Uptake of Cimetidine into Syncytial Microvillus Membrane Vesicles of Human Term Placenta\textsuperscript{1,2}

ERIC M. VAN DER AA, ALFONS C. WOUTERSE, CHRISTEL E. H. VERRUJT, JENNY H. J. COPIUS PEEREBOOM-STEHEMAN and FRANS G. M. RÜSSEL

Departments of Pharmacology (E.M.A., A.C.W., C.E.H.V., F.G.M.R.) and Toxicology (J.H.J.C.P.S.), University of Nijmegen, P.O. Box 9101, 6500 HB Nijmegen, the Netherlands
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ABSTRACT

Uptake of the \(H_2\)-receptor antagonist, cimetidine, into syncytial microvillus membrane vesicles of human term placenta was investigated to clarify whether an active transport mechanism can be responsible for the observed barrier of the human placenta for cimetidine. Imposition of an outwardly directed \(H^+\)-gradient stimulated cimetidine uptake, resulting in a small transient overshoot. The \(H^+\)-gradient-dependent peak uptake was decreased under voltage-clamped conditions by carbonyl cyanide \(p\)-trifluoromethoxy-phenylhydrazone, suggesting the presence of an organic cation-proton exchange mechanism. Uptake was partially, but significantly, inhibited by organic cation transport inhibitors, \(H_2\)-receptor antagonists and several other cationic drugs, providing further evidence for mediated uptake. \(H^+\)-gradient-dependent cimetidine uptake was saturable and characterized by a low-affinity (\(K_m\)) of 6.3 mM and \(V_{\text{max}}\) of 17.5 nmol/mg protein/10 sec. We conclude that the system cannot play an important role in the barrier function of the human placenta in the transport of cimetidine. Rather than active transport, other factors, as for instance the degree of ionization of cimetidine at physiological pH, seem to be a more likely explanation for the low clearance of cimetidine across the human placenta.

\textsuperscript{1}Department of Pharmacology, University of Nijmegen.

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ABBREVIATIONS: FCCP, carbonyl cyanide \(p\)-trifluoromethoxyphenylhydrazone; Hepes, N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid; Mes, 2-(\(N\)-morpholino)ethanesulfonic acid; \(M_0\), starting mince; NMN, \(N\)-methylnicotinamide; \(pH_i\), pH inside the vesicle; \(pH_o\), pH outside the vesicle; SMMV, syncytial microvillus membrane vesicles; TEA, tetraethylammonium; Tris, 2-amino-2-hydroxymethylpropane-1,3-diol; val, valinomycin.
concentrations of up to only 400 μM were used and no driving forces were applied.

More precise information concerning the driving forces, specificity and saturability of transport could provide better insight into the underlying mechanism of the low clearance for cimetidine across the human placenta. The present study was designed to investigate in more detail the molecular mechanisms of cimetidine transport across the human term placenta by using isolated SMMV.

Materials and Methods

Vesicle preparation. Human placental SMMV were prepared according to a modification of the procedure described by Glazier et al. (1988) as method number three. Briefly, tissue obtained within 15 min after uncomplicated delivery was minced in a Waring blender and stirred for 30 min to loosen the microvilli. After MgCl₂ aggregation and differential centrifugation, both steps repeated once, SMMV were harvested and suspended in an appropriate buffer. The protein concentration of SMMV used in this study was 10.4 ± 2.2 mg/ml (n = 23). All subsequent steps were performed at 4°C. Vesicles were frozen in N₂(l) and stored at −80°C for 4 weeks at the maximum. Protein was assayed with a Coomassie blue kit (Biorad, München, Germany). Alkaline phosphatase enrichment of SMMV, measured according to Mircheff and Wright (1976), was 22-fold as compared with starting mince (M₀: 60 ± 20, SMMV: 1360 ± 570 μmol/h/mg, n = 14).

Uptake studies. Uptake of [3H]cimetidine into SMMV was measured in quadruplicate at 37°C with a rapid filtration technique (Russel et al., 1988). Uptake was started by addition of 10 μl membrane suspension to 40 μl extravesicular medium containing radiolabeled compound. The transport conditions are given in the legends. At appropriate time intervals, the reaction was terminated by adding 2 ml of ice-cold stop solution with the same composition as the intravesicular medium, except for membrane binding studies in which the stop solutions were equal to the corresponding extravesicular medium. The samples were filtered under vacuum through a Whatman GF/F glass fiber filter and washed 3-fold. Radioactivity remaining at the filter was counted in a Beckman LS 6000 LL liquid scintillation counter. Corrections were made for nonspecific filter binding.

Data analysis. Data are presented as means ± S.D., with n representing the number of experiments with different vesicle preparations. Statistical significance of differences in cimetidine uptake was determined with the use of two-tailed, paired Student's t-test (P < .05). Curve fitting was done by least squares nonlinear regression analysis with the computer program PCNONLIN (Metzler and Weiner, 1989).

