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Mathematical model for shear stress dependent NO and adenine nucleotide production from endothelial cells

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ABSTRACT

We developed a mass transport model for a parallel-plate flow chamber apparatus to predict the concentrations of nitric oxide (NO) and adenine nucleotides (ATP, ADP) produced by cultured endothelial cells (ECs) and investigated how the net rates of production, degradation, and mass transport for these three chemical species vary with changes in wall shear stress (τ_w). These simulations provide an improved understanding of experimental results obtained with parallel-plate flow chambers and allows quantitative analysis of the relationship between τ_w, adenine nucleotide concentrations, and NO produced by ECs. Experimental data obtained after altering ATP and ADP concentrations with apyrase were analyzed to quantify changes in the rate of NO production (R_{NO}). The effects of different isoforms of apyrase on ATP and ADP concentrations and nucleotide-dependent changes in R_{NO} could be predicted with the model. A decrease in ATP was predicted with apyrase, but an increase in ADP was simulated due to degradation of ATP. We found that a simple proportional relationship relating a component of R_{NO} to the sum of ATP and ADP provided a close match to the fitted curve for experimentally measured changes in R_{NO} with apyrase. Estimates for the proportionality constant ranged from 0.0067 to 0.0321 μM/s increase in R_{NO} per nM nucleotide concentration, depending on which isoform of apyrase was modeled, with the largest effect of nucleotides on R_{NO} at low τ_w (<6 dyn/cm^2).

1. Introduction

Wall shear stress (τ_w) is the primary hemodynamic determinant of endothelial release of nitric oxide (NO) produced by endothelial nitric oxide synthase (eNOS) in endothelial cells (ECs) with subsequent effects on vascular tone [1–11]. It is well known that the NO produced due to viscous drag over ECs depends upon Ca^{2+} [6,11,12], eNOS phosphorylation [13–16], and eNOS expression [16]. The importance of purinergic signaling in the vasculature at the cellular scale (both EC and smooth muscle cells) is also recognized [17,18]. Adenine nucleotides (ATP, ADP) are known to modulate the rate of NO production (R_{NO}) by ECs [19–22], where R_{NO} is expressed as the rate of NO produced per volume of cells (10^{-9} mol/liter/s or nM/s). However, it is difficult to investigate specific mechanisms involved in NO release, since it is technically challenging to quantify τ_w-dependent R_{NO} from ECs as there are numerous shortcomings for direct and indirect measurements of NO, especially under *in vivo* conditions. To address this problem, we previously designed a parallel-plate flow chamber apparatus (Fig. 1) capable of direct *in vitro* real time measurement of NO released by ECs in response to changes in flow [10], which we have used for additional NO measurements [23,24]. Here, we further develop a computational mass transport model that allows quantitative analysis of *in vitro* experimental data obtained with parallel-plate flow chambers that could provide insight into the relationship between blood flow and production of NO by ECs *in vivo*.

2. Models for τ_w-dependent R_{NO}

Fadel et al. [25] and Plata [26] developed mass transport models which predict the spatial distribution of NO produced by ECs cultured in a parallel-plate flow chamber, based on linear and nonlinear empirical functions to represent the increase in R_{NO} with increased flow and τ_w. More recently, Andrews et al. [10] found that a hyperbolic function,

$$R_{NO} = R_{basal} + R_{max}\frac{\tau_w}{\tau_w + A} \quad (1)$$

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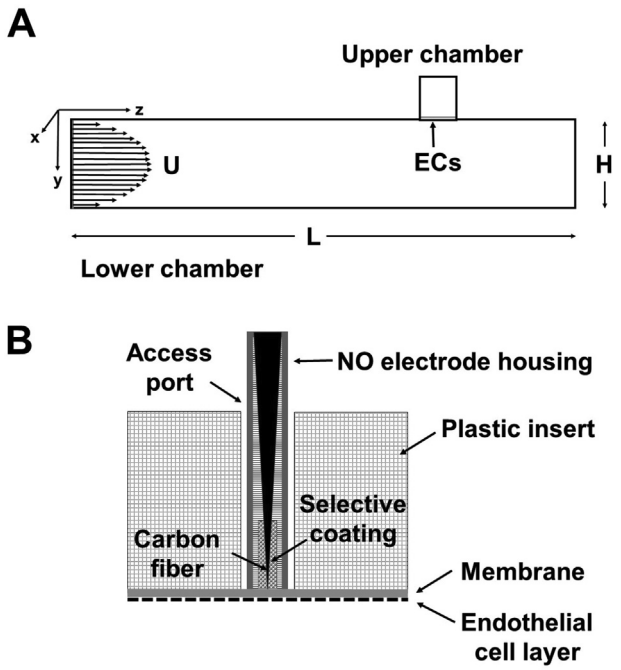

