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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 $\mu\text{M/s}$ 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 ($< 6 \text{ dyn/cm}^2$).

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Keywords

ADP; Apyrase; ATP; Endothelial Cell; Nitric Oxide; Parallel-plate Flow Chamber; Wall Shear Stress

1.1 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 (Figure 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*.

2.1 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 non-linear 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,

$$R_{\text{NO}} = R_{\text{basal}} + R_{\text{max}} \frac{\tau_w}{\tau_w + A} \quad \text{Equation 1}$$

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

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

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

2.2 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 to 30 dyn/cm²).

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).

2.2.1 Model A

ATP production with increasing τ_w is represented by a sigmoidal function

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

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/cm² (40), based upon experiments by Bodin and Burnstock (41), assuming that S_{max} occurs at $\tau_w = 25$ dyn/cm². 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.

2.2.2 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

$$S_{\text{ATP}}(\tau_w, t) = p_1 p_2 \quad \text{Equation 4}$$

where p_1 and p_2 satisfy the following ordinary differential equations

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

and

$$\frac{dp_2}{dt} = -\frac{p_2}{t_2} \quad \text{Equation 6}$$

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

$$f(\tau_w) = a_1 + \frac{a_2 \tau_w}{a_3 + \tau_w} \quad \text{Equation 7}$$

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

$$p_1(t) = \begin{cases} t_1 f(\tau_{w1}) [1 - e^{-t/t_1}]; & 0 \leq t < T_1 \\ t_1 e^{-t/t_1} [f(\tau_{w1}) [e^{T_1/t_1} - 1] + f(\tau_{w2}) [e^{t/t_1} - e^{T_1/t_1}]]; & T_1 \leq t < T_2 \end{cases} \quad \text{Equation 8}$$

and

$$p_2(t) = e^{-t/t_2} \quad \text{Equation 9}$$

Coefficients for **Model B** (Table 2) were determined by Qin *et al.* (37) by fitting Yamamoto's data (39), where τ_{w1} and τ_{w2} correspond to the two different τ_w steps that ECs were exposed to. Recently, Li *et al.* (44) used this model to describe effects of ATP on Ca^{2+} dynamics, with corrected units for the flux coefficients a_1 and a_2 ($\text{mol m}^{-2} \text{s}^{-1}$). With respect to the current simulation, the baseline NO is obtained at the first shear stress (τ_{w1}), then flow is increased to expose ECs to a second, final shear stress (τ_{w2}). The time T_1 is for the initial step and T_2 is for the final time.

2.2.3 Net Release of ATP and ADP

ATP is hydrolyzed according to an irreversible Michaelis-Menten reaction to form ADP via ectonucleotidases located at the EC surface (34–37, 45). The net flux for ATP release (F_{ATP}) is given by

$$F_{\text{ATP}} = S_{\text{ATP}}(\tau_w) - \frac{V_{\text{max,ATP}} C_{\text{ATP}}}{K_{\text{m,ATP}} + C_{\text{ATP}}} \quad \text{Equation 10}$$

The net flux for ADP release (F_{ADP}) is the summation of ADP degraded at the EC surface and the rate of ADP production due to ATP hydrolysis.

$$F_{\text{ADP}} = \frac{V_{\text{max,ATP}} C_{\text{ATP}}}{K_{\text{m,ATP}} + C_{\text{ATP}}} - \frac{V_{\text{max,ADP}} C_{\text{ADP}}}{K_{\text{m,ADP}} + C_{\text{ADP}}} \quad \text{Equation 11}$$

Rate constants for the conversion of ATP and ADP have been determined previously (46–48). Multiplying each flux by the surface area (πr^2) and dividing by the volume of ECs ($\pi r^2 \Delta_{EC}$) converts the mass fluxes to volumetric reaction rates (with the surface area canceling out)

$$R_{ATP} = F_{ATP} / \Delta_{EC} \quad \text{Equation 12}$$

$$R_{ADP} = F_{ADP} / \Delta_{EC} \quad \text{Equation 13}$$

where $\Delta_{EC} = 5 \mu\text{m}$ is the thickness of the EC monolayer.

2.2.4 Relationship between R_{NO} and Adenine Nucleotides

It is reported that NO produced by ECs is dependent upon both ATP (19, 49–52) and ADP (21). However, the empirical relationship (Equations 1 & 2) that fits our experimental data (10) does not explicitly model this dependence. Previous experimental studies relating adenine nucleotide concentrations and NO are based upon increases in eNOS activity (19, 21). Other studies use diaminofluorescein (DAF) fluorescence to detect changes in NO (51–53) or to evaluate changes in eNOS expression (54). Since no direct correlation exists between these measurements and R_{NO} , it is difficult to obtain a quantitative relationship for the change in R_{NO} with adenine nucleotide concentrations as a function of τ_w .

Andrews *et al.* (23, 24) examined the influence of adenine nucleotides on τ_w -dependent R_{NO} in our parallel-plate flow chamber, although ATP and ADP were not directly measured. Instead, NO was measured at different τ_w before and after adding 1 U/mL of apyrase (Sigma Aldrich) to the media to alter adenine nucleotide concentrations. In the presence of apyrase, a smaller increase in NO with τ_w was observed from ECs, especially in the lower τ_w range, consistent with a decrease in R_{NO} . In the current report, we quantified the change in τ_w -dependent R_{NO} with apyrase using our modified model.

2.3.1 Characterization of Apyrase Activity

Apyrase is a Ca^{2+} -activated plasma membrane-bound enzyme that catalyzes the conversion of ATP to ADP, followed by conversion of ADP to AMP and inorganic phosphate. The reaction with apyrase

affects ATP and ADP concentrations with degradation rates of

$$R_{ATP, \text{apyrase}} = -C_{ATP} C_{\text{apyrase}} \frac{k_{cat}}{K_m + C_{ATP}} \quad \text{Equation 15}$$

$$R_{ADP, \text{apyrase}} = -C_{ADP} C_{\text{apyrase}} \frac{k_{cat}}{K_m + C_{ADP}} \quad \text{Equation 16}$$

Apyrase used in these experiments (23, 24) was derived from potatoes (*S. tuberosum*). According to the manufacturer (Sigma Aldrich), the specific activity varies from batch to batch, ranging between 3.0–40.0 U/mg protein. There are at least two isoforms in different varieties of *S. tuberosum*. Since the exact composition of apyrase used experimentally was not specified, we focused our attention on two isoforms that have been previously characterized. The first isoform, Pimpernel, catalyzes hydrolysis of the γ -phosphoryl group of ATP ten times faster than the β -phosphoryl group of ADP (46, 47). The other isoform, Desiree, has a lower ATPase/ADPase ratio, and converts ADP to AMP at a rate similar to the conversion rate of ATP to ADP. Each isoform has the same molecular weight of 49 kDa (46).

