L-carnitine transport in human placental brush-border membranes is mediated by the sodium-dependent organic cation transporter OCTN2

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Lahjouji, Karim, Ihsan Elimrani, Julie Lafond, Ijaz A. Qureshi, and Grant A. Mitchell. L-carnitine transport in human placental brush-border membranes is mediated by the sodium-dependent organic cation transporter OCTN2. Am J Physiol Cell Physiol 287: C263–C269, 2004; 10.1152/ajpcell.00333.2003.—Maternofoetal transport of L-carnitine, a molecule that shuttles long-chain fatty acids to the mitochondria for oxidation, is thought to be important in preparing the fetus for its lipid-rich postnatal milk diet. Using brush-border membrane (BBM) vesicles from human term placentas, we showed that L-carnitine uptake was sodium and temperature dependent, showed high affinity for carnitine (apparent $K_m = 11.09 \pm 1.32 \mu M$; $V_{max} = 41.75 \pm 0.94 \text{pmol} \cdot \text{mg protein}^{-1} \cdot \text{min}^{-1}$), and was unchanged over the pH range from 5.5 to 8.5. L-Carnitine uptake was inhibited in BBM vesicles by valproate, verapamil, tetrathyrammonium, and pyrilamine and by structural analogs of L-carnitine, including D-carnitine, acetyl-D,L-carnitine, and propionyl-, butyryl-, octanoyl-, isovaleryl-, and palmitoyl-L-carnitine. Western blot analysis revealed that OCTN2, a high-affinity, Na+-dependent carnitine transporter, is present in placental BBM but not in isolated basal plasma membrane vesicles. The reported properties of OCTN2 resemble those observed for L-carnitine uptake in placental BBM vesicles, suggesting that OCTN2 may mediate most maternofoetal carnitine transport in humans.

Recently, a high-affinity carnitine transporter, OCTN2, was cloned from human placenta (38, 45), but its localization in the placenta is unknown. OCTN2 is unique in that it transports carnitine with high affinity in a Na+-dependent manner and transports organic cations in a Na+-independent manner (4, 21, 44). Mutations in the human OCTN2 gene cause primary systemic carnitine deficiency (SCD; OMIM 212140), an autosomal recessive disease associated with cardiomyopathy, muscle weakness, fasting hypoglycemia, and sudden death (13). The deduced OCTN2 protein has 557 amino acids, a molecular mass of 63 kDa, and 12 putative transmembrane domains.

To date, three carnitine transporters have been identified: OCTN1, OCTN2, and OCTN3. They belong to the organic cation transporter (OCT) family and differ in their affinity and capacity for carnitine transport, energization of transport, and sensitivity to inhibitors. OCT transporters are expressed in several tissues, but in most cases their intracellular localization is unknown.

Our aim was to characterize the mechanism of carnitine transport in human placenta, specifically at the brush-border membrane (BBM). The BBM forms the interface between the fetus and the maternal circulation, and BBM transport is the first step of uptake from mother to fetus. The results in this article support the hypothesis that human placental carnitine uptake is mediated by OCTN2.

MATERIALS AND METHODS

Materials. L-[Methyl-3H]carnitine hydrochloride (82 Ci/mmol) was purchased from Amersham Pharmacia Biotech (UK). D-Carnitine, L-carnitine, hexanoyl-L-carnitine, octanoyl-L-carnitine, and palmitoyl-L-carnitine were purchased from Sigma-Aldrich (Oakville, ON, Canada). Acetyl D,L-, isovaleryl-D-, and propionyl-L-carnitine were synthesized as described by Montgomery and Mamer (16). Other reagents were obtained from Sigma-Aldrich.

