Regulation of adenine nucleotide concentration at endothelium-fluid interface by viscous shear flow

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ABSTRACT The action of adenine nucleotides on vascular endothelial cells is apparently mediated by the local flow conditions. Because nucleotides are sequentially degraded from ATP → ADP → AMP → adenosine by ecto-enzymes at the endothelial surface, it has been hypothesized that the observed flow effect is caused by the flow-dependent change of nucleotide concentration at the cell surface. In this study, we have calculated the concentration profiles of adenine nucleotides at the cell surface under flow conditions encountered in an in vitro parallel-plate flow system, as has been used in several related experimental studies. When medium containing uniformly distributed ATP is perfused over endothelial monolayers, our results show that ATP concentration in the cell vicinity gradually decreases in the streamwise direction as a result of enzymatic degradation. This hydrolysis of ATP results in the generation of ADP, and ADP concentration in turn gradually increases at the cell surface. The concentration profiles of nucleotides are dependent on the levels of applied wall shear rate. As the corresponding shear stress increases from 0.1 to 30 dynes/cm², ATP concentration at the cell surface at the center of cover-slip increases from 0.66 to 0.93. Under no-flow conditions, our model predicts a steady decline of ATP concentration and a transient increase of ATP-derived ADP, comparable to the published results of previous experiments. These numerical results, combined with our recent experimental data, provide insights into the cellular mechanisms by which hemodynamic flow modulates the effects of vasoactive agents on endothelium.

INTRODUCTION Extracellular adenine nucleotides are important modulators of vascular tone and platelet functions. Nucleotides ATP and ADP can cause vasodilation, by acting at P2-purinoceptors in endothelial cells to induce release of endothelium-derived relaxing factor (EDRF) which relaxes subjacent smooth muscle cells (Burnstock and Kennedy, 1985; Furchgott, 1984). Recent work has shown that these effects of ATP and ADP on endothelial cells are mediated by the hydrolysis of phosphatidylinositol 4,5-bisphosphate and increase in cytosolic free Ca²⁺ ([Ca²⁺]i) (Pirrotton et al., 1987). In vivo, adenine nucleotides may be presented in blood as a result of secretion from activated platelets, or leakage from damaged cells within the vessel wall (Gordon, 1986). To regulate the extent and time course of nucleotide action, endothelial cells possess three separate ectonucleotidases at the luminal surface which sequentially hydrolyze ATP → ADP → AMP → adenosine. (Pearson et al., 1980; Olsson and Pearson, 1990).

Vascular endothelial cells in vivo are constantly exposed to blood flow. Previous experiments have demonstrated that hemodynamic shear stress has a profound influence on endothelial structure and functions. In vitro, fluid shear stress stimulates rapid production of prostacyclin (PGI₂) (Grabowski et al., 1985; Frangos et al., 1985), release of EDRF (Rubanyi et al., 1986), a transient increase in cytosolic free calcium concentration ([Ca²⁺]i) (Shen et al., 1992), and alteration of gene expression (Diamond et al., 1989). Chronic exposure to shear stress results in a change of endothelial cell shape and a rearrangement of cytoskeletal structure (Dewey et al., 1981; Franke et al., 1984; Levesque and Nerem, 1985). Moreover, shear stress appears to enhance the receptor-mediated binding, internalization, and degradation of low-density lipoprotein by endothelial cells (Sprague et al., 1987).

Recently, several groups including ourselves have reported that the action of adenine nucleotide ATP and ADP on endothelial cells can be modulated significantly by fluid flow conditions around endothelial monolayers (Dull and Davies, 1991; Mo et al., 1991; Shen et al., 1992). In our experiments, we have monitored the intracellular Ca²⁺ responses in fura-2-loaded endothelial cells during perfusion with ATP or ADP under defined flow conditions using a parallel-plate flow chamber, and observed that the endothelial [Ca²⁺]i responses to ATP (or ADP) are dramatically enhanced with increasing applied shear rate. Because the degradation of nucleotides by endothelium could result in a shear-dependent change of nucleotide concentration in the cell surface, it has been suggested that flow modulation of endothelial response to nucleotides may be caused in part by the concentration effect.