3.1 Mass Transport Model for Parallel-plate Flow Chamber Model

The computational domain for the modified model is based on the specific geometry of our parallel-plate flow apparatus (Figure 1), as described by Andrews *et al.* (10). ECs are grown to confluence on the bottom surface of a 2.4 cm diameter Transwell® porous membrane (10 μm thick), which is then placed at the top of the main flow chamber, exposing ECs to fluid flow. A second chamber allows a NO electrode housed in a stainless steel tube with a gas permeable membrane covering the 200 μm diameter carbon fiber sensor (World Precision Instruments ISO-NOPF®, as described by Zhang (55)) to be positioned at the center of the membrane immediately above the EC monolayer, physically separated from fluid flow. Dimensions of the flow channel (in cm) are $W = 4.57$, $L = 12.19$, $H = 0.025$. The 2-dimensional cross section through the EC monolayer is 5 μm thick and 2.4 cm long. The EC cell surface is defined at $y = 0$ with the bottom of the flow chamber located at $y = H$.

Each simulation reflects the protocols that were used experimentally (10, 23, 24). The perfusing media in the lower chamber was maintained at constant temperature (37° C) and is assumed to have the same dynamic viscosity as water ($\mu = 0.00696 \text{ dyn-s/cm}^2$). Initially, a low flow rate with $\tau_w = 0.1 \text{ dyn/cm}^2$ was maintained for a sufficient time (typically $t > 400 \text{ s}$) to generate a steady-state baseline NO at the electrode tip. A step change in pump flow was then applied to increase τ_w . Four different step changes in τ_w from the baseline were simulated (0.1 to 1, 0.1 to 6, 0.1 to 10, and 0.1 to 20 dyn/cm^2).

3.1.1 Model Assumptions

1. The flow chamber is treated as being infinitely wide ($W \gg H$), the EC monolayer thickness is $\ll$ membrane radius, and the area under the NO sensor is small ($\sim 0.007\%$ of total area), simplifying the model to two dimensions.
2. Fully developed Poiseuille flow is assumed, neglecting edge effects.
3. There is no NO dissolved in the culture medium entering the chamber (NO = zero at the entrance).
4. Excess substrates are available for NO synthesis (L-arginine, O_2), and O_2 consumption by ECs is negligible (25).
5. The model neglects any influence of negative feedback by NO on R_{NO} , and any influence of ATP feedback on ATP release.

6. The hyperbolic representation of τ_w -dependent R_{NO} (Equation 1) by Andrews *et al.* (10) was modified (Equation 2) to reflect a time lag between the increase in τ_w and the corresponding increase in R_{NO} , as suggested experimentally.
7. The concentration of ATP = 0 at the inlet of the chamber, with concentrations of ATP and ADP in the flowing media originating solely from ECs.
8. The ATP production rate depends upon the magnitude of τ_w (39), which can be represented by a single term, and intracellular ATP stores are not depleted.
9. Consumption of NO by the electrode is assumed to be negligible.

3.1.2 Governing Equations

The mathematical model for the parallel-plate flow chamber includes transport of three chemical species (NO, ATP, and ADP). Since some NO may be lost due to auto-oxidation in an oxygenated aqueous solution (56)

we include a pseudo-second order reaction ($k_{\text{auto}}C_{NO}^2$) with rate constant $k_{\text{auto}} = 2 \times 10^{-6} \text{ nM}^{-1}\text{s}^{-1}$ (57). The validity of using a pseudo-second order reaction when O_2 concentration is constant has been reviewed by Buerk (58).

3.1.3 Flowing Region (Lower Chamber)

Within the flowing media, the transport of NO is described by the general time-dependent convection/diffusion/reaction equation, including removal by auto-oxidation,

$$\frac{\partial C_{NO}}{\partial t} = D_{NO} \nabla^2 C_{NO} - k_{\text{auto}} C_{NO}^2 - u(y) \frac{\partial C_{NO}}{\partial z} \quad \text{Equation 18}$$

with model parameters as defined in Table 1.

Equations for transport of ATP and ADP can be described similarly but do not require an auto-oxidation term:

$$\frac{\partial C_{ATP}}{\partial t} = D_{ATP} \nabla^2 C_{ATP} - u(y) \frac{\partial C_{ATP}}{\partial z} \quad \text{Equation 19}$$

$$\frac{\partial C_{ADP}}{\partial t} = D_{ADP} \nabla^2 C_{ADP} - u(y) \frac{\partial C_{ADP}}{\partial z} \quad \text{Equation 20}$$

where $u(y)$ defines the parabolic velocity profile for laminar flow:

$$u(y) = 4U \left[\frac{y}{H} - \left(\frac{y}{H} \right)^2 \right] \quad \text{Equation 21}$$

The derivative of the velocity profile is used to calculate τ_w at the EC surface ($y=0$).

$$\tau_w = \mu \left. \frac{du}{dy} \right|_{y=0} = \frac{4\mu U}{H} \quad \text{Equation 22}$$

The volumetric flow rate that determines the midstream maximum velocity U at $y = H/2$ is based on experimental values of τ_w (10, 23, 24).

3.1.4 Effect of Apyrase

When apyrase is present in the medium, degradation reactions (Eqns. 15, 16) are added to the nucleotide mass transport equations.

$$\frac{\partial C_{ATP}}{\partial t} = D_{ATP} \nabla^2 C_{ATP} - u(y) \frac{\partial C_{ATP}}{\partial z} + R_{ATP,apyrase} \quad \text{Equation 23}$$

$$\frac{\partial C_{ADP}}{\partial t} = D_{ADP} \nabla^2 C_{ADP} - u(y) \frac{\partial C_{ADP}}{\partial z} + R_{ADP,apyrase} \quad \text{Equation 24}$$

3.1.5 Endothelial Cell Monolayer

As there is no fluid flow within the EC monolayer, convective transport can be neglected, and governing equations for each chemical species are:

$$\frac{\partial C_{NO}}{\partial t} = D_{NO} \nabla^2 C_{NO} - k_{auto} C_{NO}^2 + R_{NO} \quad \text{Equation 25}$$

$$\frac{\partial C_{ATP}}{\partial t} = D_{ATP} \nabla^2 C_{ATP} + R_{ATP} \quad \text{Equation 26}$$

$$\frac{\partial C_{ADP}}{\partial t} = D_{ADP} \nabla^2 C_{ADP} + R_{ADP} \quad \text{Equation 27}$$

where C_{NO} , C_{ATP} , and C_{ADP} are concentrations of NO, ATP, and ADP, and R_{NO} , R_{ATP} and R_{ADP} are the overall reaction rates for each chemical species.