Membrane vesicle preparation. BBM vesicles were isolated from normal human term placentas obtained within 1 h of delivery using the Mg$^{2+}$ precipitation method of Schmitz et al. (32). Briefly, the placentas were placed in 0.9% NaCl at 4°C, and the cord, amniochorion, and decidua were removed. Tissue obtained from the central part of the placenta was cut into 2- to 5-mm fragments and homogenized at 4°C in (in mM) 50 mannitol, 5 EGTA, and 10 Tris-HEPES (pH 7.5) with a small Waring blender (3 times for 30 s each). MgCl$_2$ was added to a final concentration of 10 mM, and the homogenate was stirred on ice for 20 min. The homogenate was centrifuged for 10 min at 2,000 g, and then the supernatant was centrifuged for 20 min at 20,000 g. The pellet was resuspended in intravesicular buffer (see below) using

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a Potter-Elvehjem apparatus and then centrifuged for 15 min at 1,900 g. The resultant supernatant was centrifuged at 30,900 g for 20 min. With a 25-gauge needle and syringe, the final BBM pellet was resuspended in intravesicular buffer (in mM: 50 Tris-HEPES, 250 KCl, and 125 mannitol, pH 7.5) at a protein concentration of 15–20 mg/ml. All centrifugation steps were performed in a Beckman J2-21 rotor. A sample of each final BBM preparation was removed for protein determination according to the method of Lowry et al. (14) using bovine serum albumin (BSA) as a standard. BBM were stored in liquid nitrogen until uptake studies. The basal plasma membrane (BPM) fraction was prepared using a discontinuous Ficoll gradient as described by Lafond et al. (11). The protocol was approved by the ethics committees of St. Justine’s Hospital. Placentas were used after informed consent was obtained from mothers after they gave birth.

Purity of BBM and BPM. Membrane purity was assessed by measuring the enrichment of marker enzymes alkaline phosphatase (8) for the BBM and Na+/K+-ATPase (15) for the BPM.

Uptake studies. Uptake of [3H]carnitine was measured by using a rapid filtration method with manifold with cellulose nitrate filters of 0.65-μm pore size from Sartorius (Göttingen, Germany). The human placental BBM were usually resuspended in a final concentration of 15–20 mg/ml in (in mM) 50 Tris-HEPES, 250 KCl, and 125 mannitol, pH 7.5. Next, a 4-μl vesicle suspension was mixed in a final volume of 50 μl of incubation medium (in mM: 50 Tris-HEPES, 100 KCl, 150 NaCl, and 125 mannitol, pH 7.5). Briefly, the reaction was started by mixing the vesicles with the incubation medium, to which the required amount of [3H]carnitine (82 Ci/mmol) had been added. The reaction was stopped by adding 1 ml of ice-cold stop solution. The solution was then filtered on 0.65-μm (Micro Filtration System) nitrogen cellulose filters and washed three times with 1 ml of nonradioactive ice-cold stop solution. Filters were dissolved in minisials by 15-min incubation with 5 ml of Filter Count (United Technologies Packard) and continuous shaking. 3H radioactivity was determined by using a Minaxi Tri-Carb series 4000 model 4450 scintillation counter (United Technologies Packard). All vials were counted for 5 min. Values presented represent the mean of triplicate or quadruplicate determinations.3H radioactivity was determined by using a Minaxi Tri-Carb series 4000 model 4450 scintillation counter (United Technologies Packard). All vials were counted for 5 min. Values presented represent the mean of triplicate or quadruplicate determinations.

Assays with xenobiotic inhibitors. Membrane vesicles were prepared as described above. The incubation medium contained (final concentrations) 1 μM L-[3H]carnitine (4 μCi/assay), 50 mM Tris-HEPES, 0.1 mM MgSO4, 100 mM KCl, 150 mM NaCl, 125 mM mannitol, pH 7.5, and 500 μM of the xenobiotic. Transport was started by adding 4 μl of membrane suspension to 46 μl of preheated incubation medium (37°C). The reaction was stopped after 8 min, which was determined to be optimal in preliminary studies.

Assays with car nitine analogs. BBM vesicles were incubated as described above in the presence of 50 μM of different acylcarnitine (acyetyl-L-carnitine, propionyl-L-carnitine, butyryl-L-carnitine, isovaleryl-L-carnitine, octanoyl-L-carnitine, and palmitoyl-L-carnitine). These analogs are known to significantly inhibit car nitine uptake via OCTN2 (21).

