Novel Organic Cation Transporter 2-Mediated Carnitine Uptake in Placental Choriocarcinoma (BeWo) Cells

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ABSTRACT
The placental transport of carnitine is significant because the fetus cannot supply itself with adequate amounts of this nutrient. Carnitine deficiencies in infants can lead to symptoms ranging from muscle weakness to sudden infant death. Objectives of this study include the characterization of novel organic cation transporter 2 (OCTN2) function in the BeWo cell line and the inhibition of placental carnitine uptake by amphetamine derivatives. BeWo cells were seeded in 12- or 24-well tissue culture plates and incubated at 37°C until monolayers were confluent. Uptake studies with radiolabeled L-carnitine and inhibitors in Hanks’ balanced salt solution were carried out in the plates at 37°C for 30 min. Uptake of L-carnitine in BeWo cells was Na+-dependent and saturable (K_m = 9.8 ± 2.4 μM, V_max = 800 ± 70 pmol/mg of protein/30 min) with a nonsaturable constant of 2.8 ± 0.3 μl/mg of protein/30 min. Among the amphetamine analogs studied, IC_{50} values ranged from 2.3 to 9.2 mM, and the inhibition of carnitine uptake was stronger for compounds having a methyl-substituted nitrogen atom. Lineweaver-Burk plots show that inhibition by tetraethylammonium and valproate was competitive; inhibition by ephedrine was not completely competitive. The observed kinetics, Western blot, and inhibition profiles indicate that high-affinity carnitine uptake in the BeWo cell line is mediated by OCTN2. Inhibition of carnitine transport by amphetamines potentially poses serious consequences for fetal development.

Although the transport of many drugs across the placenta is by simple diffusion, active and facilitative transport processes mediate the passage of certain nutrients, drugs, and xenobiotics. Transporters in the placenta assist to transfer nutrients from the mother to the fetus, eliminate waste products from the fetus, and limit the transport of certain molecules (Unadkat et al., 2004). Understanding the mechanisms of placental transporters will assist in controlling fetal drug delivery during pregnancy. Expanding knowledge regarding the function, selectivity, and regulation of these transporters is necessary to reduce the risks of fetal drug exposure when the mother must receive medication. Care must be taken because certain drugs may interfere with physiologic transport pathways and result in undesirable side effects (Ganapathy et al., 2000b).

Organic cation transporters (OCTs) play an important role in the absorption and distribution of many drugs and endogenous compounds. Although it is clear that some OCTs are active in transporting nutrients and other metabolites, the physiological functions of all membrane transporters are not yet known (Ganapathy et al., 2000b). When a drug or xenobiotic compound is recognized by these transporters, not only is cell entry possible for the compound but also the compound can inhibit the transport of physiological substrates. Active transport of organic cations is also significant to pharmaceutical research because many OCT substrates are also substrates for multidrug resistance proteins.

Novel organic cation transporter 2 (OCTN2) is a Na+-dependent carnitine transporter expressed in the placenta and other tissues (Lahjoji et al., 2001). L-Carnitine is necessary for the mitochondrial oxidation of fatty acids. Placental transport of carnitine is especially important, because the fetus is not capable of synthesizing sufficient amounts of this nutrient (Wu et al., 1999). Mutations of OCTN2 causing decreased carnitine transport result in primary carnitine deficiency; the impaired fatty acid oxidation brought about by this condition can lead to cardiomyopathy, muscle weakness, hypoketotic hypoglycemia, Reye’s syndrome, and sudden infant death (Wang et al., 2000; Lahjoji et al., 2001). Drug-induced inhibition of carnitine transport may also lead

ABBREVIATIONS: OCT, organic cation transporter; OCTN2, novel organic cation transporter 2; PBS, phosphate-buffered saline; HBSS, Hanks’ balanced salt solution; TEA, tetraethylammonium; MPP⁺, 1-methyl-4-phenylpyridinium; MES, 2-(N-morpholino)ethanesulfonic acid.
to lower plasma carnitine levels and symptoms of carnitine deficiency (Tein et al., 1993).

