Available online at www.sciencedirect.com
SCIENCE DIRECT
ELSEVIER
Life Sciences 78 (2005) 310 – 320
Life Sciences
www.elsevier.com/locate/lifescie

Functional and pharmacological mechanisms of nucleoside transport across the basolateral membrane of rabbit tracheal epithelial cells

Sharon K. Wu [a,*], Neil R. Mathias [a,1], Kwang-Jin Kim [b], Vincent H.L. Lee [a,c,2]

[a] *Department of Pharmaceutical Sciences, University of Southern California, Los Angeles, CA 90089-9121, United States*
[b] *Departments of Medicine, Physiology and Biophysics, Molecular Pharmacology and Toxicology, Biomedical Engineering, and Will Rogers Institute Pulmonary Research Center, University of Southern California, Los Angeles, CA 90089-9121, United States*
[c] *Departments of Pharmaceutical Sciences and Ophthalmology, University of Southern California, Los Angeles, CA 90089-9121, United States*

Received 14 March 2005; accepted 22 April 2005

Abstract

The role of basolateral membrane nucleoside transport in primary cultured rabbit tracheal epithelial cells (RTEC) was studied. Primary cultured RTEC were grown on permeable support at an air-interface. Transport studies were conducted in the uptake, efflux, and transepithelial transport configurations using ^{3}H-uridine as a model substrate. Time, temperature and concentration dependency of ^{3}H-uridine transport were evaluated in parallel to the metabolism of this substrate using scintillation counting and thin layer chromatography. Inhibition of ^{3}H-uridine uptake from basolateral fluid was estimated in presence of all unlabeled natural nucleosides as well as analogs and nucleobases. Functional modulation pathways of ^{3}H-uridine uptake were studied after treatment of RTEC with pharmacological levels of A23187, forskolin, tamoxifen, H89 and colchicine.

The basolateral aspect has a low-affinity and high-capacity transport system that exhibits characteristics of bi-directionality, temperature/concentration dependency, and broad specificity towards purines and pyrimidines without requiring Na^+. Basolateral equilibrative-sensitive/insensitive (*es*/*ei*) type transport machinery manifested as a biphasic dose response to nitro-benzyl-mercapto-purine-ribose (NBMPR) inhibition. In addition, a number of therapeutically relevant nucleoside analogs appeared to compete with the uptake of uridine from basolateral fluid. Short-term pre-incubation of primary cultured RTEC with the calcium ionophore A23187 inhibited basolateral uridine uptake without affecting the J_{max} and K_m. The inhibitory effect was not reversible with a protein kinase C (PKC) antagonist, tamoxifen. In contrast, basolateral uridine uptake was increased by adenylyl cyclase activator forskolin (reversible with protein kinase A (PKA) inhibitor H89), resulting in a decreased K_m, but a lower J_{max}. Uridine exit across the basolateral membrane of primary cultured RTEC occurs via a facilitative diffusion carrier, which can be modulated by intracellular Ca^{2+} levels and PKA. Information about these carriers will help improve the transportability of antitumor and antiviral nucleoside analogs in the pulmonary setting.
© 2005 Elsevier Inc. All rights reserved.

Keywords: NBMPR; Rabbit tracheal epithelial cells; Basolateral; Equilibrative nucleoside transport; Regulation