Membrane localization of OCTN2 by Western blot analysis. Rabbit polyclonal antibodies were raised (Research Genetics, Huntsville AL) against a synthetic polypeptide, QWQIQSQTRMQKDGEEPS, corresponding to amino acids 532–550 of mouse OCTN2. Whole placenta, BBM, or BPM fractions were isolated and homogenized in 4 ml of buffer containing 10 mM Tris–HCl and 50 mM mannitol (pH 7.4) using a Polytron homogenizer. The solution was then dispersed ultrasonically. After mixing in 2× sample buffer (4% SDS, 20% glycerol, 200 mM dithiothreitol, 120 mM Tris, pH 6.8, and 0.002% bromphenol blue), samples were denatured in a boiling water bath for 5 min and then resolved on 7.5% SDS-polyacrylamide gel. Proteins were transferred to a polyvinylidene difluoride membrane (Immobilon-M Millipore, Bedford, MA). The membrane was incubated for 50 min in PBS buffer (in mM: 150 NaCl, 2.5 KCl, 5 Na2HPO4, and 1.5 KH2PO4) containing 0.02% wt/vol sodium azide and 12% (vol/vol) skim milk and then was incubated overnight with polyclonal antipeptide antibody in PBS buffer containing 0.02% sodium azide and 1% BSA, washed in PBS with 0.1% Tween 20, and incubated with secondary antibody, goat anti-rabbit IgG, and horseradish peroxidase-linked whole antibody (Sigma, St. Louis, MO). The membrane was washed as described above, and the proteins were detected by chemiluminescence using BM chemiluminescence ELISA substrate (POD) (Boehringer Mannheim, Laval, QC, Canada). Molecular weights were estimated by using prestained SDS-PAGE standards, broad range (Bio-Rad Laboratories, Hercules, CA).

Statistical analyses. Results are reported as means ± SE of at least three samples. Statistical analyses were performed using analysis of variance (ANOVA) with GraphPad InStat (GraphPad Software, San Diego, CA). For the determination of uptake kinetic parameters, the GraphPad Prism version 3.00 software program was used. Inhibition studies using valproate were analyzed using linear regression of Lineweaver-Burk plots.

RESULTS

Purity of human placental membranes. For the BBM, alkaline phosphatase activities were 0.037 and 1.2 μmol-mg protein−1·min−1 (P < 0.001) in homogenate and BBM, respectively, and showed a 32-fold increase with respect to whole cell homogenate, indicating a high purity of these membranes. For the BPM, the enrichment factor for Na+/K+-ATPase was 26-fold.

Binding vs. transport of L-[3H]carnitine in placental BBM. Nonspecific binding of the substrate to the vesicle surface, which causes an overestimation of transport into the vesicle, was calculated as the uptake of L-carnitine by the vesicles at infinite osmolarity. At 50 min of incubation, increasing medium osmolality decreased L-carnitine uptake (Fig. 1), indicating that L-carnitine is taken up into an osmotically sensitive vesicular space. The relationship between uptake and the reciprocal of osmolality was linear. The intercept on the ordinate (zero intravesicular volume) is a measurement of nonspecific binding (2). The binding of L-carnitine measured in the presence of a Na⁺ gradient (50.4 pmol/mg protein) represents 36% of the L-carnitine uptake measured under standard conditions.

![Fig. 1. Effect of medium osmolarity on uptake of 1 μM L-[3H]carnitine by human placental brush-border membrane (BBM) at 37°C in the presence of a Na⁺ gradient (150 mM NaCl). The uptake of carnitine was measured after 50 min in the presence of various concentrations of cellbiose. Values represent means ± SE from 3 different experiments.](https://ajpcell.physiology.org/content/files/JCP01020033/Fig1.jpg)
Time course of L-carnitine uptake by human placental BBM vesicles. Figure 2 describes the time course of carnitine uptake in human placental BBM vesicles in the presence and absence of Na\(^+\). The presence of an inwardly directed 150 mM Na\(^+\)/H\(^+\) gradient stimulated L-[\(^3\)H]carnitine uptake (Fig. 2). The uptake was linear with time for 10 min (\(V_{\text{i}} = 6.2 \pm 1.2\) pmol mg\(^{-1}\) protein\(^{-1}\) min\(^{-1}\)) but reached saturation after 60 min. This result indicated Na\(^+\)-coupled carnitine transport in human placental BBM.

Temperature dependency of L-carnitine uptake by human placental BBM. We determined the initial rate of carnitine uptake at two different temperatures: 37\(^\circ\)C and 4\(^\circ\)C. At 4\(^\circ\)C, the initial uptake was 15 times less than it was at 37\(^\circ\)C (Fig. 3).

