Guanidine Transport in a Human Choriocarcinoma Cell Line (JAR)

Shoshana Zevin,1 Marci E. Schaner,1 Nicholas P. Illsley,2 and Kathleen M. Giacomini1,3

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Purpose. Many endogenous substances and xenobiotics are organic cations. Transplacental transport of organic cations is an important determinant of the delivery of these compounds to the fetus. The aim of this study was to determine the mechanisms of organic cation transport using the human choriocarcinoma cell line (JAR) as a model system with [14C]guanidine as a ligand.

Methods. Uptake studies of [14C]guanidine were carried out in JAR cell monolayers on day 2 after plating.

Results. [14C]guanidine uptake was temperature dependent, saturable (Km = 167 μM) and inhibited by many organic cations including amiloride, cimetidine, quinine, quindine and nicotine. [14C]guanidine uptake exhibited a counterflux phenomenon indicative of a carrier-mediated process. The uptake of [14C]guanidine was sodium and pH-independent and could be driven by an inside-negative membrane potential difference.

Conclusions. This is the first demonstration of an electrogenic guanine transporter in a human cell culture model. This transporter may play a role in the transplacental transport of many clinically used drugs and xenobiotics.

KEY WORDS: organic cation transport; placenta; guanidine; JAR cells.

INTRODUCTION

Many clinically used drugs, including a number of antihypertensive agents, antiarrhythmic agents, antihistamines and antidepressants are organic cations. These compounds may be used or indicated for use during pregnancy. Transplacental flux of organic cations is a major determinant of fetal exposure to these compounds and may involve specific transporters located in the placental plasma membranes.

An organic cation-proton antiporter for guanidine and 5-(N-methyl-N-isobutyl)amiloride (MIBA) was demonstrated in human placental microvillus membrane vesicles (1,2). This transporter is distinct from the well-described renal organic cation-proton antiporter in terms of its substrate selectivity. Notably, no tetraethylammonium (TEA) transport could be demonstrated in the microvillus membrane vesicles. Guanidine-selective organic cation transporters which either exclude or have low affinity for TEA have also been described in renal and intestinal brush border membrane vesicles from rabbit (3,4).

To date, a cell culture model for guanidine selective organic cation transporters has not been described.

The focus of this study was to develop a cell culture model for guanidine selective organic cation transport. Cultured cells are advantageous for studies of regulation and electrophysiology of transport processes. We chose the human choriocarcinoma cell line (JAR) as a model system for these studies. JAR cells can be maintained in a continuous culture and form monolayers (5,6). Several transporters, including transporters for thyroid hormone, serotonin, taurine and glycine, have been identified and characterized in JAR cells (7–11). Because several of these transporters are present in human placenta (12,13), JAR may be a suitable model for studying biologically relevant placental transport processes.

MATERIALS AND METHODS

Cell Culture

JAR cells were obtained from the Cell Culture Facility at the University of California, San Francisco and maintained in culture at 37°C in a humidified 5% CO2, 95% air atmosphere. The growth medium was RPMI-1640, supplemented with 10% fetal bovine serum, 1% penicillin-streptomycin and 1% amphotericin B. All studies were performed in cells between passages 725 and 745. For transport studies in monolayers, the cells were subcultured in 12-well plates following trypsinization with 0.05% trypsin containing 0.02% EDTA. The seeding density was 0.5 * 106 cells/well (1.3 * 104 cells/cm2). The medium was changed after 24 hours and the monolayers were used for experiments on day 2 after plating.

Uptake Measurements

To study the uptake of [14C]guanidine in the JAR cell monolayers, each monolayer was rinsed three times with a buffer containing 140 mM NaCl, 5.4 mM KCl, 1.8 mM CaCl2, 0.8 mM MgSO4, 5 mM glucose and 25 mM Hepes/TRIS (pH 7.4). We compared the uptake of guanidine using either 25 mM Hepes/TRIS (pH 7.4) or 25 mM Hepes/NaOH (pH 7.4), and found no effect of TRIS on the uptake (96.5 ± 18.2 pmol/mg protein/3 min vs 92.2 ± 3.7 pmol/mg protein/3 min). To initiate uptake, 0.5 ml of the buffer containing 20 μM of [14C]guanidine (uptake medium) was added to each well and the wells were incubated at room temperature for the given time (three minutes for most experiments). The amount of tracer added to each well was 0.54 μCi. The uptake was stopped by aspirating the uptake medium and washing the wells three times with ice-cold buffer. The cells were solubilized in 1 ml of 0.5% Triton X-100, and an aliquot of the solubilized cells was transferred to scintillation vials to determine radioactivity. Inhibition studies were carried out by adding various concentrations of the unlabeled compounds to the uptake medium. For counterflux studies, JAR cells were preincubated for 30 minutes with or without unlabeled guanidine (1 mM), and the uptake of [14C]guanidine was determined at 3 min. For kinetic studies, the uptake of [14C]guanidine was determined at 3 min in uptake medium containing various concentrations of guanidine (range 10 μM to 1 mM).

