Transport of Clonidine at Cultured Epithelial Cells (JEG-3) of the Human Placenta

Johanna Müller,[1] Reinhard Neubert,[2] and Matthias Brandsch[1,3]

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Purpose. This study was performed to characterize the uptake of the antihypertensive drug clonidine at epithelial cells of the human placenta (JEG-3) and its interaction with other cationic drugs.
Methods. Uptake of [^{3}H]clonidine into cells of the human choriocarcinoma cell line JEG-3 was investigated.
Results. Uptake of [^{3}H]clonidine into JEG-3 cells reached its maximum on the eighth day after seeding. Uptake of [^{3}H]clonidine was linear for up to 1 min, Na^+ independent, but strongly H^+ dependent. The uptake rate of clonidine was saturable with an affinity constant (K_t) of 1.1 mM and a V_{max} value of 27 nmol·min^{-1}·mg^{-1} of protein. Several cationic drugs such as clonidine, quinine, quinidine, and diphenhydramine strongly inhibited the [^{3}H]clonidine uptake with K_i values around 1 mM.
Conclusions. Clonidine is transported across the placental epithelium by a carrier mediated process.

KEY WORDS: drug delivery; JEG-3 cells; membrane transport.

INTRODUCTION

The alpha$_2$-receptor agonist clonidine is a well-known therapeutic agent for treatment of hypertension (1). Clonidine is administered orally and in transdermal therapeutic systems (TTS) (2). Clonidine also supports local analgetic effects of ropivacaine thereby decreasing its minimum local analgesic concentration (3). During pregnancy, clonidine serves as a potent antihypertensive agent especially in case of pre-eclampsia, which is the predominant cause of maternal and fetal morbidity and mortality. Edwards and co-workers (4) described alterations in sleep architecture in context with pre-eclamptic patients taking clonidine for blood pressure reduction. Even though clonidine is often administered during pregnancy, there are only a very few reports on clonidine transport at the epithelial barrier of the human placenta. Ala-Kokko *et al.* (5) compared the net transfer of clonidine and dexmedetomine across isolated perfused human placenta. In a study on guanidine transport using brush border membrane vesicles of human placenta, clonidine at a concentration of 5 mM inhibited [^{14}C]guanidine uptake (15 μM) by 47% (6). The same group has shown that at the placental epithelium, clonidine interacts at the placental epithelium with the Na^+/H^+-exchanger (7). To the best of our knowledge, there are no studies on the placental clonidine transport mechanism.

[1] Membrane Transport Group, Biozentrum of the Martin-Luther-University Halle-Wittenberg, D-06120 Halle, Germany.
[2] Institute of Pharmaceutics and Biopharmaceutics, Department of Pharmacy, Martin-Luther-University Halle-Wittenberg, D-06120 Halle, Germany.
[3] To whom correspondence should be addressed. (e-mail: brandsch@biozentrum.uni-halle.de)

MATERIALS AND METHODS

Cell Culture

JEG-3 cells (passages 32-90) obtained from B. Ugele, Medical Department of the Ludwig-Maximilian-University, Munich (Germany), were cultured in Minimal Essential Medium supplemented with 10% fetal bovine serum, 44.6 μg/ml gentamicin, and 1% sodium-pyruvate (100 mM) (Gibco Life Tech, Karlsruhe, Germany). For uptake studies, cells were subcultured as described earlier (8) in 35-mm Petri dishes following trypsinization with 0.05% trypsin containing 0.02% EDTA. The seeding density was 0.8×10^6 cells/dish. The medium was changed 24 h after seeding, and after that every second day. Monolayers were used for experiments at the seventh day.

Uptake Measurements

Monolayers were rinsed one time with buffer containing 140 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, 0.8 mM $MgSO_4$, 5 mM glucose and 25 mM MES/Tris (pH 6.0), 25 mM HEPES/Tris (pH 7.5) or 25 mM Tris/HEPES (pH 8.5). To initiate uptake, 1 ml buffer containing 3 nM [^{3}H]clonidine (specific activity 55.5 Ci/mmol, Amersham International, Freiburg, Germany) and drugs at increasing concentrations (purchased from Sigma, Deisenhofen, Germany; ICN, Eschwege, Germany; Synopharm, Halle, Germany) were added to each dish for 1 min at room temperature. Uptake was stopped by washing the dishes four times with ice-cold buffer. Cells were solubilized and prepared for scintillation counting. The protein contents were measured by the method of Bradford using the Bio-Rad reagent (Munich, Germany) (9).