Effect of Na\(^+\) concentration on carnitine transport. The rate of L-[\(^3\)H]carnitine uptake increased with increasing concentration of Na\(^+\), and L-carnitine uptake exhibited a simple hyperbolic curve as the Na\(^+\) concentration was increased (Fig. 4).

Effect of extravesicular pH. The effect of extravesicular pH on human placental BBM uptake was examined while the intravesicular pH was maintained at 7.5. The mean value for carnitine uptake was higher at extravesicular pH values of 5.5 and 6.5 than at pH 8.5, but this difference was not statistically significant (Fig. 5).

Fig. 2. Time course and Na\(^+\) dependence of L-carnitine uptake by human placental BBM at 37\(^\circ\)C. The incubation medium contained (final concentrations) 1 \(\mu\)M L-[\(^3\)H]carnitine (4 \(\mu\)Ci/assay), 125 mM mannitol, 50 mM Tris-HEPES, pH 7.5, 100 mM KCl, and 150 mM NaCl (+Na\(^+\)) or 150 mM KCl (−Na\(^+\)). Results are the means ± SE from 3 different membrane preparations. When not shown, error bars fall within the symbols used.

Fig. 3. Effect of temperature on L-carnitine uptake by human placental BBM in the presence of a Na\(^+\) gradient. Uptake was measured at the times indicated at 2 temperatures: 37\(^\circ\)C and 4\(^\circ\)C. Conditions and procedures are similar to those described in Fig. 2. Results are means ± SE from 3 different experiments.

Fig. 4. Effect of Na\(^+\) concentration on L-carnitine transport into BBM of human placenta. The membranes were incubated with 1 \(\mu\)M L-[\(^3\)H]carnitine for 8 min in presence of Na\(^+\) (as NaCl) (range 5–150 mM). Appropriate concentrations of KCl were used to maintain constant osmolality. Na\(^+\)-dependent transport was calculated by subtracting uptake measured in the absence of Na\(^+\) from that measured in the presence of Na\(^+\). Results are means ± SE from 3 different experiments.

Fig. 5. Effect of extravesicular pH on the L-carnitine transport into BBM of human placenta. The intravesicular pH was maintained at pH 7.5, and the uptake medium pH was varied from 5.5 to 8.5. Initial rates of uptake were measured in the presence of a Na\(^+\) gradient after 8 min of incubation at 37\(^\circ\)C. Values shown are means ± SE from 2 different experiments.
Kinetics of carnitine uptake by human placental BBM vesicles in the presence and absence of valproate. Figure 6A shows carnitine uptake as a function of concentration in the presence and absence of 500 μM valproate. The data indicate that placental BBM vesicle carnitine transport is a saturable process with a \( K_m \) of 11.09 ± 1.32 μM and a \( V_{max} \) of 41.75 ± 0.94 pmol·mg protein\(^{-1}\)·min\(^{-1}\).

A Lineweaver-Burk plot (Fig. 6B) revealed that valproate competitively inhibits carnitine uptake by placental BBM vesicles. The kinetic parameters of L-carnitine uptake in the presence of valproate were a \( K_m \) of 32.3 ± 3.46 μM/l (\( P < 0.05 \)) and a \( V_{max} \) of 37.6 ± 1.17 pmol·mg protein\(^{-1}\)·min\(^{-1}\) (\( P > 0.05 \)) (not significant).

Effect of structural analogs of carnitine on L-carnitine transport by human placental BBM vesicles. The uptake of L-[\(^3\)H]carnitine by human placental BBM vesicles was characterized in the presence of various carnitine analogs known to inhibit carnitine transport in other systems (2, 21, 35, 44) (Table 1). D-, Acetyl-D,L-, propionyl-D,L-, butyryl-D,L-, isovaleryl-D,L-, octanoyl-D,L-, and palmitoyl-D,L-carnitine all significantly inhibited carnitine transport by the BBM vesicles (inhibition between 29 and 75%; \( P < 0.001 \)). These results show that the transport is specific for carnitine and its acyl derivatives. Short-chain as well as long-chain acylcarnitines are effective substrates.

