). Carnitine is an endogenous compound with an obligatory role in mitochondrial fatty acid oxidation. It has been known for several years that cellular uptake of carnitine is mediated by a Na⁺-dependent, high affinity transporter located in the plasma membrane (5, 6). This transport mechanism is also responsible for reabsorption of filtered carnitine in the kidney. Genetic defects in this transport system cause primary carnitine deficiency associated with very low levels of carnitine in the circulation resulting from the inability of the kidney to reabsorb filtered carnitine (5, 6). The clinical symptoms of this disease include cardiac myopathy and skeletal myopathy. Available evidence indicates that OCTN2 is responsible for this Na⁺-dependent high affinity carnitine transport in the plasma membrane of various tissues including the kidney, heart, and skeletal muscle (2–4). Genetic defects in the octn2 gene have been identified in a number of patients with primary carnitine deficiency (7–11).

Cephaloridine is a β-lactam antibiotic that has significant structural similarity with carnitine. This antibiotic contains a quaternary nitrogen and exists as a zwitterion under physiological conditions. Therapeutic use of cephaloridine has been found to be associated with nephrotoxicity (12, 13). Studies on the mechanisms of cephaloridine-induced nephrotoxicity have shown that cephaloridine interferes with carnitine-dependent fatty acid oxidation in mitochondria (14, 15). These studies have also shown that cephaloridine increases fractional renal excretion of carnitine, suggesting that this antibiotic may interfere with the reabsorption of carnitine in the kidney (14, 15).

The present work was undertaken to identify the cellular mechanism responsible for enhanced renal excretion of carnitine during cephaloridine therapy. The Na⁺-dependent high affinity carnitine transporter is a potential target for cephaloridine. The present studies were therefore designed to investigate the interaction of cephaloridine as well as a variety of other β-lactam antibiotics with the carnitine transporter using cell lines which express the transporter constitutively and also using the cloned carnitine transporter OCTN2 following its heterologous expression in mammalian cells. The results of these studies show that cephaloridine and other β-lactam antibiotics that contain a quaternary nitrogen interact with the carnitine transporter and compete with carnitine for the transporter process. These studies also suggest that OCTN2 may

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‡ To whom correspondence should be addressed: Dept. of Medicine, Medical College of Georgia, Augusta, GA 30912. Tel: 706-721-7652; Fax: 706-721-6608; E-mail: mganapat@mail.mcg.edu.

¶ The abbreviations used are: OCTN, novel organic cation transporter; TEA, tetraethylammonium; PEPT, peptide transporter; HPCT, human proximal convoluted tubule; HPLC, high pressure liquid chromatography; Mes, 4-morpholineethanesulfonic acid.

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play a role in the intestinal and renal absorption as well as in the cellular uptake in other tissues of these antibiotics.

**EXPERIMENTAL PROCEDURES**

**Materials**—L-[3H]Carnitine (specific radioactivity, 65 Ci/mmol), acetyl-L-[3H]carnitine (specific radioactivity, 65 Ci/mmol), and propionyl-L-[3H]carnitine (specific radioactivity, 65 Ci/mmol) were obtained from Moravek Biochemicals, Inc. (Brea, CA). Ethyl-1-14C]-Tetraethylammonium (TEA) bromide (specific radioactivity, 55 mCi/mmol) was obtained from American Radiolabeled Chemicals, Inc. (St. Louis, MO). [2-14C]Glycyl-L-[1-14C] sarcosine (specific radioactivity, 109 mCi/mmol) was custom-synthesized by Cambridge Research Biochemicals (Cleveland, UK). HeLa cells (a human cervical cancer cell line) and JAR cells (a human placental choriocarcinoma cell line) were originally obtained from the American Type Culture Collection (Rockville, MD). The human proximal convoluted tubule (HPCT) epithelial cell line was kindly provided by Dr. Ulrich Hopfer (Case Western University, Cleveland, OH). Cell culture media were from either Mediatech Inc. (Herndon, VA) or Life Technologies, Inc. Lipofectin was obtained from Life Technologies, Inc. Most of the β-lactam antibiotics and other chemicals were purchased from Sigma.

