Life Sciences 188 (2017) 76–82

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Nicotine transport in lung and non-lung epithelial cells

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ARTICLE INFO

Chemical compounds studied in this article:
Nicotine (PubChem CID: 942)

Keywords:
Nicotine
pH-dependent transport
Lung
Alveolar epithelial cells
Non-lung epithelial cells

ABSTRACT

Aims: Nicotine is rapidly absorbed from the lung alveoli into systemic circulation during cigarette smoking. However, mechanism underlying nicotine transport in alveolar epithelial cells is not well understood to date. In the present study, we characterized nicotine uptake in lung epithelial cell lines A549 and NCI-H441 and in non-lung epithelial cell lines HepG2 and MCF-7.

Materials and methods: Characteristics of [^{3}H]nicotine uptake was studied using these cell lines.

Key findings: Nicotine uptake in A549 cells occurred in a time- and temperature-dependent manner and showed saturation kinetics, with a Km value of 0.31 mM. Treatment with some organic cations such as diphenhydramine and pyrilamine inhibited nicotine uptake, whereas treatment with organic cations such as carnitine and tetraethylammonium did not affect nicotine uptake. Extracellular pH markedly affected nicotine uptake, with high nicotine uptake being observed at high pH up to 11.0. Modulation of intracellular pH with ammonium chloride also affected nicotine uptake. Treatment with valinomycin, a potassium ionophore, did not significantly affect nicotine uptake, indicating that nicotine uptake is an electroneutral process. For comparison, we assessed the characteristics of nicotine uptake in another lung epithelial cell line NCI-H441 and in non-lung epithelial cell lines HepG2 and MCF-7. Interestingly, these cell lines showed similar characteristics of nicotine uptake with respect to pH dependency and inhibition by various organic cations.

Significance: The present findings suggest that a similar or the same pH-dependent transport system is involved in nicotine uptake in these cell lines. A novel molecular mechanism of nicotine transport is proposed.

1. Introduction

Nicotine is rapidly absorbed from the lungs into systemic circulation during cigarette smoking. After absorption, nicotine rapidly reaches the brain where it exerts various pharmacological and toxicological effects [1,2]. Using positron emission tomography, Berridge et al. [3] reported that nicotine concentration in the brain after a single puff of cigarette formulated with [^{11}C]nicotine reached > 50% of its maximum brain concentration within 15 s in most subjects. However, mechanism underlying pulmonary nicotine absorption is not well understood to date.

Pulmonary absorption of nicotine occurs predominantly from the alveoli because of their large surface area. The alveolar region in the lungs is lined by two types of epithelial cells, namely, type I and type II cells [4]. Type I cells have a squamous morphology and cover 90%–95% of the alveolar surface, whereas type II cells have a cuboidal morphology and cover 5%–10% of the alveolar surface. Expression of some membrane transporters is often different between type I and type II cells. For example, our and other previous studies have reported that P-glycoprotein (ABCB1) is functionally expressed in type I cells but not in type II cells [5,6], whereas PEPT2 (SLC15A2) is functionally expressed in type II cells but not in type I cells [7,8].

Recently, we examined nicotine transport in type II cells and in transdifferentiated type I-like cells by using rat primary cultured alveolar epithelial cells and found for the first time that a similar or the same transport system was involved in nicotine uptake in type I-like and type II cells [9]. Nicotine uptake in these cells was highly sensitive to extracellular pH, with high uptake being observed at high pH. In addition, nicotine uptake was potently inhibited by organic cations such as diphenhydramine and pyrilamine but was not affected by substrates and/or inhibitors of known organic cation transporters such as carnitine, 1-methyl-4-phenylpyridinium (MPP^+), and tetraethylammonium (TEA).

More recently, Tega et al. [10] reported the characteristics of nicotine transport in human alveolar epithelial cell line A549. They found that nicotine uptake in A549 cells was inhibited by some organic cations such as propranolol and pyrilamine, similar to that observed in our previous study [9]. However, Tega et al. [10] suggested that nicotine uptake in A549 cells was insensitive to changes in extracellular

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http://dx.doi.org/10.1016/j.lfs.2017.08.030
Received 12 June 2017; Received in revised form 17 August 2017; Accepted 28 August 2017
Available online 31 August 2017
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pH, which was quite different from that observed in rat primary cultured alveolar epithelial cells [9]. This discrepancy may be because of the differences in animal species (rat vs human cells); however, the exact reason is unclear.