**Table 1. Effect of carnitine structural analogs and of selected xenobiotics on L-[\(^3\)H]carnitine transport by human placental BBM**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration, μM</th>
<th>Uptake</th>
<th>Inhibition, %</th>
<th>( P ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Analogs</td>
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<tr>
<td>L-Carnitine</td>
<td>50</td>
<td>0.87±0.20</td>
<td>85</td>
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<tr>
<td>d-Carnitine</td>
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<td>1.93±0.01</td>
<td>67</td>
<td>&lt;0.001</td>
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<td>Acetyl-L-carnitine</td>
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<td>2.94±0.10</td>
<td>50</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Propionyl-L-carnitine</td>
<td>50</td>
<td>3.03±0.34</td>
<td>49</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Butyryl-L-carnitine</td>
<td>50</td>
<td>1.59±0.30</td>
<td>73</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Isovaleryl-L-carnitine</td>
<td>50</td>
<td>1.70±0.02</td>
<td>71</td>
<td>&lt;0.001</td>
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<tr>
<td>Hexanoyl-L-carnitine</td>
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<td>1.76±0.03</td>
<td>70</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Octanoyl-L-carnitine</td>
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<td>1.46±0.06</td>
<td>75</td>
<td>&lt;0.001</td>
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<tr>
<td>Palmitoyl-L-carnitine</td>
<td>50</td>
<td>4.21±0.20</td>
<td>29</td>
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<td>Xenobiotics</td>
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<tr>
<td>Verapamil</td>
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<td>1.03±0.32</td>
<td>82</td>
<td>&lt;0.001</td>
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<tr>
<td>Pyrilamine</td>
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<td>42</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Valproate</td>
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<td>3.15±0.04</td>
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<td>&lt;0.001</td>
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<tr>
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<td>40</td>
<td>&lt;0.001</td>
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<tr>
<td>Proline</td>
<td>500</td>
<td>5.64±0.16</td>
<td>NS</td>
<td></td>
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</table>

Values are means ± SE from 3 different experiments. Membrane vesicles were incubated with the indicated concentrations of each compound. The incubation medium contained (final concentrations) 1 μM L-[\(^3\)H]carnitine (4 μCi per assay), 125 mM mannitol, 50 mM Tris-HEPES pH 7.5, 100 mM KCl, and 150 mM NaCl. Initial rates of carnitine uptake were measured after 8 min of BBM incubation at 37°C in the presence of a Na\(^+\) gradient. In the control, the analog was replaced by incubation buffer. Results are given as pmol·mg protein\(^{-1}\)·min\(^{-1}\). TEA, tetraethylammonium.

Effect of xenobiotics on L-carnitine transport by membrane vesicles. Compounds known to interact with OCTN2 (44, 21) were investigated for their effect on carnitine transport by human placental BBM (Table 1). Valproate, tetraethylammonium, verapamil, and pyrilamine significantly inhibited carnitine transport by placental BBM vesicles (inhibition between 40 and 82%; \( P < 0.001 \)), whereas no significant effect was observed in the presence of L-proline (Table 1).

Detection and localization of OCTN2. Extracts of human placental BBM and BPM were analyzed by Western blotting. The polyclonal OCTN2-specific antibody detected a BBM protein with an apparent molecular mass of 80 kDa (Fig. 7). No OCTN2 immunoreactivity was observed in BPM.

**DISCUSSION**

Our study suggests that carnitine uptake by BBM vesicles may be principally mediated by OCTN2. The localization of OCTN2 in placenta has not been reported previously. We have characterized carnitine transport in the human placenta, extending previous studies and resolving certain discrepancies among them. We studied the Na\(^+\), temperature, pH, and osmolarity dependence of carnitine uptake. We also studied the kinetics of this transporter in the presence and absence of some.
OCTN2-specific inhibitors and confirmed its localization by Western blot analysis.

Na⁺, temperature, pH, and osmolarity dependence of carnitine uptake. Our study demonstrates that the uptake of L-carnitine declined with increasing external osmolarity, suggesting that L-carnitine was not only adsorbed onto vesicles but also transported into the intravesicular space (Fig. 1). This contrasts with data reported by Roque et al. (27). We performed osmolarity studies after 10 min as described by Roque et al., and like those investigators, we found no osmolarity dependence under these conditions (data not shown). This suggests that the conditions used by Roque et al. may not have permitted the reaction to continue until completion and may have lacked the sensitivity necessary to detect concentration dependence. Our data suggest that these preparations are a suitable model in which to study the characteristics of carnitine uptake across the apical membrane of the human placental syncytiotrophoblast.