Several drugs have been shown to induce carnitine deficiencies, including anticonvulsants (e.g., valproic acid) (Tein et al., 1993; Verrotti et al., 1999; Wu et al., 2004), antibiotics (e.g., cephalexin and enemite) (Ganapathy et al., 2000a; Wagner et al., 2000), and pivalic acid (Okudaira et al., 2001). Although each of these drugs causes depletion of plasma carnitine levels, the mechanisms differ. Cephalexin and enemite inhibit OCTN2-mediated carnitine uptake (Ganapathy et al., 2000a; Wagner et al., 2000), but administration of pivalic acid leads to the production of pivaloylcarnitine conjugates, the excretion of which results in lower plasma carnitine concentrations (Brass, 2002).

Unfortunately, drug abuse during pregnancy continues to be a significant problem (Huestis and Choo, 2002), some women even use illegal drugs as a means of relieving discomforts experienced in pregnancy. The interference of these drugs with placental nutrient exchange can lead to serious developmental consequences for the baby. Abuse of amphetamines during pregnancy has been linked to low birth weight, premature delivery, brain hemorrhage, infarction, cavitory lesions, and increased risks of neonatal seizures and sudden infant death syndrome (Huestis and Choo, 2002). Amphetamines have been shown to strongly inhibit the serotonin and norepinephrine transporters in the placenta (Rammamoorthy et al., 1995). These illegal drugs have also been linked to decreased mitochondrial function (Burrows et al., 2000), and it has been suggested that carnitine may play a role in attenuating the toxic effects of methamphetamine (Virmani et al., 2003).

Objectives of this study include the characterization of OCTN2 function in the BeWo chorioniccell carcinoma cell line and the inhibition of placental carnitine uptake by amphetamine derivatives. BeWo cells form monolayers in culture and serve as an in vitro model of the rate-limiting barrier for maternal-fetal exchange (Young et al., 2002).

Materials and Methods

Cell Culture. The BeWo cell line (clone b30) was obtained from Dr. Alan Schwartz (Washington University, St. Louis, MO). Passages 29 through 40 of the cells were used in this study, cultured as described previously (Young et al., 2002). Briefly, the cells were cultured in Dulbecco's modified Eagle's medium (Sigma-Aldrich, St. Louis, MO) adjusted to pH 7.4 and containing 10% heat-inactivated fetal bovine serum (Atlanta Biologicals, Norcross, GA) and 1% each of 200 mM (100×) l-glutamine, 10,000 units/ml penicillin with 10 mg/ml streptomycin, and 10 mM (100×) minimal essential medium nonessential amino acids solution (all three from Invitrogen, Carlsbad, CA). Cells were maintained at 37°C in 150-cm² flasks under 5% CO₂ and 95% relative humidity; the medium was changed every other day. Cells were harvested with trypsin-EDTA solution (Sigma-Aldrich) diluted in phosphate-buffered saline (PBS; 129 mM NaCl, 2.5 mM KCl, 7.4 mM Na₂HPO₄, and 1.3 mM KH₂PO₄, adjusted to pH 7.4).

Western Blotting. Cellular lysate from confluent monolayers of BeWo cells grown in 150-cm² flasks was obtained, and Western blotting was performed as described previously (Hamilton et al., 2001), except that the staining with Ponceau S was omitted. Electrophoresis was carried out in a 12% Tris-glycine gel (Invitrogen) at 110 V for 2 h, and the transfer to a nitrocellulose membrane (Invitrogen) was at 25 V for 2 h on ice. The primary antibody was rabbit anti-mouse OCTN2 affinity pure IgG (Alpha Diagnostic International, San Antonio, TX) at a concentration of 10 μg/ml, and the secondary antibody was peroxidase-conjugated AffiniPure goat anti-rabbit IgG (Jackson ImmunoResearch Laboratories, West Grove, PA), diluted 1:10,000. The enhanced chemiluminescence Western blotting detection reagents were obtained from Amersham Biosciences UK, Ltd. (Little Chalfont, Buckinghamshire, UK).