1 Division of Clinical Pharmacology and Experimental Therapeutics and Department of Biopharmaceutical Sciences, University of California San Francisco, San Francisco, California 94143.
2 Department of Obstetrics and Gynecology, University of Medicine and Dentistry New Jersey.
3 To whom correspondence should be addressed. (e-mail: kmg@itsa.ucsf.edu)
Protein Assay

The protein concentration in cell monolayers was measured by the method of Bradford, using the Bio-Rad reagent (14). Bovine serum albumin was used as a standard.

Studies with Ionophores

Following preincubation for 20 min with either ouabain (1 μM or 1 mM) or monensin (10 mg/l), [14C]guanidine uptake was determined at 3 min. Control cells were incubated in buffer. For studies of membrane potential, valinomycin (1 μM) dissolved in ethanol was added to an uptake medium containing either 4.5 mM KCl (i.e., in the presence of an outwardly—directed K+ gradient) or 145 mM KCl (i.e., voltage clamped). The same amount of ethanol was added to control cells. Following preincubation with 2,4-dinitrophenol (DNP) (250 μM) for 30 minutes, the uptake of [14C]guanidine at 3 min was determined in uptake medium containing DNP (250 μM).

Effect of pH on [14C]Guanidine Uptake

For pH studies, the uptake of [14C]guanidine was determined at 3 min in buffer at pH 5, 7.4 or 8. To evaluate the effect of an acidified intracellular pH, we preincubated the cells with NH4Cl (20 mM) for 20 min, then determined [14C]guanidine uptake at 3 min.

Thin Layer Chromatography

Thin layer chromatography (TLC) of [14C]guanidine associated with the cells at 3 min was carried out using methanol, chloroform and ammonium hydroxide (2:1:0.2) as a mobile phase on pre-coated cellulose plastic sheets.

Data Analysis

In general, each data point was determined at least in triplicate for each experiment. All the experiments, except the experiment evaluating the effect of days in culture on guanidine uptake, were repeated at least once on a different day using a different cell passage. Inhibitable guanidine uptake was calculated by subtracting the uptake in the presence of unlabeled guanidine (1 mM) from the total [14C]guanidine uptake in the absence of unlabeled guanidine. The data are presented as mean ± standard deviation (S.D). Statistical significance was determined by the unpaired Student’s t test. Results were considered to be statistically different at a probability of less than 0.05 (p < 0.05).

Materials

[14C]Guanidine (specific activity 56 mCl/mmole) was purchased from Moravek. The following chemicals were purchased from Sigma: guanidine, amidoride, cimetidine, clonidine, quinine, quinidine, valinomycin, ouabain, monensin, choline, NMG, TEA, nicotine and procainamide. The protein assay dye reagent was from Bio-Rad. TLC plates were from EM reagents. Falcon culture plates were used for most experiments. Cell culture supplies were purchased from the Cell Culture Facility at UCSF.

RESULTS

[14C]Guanidine accumulated with time in the JAR cell monolayers, reaching equilibrium at approximately 60 min (Fig. 1). In contrast, the uptake of [14C]TEA was negligible (Fig. 1) and not inhibited by 5 mM unlabeled TEA. [14C]Guanidine uptake was markedly inhibited by unlabeled guanidine and was also temperature dependent (Fig. 2). Uptake in the presence of 1 mM and 5 mM unlabeled guanidine was similar (17.1 ± 3.1 and 17 ± 6.5 pmol/mg protein/3 min, respectively); therefore, a concentration of 1 mM was used for inhibition of specific uptake in subsequent experiments. The total and the inhibitable uptake of guanidine was highest on the second day after plating.

Fig. 1. Time course of guanidine and TEA uptake in JAR cells. The uptake of [14C]guanidine (20 μM) and of [14C]TEA was measured at room temperature (24°C). Data represent the mean ± SEM of determinations in 6–11 wells in 3 different cultures.

Fig. 2. The effect of temperature on guanidine uptake in JAR cells. Uptake of [14C]guanidine was measured at room temperature (24°C) with (squares) and without (closed circles) unlabeled guanidine (1 mM) and at 4°C (triangles). Data represent mean ± S.D. of determinations in 7 wells in 2 different cultures. At all the time points the uptake at 4°C was significantly different from the control at 24°C (p < 0.05).