**Cell Culture and Uptake Measurements**—The JAR cells were cultured at 37 °C in EMEM 1640 medium supplemented with 10% fetal bovine serum, penicillin (100 units/ml), and streptomycin (100 μg/ml). The HPCT cells were cultured in ketamine medium, supplemented with epidermal growth factor (0.2 ng/ml), bovine pituitary extract (30 μg/ml), fetal bovine serum (10%), penicillin (100 units/ml), and streptomycin (100 μg/ml). All steps involved in uptake measurements were carried out at room temperature. The medium was aspirated, and the cells were washed once with the uptake buffer. 1 ml of uptake buffer containing radiolabeled substrate (L-carnitine, acetyl-L-carnitine, or propionyl-L-carnitine) was added to the cells and incubated for 30 min. Uptake was terminated by aspirating the buffer and subsequently washing the cells three times with fresh uptake buffer. The cells were lysed with 1 ml of 1% SDS in 0.2 N NaOH, and the lysate was transferred to scintillation vials for quantitation of radioactivity. The composition of the uptake buffer was 25 mM Hepes/Tris (pH 7.5), 140 mM NaCl, 5.4 mM KCl, 1.8 mM CaCl₂, 0.8 mM MgSO₄, and 5 mM glucose.

**Functional Expression of Human and Rat OCTN2 cDNAs in HeLa Cells**—The cloned human and rat OCTN2 cDNAs were oriented in the pSPORT plasmid in such a way that their expression was under the control of the T7 promoter. The cDNAs were heterologously expressed in HeLa cells by vaccinia virus expression system as described previously (6). Uptake measurements were made at room temperature using [3H]carnitine or [14C]TEA. The incubation time was 30 min. The procedure for uptake measurements in cDNA-transfected cells was the same as described for JAR and HPCT cells. However, in some experiments dealing with [14C]TEA uptake, measurements were made in the normal Na⁺-containing uptake buffer as well as in a Na⁺-free uptake buffer in which NaCl was replaced iso-osmotically by N-methyl-D-glucamine chloride.

**Measurement of Cephaloridine Uptake via OCTN2**—HeLa cells cultured in 24-well culture plates were transfected with pSPORT vector alone or with hOCTN2 cDNA. The transfected cDNA was functionally expressed by the vaccinia virus expression technique. Vector-transfected control cells and hOCTN2-expressing cells were then incubated with 1 mM cephaloridine in a NaCl-containing medium or in a Na⁺-free medium in which NaCl was iso-osmotically replaced by N-methyl-D-glucamine chloride. The incubation was carried out at 37 °C for 1 h. Following the incubation, the cells were washed with the uptake medium and then lysed with deionized water (150 μl per well). The lysate was centrifuged to remove cellular membranes, and the supernatant was used to measure the levels of cephaloridine. These measurements were made by HPLC (Beckman System Gold) as described previously by Wold and Turnipseed (16) with some modifications. Chromatography was carried out on a Kromasil C18 column using the solvent consisting of 85% 1Tris acetate (pH 3.5) and 15% acetonitrile. The flow rate was 1.3 ml/min. Cephaloridine was detected by ultraviolet absorption at 254 nm. The cephaloridine peak appeared at 7.2 min. Cephaloridine content was 1.3 ml/min. Cephaloridine was detected by ultraviolet absorption at 254 nm. The cephaloridine peak appeared at 7.2 min. Cephaloridine was detected by ultraviolet absorption at 254 nm. The cephaloridine peak appeared at 7.2 min.

**Functional Expression of Human PEPT1 and Rat PEPT2 cDNAs in HeLa Cells**—Human PEPT1 cDNA (17) and rat PEPT2 cDNA (18) were also expressed heterologously in HeLa cells using the vaccinia virus expression system as described previously (17–19). Uptake of [14C]glycyl sarcosine was measured with a 5-min incubation in the presence of an inwardly directed H⁺ gradient. The composition of the uptake buffer was 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.

**RESULTS**

**Interaction of β-Lactam Antibiotics with OCTN2 Constitutively Expressed in Human Placental Trophoblast Cells JAR**—The β-lactam antibiotics that contain a quaternary nitrogen atom are listed with their structures in Fig. 1. The list includes cephaloridine, cefepime, cefoselis, cefluprenam, and ceftazidime. All of these antibiotics are cephalosporins. These antibiotics were tested along with several other penicillins and cephalosporins for their ability to interact with OCTN2 constitutively expressed in JAR cells. These cells express a Na⁺-dependent carnitine transporter (21) whose characteristics are similar to those of the cloned human OCTN2 (4). Northern blot analysis has shown that JAR cells possess OCTN2 mRNA (2). Furthermore, human placenta expresses Na⁺-dependent carnitine transport activity as do the JAR placental cells (22). Therefore, Na⁺-coupled carnitine transport activity in JAR cells represents the transport function of constitutively expressed OCTN2. The interaction of β-lactam antibiotics with OCTN2 in JAR cells was hence studied by assessing the ability of these compounds (2.5 mM) to compete with carnitine (25 mM) for the transport process (Table I). The most potent inhibitors of car-