3.1.6 Membrane

The governing equations require only diffusion for each chemical species within the membrane layer, with only one reaction (auto-oxidation of NO).

$$\frac{\partial C_{NO}}{\partial t} = D_{NO} \nabla^2 C_{NO} - k_{auto} C_{NO}^2 \quad \text{Equation 28}$$

$$\frac{\partial C_{ATP}}{\partial t} = D_{ATP} \nabla^2 C_{ATP} \quad \text{Equation 29}$$

$$\frac{\partial C_{ADP}}{\partial t} = D_{ADP} \nabla^2 C_{ADP} \quad \text{Equation 30}$$

3.2 Initial and Boundary Conditions

The initial and boundary conditions applied in the parallel-plate model are identical to those used by Fadel *et al.* (25) with continuity of concentrations and mass fluxes between compartments. At the inlet of the lower chamber ($z=0$), the initial and boundary concentrations for all chemical species ($C_{i,j,k} = \text{NO, ATP, ADP}$) are set to 0.

$$C_{i,j,k} \Big|_{t=0} = C_{i,j,k} \Big|_{z=0} = 0 \quad \text{Equation 31}$$

Mass fluxes at the bottom ($y=H$) and entrance ($z=0$) of the chamber are set to zero for all chemical species.

$$\frac{\partial C_{i,j,k}}{\partial y} \Big|_{y=H} = \frac{\partial C_{i,j,k}}{\partial z} \Big|_{z=0} = 0 \quad \text{Equation 32}$$

Concentrations and mass fluxes are continuous at the interface between the EC monolayer and the flowing fluid ($y = 0$),

$$C_{i,j,k} \Big|_{y=0^-} = C_{i,j,k} \Big|_{y=0^+} \quad \text{Equation 33}$$

$$D_{EC} \frac{\partial C_{i,j,k}}{\partial y} \Big|_{y=0^-} = D_{fluid} \frac{\partial C_{i,j,k}}{\partial y} \Big|_{y=0^+} \quad \text{Equation 34}$$

and at the interface between the membrane and EC monolayer ($y = -\Delta EC$).

$$C_{i,j,k} \Big|_{y=-\Delta EC^-} = C_{i,j,k} \Big|_{y=-\Delta EC^+} \quad \text{Equation 35}$$

$$D_{membrane} \frac{\partial C_{i,j,k}}{\partial y} \Big|_{y=-\Delta EC^-} = D_{EC} \frac{\partial C_{i,j,k}}{\partial y} \Big|_{y=-\Delta EC^+} \quad \text{Equation 36}$$

There is negligible mass flux at the interface between the membrane and upper chamber ($y = -(\Delta EC + \Delta membrane)$),

$$D_{membrane} \frac{\partial C_{i,j,k}}{\partial y} \Big|_{y=-(\Delta EC + \Delta membrane)} = 0 \quad \text{Equation 37}$$

since it is covered by a plastic insert.

3.3 Dimensionless Governing Equations

Defining dimensionless variables $C^* = C/C_{ref}$, $y^* = y/H$, $z^* = z/L$, $t^* = tD/H^2$, $u^* = u(y)/U$, and $R^* = R/R_{max}$, the governing partial differential equations are converted into dimensionless form

$$\frac{\partial C^*}{\partial t^*} = \frac{\partial^2 C^*}{\partial y^{*2}} + \left(\frac{H}{L}\right)^2 \frac{\partial^2 C^*}{\partial z^{*2}} - \left(\frac{UH^2}{DL}\right) u^* \frac{\partial C^*}{\partial z^*} + \left(\frac{H^2 R_{max}}{DC_{ref}}\right) R^* \quad \text{Equation 38}$$

where the dimensionless velocity profile is

$$u^* = 4y^* (1 - y^*) \quad \text{Equation 39}$$

For the NO simulations, the dimensionless auto-oxidation loss term $-k^*C^{*2}$ is added to Equation 31, where the dimensionless rate constant is

$$k^* = \frac{kH^2C_{ref}}{D} \quad \text{Equation 40}$$

4. Results

Dimensionless partial differential equations for all species in each region of the flow chamber were solved by the finite element method using commercial software (FlexPDE 4.2[®]), which employs adaptive mesh refinement to guarantee an accuracy of 0.0001. Changes in the concentrations of NO, ATP, and ADP for τ_w ranging from 0.1 to 30 dyn/cm² were computed in the membrane, EC layer, and flowing fluid in the chamber. Results described below are predicted concentrations at the midpoint ($z = 12$ mm) for NO on the membrane under the NO electrode and for ATP and ADP in the EC monolayer. As an additional check on the accuracy of the numerical solutions, both models were also programmed in Comsol 5.1[®]. Both software programs predicted virtually identical concentration values.

4.1 Dynamic Changes in NO Electrode Measurement

The dynamic NO electrode response that is predicted for the simulation using a simultaneous step change in τ_w and R_{NO} (dashed line) at $t = 0$ s in Figure 2A is much faster than observed experimentally (circles). We previously found that the rise in NO could be fit to an exponential function with average time constants of 64, 41, and 21 s for $\tau_w = 6, 10$, and 20 dyn/cm², respectively (10). Using a ramp function (Equation 2) with a 60 s period for the increase in R_{NO} causes a slower increase in NO (solid line) following the instantaneous step change in τ_w at $t = 0$ s. There is a transient decrease in NO after increasing flow, which can be attributed to convective washout of NO until R_{NO} reaches a higher value. Predicted changes in NO at the membrane under the NO electrode are shown in Figure 2B for step changes in τ_w used experimentally. The chamber model was used to determine the values for R_{basal} , R_{max} and A that provided the best fit to the steady-state experimental NO data (squares) from Andrews *et al.* (10). For the smallest step change in τ_w (0.1 to 1 dyn/cm²), the final steady-state NO is below the baseline value, due to a convective washout effect.

With larger increases in τ_w , there is a sufficient increase in R_{NO} to increase NO above baseline. The NO value at the midpoint ($z = 12$ mm) of the membrane is not the highest value, which occurs downstream just before the EC layer ends. For example, the midpoint NO = 70.4 nM for the largest $\tau_w = 20$ dyn/cm², but is higher (NO = 89.3 nM) near the downstream edge of the membrane.

The final steady-state concentrations across the membrane, EC layer, and out into the fluid flowing in the lower chamber are shown in Figure 2C, fitted to the NO measurements (squares) reported by Andrews *et al.* (10). Parameters determined for the best fit of Equation 1 (inset Figure 2C) are $R_{\text{basal}} = 0.0318$ $\mu\text{M/s}$, $R_{\text{max}} = 2.831$ $\mu\text{M/s}$, and $A = 30.76$ dyn/cm².