Uptake Experiments. Before seeding BeWo cells in 12- or 24-well tissue culture plates, the plates (Corning Costar, Corning, NY) were prepared by coating each well in a 12-well plate with 100 μl of a 5 μg/ml poly-l-lysine (Sigma-Aldrich) solution in 28% ethanol (70 μl was added to each well in a 24-well plate). The plates dried under fluorescent light for 3 h in a sterile hood and were then exposed to ultraviolet light for 1 h. At this point, the plates could either be wrapped and refrigerated for up to 2 weeks, or preparation for cell seeding could continue under sterile conditions, with 1 ml of PBS warmed to 37°C added to each well of 12-well plates warmed to room temperature (0.5 ml of PBS was used in 24-well plates). After 30 min, the PBS was aspirated, and 3 drops of a 50 μg/ml fibronectin (Sigma-Aldrich) solution in PBS were added to each well in 12-well plates (2 drops were added in 24-well plates). Once even coating of the fibronectin solution had been verified, the plates were dried for 45 to 90 min under fluorescent light. Immediately before cell seeding, 1 ml of Dulbecco's modified Eagle's medium was added to each well (0.5 ml in 24-well plates) and then aspirated. BeWo cells were seeded in the plates as described previously (Young et al., 2002) but at a density of 12,500 cells/cm². The cells were incubated and medium was changed every other day until 100% confluent (4–6 days).

The uptake experiments in BeWo cells were carried out as described previously (Young et al., 2002). The cells were washed three times with Hanks' balanced salt solution (HBSS, Sigma-Aldrich) and then equilibrated in HBSS at 37°C for approximately 45 min. [3H]Carnitine hydrochloride (Moravek Biochemicals, Brea, CA) premixed with (or without) inhibitors in warm HBSS was added to the cells in a shaking hot box (37°C) for 30 min. The dosing solutions were then aspirated, and the cells were washed with ice-cold HBSS three times before the lysing solution (0.5% Triton X-100 in 0.2 N NaOH) was added, and the plate continued to shake in the hot box for approximately three hours. Samples of the lysate were then collected for scintillation spectrometry (LS 6000IC, Beckman Coulter, Fullerton, CA) and protein assay using a kit from Pierce Chemical (Rockford, IL) with bovine serum albumin as the standard.

The inhibitor compounds L-carnitine hydrochloride (unlabeled), D-carnitine hydrochloride, acetyl-DL-carnitine hydrochloride, tetraethylammonium (TEA) chloride, 1-methyl-4-phenylpyridinium (MPP⁺) iodide, sodium valproate, choline chloride, (+)-amphetamine hydrochloride, (+)-ephedrine hydrochloride, and (+)-nor-ephedrine hydrochloride were supplied by the National Institute on Drug Abuse (Rockville, MD). Dr. William Soine (Virginia Commonwealth University, Richmond, VA) kindly provided the chloroephedrine hydrochloride compounds.

It was difficult to adjust the pH of HBSS; therefore, pH-dependent uptake studies were carried out in Krebs-Ringer buffer (123 mM NaCl, 4.93 mM KCl, 1.23 mM MgSO₄, 0.85 mM CaCl₂, 5 mM glucose, 5 mM glutamine, 10 mM HEPES, and 10 mM MES) (Said et al., 2002). The pH was adjusted using HCl and KOH because titration with NaOH would result in increased uptake due to the Na⁺-coupled transport of carnitine by OCTN2. The sodium dependence of carnitine uptake was investigated by replacing the NaCl in Krebs-Ringer buffer with 123 mM N-methyl-d-glucamine chloride (Sigma-Aldrich). Sodium gluconate (123 mM) and 4.93 mM potassium gluconate (both from Sigma-Aldrich) replaced NaCl and KCl in Krebs-Ringer buffer to study the chloride dependence of the uptake.

The chloroephedrine compounds are stable for at least 8 h when the pH is between 5 and 6, but at pH 7.4, decomposition may occur within minutes. For these reasons, the uptake experiments involving the chloroephedrines as inhibitors were shortened to 20 min, and
these studies (and appropriate controls) were carried out in Krebs-Ringer buffer adjusted to pH 5.2. All other uptake inhibition studies were performed in HBSS for 30 min, which was verified to represent the initial rate.