Our study shows Na⁺- and temperature-dependent transport in placental BBM uptake of carnitine (Figs. 2 and 3). The uptake was high at 37°C and low at 4°C. Moreover, carnitine uptake by placental BBM was not significantly affected by pH (Fig. 5).

Kinetics and inhibition of carnitine transport. We demonstrated that L-carnitine uptake in placental BBM vesicles is a saturable process with high affinity (Kᵡ = 11.09 ± 1.32 μM) (Fig. 6A) similar to the known properties of OCTN2. Similar high-affinity transport of carnitine was observed in human kidney cells (38), in HLF cells (47), and in Caco-2 cells (3). The affinity constant obtained in our study, Kᵡ = 11.09 ± 1.32 μM, is also close to that reported for the Na⁺-dependent carnitine transport in human placental choriocarcinoma cells (Kᵡ ~12.3 μM) (23).

Many of the properties observed for human placental L-carnitine transport resemble those reported for OCTN2, including sodium and temperature dependence (Figs. 2 and 3) and high affinity for carnitine. The Kᵡ for L-carnitine transport in human placental BBM, 11.09 ± 1.32 μM, is of the same order as that reported for human kidney OCTN2 (~4.3 μM) (38).

An apparent discrepancy between our results and those of Roque et al. (27) is Roque et al.’s conclusion regarding carnitine binding but not transport in BBM. We think that the difference in our conclusions is due to technical reasons. Using 10-min incubation to study the effect of osmolarity on L-carnitine uptake and/or binding as described by Roque et al. (27), we reproduced their results, finding no change of carnitine binding and/or uptake with osmolarity (data not shown). In contrast, a 50-min incubation period (Fig. 1) allows transport to proceed sufficiently to reveal a clear effect of medium osmolarity on L-carnitine handling, confirming that L-carnitine is transported into BBM vesicles and is not simply bound to their exterior.

To better define L-carnitine transport via OCTN2, we examined some structural analogs of carnitine, such as short-chain fatty acid esters of carnitine and compounds that inhibit L-carnitine transport via OCTN2 (4, 21, 25, 35, 37, 44). Short-chain acyl esters of L-carnitine are used in the treatment of a wide range of disorders (34, 42) and also can accumulate abnormally in several inborn errors of metabolism (26). As shown in Table 1, the short-chain acyl esters of L-carnitine significantly inhibited carnitine uptake by human placental BBM. Our results also show that carnitine uptake was significantly inhibited by verapamil, valproate, pyrilamine, and tetraethylammonium (Table 1) as previously reported for OCTN2-mediated L-[³H]carnitine uptake in both humans and rats (21, 44). These drugs are sometimes used in pregnancy, such as verapamil for fetal tachyarrhythmia (7) and valproate for maternal epilepsy. Of note, valproate is a teratogen (9), and the therapeutic level of valproate, 300–700 μM, coincides with that used in our study (500 μM). Valproate is known to interfere with carnitine-related metabolic processes and can induce carnitine deficiency in cells (21, 24, 40) and patients (1, 41).

Different modes of inhibition have been described for the effect of drugs on carnitine transport. For valproate, we demonstrated a competitive inhibition of L-carnitine uptake in human placental BBM, with decrease of the affinity for carnitine in the presence of valproate (Kᵡ = 32.30 ± 3.46 μM) (Fig. 6, A and B). The maximum velocity of L-carnitine transport was not affected (37.63 ± 1.17 pmol·mg protein⁻¹·min⁻¹). Previous studies using HEK-293 cells transfected by human OCTN2 (21) showed competitive inhibition of L-carnitine transport by valproate. Recently, it was reported (17) that valproate is transported by a proton-dependent transporter in human placental BBM vesicles with a Kᵡ of 1.04 mM. Of note, other studies suggest that the inhibition of OCTN2 by verapamil may involve both competitive and non-competitive inhibition (19).