4.2 Dynamic Changes in ATP and ADP at EC Surface

Predicted dynamic changes in nucleotide concentrations in the EC monolayer following step changes in τ_w at $t = 1$ min for both **Model A** and **Model B** are shown for ATP in Figure 3, and for ADP in Figure 4. For **Model A**, both ATP (Figure 3A) and ADP (Figure 4A) increase rapidly after the increase in τ_w , then reach a steady state. In contrast, **Model B** predicts a much slower and smaller increase, reaching peak concentrations (open circles) at $t \sim 45$ s for both ATP (Figure 3B) and ADP (Figure 4B) following the step increase in τ_w at $t = 1$ min. The concentrations for both ATP and ADP then slowly decline, falling to $\frac{1}{2}$ of the peak (closed circles) at $t \sim 4.6$ min, or 3.6 min after the step increase in τ_w . This decline is due to the receptor desensitization included in **Model B**. In addition to the difference in time responses, another major difference between the two ATP-release models is the lower concentrations of ATP and ADP in the EC monolayer simulated for **Model B**, compared with **Model A**. In addition, Model A predicts a biphasic response to shear stress for both ATP and ADP concentrations. At the center of the EC monolayer ($z = 12$ mm) under the NO electrode, **Model A** predicts that the ATP is highest for $\tau_w \sim 15$ dyn/cm² (76.0 nM at membrane, 66.3 nM at EC surface Figure 3C) and the highest ADP for $\tau_w \sim 12$ dyn/cm² (0.817 nM at membrane, 0.705 nM at EC surface, Figure 4C). **Model A** predicts that the net production rate for ATP (R_{ATP}) reaches a plateau as τ_w increases over the range reported here (Figure 3D), whereas R_{ADP} reaches a maximum around $\tau_w = 15$ dyn/cm² and then declines (Figure 4D). Nucleotide concentrations in the EC monolayer also depend on convective transport, which increases with τ_w . As a result, at the high shear rates studied here, both ATP and ADP concentrations predicted by **Model A** gradually decline after reaching their peak values (Figures 3C and 4C). However, Model B predicts that R_{ATP} and R_{ADP} increase progressively over the entire τ_w range examined (Figures 3D and 4D). At very high τ_w (>650 dyn/cm²) R_{ATP} exceeds 500 nM/s. The biphasic response for both species predicted by **Model A** is not seen for **Model B** (Figures 3C and 4C). This observation suggests that increased nucleotide production rates at higher τ_w in **Model B** exceed the effect of depletion due to convective transport and thus both ATP and ADP concentrations in the EC monolayer continue to increase with τ_w .

Additional detail for the simulation of **Model A** with the maximum predicted ATP at the midpoint ($z = 12$ mm) for $\tau_w = 15$ dyn/cm² is shown in Figure 5A (not to scale, since $y \ll z$), with a magnified color map of ATP concentrations in the membrane and EC layer and a section of the flow chamber. The change in ATP on the EC surface from $0 < z < 24$ mm is

shown in Figure 5B, and the ATP gradient out into the flowing stream is shown at 2 locations in Figure 5C. The majority of ATP is transported downstream in a relatively thin layer of the flow stream near the EC surface. The predicted ATP at the EC surface increases progressively, reaching 76 nM at the center of the EC monolayer ($z = 12$ mm), with the highest concentration (93 nM) located farther downstream at $z = 23.8$ mm, which is about 17 nM higher than at the center. The ATP concentration along the top of the chamber ($y = 0$) then decreases to ~ 17 nM at the exit. The ATP concentration gradient into the flow chamber ($y > 0$) falls below < 1 nM at $y \sim 40$ μ m from the surface, and is negligible in the middle and outer flow regions. Qualitatively similar predictions for the spatial distribution of ADP were found. ATP and ADP concentrations exiting the chamber will be very low due to dilution as they mix into the bulk flow.

4.3 Effect of apyrase on ATP and ADP concentrations

We performed simulations to evaluate the effect of constant apyrase concentration = 1 U/mg protein in the media using different parameters (K_m , k_{cat} , Table 3) and different ATPase and ADPase activities for each enzyme isoform (Table 4). The ATP and ADP concentrations were computed across the center of the endothelial monolayer under the location on the membrane where the NO electrode is positioned, in order to model effects of ATP and ADP on R_{NO} . As expected, apyrase reduced ATP concentrations simulated for all τ_w with both models, as summarized in Table 5 and compared with ATP results without apyrase (first column). Reductions in ATP with apyrase are compared with the maximum (**Model A**) or peak ATP (**Model B**) without apyrase in Figures 6A and 6B over the τ_w range. For both models, the lowest ATP concentrations are predicted for Desiree with parameter set C, whereas Pimpernel with parameter sets A and B had the smallest effect on ATP. Changes in ADP are compared with the maximum (**Model A**) or peak ADP (**Model B**) without apyrase in Figures 6A and 6B over the τ_w range. In all cases, there is an increase in ADP above the value predicted without apyrase, with the largest increases for Desiree with parameter sets A and B. Farther downstream from the EC monolayer, the exiting ATP and ADP concentrations will be very low since there is more time for further degradation reactions catalyzed by apyrase in the media as well as dilution due to mixing into the bulk flow.

4.4 Effect of apyrase on R_{NO}

Values for R_{NO} calculated from experimental NO data measured with (red circles) and without apyrase (blue circles) in the media (23, 24) are shown in Figure 7 as a function of τ_w , using the same modeling approach previously described by Andrews *et al.* (10).

To more accurately model R_{NO} by including the effects of adenine nucleotides, we hypothesize that a component of R_{NO} at a given τ_w is directly proportional to the total nucleotide concentration (ATP + ADP), modifying Equation 1 to

$$R_{NO} = R_{basal} + R_{max} \frac{\tau_w}{\tau_w + A} + \beta [ATP + ADP]_{\tau} \quad \text{Equation 41}$$

Assuming that the τ_w -dependent parameters (R_{basal} , R_{max} , A) remain completely independent of the nucleotide concentrations, we estimated the proportionality constant β (units: μ M/s NO production per nM of nucleotide concentration) for the nucleotide

component from the difference in R_{NO} measured experimentally before (untreated) and after apyrase.

$$\beta = \left\{ R_{NO (untreated)} - R_{NO (apyrase)} \right\} / \left\{ [ATP + ADP]_{\tau (untreated)} - [ATP + ADP]_{\tau (apyrase)} \right\} \quad \text{Equation 42}$$

Simulations were performed for both **Model A** and **Model B** using parameters for each isoform (Table 4), then substituting [ATP and ADP] values from Table 5 in Equation 42 for each experimental τ_w . Estimated values for β are listed in Table 6.