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**FIG. 1.** Structures of cephalosporins containing a quaternary nitrogen.
TABLE I
Inhibition of carnitine uptake in JAR cells by β-lactam antibiotics

| β-Lactam antibiotic | [3H]Carnitine uptake (fmol/mg of protein/30 min) % |
|---------------------|-----------------------------------------------|
| Control             | 235 ± 22                                      |
| Cephalixin          | 223 ± 9                                       |
| Cephradine          | 193 ± 13                                      |
| Cyclacillin         | 171 ± 4                                       |
| Cefadroxil          | 235 ± 12                                      |
| Cefoselis           | 235 ± 10                                      |
| Penicillin          | 227 ± 16                                      |
| Ampicillin          | 231 ± 12                                      |
| Cefaloridine        | 25 ± 8                                        |
| Cefepime            | 49 ± 1                                        |
| Cefuprenam          | 147 ± 8                                       |
| Cefoselis           | 154 ± 4                                       |
| Cefazidime          | 206 ± 10                                      |

The presence of Na⁺ in the incubation period of 30 min. The concentration of β-lactam antibiotics was 2.5 mM. Uptake of [3H]carnitine measured in the presence of an excess amount (25 mM) of unlabeled carnitine was used to adjust for the diffusional component. Data are means ± S.E. for four independent measurements.

OCTN2–mediated Transport of β-Lactam Antibiotics

The inhibition of carnitine transport caused by these antibiotics was in the range of 40–90%. Other antibiotics with a less, but significant, inhibitory potency were cyclacillin, cephradine, and ceftazidime. The inhibition in these cases ranged between 20 and 30%. The remaining antibiotics had negligible ability to interact with OCTN2. The dose-response relationship for the inhibition of carnitine transport by cephaloridine and cefepime is given in Fig. 2. The IC₅₀ values (i.e. concentration necessary for 50% inhibition) for cephaloridine and cephalosporins were 0.18 ± 0.02 and 1.0 ± 0.1 mM, respectively. These IC₅₀ values are very close to inhibition constants (Kᵢ) because the concentration of carnitine used in these inhibition experiments was severalfold lower than the Michaelis-Menten constant for carnitine (see below).

OCTN2 also transports the acetyl esters of carnitine (4, 21, 22). Therefore, we tested the ability of cephaloridine to inhibit the transport of acetyl-L-carnitine and propionyl-L-carnitine in JAR cells to provide additional evidence for the interaction of this cephalosporin with OCTN2 (Fig. 3). The transport of these acetyl esters of carnitine was found to be inhibited about 70% by 1 mM cephaloridine. Cefadroxil was one of the cephalosporins that did not have any significant inhibitory effect on carnitine transport. This cephalosporin was found to have no effect on the transport of acetyl-L-carnitine and propionyl-L-carnitine.

The kinetic nature of the inhibition was analyzed with cephaloridine (Fig. 4). Carnitine transport in JAR cells was saturable with a Michaelis-Menten constant (Kₑ) of 12.6 ± 1.4 μM and a maximal velocity (Vₘₐₓ) of 133 ± 5 nmol/mg protein/30 min. The presence of 0.3 mM cephaloridine caused a 2-fold increase in the Kₑ value (23.1 ± 1.1 μM) without affecting the Vₘₐₓ (139 ± 3) nmol/mg protein/30 min. These results show that cephaloridine competes with carnitine for the same binding site on the transporter.

OCTN2 in the kidney participates in the reabsorption of filtered carnitine. Brush border membrane vesicles isolated from renal cortex have been shown to possess Na⁺-dependent high affinity carnitine transport activity (22–24). We have shown recently that the HPCT cell line, derived from human kidney proximal tubules, expresses OCTN2 (2, 25). We investigated the interaction of cephaloridine and other β-lactam antibiotics containing a quaternary nitrogen with the carnitine transporter expressed constitutively in these renal epithelial cells. The results were very similar to those obtained with JAR cells (data not shown). Cephaloridine, cepfime, cefoselis, and cefuprenam were found to inhibit carnitine transport in these cells with the same potency as found in JAR cells. In addition, the inhibition by cephaloridine was competitive.