Fetal valproate syndrome is a characteristic cluster of malformations and intellectual disabilities reported in children exposed to valproate during fetal life (9). The relationships, if any, of OCTN2-mediated carnitine transport to fetal valproate syndrome are speculative but merit further study. Similarly, OCTN2-mediated placental transport may be pertinent to the therapeutic use of acylcarnitines or the accumulation of acylcarnitines in maternal or fetal inborn errors of organic acid metabolism.

Immunohistochemical identification and localization. To confirm that the uptake of L-carnitine in human placenta is mediated by OCTN2, a mouse OCTN2-specific antibody was produced and used against a total homogenate of human placenta and against human placental BBM and BPM vesicles (Fig. 7). Immunoblotting of total homogenate and of the BBM preparation showed an immunoreactivity to the antibodies against OCTN2 (Fig. 7). However, no reactivity was observed in BPM preparation. The molecular mass (80 kDa) of the reactive band differs from that deduced for OCTN2 (63 kDa), perhaps because of atypical proprieties of migration related to post-translational modification such as glycosylation, or perhaps
representing a previously unreported OCTN2 isoform. Similar differences in molecular mass between that predicted from primary structure and that observed on electrophoretic migration were reported in mice by Tamai et al. (37), who used antibodies against mouse OCTN2 and obtained bands of apparent molecular mass between 70 and 80 kDa in several mouse tissues.

Our Western blotting results are in accord with many studies showing that OCTN2 is localized in BBM of other epithelial tissues, including mouse and rat kidney (36, 12), chicken intestine (2), and human intestinal Caco-2 cells (3).

The absence of reactivity with the anti-OCTN2 antibody in our human placental BPM vesicles supports the notion that placental OCTN2 is confined to BBM (Fig. 7). In BPM, carnitine may diffuse or be transported by another molecule. To our knowledge, carnitine uptake has not been described in placental BPM of any species. It will be interesting to compare l-carnitine uptake in the BBM and the BPM. Transport mechanisms frequently differ between BBM and BPM, such as those for lactate (6) and l-tryptophan (10).

Our study is consistent with a major role for OCTN2 in human placental carnitine transport. We cannot exclude a role for other transporters. For instance, the OCTN transporter subfamily includes two other members: OCTN1 and OCTN3. Each can transport carnitine, although with characteristics different from those of OCTN2 (46, 37). Human OCTN1 is expressed in several tissues, including placenta, and transports carnitine in a Na\(^+\)-dependent manner, although the affinity of human OCTN1 for carnitine has not been reported (39, 37, 46). Rat intestinal OCTN1 reportedly interacts with carnitine with low affinity and in a Na\(^+\)-independent manner (43). In mice, OCTN1 mediates Na\(^+\)-dependent carnitine uptake with low affinity, although its expression in placenta has not been studied (37). OCTN3 has been cloned only in mice (37) and mediates Na\(^+\)-independent carnitine uptake. In mice, it is expressed predominantly in testis and weakly in kidney, but its expression in the placenta was not described. Because we found that carnitine uptake by human placental BBM vesicles was Na\(^+\)-dependent, it is unlikely that OCTN3 plays a role in carnitine transport by placental BBM. We cannot exclude some role of OCTN1 or other transporters in carnitine uptake by human placental BBM vesicles.

We used l-proline to test whether l-carnitine is transported by the ATB\(^{0, +}\) system, which transports both proline and carnitine (18) and is principally expressed in intestine, lung, and mammary gland. ATB\(^{0, +}\) is a Na\(^+\)-dependent transporter with a low affinity for carnitine (\(K_m\) = 0.83 mM). Because proline did not inhibit carnitine uptake, and in view of the high affinity of placental carnitine transport (Fig. 6), there is no evidence in favor of ATB\(^{0, +}\)-mediated carnitine uptake in human placental BBM.

In summary, the properties of carnitine transport in BBM vesicles and the demonstration herein of immunoreactive OCTN2 in human placental BBM points to a hypothesis that OCTN2 may mediate most and possibly all of maternofetal carnitine transport. Because OCTN2 is multifunctional and mediates the transport of many drugs, we speculate that this transporter is also involved in the transfer of these drugs from mother to fetus. These results thus have potential clinical implications for maternofetal nutrient transfer and for the pharmacology and transfer of cationic drugs in the fetus.

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