Parameters (R_{basal} , R_{max} , A) listed in Table 6 for the τ_{O} -dependent component were found after removing the nucleotide component ($\beta[ATP + ADP]\tau$) from the experimental data at each τ_w , and fitting a hyperbolic function. The resulting overall curve fit to the experimental data was obtained by adding the two components. To assess which apyrase isoform provided the best match, the minimum square root of the mean sum of squared errors (rmse) was calculated for each simulation (Table 6).

Very good matches were obtained with **Model B** for all of the Desiree isoforms. Simulations for Desiree with parameter set B are shown for **Model A** (Figure 7A) and for Desiree with parameter set A for **Model B** (Figure 7B). The nucleotide components are shown at the bottom of Figures 7A and 7B (solid curves, closed squares for untreated, open squares for apyrase), along with the τ_{O} -dependent component (green dashed curves). In general, the match for the simulation with **Model B** was closer than determined with **Model A** for all 3 parameter sets for the Desiree isoform. Both of the Pimperal isoforms provided the poorest match for **Model A**.

4.5 Sensitivity Analysis

To examine the sensitivity of the models to parameters and critical dimensions, we performed additional simulations with $\pm 5\%$ variation in the individual values, holding all other model parameters or variables constant. Relative changes in the predicted maximum concentrations at the center location ($z = 12 \text{ mm}$) on the membrane are shown in Figure 8 for all three species for simulations conducted with $\tau_w = 10 \text{ dyn/cm}^2$. For the NO simulation, a 5% increase in the thickness of the EC layer (ℓ_{EC}) had the largest effect ($\sim 5.3\%$), followed by an increase in the chamber height ($\sim 5.1\%$) and R_{max} ($\sim 5.0\%$). A 5% increase in τ_w had a small effect ($\sim 1.9\%$), while increasing the size of the Transwell® membrane (L) by 5% had the smallest effect ($\sim 1.6\%$). Increasing the diffusion coefficient by 5% causes a modest decrease ($\sim 3.1\%$).

Similar sensitivity trends were found for the two models of ATP release. **Model A** was more sensitive to τ_w (Figure 8B, $\sim 2.9\%$ for ATP, $\sim 1.8\%$ for ADP) than **Model B** (Figure 8C, $\sim 1.7\%$ for ATP, $\sim 0.6\%$ for ADP). The maximum values for ADP were almost two times more sensitive to the reaction rate (R_{ADP}), thickness of the EC monolayer (ℓ_{EC}), height of the chamber (H), and diffusion coefficients (D_{ATP} , D_{ADP}) than the ATP parameter sensitivity. Increasing the diffusion coefficient causes a modest decrease in ATP ($\sim 3.3\%$ for both **Model A** and **Model B**) but a larger decrease in ADP (-5.7% for **Model A**, -5.6% for **Model B**). For both **Model A** and **Model B**, increasing the length (L) of the Transwell® membrane by 5% causes a slightly larger increase in ADP ($\sim 2.6\%$ for both models) than for

ATP (~1.5% for **Model A**, ~1.4% for **Model B**). Since the ADP levels are low, they have little effect on R_{NO} .

For the best fit of **Model A** (Figure 7A), varying the estimated parameters by $\pm 5\%$ increases the rmse by ~0.2% for R_{basal} , by 14.2 to 16.8% for R_{max} , by 11.4 to 12.0% for A , and by 3.2 to 28.0% for β . Similarly, for the best fit of **Model B** (Figure 7B), the parameter variations increase rmse by ~0.2% for R_{basal} , 6.5 to 22% for R_{max} , 8.7 to 11.7% for A , and 3.2 to 3.8% for β . The fit is most sensitive to R_{max} , then A , then β , and least sensitive to R_{basal} .

5.1 Discussion

Andrews *et al.* (24) propose that the mechanism for τ_w -induced NO production is in part due to the release of ATP in nM-to- μ M concentrations, with subsequent activation of purinergic receptors in the plasma membrane and changes in intracellular Ca^{2+} . To our knowledge, Andrews *et al.* (24) are the first to report on the role of capacitive Ca^{2+} entry (CCE) in the mechanism of the acute τ_w -induced NO response. ATP stimulates a Ca^{2+} response through both the release from endoplasmic reticulum (ER) stores as well as influx through store-operated channels (SOCs). Studies of purinergic receptors with human umbilical vein ECs (HUVEC) found an EC_{50} for ATP around 3.05 μ M for peak Ca^{2+} responses (59). Our predicted responses for both ATP release models would be in the lower 2% of the Ca^{2+} response curve reported for HUVECs. Raqeeb *et al.* (59) also found an increase in R_{NO} with 2 μ M ATP under static conditions (no shear) based on an approximately 27% increase in DAF fluorescence. Our previous experimental data (10) demonstrates much larger τ_w -dependent changes in R_{NO} . More experimental data on changes in R_{NO} with ATP is needed to develop a more quantitative relationship. Also, experimental data are currently lacking for changes in R_{NO} with ADP.

In addition to the studies showing reduced R_{NO} with apyrase, Andrews *et al.* (24) also conducted a study blocking purinergic receptors with suramin (200 μ M for 10 min), which attenuated the τ_w -dependent NO response by 60% following a step change in τ_w from 0.1 to 10 dyn/cm² compared with the response for untreated ECs. Andrews *et al.* (24) also inhibited SOCs using SKF-96365, which caused a 40% reduction in NO responses to $\tau = 10$ dyn/cm². When apyrase was added to the medium, the degree of R_{NO} inhibition was greater at lower τ_w , which can be explained by the diffusion limited reaction of apyrase with ATP. Also, there are higher rates of ATP release at higher shear stresses. Suramin, which would not be diffusion limited, showed greater attenuation of the NO response at 10 dyn/cm² than seen with apyrase.

A reduction in EC NO production following exposure to apyrase has also been reported by Kiefmann *et al.* (60) from *in vivo* studies in rat lung. They showed that lung expansion augments Ca^{2+} oscillations and increased ATP release from the pulmonary vasculature. Microinjections of ATP directly into lung alveoli caused localized Ca^{2+} oscillations. Intra-alveolar infusion of apyrase (50 U/ml) completely blocked cytosolic Ca^{2+} oscillations and significantly attenuated EC NO production (evaluated by DAF fluorescence). Increased NO production with ATP was also seen when the lung alveoli were infused with Ca^{2+} -free

solution, which the authors interpreted as evidence that there was no role for CCE. A quantitative relationship between ATP and R_{NO} is not possible from these observations.