The purpose of this study is to examine the influence of fluid flow in regulating nucleotide concentration in the vicinity of endothelial monolayers. We have developed a mathematical model for the transport of nucleotides to endothelium under flow conditions encountered in an in vitro parallel-plate system, as has been used in numerous related experimental studies. The calculated results indicate that the nucleotide concentration at the endothelial surface changes slightly with shear stress over...
must balance its degradation by endothelial cells. Assuming the kinetic characteristics of endothelial ectonucleotidases follow an irreversible Michaelis–Menten expression (Southerland), the boundary condition can be written as

$$D \frac{\partial c}{\partial y} = \frac{V_{\text{max}} c}{K_m + c} \quad \text{at} \quad y = 0,$$

where $V_{\text{max}}$ is the maximum enzyme reaction velocity and $K_m$ is the Michaelis constant for the enzyme. At low nucleotide concentration ($c < K_m$), Eq. 3 can be simplified to the following linear form:

$$D \frac{\partial c}{\partial y} = \frac{V_{\text{max}} c}{K_m}.$$

By introducing the following nondimensionalized variables:

$$x^* = \frac{x}{h}, \quad y^* = \frac{y}{h}, \quad t^* = \frac{t}{T},$$

$$c^* = \frac{c}{c_0}, \quad Pe = \frac{\nu h}{D},$$

$$Da = \frac{h V_{\text{max}}}{D K_m}, \quad St = \frac{h}{Tv},$$

Eq. 2 becomes

$$St \frac{\partial c^*}{\partial t^*} + 6 y^* (1 - y^*) \frac{\partial c^*}{\partial x^*} = \frac{1}{Pe} \frac{\partial^2 c^*}{\partial y^* 2}$$

with the nondimensionalized boundary conditions

$$\frac{\partial c^*}{\partial y^*} = 0, \quad \text{at} \quad y^* = 1,$$

$$\frac{\partial c^*}{\partial y^*} = \frac{1}{Da} c^*, \quad \text{at} \quad y^* = 0,$$

$$c^* = 1, \quad \text{at} \quad x^* = 0,$$

where $c_0$ is the original concentration of nucleotide; $T$ is the characteristic time, which is primarily determined by the diffusion process of chemical species and is given by $h^2/D$; $Pe$ is the Peclet number, representing the ratio of mass transport by convection to mass transport by diffusion; $Da$ is the Damkohler number, representing the ratio of reaction velocity to diffusion velocity; and $St$ is the Strouhal number. The solution of the above equations gives the concentration profiles of the original nucleotide substrate in the channel.

The hydrolysis of ATP results in generation and accumulation of ADP. This ATP-derived ADP is in turn catalyzed to AMP and finally to adenosine. ADP is about equally (or more) active as ATP to endothelial cells (e.g., in promoting $[Ca^{2+}]_i$ responses and EDRF release) (Pittotton et al. 1987; Olsson and Pearson, 1990), while AMP and adenosine are much less effective. The governing equation for calculating ADP concentration is the
same as Eq. 2 except for the coefficients. However, the boundary conditions (nondimensionalized) now become

\[
\frac{\partial c_T^*}{\partial y^*} = 0 \quad \text{at} \quad y^* = 1,
\]

\[
\frac{\partial c_D^*}{\partial y^*} = -\left( \frac{D_T}{D_D} \right) \frac{\partial c_D^*}{\partial y^*} + \frac{1}{Da_D} \cdot c_D^* \quad \text{at} \quad y^* = 0,
\]

\[
c_D^* = 0 \quad \text{at} \quad x^* = 0, \quad (6)
\]

where \(c_T\) and \(D_T\) are the concentration and diffusion coefficient for ATP, respectively; and \(c_D\) and \(D_D\) are those for ADP; \(Da_D\) is the Damkohler number for ecto-ADPase. The first term on the right-hand side of Eq. 6 represents generation of ADP, which is equal to the amount of ATP hydrolysis, and the second term represents degradation of ADP by ecto-ADPase.

Several groups have previously investigated the kinetic characteristics of endothelial ectonucleotidases of cultured cells from a variety of animal species (Pearson et al., 1980; Cusack et al., 1983; Coade and Pearson, 1989). The data used in our numerical model are cited from the paper of Cusack et al. (1983) on the basis of pig aortic endothelial cells, as listed in Table 1. In addition, the diffusion coefficient of nucleotides used in our calculation is based on the data given by Adams and Feuerstein (1983), and Folie and McIntire (1989). However, the kinetic data reported by Cusack et al. may be diffusion limited because the measurement was probably performed under static conditions. To address this potential limitation, we have examined the sensitivity of our numerical solutions to the enzymatic parameters. The results at three different reaction rates are presented in the next section.

The above equations were solved numerically. The computation code developed in this study employed the linear finite element in conjunction with the linear Euler backward difference technique. The following solutions are given in the next section: (1) steady-state concentration profiles of the original substrates (ATP and ADP) in the presence of flow; (2) steady-state concentration profiles for the intermediate product ADP; and (3) time-course of nucleotide degradation under no-flow conditions.