**Data Analysis.** The saturable uptake data (picomoles per 30 min per milligram of protein) were fit to the following equation:

\[
V = \frac{V_{\text{max}} \times C}{K_m + C} + k \times C
\]  

where \( V \), \( V_{\text{max}} \), \( C \), \( K_m \), and \( k \) represent the uptake rate, maximum uptake rate, carnitine concentration, the half-saturation concentration (Michaelis constant), and the nonsaturable constant, respectively. 

Uptake in the presence of inhibitors was calculated as percentage of control (uptake in the absence of any inhibitor). The \( IC_{50} \) values (inhibitor concentration at which inhibition is 50%) were calculated from the following equation:

\[
\text{Percentage of control} = \min \left(1 + \frac{100\% - \text{min}}{1 + 10^{\log IC_{50} - \log I}} \times \text{Hillslope}\right)
\]

where \( I \) is the inhibitor concentration, \( \text{min} \) is the minimum uptake (as \( I \) approaches \( \infty \)), and Hillslope is the slope factor. Regression was performed using SigmaPlot software (SPSS Inc., Chicago, IL). Errors associated with reported values represent the standard deviation calculated from three or four determinations.

To assess the competitive nature of certain inhibitors, Lineweaver-Burk plots were generated. In cases of competitive inhibition, \( K_i \) values were determined from the slopes of the plots, according to the following equation:

\[
V = \frac{V_{\text{max}} \times C}{\left(1 + \frac{I}{K_i}\right) \times K_m + C}
\]

However, where the inhibition was not purely competitive, both \( K_i \) and \( K_i' \) values were obtained from the following equation:

\[
V = \frac{V_{\text{max}} \times C}{\left(1 + \frac{I}{K_i}\right) \times K_m + \left(1 + \frac{I}{K_i'}\right) \times C}
\]

**Results**

The Western blot in Fig. 1 demonstrates the expression of OCTN2 in BeWo cells. Bands occurred between 50 and 64 kDa, which is in agreement with the calculated molecular weight of hOCTN2 (62.7 kDa). Similar bands also occurred in the cell lysate of Calu-3 cells (a human submucosal gland cell line) analyzed simultaneously.

The uptake of \(^3\text{H}\)l-carnitine with varying pH is shown in Fig. 2. The uptake of l-carnitine was independent of pH in the range of 6.5 to 8.0, but below pH 6.5, the uptake decreased as the pH decreased. l-Carnitine uptake in BeWo cells was dependent on the presence of sodium ions, as the uptake decreased significantly (\( p < 0.001 \)) when the NaCl in the transport buffer was replaced by N-methyl-D-glucamine chloride (Fig. 3). In the presence of Na\(^+\), administration of 10 nM \(^3\text{H}\)l-carnitine resulted in an uptake of 0.582 ± 0.066 pmol/30 min/mg of protein, and in the absence of sodium, this uptake was reduced to 0.385 ± 0.008 pmol/30 min/mg of protein. No significant dependence on chloride ion was observed, however. The replacement of NaCl and KCl with sodium gluconate and potassium gluconate resulted in an uptake that was 107.7 ± 7.2% of control.

l-Carnitine uptake in BeWo cells was saturable, as shown in Fig. 4. The \( K_m \) value was determined to be 9.8 ± 2.4 \( \mu \text{M} \), with a \( V_{\text{max}} \) of 800 ± 70 pmol/30 min/mg of protein. The uptake was linear up to 30 min (data not shown); therefore, all reported values correspond to the initial rate. A nonsaturable constant was also obtained; the value for \( k \) from eq. 1 was found to be 2.8 ± 0.3 \( \mu \text{mol}/30 \text{ min/mg of protein} \).