Phosphorylation of eNOS was also measured by Andrews *et al.* (23, 24), demonstrating that Ca^{2+} from extracellular sources is required. Under static conditions, ATP stimulation in the presence of Ca^{2+} caused a rapid increase in phosphorylation that peaked at 3 min followed by a sustained elevation through 10 min. For experiments with flow under control conditions (no apyrase), there was a statistically significant increase in eNOS phosphorylation in ECs exposed to τ_w for 3 minutes, but not for ECs exposed for a shorter time (1 min). Flow caused a 20% increase in eNOS phosphorylation for control ECs while degradation of endogenous ATP with apyrase showed a 15% decrease in eNOS phosphorylation after 3 min of τ_w . However, the mechanisms by which ATP influences R_{NO} are not well understood. ATP has been demonstrated to increase eNOS phosphorylation at the Ser¹¹⁷⁷ site (19). ADP appears to also trigger an increase in eNOS phosphorylation at Ser¹¹⁷⁷ and Ser⁶³⁵ as well as dephosphorylation at Ser¹¹⁶ (21). In cultured fetal bovine aortic ECs, extracellular ATP in the media during laminar flow was found to significantly increase NO-dependent signaling (5). However, this may not necessarily be due to τ_w -dependent R_{NO} , but may involve another activation pathway (22).

Other mechanisms that increase R_{NO} have been identified (61, 62), although they may not necessarily be independent of adenine nucleotides. Kumar *et al.* (63) report that protein kinase C- δ (PKC δ) and catalase are two previously unidentified molecules that are important in regulating τ_w -mediated NO signaling in pulmonary artery ECs. They found that acute increases in τ_w stimulate NO generation through the inhibition of PKC δ signaling. The decrease in PKC δ in turn leads to a decrease in phosphorylation of eNOS at Thr⁴⁹⁵ and an increase in eNOS phosphorylation at Ser¹¹⁷⁷. There is evidence that phosphorylation at Ser⁶³³ increases NO production (62), but this can occur without the need for an increase in intracellular Ca^{2+} (64, 65), as would occur with increased adenine nucleotide concentrations.

A number of studies suggest that red blood cells (rbc) release ATP, especially in response to hypoxic or hypercapnic conditions *in vivo* (66, 67), and may therefore play a role in modulating NO and blood flow. This mechanism is controversial, since a recent study suggests that the source of ATP and ADP from rbc is due to hemolysis and release of internal contents (68). However, another study using rbc incubated with hydroxyurea, the FDA approved therapy for sickle cell disease, contends that ATP release from rbc is due to mechanisms other than hemolysis (69). This study reports that rbc incubated with hydroxyurea had an over two-fold greater release rate for ATP compared with normal rbc, and caused a 1.34 times higher rate of NO production from cultured ECs, compared with measurements using normal rbc. The actual concentrations of ATP (determined by luciferin–luciferase assay) and NO (determined by DAF fluorescence) in this study are not known, so it is not possible to obtain a quantitative relationship between ATP and R_{NO} . Hemolysis appears to account for significant measurement errors in determining human plasma ATP (70), since rbc normally contain high concentrations of ATP (mM levels). Gorman *et al.* (70) report than normal ATP levels in human blood plasma are 28 nM, much lower than the ~ 1 μ M levels (or higher) that are reported in other studies (see their Table 1). Our simulation predicts that ATP levels in the endothelial layer are < 100 nM, and not in the

μM range. Similar ATP concentrations ($< 150 \text{ nM}$) at the endothelial surface have been predicted from simulations using realistic blood vessel geometries by Comerford *et al.* (36) and by Boileau *et al.* (71), based on the John and Barakat (34) model (**Model A**) for ATP release, for which R_{ATP} saturates $\sim \tau_w = 35 \text{ dyn/cm}^2$. Although **Model B** has not been investigated, higher ATP values would be generated for simulations at high τ_w , which can occur with stenosed blood vessels. However, it may not be valid to extrapolate **Model B** for high τ_w conditions since the model is based on an analysis of data obtained at a lower τ_w range.

It should be recognized that we have focused only on the endogenous production of ATP by ECs to model effects on NO production in a simple, *in vitro* system. More complicated modeling would be necessary for *in vivo* models of blood vessels, especially for dynamic models that include changing diameters and τ_w with vascular regulation by NO. The release of ATP by sympathetic nerves as a co-transmitter with noradrenaline, or other vasoactive substances produced by ECs, or the interaction of the adenine nucleotides with their receptors would also need to be considered (18). Effects of pulsatile and steady-state flow on nucleotide transport has been modeled in realistic geometries for branching arteries (72, 73) although the τ_w -dependent production of adenine nucleotides was neglected. A model incorporating eNOS has been developed that identifies the anatomical locations where τ_w is low and NO may be deficient, especially in the vascular wall at bifurcations and bends (36). These regions were also predicted to have lower values for ATP and ADP at the EC surface. However, the eNOS activation model by Comerford *et al.* (36) lacked NO data to validate their model by comparing simulations with experimental results. While the parallel-plate flow chamber may have limitations, it remains a useful experimental tool for studying different mechanisms for τ_w -dependent R_{NO} .

5.2 Model Limitations

We used a 2-dimensional model with simplified relationships to model the increase in R_{NO} and adenine nucleotides with τ_w in this study. A more complicated, 3-dimensional model could be developed to more accurately represent the circular geometry of the membrane and EC monolayer, which would then include possible effects of concentration gradients for all 3 chemical species due to edge effects and production from a smaller volume of ECs farther from the midpoint. We anticipate that this effect will be negligible due to the large distance from the center to the circular edge. Another refinement in the modeling would be to include possible depletion of NO by the electrochemical sensor at the measurement site. This is an especially critical issue for *in vivo* measurements of NO in tissue, as investigated by Bohlen (74), who demonstrates that significant depletion of NO around the tip can occur for electrochemical sensors with diameters of $30 \mu\text{m}$ or greater. However, this may not be a problem if the sensor has a sufficiently thick membrane that limits diffusion.

More elaborate modeling that incorporates purinergic receptor-mediated Ca^{2+} dynamics would require further development. Koo *et al.* (11) developed a much more complex, integrated model for R_{NO} incorporating major mechanisms of τ_w -dependent eNOS activation, including transcriptional regulation by key proteins, eNOS phosphorylation by key kinases, and interactions with protein partners (*e.g.*, caveolin, calmodulin, heat shock

proteins). They computed a relative increase in cumulative R_{NO} , as well as relative contributions from each mechanism. However, it would be necessary to couple this model with geometry-specific mass transport to quantify the concentration of NO in a biological system. They did not model the effects of adenine nucleotides.