Interaction of β-Lactam Antibiotics with Cloned Human OCTN2—The cloned human OCTN2 was expressed in HEK cells heterologously, and its interaction with β-lactam antibiotics was studied (Table II). Among several β-lactam antibiotics tested, only those that contain a quaternary nitrogen atom inhibited human OCTN2-mediated carnitine transport. These antibiotics were cephaloridine, cepfime, cefuprenam, and cefoselis. The only exception was ceftazidime that did not inhibit carnitine transport. All other β-lactam antibiotics tested did not have any significant effect on carnitine transport mediated by human OCTN2. Among the four inhibitors, cephaloridine and cepfime were more potent than cefuprenam and cefoselis. These results with cloned human OCTN2 are similar to those obtained with constitutively expressed OCTN2 in JAR cells and HPCT cells.

Fig. 5 describes the dose-response relationship for cephaloridine, cepfime, cefoselis, and ceftazidime. The first three β-lactam antibiotics inhibited human OCTN2-mediated carnitine transport with IC₅₀ values 0.23 ± 0.05, 1.7 ± 0.2, and 6.4 ± 1.0 mM, respectively. Cefazidime did not inhibit carnitine transport at least up to a concentration of 10 mM. The inhibition caused by cephaloridine was competitive (Fig. 6). In the absence of the inhibitor, human OCTN2-mediated carnitine transport was saturable with a Kₑ value of 3.5 ± 0.3 μM and a Vₘₐₓ of 1.17 ± 0.04 nmol/10⁶ cells/30 min. The presence of cephaloridine (0.3 mM) increased the Kₑ value 2-fold (8.5 ± 1.4 μM) without affecting the Vₘₐₓ significantly (0.98 ± 0.08 nmol/10⁶ cells/30 min).

Na⁺ Dependence of the Interaction of β-Lactam Antibiotics with OCTN2—OCTN2 is an organic cation/carnitine transporter that transports organic cations in a Na⁺-independent manner and zwitterionic carnitine and acylcarnitines in a Na⁺-dependent manner (4). Therefore, it was of interest to evaluate the Na⁺ dependence of the interaction of the inhibitory β-lactam antibiotics with OCTN2. For these experiments, the organic cation TEA was used as the substrate of OCTN2 for transport measurements. The transport of TEA via OCTN2 is Na⁺-independent and therefore its transport can be studied in the presence or absence of Na⁺. The Na⁺ dependence of the interaction of β-lactam antibiotics was investigated by assessing the relative ability of these compounds to inhibit OCTN2-mediated TEA transport in the presence and absence of Na⁺. Rat OCTN2 was chosen for these studies rather than human OCTN2 because the former transports TEA and carnitine equally well, whereas the latter transports TEA at a much lower rate (4). First, we investigated whether the β-lactam antibiotics with a quaternary nitrogen atom interacts with rat OCTN2 by assessing their ability to inhibit the Na⁺-coupled carnitine transport in HeLa cells expressing rat OCTN2 heterologously (Table III). Cephaloridine and cepfime at a concentration of 2.5 mM were found to inhibit carnitine transport by 70–75%. Cefuprenam and cefoselis were comparatively less potent inhibitors, causing 25–30% inhibition. Cefazidime did not have any effect. Similarly, cefadroxil, which does not possess a quaternary nitrogen atom, did not have any effect. These results are qualitatively similar to those obtained with human OCTN2. There may, however, be some differences in the affinity for these β-lactam antibiotics between rat OCTN2 and human OCTN2. Based on the relative magnitude of inhibition, it appears that rat OCTN2 may exhibit less affinity for these β-lactam antibiotics than human OCTN2.