### RESULTS

The longitudinal delivery by flow and hydrolysis of nucleotide substrate ATP by endothelial ecto-ATPase at endothelial cell surface results in the streamwise development of a diffusion boundary layer with reduced ATP concentration in the vicinity of the cell monolayer. As the boundary layer grows with the streamwise distance, the nucleotide concentration at the cell surface gradually decreases. Fig. 2A shows the steady-state ATP concentration profiles at the cell surface as function of the nondimensionalized distance (\(x^*\)) away from the point where ATP encounters endothelium under three different flow rates (corresponding to wall shear stress of 0.1, 1.0, and 10 dynes/cm²). (A) Concentration distribution at the endothelial cell surface as functions of \(x^*\). (B) Concentration distribution in the medium at the center of coverslip (\(x^* = 50\)) as functions of \(y^*\).
10.0 remains almost unchanged (\(=1.0\)) with shear stress, indicating that only a very small amount of ATP has been converted to AMP and adenosine. The kinetic characteristics of endothelial ectonucleotidases vary among different animal species. In addition, the original measurements of ectonucleotidases of endothelial cells appeared to be performed under static conditions, and the efficiency of nucleotidases could have been underestimated because of diffusion limitation. Thus, we have estimated how nucleotide concentration profiles vary depending on the efficiency of ectonucleotidases, which is fully represented by the Damkohler number \(Da\) in our model. Fig. 5 (A) and (B) show the concentration of nucleotides at the cell surface in the middle of coverslip (\(x^* = 50\)) as functions of shear stress when Damkohler numbers for both ATP and ADP are 5 or 10 times greater than the normal number. At high Damkohler numbers, ATP concentration is significantly reduced at all shear stress levels over the entire range examined and varies near logarithmically with shear stress. In addition, we see that most of the hydrolyzed ATP remains as ADP in the channel and the total amount of ATP and ADP varies with shear stress in a similar fashion as ATP.

We have next numerically simulated the time-course of ATP catabolism under no-flow conditions (in static medium). Both the bulk phase and cell surface concentration of ATP and ATP-derived ADP is plotted as a function of time (Fig. 6). The bulk ATP concentration steadily declined with time, with a half-life of \(\sim 4\) min. The ATP concentration at the cell surface is lower than the bulk concentration over the entire time-course. The concentrations (corresponding shear stress: 0.1, 1.0, and 10 dynes/cm\(^2\)). At a shear stress of 0.1 dynes/cm\(^2\), ATP concentration drops from 1.0 to 0.59 across the full length of the monolayer (2.5 cm). As the shear stress increases, the longitudinal delivery of ATP overcomes the hydrolysis of ATP by endothelial cells and the concentration decrease becomes less significant (Fig. 2 A). Fig. 2 B shows the ATP concentration profiles above the center of the monolayer (\(x = 1.25\) cm) as a function of the perpendicular distance (\(y^*\)). As expected, the concentration gradually decreases as the cell surface is approached.

The hydrolysis of original substrate ATP results in generation and accumulation of ADP. ADP is nearly as effective as ATP in stimulating release of EDRF and increase in [Ca\(^{2+}\)] in most endothelial cells (Pirotton et al., 1987). Therefore, we have estimated the concentration profiles of ATP-derived ADP by solving Eq. 2 in conjunction with the boundary condition (Eq. 6). The concentration profiles of ATP-derived ADP, along with ATP and total addition of ATP and ADP at the cell surface, as a function of \(x^*\) for three given shear rates, are shown in Fig. 3. As is clear from the figure, most hydrolyzed ATP remains as ADP in the channel over the shear stress range examined.

Fig. 4 shows the concentration of ATP, ADP, and addition of ATP and ADP at the endothelial cell surface at the center of coverslip (\(x = 1.25\) cm) as a function of the applied shear stress over a range of 0.1–30 dynes/cm\(^2\). As shear stress increases from 0.1–30 dynes/cm\(^2\), ATP concentration gradually increases from 0.66–0.93, while the concentration of ATP-derived ADP decreases from 0.28–0.06. The total concentration of ATP and ADP remains almost unchanged (\(=1.0\)) with shear stress, indicating that only a very small amount of ATP has been converted to AMP and adenosine.
hydrolysis of ATP results in a transient accumulation of ADP, which reaches the maximum (0.4) in about 6 min, followed by a steady decline of ADP concentration as a result of its hydrolysis by ecto-ADPase. These numerical results are in a good agreement with the experimental data previously reported (Cusack et al., 1983).