The uptake of \(^3\text{H}\)l-carnitine was strongly inhibited by unlabeled l-carnitine, its stereoisomer (d-carnitine), and acetyl-DL-carnitine. Figure 5 shows that the inhibition by the organic cations TEA and MPP\(^+\) and by sodium valproate was not as strong as that of the carnitine analogs. This figure shows two distinct regions of inhibitory efficacy, and Table 1 shows that the \( IC_{50} \) values are in the micromolar range for the carnitines and in the millimolar range for the other compounds. At the highest concentration of choline studied (30 mM), choline only inhibited the carnitine uptake to 57 ± 9% of control. This incomplete inhibition profile precluded the determination of an \( IC_{50} \) value for choline.

The amphetamine derivatives studied also inhibited the carnitine uptake in BeWo cells, with \( IC_{50} \) values similar to those of TEA and MPP\(^+\). Table 2 lists these drugs in order of increasing \( IC_{50} \) values, which range from 2.3 mM (methamphetamine) to 9.2 mM (nor-\( \psi \)-ephedrine). The structural substituent assignments \( R_1 \), \( R_2 \), and \( R_3 \) in the table correspond to Fig. 6.

Lineweaver-Burk plots in Figs. 7 and 8 show that inhibition of carnitine uptake in BeWo cells is competitive for TEA.
and valproate, respectively. In the presence of 5.0 mM TEA, the $K_m$ value for carnitine uptake increased from 9.8 ± 2.1 to 50.3 ± 12.6 μM, whereas the $V_{max}$ did not change significantly (770 ± 180 pmol/30 min/mg of protein in the absence of TEA, and 920 ± 210 pmol/30 min/mg of protein in presence of 5.0 mM TEA). Similarly, 5.0 mM sodium valproate caused the $K_m$ value to increase from 17.8 ± 8.3 to 34.6 ± 12.7 μM and the $V_{max}$ to go from 490 ± 100 to 560 ± 90 pmol/15 min/mg of protein. The $K_i$ values were determined to be 1.2 ± 0.5 mM for TEA and 2.0 ± 0.7 mM for valproate.

Whereas a similar concentration of ephedrine (4.5 mM) also caused the $K_m$ value to increase from 9.8 ± 2.1 to 15.2 ± 6.9 μM, there was also an accompanying decrease in the $V_{max}$ value, from 770 ± 180 to 570 ± 360 pmol/30 min/mg of protein (the same control experiment was used for both TEA and ephedrine). The decrease in $V_{max}$ was more pronounced in the presence of 9.0 mM ephedrine, where the $V_{max}$ for carnitine uptake in BeWo cells was only 380 ± 50 pmol/30 min/mg of protein. This decrease in $V_{max}$, together with the intersection of the lines occurring slightly to the left of the $y$-axis in Fig. 9, suggests that the inhibition of carnitine uptake in BeWo cells by ephedrine is not completely competitive. For ephedrine, the $K_i$ value was found to be 3.6 ± 0.9 mM, and $K_i$ was 6.7 ± 3.0 mM, suggesting mixed inhibition.

Discussion

In the BeWo choriocarcinoma cell line, OCTN2 mediates the uptake of L-carnitine. This protein is present in BeWo cells, as demonstrated by Western blot, and the observed pH dependence, kinetics, and inhibition profiles are in good agreement with the literature. The pH dependence of carnitine uptake by BeWo cells corresponds well with the results shown by Ohashi et al. (1999) in hOCTN2-transfected HEK293 cells. For the concentration-dependent uptake of carnitine in BeWo cells, we present a $K_m$ value of 3.6 ± 0.9 μM, whereas Tamai et al. (1998) report a $K_m$ value of 4.3 μM in OCTN2-transfected HEK293 cells, and a $K_m$ value of 12.6 μM in JAR cells is reported by Ganapathy et al. (2000a).

The observed Na$^+$-dependent carnitine uptake with no Cl$^-$ dependence agrees with results observed in JAR cells (Prasad et al., 1996), where the replacement of NaCl with LiCl resulted in a 43% reduction in carnitine uptake. Prasad et al. (1996) saw reductions that are more pronounced when NaCl was replaced with KCl and choline chloride, which could be attributed to the combination of sodium dependence with electrogenic effects and substrate inhibition, respectively.