Data Analysis

In general, each data point was determined at least in triplicate for each experiment. Data are presented as mean ± SE. Statistical analyses were done with the *U* test by Mann and Whitney. The kinetic constants were calculated by nonlinear regression of the Michaelis–Menten plot and confirmed by linear regression of the Eadie–Hofstee plot. The calculated parameters are shown with their SE. Inhibition constants (K_i) were calculated from the IC_{50} values (i.e., the concentration of the unlabeled compound necessary to inhibit 50% of specific [^{3}H]clonidine uptake) using the K_t value of 1.1 mM obtained in this study.

RESULTS AND DISCUSSION

First, we measured the [^{3}H]clonidine uptake in JEG-3 cells as a function of days after seeding and of incubation time. Uptake per milligram of protein increased slightly from day 2 to 10, reaching a plateau at day 7–8. Therefore, day 7 after seeding was chosen for the following experiments. With regard to the time-dependency, the uptake of [^{3}H]clonidine was linear for up to 2 min (data not shown). A 1 min uptake was chosen for further characterization.

Figure 1 illustrates the pH- and sodium dependence of [^{3}H]clonidine uptake into JEG-3 cells. [^{3}H]Clonidine uptake was found to be 10–fold stimulated by changing the extracellular pH from 6.0 to 8.5. Replacement of sodium chloride by choline chloride had no effect on the clonidine uptake. This

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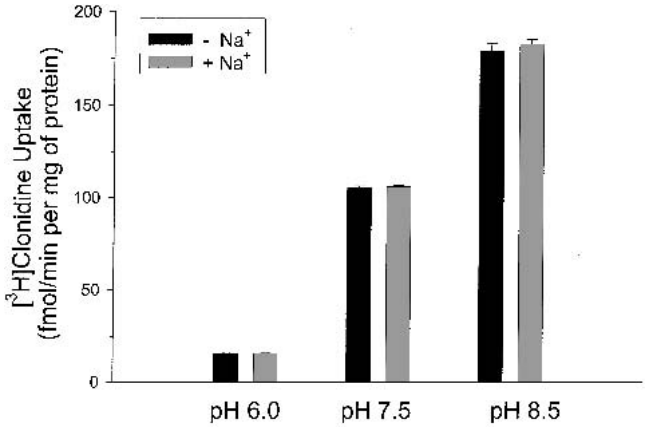

Fig. 1. pH- and sodium-dependent uptake of [^{3}H]clonidine in JEG-3 cells. Uptake of [^{3}H]clonidine (3 nM) was measured for 1 min at room temperature. Sodium chloride was iso-osmotically replaced by choline chloride. Values represent means ± SE, n = 3.

marked pH sensitivity could be explained by protonation degree of the drug (10).

The dependence of the clonidine uptake on the substrate concentration was measured to determine the kinetic parameters of the transport system in the range of 3 nM to 10 mM (Fig. 2). The nonmediated transport component, which represents simple diffusion of the tracer plus binding, was determined by measuring the uptake of [^{3}H]clonidine in the presence of excess amount (20 mM) of unlabeled clonidine. This component was 19% of the total [^{3}H]clonidine uptake at 3 nM. The relationship between carrier-mediated uptake rate and substrate concentration was found to be hyperbolic. When the results were expressed in the form of an Eadie–Hofstee plot (uptake rate/substrate concentration vs. uptake rate), a straight line (r^2 = 0.98) was obtained. The maximal transport velocity (V_{max}) was 27.5 ± 1.7 nmol/min per mg of protein. The apparent Michaelis–Menten constant of clonidine transport (K_t) was 1.1 mM ± 0.1 mM corresponding well to the K_t value 1.3 mM for clonidine uptake at cultured brain microvessel endothelial cells (10).