The concentration profiles of ADP at the cell surface under various flow conditions were also computed when ADP is used as the original substrate (Fig. 7). The data show that the degradation of ADP is less significant than that of ATP under same flow conditions. The hydrolysis of ADP results in generation of AMP which does not cause $[Ca^{2+}]_{i}$ response in endothelium (Pirotton et al., 1987).

**DISCUSSION**

In the previous experimental studies (Shen et al., 1992), we have demonstrated that the action of nucleotides on endothelium can be significantly modulated by fluid flow. The aim of this study was to determine the capacity of endothelial ectonucleotidases to regulate the concentration distribution of adenine nucleotides in the vicinity of the flow-endothelium interface. When the medium containing nucleotide ATP is perfused over endothelial monolayers in a parallel-plate flow chamber, our computational results indicate that its concentration at the cell surface gradually decreases with the streamwise distance. This decrease in ATP concentration is dependent on the shear rate induced by the perfusion flow. In our model system, as the corresponding shear stress increases from 0.1–30 dynes/cm² (Shen et al., 1992), the ATP concentration at the center of the coverslip (1.25 cm from the starting point where medium encounters cells) is increased by approximately 30%.

The major pathway of nucleotide catabolism has been previously shown to be sequential hydrolysis from ATP → ADP → AMP → adenosine by three separate ectoenzymes (Cusack et al., 1983). Because ADP is about as effective as ATP in causing several responses in endothelial cells, including release of EDRF, production of PGI₂, and increases in $[Ca^{2+}]_{i}$ (Pirotton et al., 1987), we have calculated the concentration profile of ADP, the intermediate product of the catabolism reaction. Our results (Fig. 4A) indicate that ADP is the dominant prod-
FIGURE 7 ADP concentration profiles at the cell surface when medium containing original substrate ADP is perfused through the channel at three different flow rates (corresponding to wall shear stress of 0.1, 1.0, and 10 dynes/cm²).

The results presented in this paper are the steady-state nucleotide concentration profiles. After the initial application of shear stress, the characteristic time to reach the steady-state profile is primarily determined by the diffusion process and can be estimated using the following formula:

$$\tau \approx \frac{\delta^2}{D}$$

where $\delta$ is the boundary layer thickness, and $D$ is the diffusion coefficient of ATP. On the basis of Fig. 2 B, the boundary layer thickness is approximately 0.1–0.5h in the shear stress range of 0.1–30 dynes/cm², and the characteristic time was estimated between 3–66 seconds. This transient time is significantly less than the time period during which shear stress was applied in most of our experimental studies. Therefore, the steady-state results are the most relevant to the conditions encountered in the in vitro experiments reported previously (Shen et al., 1992).

To verify our mathematical model, we have numerically simulated the time-course of ATP degradation in static medium. Our results show a steady decline of ATP, with a half-life about 4 min, and a transient accumulation of ATP-derived ADP in the medium, reaching the maximum concentration in about 6 min, followed by a decline as a result of its hydrolysis by ecto-ADPase. These numerical results are essentially the same as those obtained previously by experiments (Cusack et al., 1983), indicating that our model can correctly predict the basic patterns of nucleotide catabolism by endothelial cells.

The mathematical model developed in this study is based on previously published data of the kinetic characteristics of ectonucleotidases in cultured pig aortic endothelial cells. Because the kinetic parameters could vary among the cells from different animal species, we have investigated how nucleotide concentration profiles change, depending on the efficiency of nucleotidases. At large Damkohler numbers (corresponding to high efficiency of enzymatic degradation), although ATP concentration becomes low over the entire shear stress range examined, the concentration does not change significantly as shear stress varies. At the cell surface, ATP concentration increases roughly logarithmically with shear stress.

Modulation of the effects of adenine nucleotides on endothelial cells by fluid flow has been demonstrated in recent experiments (Dull and Davies, 1991; Mo et al., 1991; Shen et al., 1992). In a previous experimental study carried out in the apparatus modeled in this report, we have measured intracellular $\text{Ca}^{2+}$ responses of bovine aortic endothelial cells (BAEC) during perfusion with medium containing ATP or ADP (0.1–1.0 μM) under defined flow conditions, and observed that perfusion with ATP or ADP evokes repetitive oscillations in $[\text{Ca}^{2+}]_i$ in single BAEC. The frequency of these oscillations increases with level of applied fluid shear stress. Comparison of the experimental data (Shen, J., M. A. Gimbrone, Jr., F. W. Luscinskas, and C. F. Dewey, Jr., unpublished observations) to the numerical results suggests that a change of concentration due to degradation of nucleotides alone cannot account for the observed change of $[\text{Ca}^{2+}]_i$ response induced by applied fluid flow. From our experimental results, the frequency of $[\text{Ca}^{2+}]_i$ oscillations increases nearly linearly with shear stress, while the concentration of ATP and ADP at the
cell surface changes much less significantly with shear stress, as shown in Fig. 4. This conclusion is consistent with the direct observation that shear-dependent $[Ca^{2+}]$, responses can be induced with nonhydrolyzable nucleotide analogues ATPγS and ADPβS (Shen et al., 1992).