Therefore, in the present study, we further examined the characteristics of nicotine transport in A549 cells and another lung cell line NCI-H441, especially with respect to pH sensitivity. NCI-H441 is a human distal lung epithelial cell line with alveolar type II and/or club cell-like phenotype [11,12]. We also examined the characteristics of nicotine uptake in widely used non-lung epithelial cell lines HepG2 and MCF-7 for comparison, in order to obtain the information concerning the distribution of nicotine transport system in various types of cells.

2. Materials and methods

2.1. Materials

Fetal bovine serum (FBS) and Dulbecco's modified Eagle's medium (DMEM) were purchased from MP Biomedicals (Solon, Ohio). Insulin–transferrin–sodium selenite (ITS) supplement was purchased from Roche AG (Basel, Switzerland). RPMI-1640 medium, trypsin-ethylenediaminetetraacetic acid, and penicillin–streptomycin were purchased from Thermo Fisher Scientific Inc. (Waltham, Massachusetts). [^3H]Nicotine was purchased from American Radiolabeled Chemicals, Inc. (St. Louis, Missouri). L-Carnitine, choline chloride, diphenhydramine, verapamil, and *p*-aminohippurate (PAH) were purchased from Nacalai Tesque Inc. (Kyoto, Japan). Dexamethasone (DEX), nicotine, propranolol, pyrilamine maleate, pyrimethamine, quinidine, quinine, and cotinine were purchased from Wako Pure Chemical Industries (Osaka, Japan). Procainamide, MPP^+, and valinomycin were purchased from Sigma-Aldrich (St. Louis, Missouri). TEA chloride and clonidine hydrochloride were purchased from Tokyo Kasei Kogyo Company (Tokyo, Japan). All other chemicals were of the highest grade commercially available.

2.2. Cell culture

A549 and HepG2 cells were provided by RIKEN BioResource Center (Tsukuba, Japan) through the National BioResource Project of the Ministry of Education, Culture, Sports, Science, and Technology. NCI-H441 cells were obtained from American Type Culture Collection (Manassas, Virginia), and MCF-7 cells were obtained from Japan Health Sciences Foundation (Tokyo, Japan). A549, HepG2, and MCF-7 cells were cultured in DMEM containing 10% FBS, 100 IU/mL penicillin, and 100 μg/mL streptomycin. NCI-H441 cells were cultured in RPMI-1640 medium supplemented with 5% FBS, 1% sodium pyruvate, 100 IU/mL penicillin, and 100 μg/mL streptomycin. All the cells were maintained at 37 °C in a humidified atmosphere of 5% CO_2.

For experiments, A549, HepG2, and MCF-7 cells were seeded in 24-well plates and were cultured for 6–7 days. NCI-H441 cells were seeded in a 24-well plate at a density of 10×10^4 cells/well. At 24 h after seeding, the medium was replaced with RPMI-1640 medium containing DEX (200 nM) and ITS in addition to the abovementioned supplements, and the cells were cultured for 13 days [12].

2.3. Uptake of [^3H]nicotine in A549 cells

Time and concentration dependence of [^3H]nicotine uptake in A549 cells was determined, as described previously [9]. Briefly, the time dependence of nicotine uptake was determined by incubating A549 cells with [^3H]nicotine (50 μM) in HEPES buffer (145 mM NaCl, 3 mM KCl, 1 mM $CaCl_2$, 0.5 mM $MgCl_2$, 5 mM glucose, and 5 mM HEPES [pH 7.4]) at 37 °C or 4 °C for indicated time periods after preincubation with HEPES buffer for 10 min at each temperature. Generally, one cigarette contains 0.1–1.0 mg nicotine. If all the nicotine in one cigarette is dissolved in the alveolar fluid (about 40 mL) in human lungs after smoking, the concentration of nicotine would be 15–150 μM. Therefore, we used 50 μM nicotine in this study. The concentration dependence of nicotine uptake was determined by incubating A549 cells with HEPES buffer (pH 7.4) containing [^3H]nicotine (50 μM) and various concentrations of unlabeled nicotine for 15 s at 37 °C. Curve fitting was performed using nonlinear regression analysis with a Michaelis–Menten-type equation, as described previously [9].