Fig. 1. (A) Diagram of parallel-plate flow apparatus used in experiments to measure NO produced from cultured ECs (Andrews et al. [10,23,24]) with (B) detail for NO electrode in upper chamber (not to scale) placed over a 2.4 cm diameter Transwell® membrane (thickness = 10 μm) with a circular disk of ECs (thickness 5 μm, radius 2.4 cm) on the surface facing the lower chamber. The computational domain used in the mass transport model simulations is based upon chamber geometry, where L = chamber length, H = height, and U = fluid velocity.

provided the best fit to experimental steady-state NO measurements with bovine aortic ECs at different values of τ_w in our parallel-plate flow chamber. Rather than using an instantaneous increase in R_{NO} following a step increase in τ_w at $t = t_1$, we used a ramp function to simulate a gradual, linear increase in NO production over the time period from t_1 to t_2

$$R_{NO} = R_{basal} + R_{max}\,[ramp(t_1, t_2)]\frac{\tau_w}{\tau_w + A} \tag{2}$$

to more closely match experimental observations [10,23,24] for the dynamic NO response.

2.1. Models for adenine nucleotide transport and τ_w-dependent release

Computational and bench-top studies demonstrate the importance of adenine nucleotides in endothelial biology [27–30], and mass transport models for parallel-plate geometry have been developed. The mass transport model by Nollert et al. [31,32] predicts that, when ATP is in the perfusing media, convection can increase ATP concentration at the EC surface and affect intracellular Ca^{2+}. Since ADP is also potent in mobilizing EC intracellular Ca^{2+}, Shen et al. [33] extended the model by including ADP, and demonstrated that the combined nucleotide EC surface concentration in a parallel-plate flow chamber varies over a wide range of τ_w (0.1–30 dyn/cm^2).

Mathematical models have also been developed to describe τ_w-dependent endogenous release of adenine nucleotides from ECs. The model originally developed by John and Barakat [34] (**Model A**), has been used by many others [35–38]. Another model (**Model B**) was developed by Qin et al. [37] by fitting experimental results obtained by Yamamoto et al. [39]. In the current report, we compare predicted concentrations obtained with these two different ATP-release models and analyze recent experimental data from our laboratory to obtain a quantitative description for the change in R_{NO} with τ_w-dependent adenine nucleotide concentrations [23,24].

2.1.1. Model A

ATP production with increasing τ_w is represented by a sigmoidal function

$$S_{ATP}(\tau_w) = S_{max}\left[1 - e^{-\left(\frac{\tau_w}{\tau_0}\right)}\right]^3 \tag{3}$$

where τ_0 is a reference shear stress, and S_{max} is the maximum ATP flux (Table 1). Using the same criteria as Choi et al. [35], τ_0 was set to 4 dyn/cm^2 [40], based upon experiments by Bodin and Burnstock [41], assuming that S_{max} occurs at $\tau_w = 25$ dyn/cm^2. The value for S_{max} was estimated by John and Barakat [34], based on experiments by Milner et al. [42] with ECs cultured on micro-carrier beads.

2.1.2. Model B

Parallel-plate flow chamber studies by Yamamoto et al. [39] demonstrated that, following a step increase in τ_w, ATP produced by ECs reaches a maximum, then slowly decreases with time as the higher τ_w is sustained. Qin et al. [37] modified the John and Barakat [34] model, using the product of two state variables to better represent experimentally observed dynamic behavior. The first state (p_1) represents the effects of τ_w and the probability of open states of all possible ATP production pathways. The second state (p_2) represents activation levels of ATP production pathways and includes the phenomenon described by Qin et al. [37] as "receptor desensitization" (a reduction in the response with a longer stimulus). As Xiang et al. [43] note, this is a ubiquitous feature of cells that are exposed to a constant stimulus. The τ_w-dependent production of ATP from ECs is given by

$$S_{ATP}(\tau_w, t) = p_1 p_2 \tag{4}$$

where p_1 and p_2 satisfy the following ordinary differential equations

$$\frac{dp_1}{dt} = f(\tau_w) - \frac{p_1}{t_1} \tag{5}$$

and

$$\frac{dp_2}{dt} = -\frac{p_2}{t_2} \tag{6}$$

where t_1 and t_2 are time delay constants. The function in Equation (5) is

$$f(\tau_w) = a_1 + \frac{a_2\tau_w}{a_3 + \tau_w} \tag{7}$$

where coefficients (a_1, a_2, a_3) were determined from experimental data [39]. Time-dependent changes in state variables after exposing the EC monolayer to a single step change in τ_w are