Abbreviations: RTEC, rabbit tracheal epithelial cells; NBMPR, nitro-benzyl-mercapto-purine-ribose; *ei*, equilibrative NBMPR-insensitive; *es*, equilibrative NBMPR-sensitive; PKA, protein kinase A; PKC, protein kinase C; A23187, a calcium ionophore; H89, a PKA inhibitor; HBSS, Hank's Balanced Salt Solution; S-MEM, Ca^{2+}-free minimum essential medium; FBS, fetal bovine serum; DNase I, Deoxyribonuclease I; BSA, bovine serum albumin; TLC, thin layer chromatography; DMSO, dimethyl sulfoxide; 5-FU, 5-fluorouridine; IDU, 5-iodo-2′-deoxyuridine; 2′-dU, 2′-deoxyuridine; 3′-dA, 3′-deoxyuridine; ddA, 2′,3′-dideoxyadenosine; ddI, 2′,3′-dideoxyinosine; AZT, 3′-azidothymidine; Ara-C, cytosine arabinoside; Ara-A, adenosine arabinoside; ACV, acyclovir; R_f, retention factor; cAMP, cyclic adenosine 3′,5′-monophosphate; $[Ca^{2+}]_i$, intracellular Ca^{2+} concentration; MT, microtubule; IC_{75}, effective concentration at which 75% of uridine uptake remains; IC_{25}, effective concentration at which 25% of uridine uptake remains; K_m, Michaelis-Menten constant; J, total flux; J_{max}, maximal uptake rate; P_{app}, apparent permeability coefficient; J_{ss}, steady-state flux; C_0, initial dosing concentration; A, area of cell monolayers; $K_d \cdot C$, total non-specific diffusional component; K_a, affinity constant.

* Corresponding author. Present address: Cardinal Health Inc., 9250 Trade Place, San Diego, CA 92126, United States. Tel.: +1 858 547 7842; fax: +1 858 635 6296.
E-mail address: sharon.k.wu@cardinal.com (S.K. Wu).
[1] Present address: Bristol-Myers Squibb Co. Pharmaceutical Research Institute, One Squibb Drive, PO Box 191, Bldg. 105/1157, New Brunswick, NJ 08903, United States.
[2] Present address: Food and Drug Administration, 5515 Security Lane, Room 1023, Rockville, MD 20852, USA.

0024-3205/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.lfs.2005.04.066

Introduction

Nucleoside transporters are known for their role in salvage pathways of biological systems that lack physiological mechanisms of nucleoside biosynthesis or degradation. Furthermore, their regulatory function in purinergic signaling pathways is recognized (Ralevic and Burnstock, 2003). At least 7 functionally distinct nucleoside transport processes have been characterized (Cass et al., 1999; Ritzel et al., 2001; Baldwin et al., 2004). Na^+-independent equilibrative nucleoside transporters (ENTs) mediate the bi-directional, concentration gradient-driven transport of substrates. These transporters generally have a low affinity for substrates, which distinguishes them from the Na^+-coupled concentrative nucleoside transporters (CNTs). In CNTs, the driving force for the transport of substrates is the coupling of the uphill flux of nucleosides to a downhill Na^+ gradient (Ritzel et al., 2001; Baldwin et al., 2004).

ENTs, like several other facilitated diffusion carriers (glucose (Takata, 1996), amino acid (Gandhi et al., 2004), and peptide transporters (Terada et al., 1999)), are the primary mediators of the exit of intracellularly accumulated nucleosides across the basolateral membrane of absorptive epithelia. Therefore, basolateral ENTs play an important role in the overall transepithelial transport of nucleosides or their efflux from excess intracellular pools. Two types of ENTs, *es*-type and *ei*-type, have been characterized in considerable detail by their sensitivity to the classic nucleoside transport inhibitor nitro-benzyl-mercapto-purine-ribose (NBMPR) (Baldwin et al., 2004). Na^+-independent ENTs exhibit a broad substrate specificity for purines, pyrimidines, and a wide range of analogous xenobiotics. Specifically, the *ei*-type transporters have been shown to transport nucleobases such as hypoxanthine (Baldwin et al., 2004). Hence, ENTs are considered prospective drug targets for a variety of antitumor and antiviral nucleoside analogs due to their relatively broad substrate specificity (Pastor-Anglada et al., 1998). For example, ribavirin (Virazole®), a synthetic nucleoside antiviral agent for oral inhalation therapy against respiratory syncytial virus, inhibits uridine uptake by the human intestinal *es* transporter (Lum et al., 2000).