Although O_2 is required by eNOS to produce NO, O_2 transport was not included in our model, since Fadel *et al.* (25) previously demonstrated that O_2 consumption by ECs in their model did not significantly influence the spatial distribution of NO in the chamber. However, O_2 transport is an important consideration, especially for *in vivo* models, where the nonlinear oxyhemoglobin reaction must be used and the O_2 -dependence of eNOS should not be neglected. The viscosity of blood also requires more complex modeling to represent variations in τ_w throughout a network of blood vessels. We recognize that our estimated values for R_{NO} are lower than some other literature values estimated from *in vivo* studies or with excised blood vessels. However, they are almost one order of magnitude higher than the $<0.1 \mu M/s$ range predicted from a theoretical model by Chen *et al.* (75), who compared their model predictions with literature values (their Table 3). Rates as high as $68 \mu M/s$ have been estimated from a mass transport model analysis by Vaughn *et al.* (76) using experimental data reported by Malinski *et al.* (77). A possible explanation for differences reported for *in vitro* and *in vivo* R_{NO} may be lower eNOS expression in cultured ECs, especially if they are not cultured under continuous shear.

5.3 Conclusions

The two τ_w -dependent adenine nucleotide release models investigated in this study have differences in their predictions for R_{NO} sensitivity to adenine nucleotides. Using either **Model A** or **Model B**, our simulations suggest that the effect of increasing (ATP + ADP) on R_{NO} is greater in the low τ_w range, compared with effects of nucleotides at higher τ_w . For **Model A**, there are progressively smaller increases in the rates of ATP and ADP release at low τ_w , until total nucleotide concentration reaches a peak around $\tau_w = 15 \text{ dyn/cm}^2$, then declines with higher τ_w , as expected from the τ_w -dependent changes shown previously for ATP (Figure 3c) and ADP (Figure 4C). Although predicted (ATP + ADP) values are lower for **Model B** and do not reach a peak value in the τ_w range investigated, the rate of increase in total nucleotide concentration becomes progressively smaller as τ_w increases. Along with progressively smaller increases in the rates of ATP and ADP release, convective effects in the chamber also help to maintain relatively constant ATP and ADP at higher τ_w , and causes a small reduction in (ATP + ADP) for **Model A** with higher flow rates. For either **Model A** or **Model B** in the absence of apyrase, the slopes for $R_{NO, \text{nucleotide}}$ (Figures 7A and 7B) are much greater at low τ_w ($< 6 \text{ dyn/cm}^2$) compared with slopes for $\tau_w > 10 \text{ dyn/cm}^2$. The effect of altering adenine nucleotide concentrations with apyrase significantly reduces $R_{NO, \text{nucleotide}}$, but this relationship remains more sensitive in the low $\tau_w < 6 \text{ dyn/cm}^2$ range. The mass transport model predictions demonstrate a greater effect with apyrase at lower τ_w , consistent with our experimental studies.

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Highlights of: Mathematical Model for Shear Stress Dependent NO and Adenine Nucleotide Production from Endothelial Cells

- Our mass transport model for a flow chamber predicts changes in endothelial cell NO, ATP, and ADP with shear stress (τ).
- A fraction of τ -dependent NO production (R_{NO}) is linearly proportional to (ATP + ADP).
- R_{NO} sensitivity to nucleotide concentration is greater at low τ .
- Model predictions are consistent with experimental studies.

Figure 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.

Figure 2.

(A) Comparison of normalized experimental NO measurement (circles) for τ_w from 0.1 to 20 dyn/cm² with dynamic simulation using a step increase in R_{NO} (dashed line) or a 60 s duration ramp increase in R_{NO} (solid line). (B) Dynamic changes in the NO electrode measurement predicted for different step changes in wall shear rate (τ_w) by increasing the rate of fluid flow at $t = 200$ s (baseline $\tau_w = 0.1$ dyn/cm²) and steady-state experimental values from Andrews *et al.* (10) (denoted by squares). (C) Steady-state NO concentration across the membrane and EC layer, and into the flowing fluid in the lower chamber obtained after fitting experimental data (squares) measured at different τ_w (10). Inset: hyperbolic curve fit for increase in R_{NO} (circles) with τ_w determined from experimental data.

Figure 3.

Dynamic change in ATP concentration predicted by **Model A**(A) and predicted by **Model B** (B) for different step changes in τ_w above baseline of 0.1 dyn/cm^2 . (C) Comparison of maximum ATP reached above baseline in the endothelial layer between the membrane (upper curves, solid lines) and the endothelium/fluid interface (lower curves, dashed lines) for both models as a function of the final τ_w . (D) Increase in the net ATP production rate (R_{ATP}) with τ_w for both models.

Figure 4.

Dynamic change in ADP concentration predicted by **Model A**(A) and predicted by **Model B**(B) for different step changes in τ_w above baseline of 0.1 dyn/cm². (C) Comparison of maximum ADP reached above baseline in the endothelial layer between the membrane (upper curves, solid lines) and the endothelium/fluid interface (lower curves, dashed lines) for both models as a function of the final τ_w . (D) Increase in the net ADP production rate (R_{ADP}) with τ_w for both models.

Figure 5.

(A) Color map of spatial ATP concentration distribution in the flow chamber predicted by **Model A** for $\tau_w = 15 \text{ dyn/cm}^2$. (B) ATP concentration along the $y = 0$ axis, including the surface of the EC monolayer from proximal to distal edge ($0 < z < 24 \text{ mm}$), with circles indicating values at the center ($z = 12 \text{ mm}$) and the highest predicted value at $z = 23.6 \text{ mm}$, near the downstream edge of the EC layer. (C) ATP gradients out into the flowing fluid at the midpoint and near the end of the EC monolayer.

Figure 6.

Effects of apyrase isoforms for 5 different parameter sets (Table 4) on **(A)** maximum ATP and ADP predicted for **Model A**, and **(B)** peak ATP and ADP predicted for **Model B**. Values in Table 5 for each τ_w are indicated by circles.

Figure 7.

A comparison between experimental results and model predictions. Experimental data determined from NO measurements for parallel-plate flow experiments by Andrews *et al.* (23, 24) are shown by blue circles (no apyrase in media) and red circles (with apyrase in media 1 U/mg protein). **(A)** Predicted effects of apyrase isoform Desiree with parameter set B (Table 4) on the $R_{NO,nucleotide}$ components (solid curves; untreated, solid squares; apyrase, open squares) and the τ_w -dependent component (green dashed line). The resulting match of equation 41 to untreated data (blue curve) and apyrase data (red curve) using **Model A**, and **(B)** using **Model B** for apyrase isoform Desiree with parameter set A.

Figure 8.

Sensitivity analysis for (A) NO model, (B) **Model A**, and (C) **Model B** for ATP release and ADP production. Relative changes in predicted values for concentrations at the center location are shown for $\pm 5\%$ variations in parameters or dimensions, holding all other parameters and dimensions constant.

Table 1

Definitions and values for parallel-plate chamber model parameters.