Fig. 7 describes the relative potency of cephaloridine to in-
hibit rat OCTN2-mediated TEA transport in the presence and absence of Na\(^+\). Cephaloridine inhibited TEA transport with an IC\(_{50}\) value of 0.79 ± 0.10 mM in the presence of Na\(^+\). The inhibitory potency decreased almost 10-fold in the absence of Na\(^+\). The IC\(_{50}\) value in the absence of Na\(^+\) was 10 mM. These data show that the interaction of cephaloridine with rat OCTN2 is Na\(^+\)-dependent. However, when similar experiments were done with cefepime and cefoselis, it was found that the potency of these compounds to inhibit rat OCTN2-mediated TEA transport was not affected markedly by Na\(^+\) (Fig. 8). The IC\(_{50}\) values for cefepime were 2.1 ± 0.2 mM in the presence of Na\(^+\) and 3.1 ± 0.5 mM in the absence of Na\(^+\). Similarly, the IC\(_{50}\) values for cefoselis were 6.4 ± 0.2 and 8.8 ± 0.6 mM in the presence and absence of Na\(^+\). This indicates that the interaction of cefepime and cefoselis with rat OCTN2 is predominantly Na\(^+\)-independent. The observed differences between cephaloridine and the other two inhibitors in terms of Na\(^+\) dependence are interesting because all three antibiotics exist predominantly as zwitterions at pH 7.5.

Transport of Cephaloridine via Human OCTN2—The findings that cephaloridine and other \(\beta\)-lactam antibiotics such as cefepime, cefoselis, and ceftuprenam inhibit OCTN2-mediated transport of carnitine and TEA do not necessarily mean that these antibiotics are transportable substrates for OCTN2. These findings do not rule out the possibility that cephaloridine and other \(\beta\)-lactam antibiotics inhibit OCTN2 transport function without themselves being transported. Therefore, we studied the ability of OCTN2 to transport cephaloridine directly. Human OCTN2 was expressed heterologously in HeLa cells. Vector-transfected cells served as control. The cells were incubated with 1 mM cephaloridine for 1 h in the absence or presence of Na\(^+\). The cellular content of cephaloridine was below detectable levels in the presence as well as in the absence of Na\(^+\), showing that there was negligible OCTN2 activity in control cells. However, the cellular content of cephaloridine in vector-transfected cells was below detectable levels in the presence of Na\(^+\), and OCTN2 activity was detectable in the presence of cephaloridine. This indicates that human OCTN2 mediates the cellular uptake of cephaloridine.
in a Na\(^+\)-dependent manner. The Na\(^+\) dependence of the interaction of cephaloridine with OCTN2 corroborate these findings.

**Interaction of Peptide Transporters with β-Lactam Antibiotics Containing a Quaternary Nitrogen**—The H\(^+\)-coupled peptide transporters PEPT1 and PEPT2 transport several β-lactam antibiotics (26–30). Penicillins as well as cephalosporins are recognized as transportable substrates by these transporters. Many of the β-lactam antibiotics not recognized by OCTN2 in the present study have been shown to be excellent substrates for PEPT1 and PEPT2. However, the interaction of β-lactam antibiotics containing a quaternary nitrogen has not been studied with the peptide transporters. Since this group of β-lactam antibiotics is recognized by OCTN2 as transportable substrates, we investigated the interaction of these compounds with the peptide transporters. PEPT1 and PEPT2 were expressed heterologously in HeLa cells, and their transport function was monitored by measuring the transport of the dipeptide glycylsarcosine in the presence of an inwardly directed H\(^+\) gradient, the driving force for these transporters. The interaction of β-lactam antibiotics with the peptide transporters was studied by assessing the ability of these compounds to inhibit glycylsarcosine transport in PEPT1- and PEPT2-expressing cells (Table IV). Interestingly, the β-lactam antibiotics with a quaternary nitrogen did not interact with the peptide transporters to any significant extent. The only exception was cefoselis which, at a concentration of 2.5 mM, caused a small but significant inhibition (20–25%). As a control, we studied the interaction of cefadroxil with the peptide transporters. This compound does not contain a quaternary nitrogen but has been shown to be a transportable substrate for PEPT1 and PEPT2 (26–30). As expected, cefadroxil caused a 40% inhibition of PEPT1-mediated glycylsarcosine transport. The inhibition was almost 100% in the case of PEPT2. These studies show that cephaloridine and other β-lactam antibiotics with a quaternary nitrogen are not recognized by OCTN2 as transportable substrates.