Fluid flow can cause a variety of responses in vascular endothelium when ATP-containing medium (Medium 199, 1.8 μM) is used as perfusion fluid in the experiments (Ando et al., 1988; Frangos et al., 1985; Nollert et al., 1991). As recently reported by Nollert et al. (1991), these responses also appear not to be fully accounted for by the shear-dependent ATP transport model alone. However, it is unclear whether the observed responses are caused by the direct mechanical shear stress effect or a complex interplay between vasoactive agonist and hemodynamic flow. On the basis of our recent studies on $[Ca^{2+}]$, (Shen et al., 1992), it appears that endothelial cells respond to fluid flow very differently, depending on whether ATP is present in the medium. In the absence of agonist, an increase in shear stress stimulates a transient, relatively low amplitude increase in $[Ca^{2+}]$, while, in the presence of ATP, application of steady flow evokes large repetitive oscillations in $[Ca^{2+}]$.

Recently, Nollert and McIntire (1992) also have investigated this problem and have found a similar change of ATP concentration as a function of shear stress. However, in the interpretation of their data, they apparently did not consider the generation of the intermediate product ADP, which is almost equally effective in endothelial cells as ATP. In addition, no experimental results show the responses of endothelial cells under flow conditions with multiple levels of shear stresses to compare with the theoretical prediction. These differences may contribute to the discrepancy in the conclusions of their report, compared with ours. On the basis of our theoretical and experimental results, it appears that the change of nucleotide concentration due to enzymatic degradation at the endothelial cell surface is not the primary mechanism for the observed flow modulation of nucleotide action on endothelium, although it may contribute to this phenomenon. On the other hand, it is also possible that the modulation may be caused by a direct influence of flow on the interaction between ATP (or ADP) with P2-purinoceptors. To date, no direct evidence has been obtained as indicating the influence of flow on endothelial P2-receptors. However, a previous study by Sprague et al. (1987) has demonstrated that laminar fluid shear stress can alter the binding, internalization, and degradation of low-density lipoproteins (LDL) by endothelial cells, indicating that shear stress can modulate endothelial surface receptor expression and function.

Whatever the mechanism responsible, flow modulation of the effects of adenine nucleotides on vascular endothelial cells may have important physiological implications. Nucleotides may be presented at the vessel wall–blood interface due to release from endothelial cells, smooth muscle cells, and stimulated platelets under various pathophysiologial conditions (Gordon, 1986; Olsson and Pearson, 1990). Earlier studies have shown that ATP stimulates the release of EDRF and PG12 from endothelial cells which, in turn, can induce vasodilation (Burnstock and Kennedy, 1985). On the basis of our observations, the ability of ATP to promote EDRF and PG12 release is likely to be influenced and enhanced by local hemodynamic flow. This effect may be particularly important at low concentrations of ATP relevant to physiological conditions in vivo, and could be involved in the flow-induced regulation of vascular tone. Although the computational results presented in this paper are limited to the flow conditions encountered with exogenous ATP in an in vitro apparatus, the analysis can be extended to in vivo conditions if one takes into account the possibility of alternative sources (local versus systemic) for the ATP.

To conclude, we have calculated the concentration profiles of adenine nucleotides and estimated the effects of viscous shear flow in regulating the concentration at the endothelium–fluid interface. On the basis of enzymatic characteristics of endothelium reported previously (Cusack et al., 1983), our results demonstrate that nucleotide ATP and ADP concentration in the vicinity of endothelial monolayer is not significantly different from bulk concentration and only changes slightly with increasing shear stress. Even at assumed large Damkohler numbers (corresponding to increasing ectonucleotidase efficiency), nucleotide concentration does not vary significantly with shear stress. Together with other experimental results, it appears that the transport model cannot fully account for the observed flow modulation of endothelial responses to nucleotides, although it may partially contribute to this phenomenon.

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REFERENCES