Evidence of different mechanisms in apical vs. basolateral nucleoside transport has been demonstrated in rabbit kidney (Williams and Jarvis, 1991), and rat and rabbit intestine (Chandrasena et al., 1997). Nucleosides enter across the apical membrane driven by coupling to the Na^+ gradient, and exit across the basolateral membrane via a Na^+-independent facilitated transport system. Specialized nucleoside transport processes have been unequivocally identified in various tissues and cells, including intestine (Scharrer and Grenacher, 2001), liver (Choi et al., 2000), kidney (Dragan et al., 2000), placenta (Griffiths et al., 1997) and choroid plexus (Anderson et al., 1999). In human, pig, guinea pig, and rat lung membrane preparation nucleoside transport processes exhibit a high-affinity binding of the nucleoside transport inhibitors ^{3}H-dipyridamole and ^{3}H-NBMPR (apparent K_a range of 0.3–2 nM), suggesting the presence of *es*-type nucleoside transport systems in lung tissues (Kwong et al., 1993). Furthermore, recent reports on the role of equilibrative nucleoside transporters in human non-small cell lung carcinoma therapy resistance highly emphasizes the importance of studying the mechanisms which regulate basolateral membrane permeability of airway epithelia in absorption of anticancer nucleoside analog drugs from the systemic circulation (Achiwa et al., 2004).

An enhanced understanding of the post-translational modulation of nucleoside transport has pharmacological advantages, as it will improve the transportability of antitumor and antiviral nucleoside analogs. Regulation of transporters by second messengers has been reported in the glucose transporter (Braiman et al., 1999) and peptide transporter (Wenzel et al., 1999), although their regulatory mechanisms vary greatly. In undifferentiated Neuro-2A cells, the Na^+-dependent adenosine transport was not modulated by any known second messenger (e.g. Ca^{2+} or cAMP), but forskolin and A23187 (Sen et al., 1999) inhibited the *es*-type facilitated diffusion component. Cell types co-expressing different transporter isoforms can exhibit diverging modulation patterns, which probably evolved to fulfill cell-specific requirements.

The aims of the present study were to determine whether the shuttling of uridine across the basolateral membrane of primary cultured rabbit tracheal epithelial cells (RTEC) is mediated via a facilitative diffusion mechanism, and to elucidate the modulation mechanisms involved in basolateral nucleoside transport by manipulating different intracellular signaling pathways. ^{3}H-uridine uptake experiments from apical and basolateral fluids of primary cultured RTEC were used to functionally characterize nucleoside transport processes, since uridine is a known universal substrate across a spectrum of nucleoside transporter families (Baldwin et al., 2004). Substrate specificity of basolateral uridine uptake was assessed in the presence of various nucleoside analogs. Basolateral nucleoside transport activity was further assessed by studying ^{3}H-uridine uptake in primary cultured RTEC monolayers incubated with pharmacological agents that are known to act along signaling pathways leading to protein kinase C (PKC) and/or protein kinase A (PKA) activation. We also evaluated the role of cytoskeletal rearrangement in the regulation of basolateral nucleoside transport.

Materials and methods

Male, Dutch-belted pigmented rabbits, weighing 2.5–3.0 kg, were purchased from Irish Farms (Los Angeles, CA). The investigations utilizing rabbits described in this report conform to the Guiding Principles in the Care and Use of Animals (DHEW Publication, NIH 80-23). HBSS, S-MEM, FBS, penicillin, streptomycin, gentamicin, L-glutamine, and fungizone were purchased from GibcoBRL (Grand Island, NY). Protease XIV, DNase I, NBMPR, DMSO, forskolin, colchicine, tamoxifen and BSA were purchased from Sigma Chemical Co. (St. Louis, MO). Calcimycin (A23187) and H89 were purchased from Research Biochemicals International (Natick, MA). PC-1 medium was used for cultures of

Download English Version:

https://daneshyari.com/en/article/9012619

Download Persian Version:

https://daneshyari.com/article/9012619

Daneshyari.com