**TABLE II**

| β-Lactam antibiotic | [\(^3\)H]Carnitine uptake pmol/10\(^6\) cells/30 min | % |
|---------------------|-----------------------------------------------|---|
| Control             | 2.82 ± 0.27                                   | 100 |
| Cephalaxin          | 2.73 ± 0.28                                   | 97  |
| Cephradine          | 3.01 ± 0.22                                   | 107 |
| Cyclacillin         | 2.84 ± 0.24                                   | 94  |
| Cefdinir            | 2.89 ± 0.14                                   | 103 |
| Cefoxime            | 3.72 ± 0.17                                   | 97  |
| Cefadroxil          | 3.76 ± 0.21                                   | 98  |
| Penicillin          | 2.90 ± 0.29                                   | 103 |
| Ampicillin          | 2.78 ± 0.32                                   | 99  |
| Cephaloridine       | 0.37 ± 0.06                                   | 13  |
| Cefepine            | 0.76 ± 0.08                                   | 27  |
| Cefluprenam         | 2.09 ± 0.02                                   | 74  |
| Cefoselis           | 2.25 ± 0.04                                   | 80  |
| Ceftazidime         | 3.01 ± 0.33                                   | 107 |

**FIG. 4.** Kinetics of cephaloridine-induced inhibition of carnitine transport in JAR cells. Transport of carnitine in JAR cells was measured in the absence (○) or presence (●) of 0.3 mM cephaloridine over a carnitine concentration range of 2.5–100 μM. Inset, Eadie-Hofstee plot. V\(_t\), carnitine transport in pmol/mg protein/30 min; S, carnitine concentration in μM.

**FIG. 5.** Influence of cephaloridine, cefepime, cefoselis, and ceftazidime on human OCTN2-mediated carnitine transport. The cloned human OCTN2 cDNA was expressed functionally in HeLa cells using the vaccinia virus expression technique. Transport of [\(^3\)H]carnitine (20 nM) in these cells was measured in a NaCl-containing buffer in the presence of increasing concentrations of cephaloridine (○), cefepime (●), cefoselis (○), and ceftazidime (▲). Endogenous carnitine transport activity was measured under identical conditions in cells transfected with pSPORT vector alone. Transport activity in cDNA-transfected cells was adjusted for endogenous activity to calculate cDNA-specific activity. Results are given as percent of control cDNA-specific transport measured in the absence of inhibitors (2.67 ± 0.21 pmol/10\(^6\) cells/30 min).


**OCTN2-mediated Transport of β-Lactam Antibiotics**

**FIG. 6. Kinetics of cephaloridine-induced inhibition of human OCTN2-mediated carnitine transport.** Human OCTN2 cDNA was expressed in HeLa cells heterologously. Carnitine transport in these cells was measured in the absence (○) or presence (□) of 0.3 mM cephaloridine over a carnitine concentration range of 2.5–50 μM. Results represent only the cDNA-specific transport after adjustment for endogenous activity measured in vector-transfected cells. *Inset,* Eadie-Hofstee plot. V, carnitine transport in pmol/10⁶ cells/30 min; S, carnitine concentration in μM.

**TABLE III**

| β-Lactam antibiotic | [3H]Carnitine uptake pmol/10⁶ cells/30 min | % |
|---------------------|------------------------------------------|---|
| Control             | 1.22 ± 0.06                              | 100 |
| Cephaloridine       | 0.31 ± 0.01                              | 25  |
| Cefepime            | 0.34 ± 0.04                              | 28  |
| Cefteprenam         | 0.93 ± 0.05                              | 76  |
| Cefoselis           | 0.87 ± 0.09                              | 71  |
| Ceftazidime         | 1.30 ± 0.06                              | 107 |
| Cefadroxil          | 1.20 ± 0.16                              | 98  |

nitrogen are recognized as substrates by OCTN2 but not by the peptide transporters.

**DISCUSSION**

β-Lactam antibiotics are widely used in humans as therapeutic agents. This group of antibiotics includes penicillins and cephalosporins. Cellular processes that influence the oral bioavailability and half-life in systemic circulation of these drugs are important determinants of their therapeutic efficacy. Intestinal transport systems with the potential to mediate the oral absorption of these drugs have been the focus for the past several years to understand the means by which the oral bioavailability of these drugs could be enhanced. Kidney plays an important role in determining the half-life of therapeutic agents in systemic circulation by influencing their reabsorption and/or secretion. Thus, renal transport systems that have the potential to mediate the renal absorption and/or secretion of β-lactam antibiotics have also been the target of research for several years with the aim to understand the cellular processes in the kidney that influence the systemic half-life of these drugs. These studies on the intestinal and renal handling of β-lactam antibiotics have so far identified five different transport systems that interact with these drugs (26–32). They are the H⁺-coupled peptide transporters PEPT1 and PEPT2, H⁺-coupled monocarboxylate transporter MCT1, the anion exchanger OAT1, and the organic cation/H⁺ antiporter. The peptide transporters and the monocarboxylate transporter participate in the intestinal and renal uptake of β-lactam antibiotics across the brush border membrane, whereas the organic cation/H⁺ antiporter in the brush border membrane and the anion exchanger in the basolateral membrane participate in the renal excretion of these drugs.

