Journal of Theoretical Biology 363 (2014) 427–435

Contents lists available at ScienceDirect

Journal of Theoretical Biology

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

Effects of airway surface liquid height on the kinetics of extracellular nucleotides in airway epithelia

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HIGHLIGHTS

- A mathematical model is proposed for the biochemical network of extracellular nucleotides in airway epithelia, which includes diffusion of nucleotides in the airway surface liquid.
- The model predicts the kinetics of ATP, ADP, AMP, ADO and INO after hypotonic challenge.
- Due to a sharp concentration gradient, nucleotide concentrations near the epithelial surface are much higher than volume-averaged concentrations.
- CNT3 inhibition leads to the greatest availability of ADO for A_{2B} activation.
- NTPDase1/highTNAP inhibition leads to the greatest availability of ATP for $P2Y_2$ activation.

ARTICLE INFO

Article history:
Received 13 December 2013
Received in revised form
7 July 2014
Accepted 18 August 2014
Available online 24 August 2014

Keywords:
Purinergic signaling
Mucociliary clearance
Bronchial epithelium
Mathematical model
Enzyme inhibition

ABSTRACT

Experimental techniques aimed at measuring the concentration of signaling molecules in the airway surface liquid (ASL) often require an unrealistically large ASL volume to facilitate sampling. This experimental limitation, prompted by the difficulty of pipetting liquid from a very shallow layer (~15 μm), leads to dilution and the under-prediction of physiologic concentrations of signaling molecules that are vital to the regulation of mucociliary clearance. Here, we use a computational model to describe the effect of liquid height on the kinetics of extracellular nucleotides in the airway surface liquid coating respiratory epithelia. The model consists of a reaction–diffusion equation with boundary conditions that represent the enzymatic reactions occurring on the epithelial surface. The simulations reproduce successfully the kinetics of extracellular ATP following hypotonic challenge for ASL volumes ranging from 25 μl to 500 μl in a 12-mm diameter cell culture. The model reveals that [ATP] and [ADO] reach 1200 nM and 2200 nM at the epithelial surface, respectively, while their volumetric averages remain less than 200 nM at all times in experiments with a large ASL volume (500 μl). These findings imply that activation of $P2Y_2$ and A_{2B} receptors is robust after hypotonic challenge, in contrast to what could be concluded based on experimental measurements of volumetric concentrations in large ASL volumes. Finally, given the central role that ATP and ADO play in regulating mucociliary clearance, we investigated which enzymes, when inhibited, provide the greatest increase in ATP and ADO concentrations. Our findings suggest that inhibition of NTPDase1/highTNAP would cause the greatest increase in [ATP] after hypotonic challenge, while inhibition of the transporter CNT3 would provide the greatest increase in [ADO].

1. Introduction

Extracellular nucleotides are essential in protecting the respiratory tract against inhaled pathogens due to their key role in regulation of mucociliary clearance. By activating the membrane receptors A_{2B} and $P2Y_2$, the concentrations of extracellular adenosine (ADO) and adenosine triphosphate (ATP) regulate mucus secretion rate, ciliary beating frequency, mucus hydration, and mucus viscosity (Schmid et al., 2011). A quantitative understanding of the concentrations of ADO and ATP may be important for the development of new therapies for respiratory pathologies, especially cystic fibrosis (Zuo et al., 2008; Garcia et al., 2011, 2013; Herschlag et al., 2013). Individuals with this genetic disease have chronic bacterial lung infection due to impaired mucociliary clearance, that is caused by mucus dehydration and excessive mucus secretion (Boucher, 2007, 2007).

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http://dx.doi.org/10.1016/j.jtbi.2014.08.030
0022-5193/

The biochemical network that regulates the concentrations of extracellular ATP and ADO was recently described quantitatively by a computer model (Zuo et al., 2008; Garcia et al., 2011) based on experimental data collected in cultures of human respiratory epithelia with airway surface liquid (ASL) at a height of ~1500 μm. Although this model reproduced its target experimental data, it cannot be used to investigate the regulation of extracellular nucleotides *in vivo*, because *in vivo* the ASL height is approximately 15 μm (Harvey and Schlosser, 2009). Aiming to describe the behavior of the system *in vivo*, we improved the computer model by including diffusion and using experimental data from the literature where the extracellular ATP concentration was investigated for ASL heights ranging from 14 μm to 2730 μm (Okada et al., 2006). The new version of the model, proposed in this work, consists in the solution of the reaction–diffusion equation in cylindrical coordinates, with boundary conditions that express nucleotide secretion, nucleoside absorption, and the reactions catalyzed by ectonucleotidases on the epithelial surface.

The model successfully predicts the behavior of ATP concentration at the epithelial surface after hypotonic challenge. On the cell surface, where the $P2Y_2$ and A_{2B} receptors are located, the model shows that ATP concentration reaches the range for $P2Y_2$ receptor activation (~1 μM Mason et al., 1991) in response to hypotonic-shock induced ATP secretion. In addition, the model predicts the kinetics of adenosine diphosphate (ADP), adenosine monophosphate (AMP), ADO and inosine (INO), for which no experimental data is available after hypotonic challenge. Finally, and most importantly, the model was used to predict which enzymes when inhibited provide the greatest increase in ATP and ADO concentrations, thus identifying the best targets for therapeutic modulation of mucociliary clearance.