Effect of organic compounds on nicotine uptake in A549 cells was examined by incubating the cells with [^3H]nicotine (50 μM) in HEPES buffer (pH 7.4) at 37 °C in the presence or absence of the following organic compounds: 1 mM clonidine, 3–1000 μM diphenhydramine, 1 mM propranolol, 10–1000 μM pyrilamine, 1 mM quinidine, 1 mM quinine, 1 mM verapamil, 1 mM carnitine, 1 mM choline, 1 mM MPP^+, 1 mM procainamide, 100 nM pyrimethamine, 1 mM TEA, 1 mM cotinine, and 1 mM PAH. IC_{50} values of diphenhydramine and pyrilamine on [^3H]nicotine uptake were estimated through curve fitting to the Hill equation [9].

Effect of extracellular pH on [^3H]nicotine uptake was determined by incubating the cells with MES buffer (145 mM NaCl, 3 mM KCl, 1 mM $CaCl_2$, 0.5 mM $MgCl_2$, 5 mM glucose, and 5 mM MES [pH 6.0]), HEPES buffer [pH 7.0 or 8.0], or carbonate-bicarbonate buffer (0.1 M sodium carbonate and 30 mM NaCl/0.1 M sodium bicarbonate and 60 mM NaCl, 3 mM KCl, 1 mM $CaCl_2$, 0.5 mM $MgCl_2$, and 5 mM glucose [pH 9, 10, or 11]) containing [^3H]nicotine (50 μM) for 15 s at 37 °C.

Effect of intracellular pH on [^3H]nicotine uptake in A549 cells was determined by treating the cells with NH_4Cl, as reported previously [13]. Briefly, A549 cells were preincubated with HEPES buffer (pH 7.4) in the absence (control, acute) or presence (Pre) of 30 mM NH_4Cl for 20 min. Next, the cells were incubated with [^3H]nicotine (50 μM) in HEPES buffer in the absence (control, Pre) or presence (acute) of 30 mM NH_4Cl for 15 s. Effect of valinomycin, a potassium ionophore, on [^3H]nicotine uptake in A549 cells was determined by preincubating the cells with HEPES buffer (pH 7.4) in the absence (control, Co.) or presence (Pre, Pre + Co.) of 18 μM valinomycin, followed by incubation with [^3H]nicotine in the absence (control, Pre) or presence (Co., Pre + Co.) of 18 μM valinomycin.

After the incubation, the cells were solubilized with 0.1 M NaOH, and cell lysates were used for performing radioactivity analysis and protein assay. [^3H]Nicotine was measured by adding Ultima GOLD (Perkin Elmer, Massachusetts) to the cell lysates, and radioactivity was measured by performing liquid scintillation counting with LSC5100 (Hitachi Aloka Medical, Ltd., Tokyo, Japan). Protein concentrations were measured using Lowry method, with bovine serum albumin as a standard.

2.4. Uptake of [^3H]nicotine in NCI-H441, HepG2, and MCF-7 cells

To examine the time and pH dependence of nicotine uptake in NCI-H441, HepG2, and MCF-7 cells, the cells were preincubated with HEPES buffer (pH 7.4), followed by incubation with MES (pH 6.0) or HEPES (pH 8.0) buffer containing [^3H]nicotine (50 μM) for indicated time periods at 37 °C. For performing inhibition studies, each cell line was incubated with [^3H]nicotine (50 μM) in HEPES buffer (pH 7.4) for 15 s at 37 °C in the presence or absence of different organic compounds mentioned above.

2.5. Statistical analysis

Data are expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed using one-way ANOVA followed by Tukey's test for multiple comparisons. A p value of < 0.05 was considered statistically significant.

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