Symbol	Value	Units	Definition
C_{ADP}	0 to 1	nM	Concentration of ADP
C_{ATP}	0 to 100	nM	Concentration of ATP
D_{ADP}	257	$\mu\text{m}^2 \text{s}^{-1}$	Diffusion coefficient for ADP
D_{ATP}	236	$\mu\text{m}^2 \text{s}^{-1}$	Diffusion coefficient for ATP
D_{NO}	3300	$\mu\text{m}^2 \text{s}^{-1}$	Diffusion coefficient for NO
K_{ADP}	155	μM	Michaelis constant for ADP
K_{ATP}	475	μM	Michaelis constant for ATP
k_{auto}	2×10^{-8}	$\text{nM}^{-1} \text{s}^{-1}$	Pseudo-second order reaction rate for auto-oxidation of NO
NO	0 to 100	nM	Concentration of nitric oxide
μ	0.00696	dyn s cm^{-2}	Dynamic viscosity of water at 37° C
$V_{\text{max,ATP}}$	0.1×10^{-6}	$\text{mol m}^{-2} \text{s}^{-1}$	Maximum enzyme reaction velocity for ATP hydrolysis
$V_{\text{max,ADP}}$	0.8×10^{-6}	$\text{mol m}^{-2} \text{s}^{-1}$	Maximum enzyme reaction velocity for degradation of ADP
S_{max}	1×10^{-9}	$\text{mol m}^{-2} \text{s}^{-1}$	Maximum ATP flux from ECs

Table 2

Definitions and parameter values for ATP production by endothelial cells (Eqns. 4–9) from Qin *et al.* (37) and Li *et al.* (44).

Symbol	Value	Units	Definition
a_1	0.65×10^{-13}	$\text{mol m}^{-2} \text{s}^{-1}$	Dynamic model flux parameter
a_2	2.79×10^{-10}	$\text{mol m}^{-2} \text{s}^{-1}$	Dynamic model flux parameter
a_3	69.6	dyn/cm^2	Dynamic model flux parameter
t_1	17.4	s	Time delay constant
t_2	218.9	s	Time delay constant

Table 3

Reaction constants (Eqns. 13, 14) for the rate of conversion of adenine nucleotides for 2 different isoforms of apyrase at 37°C.

Isoform	Substrate	K_m (mM)	k_{cat} (s^{-1})
Pimperal	ATP	0.172	920.74
	ADP	0.264	105
Desiree	ATP	0.0635	2202.5
	ADP	0.31	2076.3

Table 4

Parameters for 5 simulations using the two isoforms of apyrase and corresponding concentrations of ATPase and ADPase within the range of specific activities indicated by the manufacturer (Sigma Aldrich).

Isoform	Parameter set	ATPase activity (U/mg protein)	[ATPase] (mol/m ³)	[ADPase] (mol/m ³)
Pimpernel	A	40	5.10×10^{-4}	5.10×10^{-3}
Pimpernel	B	30	1.02×10^{-3}	1.02×10^{-2}
Desiree	A	40	5.10×10^{-4}	5.10×10^{-4}
Desiree	B	20	1.02×10^{-3}	1.02×10^{-3}
Desiree	C	3	6.80×10^{-3}	6.80×10^{-3}

Table 5

Predicted maximum (**Model A**) or peak (**Model B**) ATP and ADP concentrations in the EC layer with and without apyrase for step changes in τ_w used experimentally.

Model A	No apyrase		Pimperial			
			Parameter set A		Parameter set B	
τ_w	ATP (nM)	ADP (nM)	ATP (nM)	ADP (nM)	ATP (nM)	ADP (nM)
0.1 to 1	2.13	0.039	0.58	0.223	0.45	0.167
0.1 to 6	49.33	0.648	22.31	8.01	17.17	6.32
0.1 to 10	69.70	0.817	36.41	12.06	28.18	10.22
0.1 to 20	71.88	0.720	44.90	12.39	35.48	12.06

	Desiree					
	Parameter set A		Parameter set B		Parameter set C	
τ_w	ATP (nM)	ADP (nM)	ATP (nM)	ADP (nM)	ATP (nM)	ADP (nM)
0.1 to 1	0.30	0.229	0.25	0.171	0.17	0.074
0.1 to 6	11.61	8.71	9.61	6.47	6.60	2.80
0.1 to 10	19.00	14.19	15.69	10.60	10.89	4.55
0.1 to 20	24.00	17.29	19.85	13.35	13.80	5.75

Model B	No apyrase		Pimperial			
			Parameter set A		Parameter set B	
τ_w	ATP (nM)	ADP (nM)	ATP (nM)	ADP (nM)	ATP (nM)	ADP (nM)
0.1 to 1	5.35	0.098	3.06	0.586	2.73	0.454
0.1 to 6	19.29	0.247	8.77	3.16	6.76	2.50
0.1 to 10	26.32	0.309	13.77	4.60	10.67	3.89
0.1 to 20	38.10	0.380	23.77	6.57	18.77	6.40

	Desiree					
	Parameter set A		Parameter set B		Parameter set C	
τ_w	ATP (nM)	ADP (nM)	ATP (nM)	ADP (nM)	ATP (nM)	ADP (nM)
0.1 to 1	2.38	0.608	2.25	0.464	2.32	0.226
0.1 to 6	4.57	3.45	3.78	2.57	2.82	1.11
0.1 to 10	7.19	5.40	5.94	4.04	4.12	1.73
0.1 to 20	12.70	9.18	10.50	7.09	7.31	3.05

Table 6

Estimated parameters for Equation 41 from simulations for **Model A** and **Model B** with each apyrase isoform (Table 4) with calculated minimum square root of the mean sum of squared errors (rmse) for the match to experimental data.

Apyrase isoform	Parameters (Equation 41)				rmse (μM)	
Model A	β ($\mu\text{M/s/nM}$)	R_{basal} ($\mu\text{M/s}$)	R_{max} ($\mu\text{M/s}$)	A (dyn/cm^2)	Untreated	Apyrase
Desiree A	0.01021	0.1166	30.66	364.1	0.0930	0.0669
Desiree B	0.00849	0.1070	10.69	103.6	0.0919	0.0663
Desiree C	0.00666	0.0969	7.659	61.81	0.0901	0.0668
Pimperal A	0.01773	0.0608	6.87×10^6	1.33×10^9	0.1643	0.0692
Pimperal B	0.01200	0.1113	1.87×10^7	2.56×10^8	0.1010	0.3376
Model B						
Desiree A	0.01845	0.0560	4.550	37.66	0.0733	0.0602
Desiree B	0.01531	0.0590	4.840	36.32	0.0748	0.0615
Desiree C	0.01196	0.0625	5.199	35.61	0.0770	0.0633
Pimperal A	0.02174	0.0717	3.662	28.37	0.1044	0.0924
Pimperal B	0.03211	0.0440	4.149	64.75	0.0694	0.0594