Our observed nonsaturatable component of carnitine uptake may be due to another transporter present in BeWo cells with a lower affinity for carnitine than that of OCTN2. The fact that sodium replacement caused the uptake to decrease by only 34% also suggests significant participation of some non-OCTN2-mediated carnitine transport. However, the sodium replacement experiment does not represent a total sodium-depleted state, but rather a sodium-deficient state, which may account for the lack of a more complete reduction in carnitine uptake. Further experimentation will be required to precisely define the fraction of transport attributable to OCTN2. Considering that other researchers have shown multiple OCT forms that trans-
TABLE 1
Inhibition of [3H]-L-carnitine uptake in BeWo cells

Uptake of 10 nM [3H]-L-carnitine in BeWo cells was determined at 37°C for 30 min in the presence or absence (control) of inhibitors. Data were corrected for protein content and expressed as percentages of the control uptake. IC50 values for each inhibitor were obtained by fitting the data presented in Fig. 6 as described in the text. The error represents the standard deviation from three or four IC50 determinations. Inhibitors are listed in order of increasing IC50 values. Each point and error bar represents the average and standard deviation for three to four determinations of uptake corrected for protein content.

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>IC50 (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Carnitine</td>
<td>3.7 ± 0.9</td>
</tr>
<tr>
<td>Acetyl-L-carnitine</td>
<td>15.7 ± 1.8</td>
</tr>
<tr>
<td>d-Carnitine</td>
<td>16.5 ± 1.0</td>
</tr>
<tr>
<td>TEA</td>
<td>1.2 ± 0.4</td>
</tr>
<tr>
<td>MPP+</td>
<td>1.7 ± 0.4</td>
</tr>
<tr>
<td>Sodium valproate</td>
<td>2.6 ± 0.5</td>
</tr>
<tr>
<td>Choline</td>
<td>ND+</td>
</tr>
</tbody>
</table>

*Not determined. The inhibition profile for choline did not allow fitting of an IC50 value (see text).

TABLE 2
Inhibition of [3H]-L-carnitine uptake in BeWo cells by amphetamine analogs

Uptake of 10 nM [3H]-L-carnitine in BeWo cells was determined at 37°C for 30 min in the presence or absence (control) of the amphetamine derivatives listed. The assignments for substituents R1, R2, and R3 correspond to the template structure shown in Fig. 6. Data were corrected for protein content and expressed as percentages of the control uptake. IC50 values for each inhibitor were obtained by fitting the data as described in the text. The error represents the standard deviation from three or four IC50 determinations. The compounds are listed in order of increasing IC50 values.

<table>
<thead>
<tr>
<th>Compound</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>IC50 (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(+)-Methamphetamine</td>
<td>H</td>
<td>H</td>
<td>CH3</td>
<td>2.3 ± 0.5</td>
</tr>
<tr>
<td>(+)-Ephedrine</td>
<td>H</td>
<td>OH</td>
<td>CH3</td>
<td>2.7 ± 1.3</td>
</tr>
<tr>
<td>(+)-β-Ephedrine</td>
<td>OH</td>
<td>H</td>
<td>CH3</td>
<td>4.2 ± 0.7</td>
</tr>
<tr>
<td>(+)-Chloroephedrine</td>
<td>H</td>
<td>Cl</td>
<td>CH3</td>
<td>4.8 ± 0.5</td>
</tr>
<tr>
<td>(+)-Chloroephedrine</td>
<td>Cl</td>
<td>H</td>
<td>CH3</td>
<td>5.0 ± 2.6</td>
</tr>
<tr>
<td>(+)-Amphetamine</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>6.0 ± 1.0</td>
</tr>
<tr>
<td>(+)-Nor-ephedrine</td>
<td>OH</td>
<td>H</td>
<td>H</td>
<td>7.1 ± 5.8</td>
</tr>
<tr>
<td>(+)-Nor-β-ephedrine</td>
<td>OH</td>
<td>H</td>
<td>H</td>
<td>9.2 ± 3.9</td>
</tr>
</tbody>
</table>
take, a very high concentration of choline (40 mM) did inhibit the uptake by 27%. Similarly, our results show that no substantial inhibition of carnitine uptake in BeWo cells was observed at concentrations below 1 mM choline, but 30 mM choline inhibited the uptake by 43%.