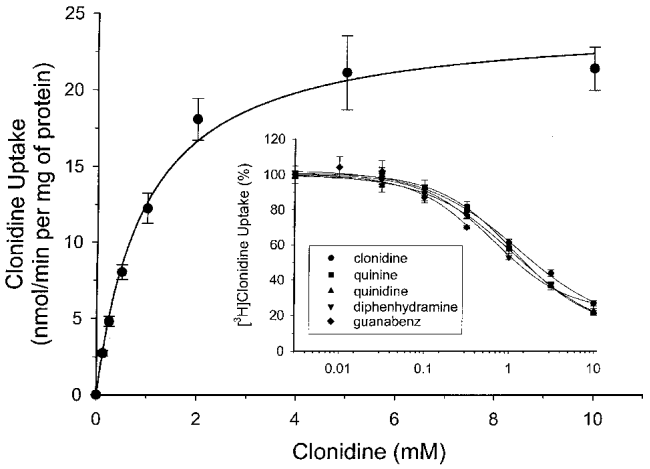

Fig. 2. Kinetics of carrier-mediated clonidine uptake in JEG-3 cells. Uptake of [^{3}H]clonidine (3 nM) was measured at room temperature, pH 7.5, for 1 min. Uptake buffer contained [^{3}H]clonidine and unlabeled clonidine (0–10 mM). Nonmediated simple diffusion was determined by measuring the uptake of [^{3}H]clonidine in the presence of an excess amount of unlabeled clonidine (20 mM) and subtracted from total uptake. Inset: Inhibition of [^{3}H]clonidine uptake (3 nM, pH 7.5, t = 1 min) by cationic drugs (0–10 mM). Values represent means ± SE, n = 4.

We next investigated the substrate specificity of the system that is responsible for the clonidine uptake in JEG-3 cells. This was done by measuring the ability of several physiologically and pharmacologically relevant drugs to inhibit the uptake of [^{3}H]clonidine (3 nM, pH 7.5) either at a fixed concentration of 1 mM or in the range of 0 to 10 mM to determine the inhibitory constants (K_i). [^{3}H]Clonidine uptake was inhibited by several therapeutically relevant drugs (Table I). Quinidine, quinine, verapamil, diphenhydramine, and clonidine itself (all 1 mM) reduced the [^{3}H]clonidine uptake (3 nM) to 50–60%. From data obtained in competition assays (Fig. 2, inset), the following K_i values ± SEM were calculated by nonlinear regression: clonidine 1.2 ± 0.3 mM, quinine 1.1 ± 0.1 mM, quinidine 1.1 ± 0.1 mM, guanabenz 0.6 ± 0.1 mM, and diphenhydramine 0.7 ± 0.05 mM.

Several transport systems have been described at the epithelium of the human placenta for organic cations, vitamins, and amino acids that are relevant for drug transport (11). The transport system described here for the apical membrane of human placenta cells might be identical or very similar to the system responsible for clonidine transport at the blood–brain barrier (10). It does not belong to the familiy of organic cation transporters OCT1-3. Clonidine interacts with choline transport systems (12), however, neither choline nor the prototypical organic cation methylphenyl pyridinium$^+$ inhibit clonidine transport (Table I).

The clonidine transport system in JEG-3 cells might be the pH-dependent transport system Inui and co-workers described functionally at intestinal (Caco-2) cells for diphen-

Table I. Effect of Different Cationic Drugs on the [^{3}H]Clonidine Uptake in JEG-3 Cells

Compound	[^{3}H]Clonidine uptake (%)
Control	100 ± 4
Clonidine	61 ± 2.0*
Atropine	92 ± 1.5*
Quinine	58 ± 0.4*
Quinidine	53 ± 1.5*
Cimetidine	103 ± 0.8
Ranitidine	99 ± 0.8
Diphenhydramine	53 ± 2.3*
Tetraethylammonium	94 ± 1.1*
Cephalexin	98 ± 1.8
Tryptamine	87 ± 1.9*
Carnitine	99 ± 1.2
Procainamide	101 ± 4
Thiamine	97 ± 1.1
Guanidine	100 ± 13
Methylphenyl pyridinium$^+$	97 ± 1.7
Guanabenz	66 ± 2.4*
Etilefrine	103 ± 2
Butylscopolamine	98 ± 0.9
Verapamil	49 ± 1.2*
Choline	97 ± 2.4

Uptake of [^{3}H]clonidine (3 nM, pH 7.5, t = 1 min) was measured in the absence or presence of unlabeled cationic drugs (all 1 mM, except cimetidine: 5 mM, guanabenz: 0.3 mM). Data are means ± SE, n = 3 to 6.

* Significantly different from control with $p \leq 0.05$.

hydramine and chlorpheniramine (13–15). Physiological substrates of the transport system are perhaps hitherto unidentified endogenous tertiary amines. At the placenta, it is not yet known whether the system is localized at the apical or basolateral membrane or both. From studies using perfused human placenta, it has been concluded that clonidine is transferred from the maternal to the fetal blood (5). The transport system for clonidine described at endothelial cells of the blood–brain barrier and the transport system for diphenhydramine at Caco-2 cells are localized both at the apical and the basolateral membrane of both cell types (10,15).

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