Chemicals. [3H]-t-alanine (77 Ci/mmol) was obtained from New England Nuclear (Dreieich, Germany) and [3H]cimetidine (21 Ci/mmol) from Amersham (Aylesbury, U.K.). Cimetidin was generously donated by Smith, Kline & French Laboratories (Herts, U.K.), nizatidine by Eli Lilly & Company (Indianapolis, IN) tri-methoprim by Bergel (the Netherlands). Mezipiperphenidol and famotidine were generously donated by Merck, Sharpe & Dohme (Rahway, NY). NMN and TEA were purchased from Janssen Chimica (Beerse, Belgium). All other chemicals were purchased from Sigma (St Louis, MO), Merck (Darmstadt, Germany) or Boehringer (Mannheim, Germany). GF/F filters were obtained from Whatman Int. Ltd (Maidstone, U.K.).

Results

H⁺-gradient-dependent uptake of cimetidine. The outwardly directed proton gradient (pHᵢ = 6.0/pHₑ = 7.4) stimulated cimetidine (20 μM) uptake into SMMV, resulting in a small, but significant, transient overshoot (peak vs. equilibrium = 1.3) as compared with uptake in the absence of a H⁺-gradient (pHᵢ = pHₑ = 7.4) (fig. 1). The difference in equilibrium uptake at 60 min is probably due to a pH-dependent increase in vesicle volume, but this should have minor effects on initial uptake values.

Effect of ionophores on H⁺-gradient uptake. To determine whether the stimulated uptake of cimetidine in the presence of a proton gradient was due to the activity of an organic cation-proton antiport system or a H⁺-diffusion potential, the effect of the proton ionophore FCCP was evaluated. FCCP causes an enhanced H⁺-flux down its concentration gradient, which lowers the availability of H⁺ for a possible cation-proton exchanger and consequently decreases the uptake of the cation. The FCCP-induced H⁺-flux also renders the inside of the vesicle more negative resulting in an increase of uptake of the cation if uptake is dependent on a H⁺-diffusion potential. As shown in table 1, FCCP did not increase the H⁺-gradient-dependent initial and peak uptake rates. These results show that an inside negative H⁺-diffusion potential is unlikely to be a driving force for cimetidine uptake. However, in voltage-clamped vesicles (equal amounts of potassium at both sides of the membrane in presence of the K⁺-ionophore valinomycin), in which peak and equilibrium uptakes were not significantly altered, FCCP was able to decrease cimetidine uptake, resulting in a diminished overshoot. The decreased uptake suggests an organic cation-proton exchanger responsible for the small transient overshoot.

Determination of membrane binding. Figure 2 shows that the uptake of 20 μM cimetidine was inversely related to medium osmolarity (R² > .81 of individual regression lines), indicating transport into an osmotically responsive intravesicular space. Because of the large variation in the uptake data between different placentas, only a rough estimate could be made of the actual intravesicular uptake, which accounted for 77 ± 27% of total uptake.

Effect of organic cations. The inhibitory effect of various organic cations on cimetidine uptake was investigated. Table 2 shows the initial H⁺-gradient-dependent uptake of 20 μM [3H]cimetidine as percentage of representative control uptake in the presence of 1 or 5 mM inhibitor. The prototypic
TABLE 1
Effect of H+-gradients and ionophores on cimetidine uptake

<table>
<thead>
<tr>
<th>Condition</th>
<th>Uptake 10 sec</th>
<th>60 sec</th>
<th>60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. pH_i = 7.4/PH_o = 7.4</td>
<td>47 ± 9</td>
<td>67 ± 23</td>
<td>78 ± 21</td>
</tr>
<tr>
<td>pH_i = 6.0/PH_o = 7.4</td>
<td>87 ± 9</td>
<td>176 ± 34</td>
<td>148 ± 26</td>
</tr>
<tr>
<td>pH_i = 6.0/PH_o = 7.4 + FCCP</td>
<td>84 ± 16</td>
<td>172 ± 36</td>
<td>154 ± 18</td>
</tr>
<tr>
<td>B. pH_i = 6.0/PH_o = 7.4 + val + FCCP</td>
<td>57 ± 12</td>
<td>108 ± 34</td>
<td>130 ± 35</td>
</tr>
</tbody>
</table>

Fig. 2. Effect of increasing osmotic pressure on uptake at 30 min of 20 μM [3H]cimetidine into SMMV. Vesicles were suspended in 300 mM mannitol and 10 mM Hepes-Tris, pH = 7.4. Extravesicular media consisted of 10 mM Hepes-Tris, pH = 7.4, and increasing concentrations of mannitol. Values of three placentas are expressed individually as picomoles per milligram protein versus reciprocal osmolarity of the final concentration.