The presence of two recognition sites on the carnitine transporter was suggested previously (Behmer and Melstad, 1980), and Ohashi et al. (2002) recently proposed a model of carnitine recognition by OCTN2, with TEA and valproate as the representative substrates for the cationic and anionic binding sites (Ohashi et al., 2002). Consistent with this model, the Lineweaver-Burk plots here show that both TEA and valproate competitively inhibit carnitine uptake in BeWo cells. The \( K_i \) values regressed from these plots are in good agreement with the IC\(_{50}\) values obtained from separate experiments.

Several mechanisms have been hypothesized for valproate-induced carnitine deficiency, including elimination of valproyl-carnitine esters, inhibition of mitochondrial fatty acid oxidation upon interactions with enzymes, and impaired carnitine transport (Tein et al., 1993; Raskind and Chaar, 2000). Whereas all of these mechanisms are possible, the competitive inhibition of OCTN2-mediated carnitine uptake by valproate in BeWo cells suggests that the latter mechanism is of significance. Administration of valproic acid during pregnancy may reduce fetal carnitine stores, which will be necessary for the newborn to metabolize the fatty acids present in milk.

Amphetamines strongly inhibit the serotonin and norepinephrine transporters in placenta (Ganapathy et al., 1999), and some inhibition of \( \text{oOCTN2} \)-mediated TEA uptake by amphetamine and methamphetamine was observed in HRPE cells (Wu et al., 1999). The extent of the interference of these drugs of abuse with the placental carnitine transporter has been examined in this study.

All of the amphetamine derivatives studied inhibited placental carnitine uptake, with IC\(_{50}\) values between 2.3 and 9.2 mM. Among the amphetamine analogs studied, the inhibitor is stronger if a methyl group is present on the nitrogen atom (substituent \( R_3 \) in Fig. 6). This inhibition pattern suggests that OCTN2 recognizes a substituted nitrogen more readily than an unsubstituted nitrogen at its cationic binding site. Carnitine has three methyl groups on its nitrogen atom.

During the inhibition studies with these drugs, it was interesting to note that at high concentrations, methamphetamine (16 mM), \( \psi \)-ephedrine (16 mM), amphetamine (8 mM), and nor-\( \psi \)-ephedrine (20 mM) were toxic to the BeWo cells, as cell detachment from the experimental plates was observed and as verified by trypan blue exclusion. However, ephedrine and norephedrine, the only compounds having a hydroxyl group in the \( R_2 \) position, were not cytotoxic to a concentration of 16 mM.

Although these drugs of abuse inhibit placental carnitine uptake, the affinity of OCTN2 for amphetamines is much lower than that of the serotonin and norepinephrine transporters. \( K_i \) values for the inhibition of serotonin uptake by amphetamines are in the micromolar range (Ganapathy et al., 1999). Whereas placental monoamine transporters are primary targets for amphetamines, OCTN2 may be an alternative target for adverse pharmacological effects. Valproic acid uptake in BeWo cells is mediated by a transport system with a \( K_m \) value of 200 \( \mu \)M (Uotoguchi and Audus, 2000). Nevertheless, valprolate competitively inhibits OCTN2 in placenta, and valproate-induced carnitine deficiency is of clinical concern.

This study has shown that high concentrations of amphetamines inhibit OCTN2-mediated carnitine uptake in BeWo cells, an in vitro model of human trophoblasts. Methamphetamine has also been shown to interfere with mitochondrial function, a physiological domain of carnitine (Burrows et al., 2000; Virmani et al., 2003). Although this study does not establish whether amphetamine abuse during pregnancy leads to symptoms of carnitine deficiency, it is of potential concern. This study shows high selectivity and specificity of BeWo OCTN2 for carnitine analogs, and the structure-activity information indicates the preference of OCTN2 for cations with substituted nitrogen atoms.

References


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