Here we report on the identification of a new transporter that is involved in the cellular transport of β-lactam antibiotics. Our data show that OCTN2, an organic cation/carnitine transporter, is able to mediate the transport of certain cephalosporin antibiotics such as cephaloridine, cefepime, cefteprenam, and cefoselis. These studies were done not only using human cell lines that constitutively express OCTN2 but also using the cloned human and rat OCTN2 following their functional expression in mammalian cells. The interaction of these and other β-lactam antibiotics with OCTN2 was studied by assessing the ability of these drugs to inhibit OCTN2-mediated transport of carnitine, acylcarnitine esters, and tetraethylammonium. Direct evidence for transport has been obtained by measuring OCTN2-mediated cephaloridine uptake into cells.

The present data demonstrating the transport of β-lactam antibiotics by OCTN2 are of immense pharmacological and clinical relevance for a number of reasons. OCTN2 is the first Na⁺-coupled transporter identified so far that is involved in cellular uptake of β-lactam antibiotics. The previously identified transport systems, namely the peptide transporters, the monocarboxylate transporter MCT1, the anion exchanger OAT1, and the organic cation/H⁺ antiporter are all dependent on a transmembrane H⁺ gradient or anion gradient rather than a transmembrane Na⁺ gradient. In addition, OCTN2 is ubiquitously expressed in human tissues, including the kidney, intestine, heart, skeletal muscle, immune cells, brain, and placenta. In contrast, the peptide transporters, the anion exchanger, and the organic cation/H⁺ antiporter exhibit only a restricted tissue distribution pattern. The monocarboxylate transporter is widely expressed, but its participation in the transport of β-lactam antibiotics is very limited. The only β-lactam antibiotic that has been shown to be transported via the monocarboxylate transporter is cefdinir (31). The dependence of OCTN2 on a transmembrane Na⁺ gradient as the driving force is functionally significant in relation to the expression of the transporter in different tissues. Under physiological condi-
tions, a transmembrane H⁺ gradient or anion gradient is quantitatively important only in the intestine and kidney. The H⁺-coupled peptide transporters that are expressed predominantly in these two tissues derive their energy from the H⁺ gradient. In contrast, a transmembrane Na⁺ gradient is present in every mammalian cell. Therefore, the widespread occurrence of OCTN2 in mammalian tissues and its dependence on a Na⁺ gradient as the driving force make this transporter an excellent conduit for cellular uptake of β-lactam antibiotics driven by a Na⁺ gradient. Furthermore, the substrate specificity of OCTN2 versus peptide transporters in terms of β-lactam antibiotics is complementary to each other. Most β-lactam antibiotics that are recognized by PEPT1 and PEPT2 are not substrates for OCTN2. Similarly, those β-lactam antibiotics that interact with OCTN2 are not recognized by the peptide transporters.

Detailed investigations of the structural requirements of β-lactam antibiotics by PEPT1 and PEPT2 have established the following structural features as necessary for recognition: (a) sterical resemblance to the tripeptide backbone; (b) the presence of an amino group in the substituent of the penam (penicillins) or cepham (cephalosporins) nucleus; and (c) the presence of a free carboxylic acid group in the thiazolidine (penicillins) or dihydrothiazine (cephalosporins) ring (26–30).