2. Materials and methods

2.1. Motivation

Zuo and coworkers (Zuo et al., 2008; Garcia et al., 2011) proposed a mathematical model that describes the kinetics of nucleotide and nucleoside ASL concentrations by coupled differential equations. It takes into account that ATP, ADP and AMP are secreted by the respiratory epithelium to the ASL, where they are metabolized by ecto-enzymes and converted into the nucleosides ADO and INO which in turn are absorbed by the respiratory epithelium (Fig. 1).

The original model was based on experimental data obtained by off-line measurements after pipette sampling of human bronchial epithelial cell cultures with ASL volume of 350 μl, which corresponds to a liquid height of ~1500 μm (Zuo et al., 2008). To construct the model, the authors assumed that the influence of diffusion could be neglected in determining the concentration of extracellular purines.

There was a logical inconsistency in the original model as it relied on the ASL height *in vivo* (~15 μm Harvey and Schlosser, 2009) to neglect the diffusion, but aimed to reproduce and analyze the kinetics of cell culture experiments with a large ASL volume (350 μl), where the assumption of no vertical gradients fails. We performed some analytical calculations (see Supporting Material B) that show that diffusion and ATP degradation occur at similar time scales, and therefore it is essential to take diffusion into account in order to accurately reproduce the kinetics of extracellular nucleotides in cell culture experiments involving large ASL volumes. Thus, in spite of reproducing the *in vitro* data for volumetric-average concentrations at ASL height of ~1500 μm, it was unclear whether the original model reproduced the kinetic behavior *in vivo*, where ASL height is ~15 μm.

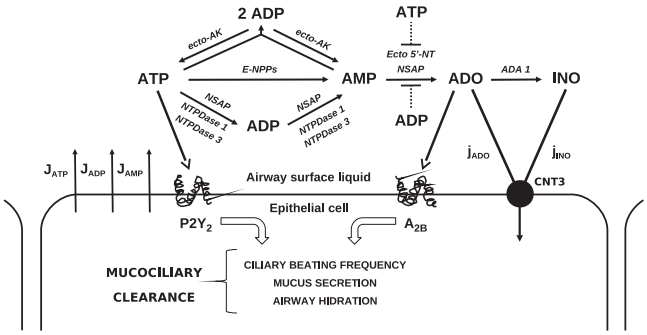

Fig. 1. Nucleotide transport and metabolism in the airway surface liquid (ASL). Respiratory epithelia release ATP, ADP and AMP at rates J_{ATP}, J_{ADP} and J_{AMP}, respectively. The nucleotides are dephosphorylated by ecto-enzymes into ADO and INO, which are absorbed by the transporter CNT3 at rates j_{ADO} e j_{INO}, respectively. ATP and ADO are signaling molecules that regulate mucociliary clearance by activating the receptors $P2Y_2$ and A_{2B}. The abbreviations of the enzymes included in the model are: NTPDase (Nucleoside triphosphate diphosphohydrolases), NSAP (tissue non-specific alkaline phosphatase), E-NPPs (Ecto-Nucleotide pirophosphatase / phosphodiesterases), ecto 5'-NT or CD73 (ecto-5'-nucleotidase), ecto-AK (ecto adenilase kinase), ADA1 (adenosine deaminase 1).

2.2. Experimental data

Okada and coworkers (Okada et al., 2006) performed a series of *in vitro* experiments to investigate the extracellular ATP concentration at an ASL height similar to the ASL height *in vivo*. To simulate the native human airway epithelia they used a well differentiated primary human bronchial epithelial cell culture.

They accessed ATP concentration after hypotonic challenge for ASL volumes ranging from 25 μl to 500 μl using three different techniques, namely (1) pipette or micro-sampling followed by off-line luminometry; (2) real-time luminometry with soluble luciferase; (3) real-time luminometry with cell-attached SPA-luc. The last technique measures ATP concentration on the cell surface, while the other two techniques provide an average ATP concentration throughout the ASL volume. Since the $P2Y_2$ receptors are located at the cell surfaces, physiologically the most important measurement is the concentration at the cell surface.

Their experimental data show that the volumetric-average ATP concentration after hypotonic challenge depends on ASL height (Fig. 2). Their data also show that the volumetric-average ATP concentration is much lower than the concentration near the cell surface. Therefore, the assumption by Zuo et al. (2008) that nucleotide concentration is uniform in the ASL is not valid.

2.3. Model equations

To reproduce the experimental data by Okada et al. (2006) and solve the inconsistency of the original model, diffusion was added to the mathematical model of Zuo and collaborators (Zuo et al., 2008) (Fig. 1).

Our model consists in solving the diffusion equation

$$\frac{\partial c}{\partial t} = D\nabla^2 c, \tag{1}$$

with boundary conditions that take into account the reactions that occur at the cell surface. Here c is the ASL concentration of a chemical specie ([ATP], [ADP], [AMP], [ADO] or [INO]; units of mol/m^3), t is the time (unit of s) and $D = 4.6 \times 10^{-10}$ m^2/s is the diffusion constant of ATP (Hubley et al., 1996). Experimental data (Bowen and Martin, 1964) suggest that the diffusion coefficient is nearly identical for all nucleotides and nucleosides. Bowen and Martin reported that the diffusion coefficient is $D = 4.3 \times 10^{-10}$ m^2/s for ATP, $D = 4.0 \times 10^{-10}$ m^2/s for ADP, $D = 4.3 \times 10^{-10}$ m^2/s for AMP, $D = 5.2 \times 10^{-10}$ m^2/s for ADO, and $D = 5.2 \times$