TABLE 2
Effect of various organic cations on cimetidine uptake

<table>
<thead>
<tr>
<th>Organic Cation</th>
<th>mM</th>
<th>% Uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>Mepiperphenidol</td>
<td>1</td>
<td>55 ± 19*</td>
</tr>
<tr>
<td>Amiloride</td>
<td>1</td>
<td>85 ± 2</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>5</td>
<td>76 ± 15*</td>
</tr>
<tr>
<td>Famotidine</td>
<td>1</td>
<td>54 ± 20*</td>
</tr>
<tr>
<td>Nizatidine</td>
<td>1</td>
<td>74 ± 13*</td>
</tr>
<tr>
<td>TEA</td>
<td>1</td>
<td>69 ± 12*</td>
</tr>
<tr>
<td>NMN</td>
<td>1</td>
<td>67 ± 14*</td>
</tr>
<tr>
<td>Guanidine</td>
<td>1</td>
<td>71 ± 10*</td>
</tr>
<tr>
<td>Choline</td>
<td>1</td>
<td>74 ± 16*</td>
</tr>
<tr>
<td>Nizatidine</td>
<td>1</td>
<td>100 ± 2</td>
</tr>
<tr>
<td>Choline</td>
<td>1</td>
<td>102 ± 5</td>
</tr>
</tbody>
</table>

Fig. 3. Concentration-dependent uptake of [3H]cimetidine at 10 sec into SMMV in the presence of an outwardly directed H+-gradient. Experimental conditions were the same as described in the legend of figure 1. Cimetidine concentrations ranged from 10 μM to 20 mM. Values are expressed as nanomoles per milligram protein (n = 3).

Discussion

In human in vitro studies and in the artificially perfused human placental cotyledon, no evidence was found for mediated transport of cimetidine (Howe et al., 1981; Ching et al., 1987). It was concluded that transfer of cimetidine across the human placental trophoblast occurs by passive diffusion. However, if diffusion transfer quantitatively exceeds uptake via a mediated pathway, the latter will be masked and not detected in these type of studies. Therefore, isolated membrane vesicles are a more appropriate tool to investigate whether mediated transport occurs across the plasma membrane.

Our results in human placental SMMV show that cimetidine transfer across the syncytiotrophoblast membrane of the human term placenta will be mainly due to passive diffusion, confirming the results obtained by others (Howe et al., 1981, Ching et al., 1987, Schenker et al., 1987). However, a small, but significant part of total cimetidine uptake into SMMV could be attributed to mediated uptake, because uptake was pH-dependent, inhibitable by several organic cations and saturable. Because the proton ionophore, FCCP, decreased H+-gradient-dependent cimetidine peak uptake...
under voltage-clamped conditions, we suggest the presence of an organic cation-proton antiport system for cimetidine in human placental SMMV, responsible for the small part of mediated uptake. Cimetidine itself has been reported to interfere with the human placental Na+/H+ -exchanger present in the syncytiotrophoblast membrane, in a reversible manner and competitive with respect to Na+ (Ganapathy et al., 1986). It seems possible that cimetidine, in the presence of a H+ - gradient, is transported by this Na+/H+ -exchanger. The inhibition of cimetidine uptake we observed in presence of the Na+/H+ -exchange inhibitor amiloride would support such a view. However, this cationic drug is not a selective inhibitor, because it also inhibits organic cation transport in human placental and rabbit renal cortical brush-border membrane vesicles (Ganapathy et al., 1988; Miyamoto et al., 1989). The inhibition of guanidine uptake into renal cortical brush-border membrane vesicles by cimetidine (Miyamoto et al., 1989) has not been found in human placental brush-border membrane vesicles (Ganapathy et al., 1988). Because guanidine did not inhibit cimetidine uptake significantly in our experiments, the guanidine analog cimetidine is not likely to be transported by the guanidine-proton antiport system present in human placental SMMV (Ganapathy et al., 1988). Cimetidine did inhibit membrane potential-dependent choline uptake into SMMV (van der Aa et al., 1994). In contrast, choline was not able to inhibit cimetidine uptake significantly in the present study. Therefore it is also unlikely that cimetidine is transported by the choline carrier.

The affinity of the system is low, as can be concluded from a $K_m$ of 6.3 mM characterizing the concentration-dependent uptake of cimetidine. The clinical relevance of this low-affinity system with respect to the use of H2-receptor antagonists during pregnancy seems therefore limited in terms of a possible accumulation into the trophoblast or extensive elimination from fetal to maternal circulation. Such a low-affinity transport system in microvillus membranes would only make sense in combination with a high-affinity system in basal membranes. From in vivo and perfused cotyledon experiments no evidence is available for the presence of such a system (Howe et al., 1981; Schenker et al., 1987).

We therefore conclude that the system cannot be an important factor in the barrier function of the human placenta in the transport of cimetidine as observed by Ching et al. (1987). Thus, active transport can indeed be ruled out in maintaining such a barrier. Other factors, as for instance the degree of ionization of cimetidine (pK_a = 6.8) at physiological pH, are probably of more importance to the low clearance of cimetidine across the human placental trophoblast.

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References


Send reprint requests to: F. G. M. Russel, Ph.D., Department of Pharmacology, Faculty of Medical Sciences, University of Nijmegen, P.O. Box 9101, 6500 HB Nijmegen, the Netherlands.