The β-lactam antibiotics that do not possess the amino group are not recognized by the peptide transporters. It appears that PEPT1 and PEPT2 accept β-lactam antibiotics as substrates only as zwitterions. The antibiotics that meet these structural criteria include cyclacillin, cefadroxil, cephradine, cephalexin, ampicillin, and cefaclor. Interestingly, none of these is a substrate for OCTN2. The antibiotics that interact with OCTN2 include cephaloridine, cefoselis, cefepime, and cefluprenam. All of these are cephalosporins. These compounds do not possess an amino group in the substituent of the cepham nucleus that is obligatory for recognition by PEPT1 and PEPT2. Instead, they possess a quaternary nitrogen attached to the dihydrothiazine ring. They do, however, exist as zwitterions at physiological pH due to the presence of the carboxylic acid group attached to the dihydrothiazine ring. Ceftazidine that contains a quaternary nitrogen but exists as an anion due to the presence of two carboxylic acid groups (one attached to the dihydrothiazine ring and the other in the substituent of the cepham nucleus) is not recognized by OCTN2 to any significant extent. It appears that OCTN2 favors zwitterionic form of the cephalosporins containing a quaternary nitrogen and excludes anionic form even in the presence of a quaternary nitrogen. Interestingly, none of the β-lactam antibiotics that are sub-

**FIG. 7.** Influence of Na⁺ on cephaloridine-induced inhibition of rat OCTN2-mediated TEA transport. The cloned rat OCTN2 cDNA was expressed heterologously in HeLa cells. Transport of [14C]TEA (20 μM) was measured in the presence of NaCl (○) or in the presence of N-methyl-D-glucamine chloride instead of NaCl (●). Concentration of cephaloridine was varied over the range of 0–10 mM. Data represent only cDNA-specific activity after adjustment for endogenous activity measured in vector-transfected cells under identical conditions. Results are given as percent of corresponding control transport measured in the absence of cephaloridine.

**FIG. 8.** Influence of Na⁺ on cefoselis- and cefepime-induced inhibition of rat OCTN2-mediated TEA transport. Experiments were done as described in the legend to Fig. 7. Key: ○, presence of Na⁺; ●, absence of Na⁺.
OCTN2 makes the Na⁺-binding site unavailable for Na⁺.

The therapeutic and clinical significance of the present studies is severalfold. Cefepime and cefoselis are widely used currently in humans as antibacterial agents. These antibiotics have a broad spectrum of action against Gram-negative as well as Gram-positive bacteria and hence are classified as the fourth generation antibiotics. These drugs are substrates for OCTN2 but not for peptide transporters. Therefore, OCTN2 is likely to play a critical role in the disposition and therapeutic efficacy of these drugs. Interestingly, the oral bioavailability of cefepime, cefoselis, and cephaloridine is very low, and these drugs are therefore administered intravenously rather than orally. Even though OCTN2 is expressed in the intestine, it is likely that the expression levels are very low at least in adults. The observations that oral bioavailability of carnitine is also very low support this contention. On the other hand, OCTN2 is expressed at high levels in the kidney, heart, skeletal muscle, and brain. Therefore, OCTN2 is likely to mediate the effective reabsorption of these drugs in the kidney and also to influence the disposition of these drugs in the other tissues expressing OCTN2. We speculate that OCTN2 in the kidney enhances the systemic half-life of these drugs by preventing their elimination in the urine and consequently increases their therapeutic efficacy. The present findings that cephaloridine is a substrate for OCTN2 also explain the development of carnitine deficiency in humans during cephaloridine therapy. Administration of cephaloridine is expected to compete with carnitine for renal reabsorption via OCTN2. This would result in increased urinary loss of carnitine leading to decreased blood levels of carnitine. Cephaloridine is not used currently in humans due to nephrotoxicity associated with its therapeutic use. We speculate that cefoselis and cefepime also interfere with the renal absorption of carnitine, but the interference may be much smaller in magnitude compared with that seen with cephaloridine due to the differences in their affinities for OCTN2.

Another important clinical aspect of the present studies is related to the area of pharmacologic genomics. Mutations in the octn2 gene have been shown to be responsible for the genetic disorder primary systemic carnitine deficiency (7–11). Since the β-lactam antibiotic substrates and carnitine seem to compete for the same substrate-binding site on OCTN2, it is very likely that the mutations causing carnitine deficiency are also associated with a loss of ability to transport the β-lactam antibiotic substrates. Patients with the disease usually die at less than 10–15 years of age if left untreated but live considerably longer if treated with oral supplementation of carnitine. We speculate that these patients may exhibit impaired handling of the fourth generation β-lactam antibiotics in various tissues including kidney due to defective OCTN2. This might lead to increased renal clearance of these antibiotics and hence to decreased systemic half-life and therapeutic efficacy. Thus, patients with primary systemic carnitine deficiency may have decreased therapeutic response to these antibiotics.

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