Mathematical model for shear-induced cytosolic calcium oscillations: the role of (ca$^{2+}$)$_i$ in cytoskeleton organisations

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Abstract

The inherent role of [Ca$^{2+}$]$_i$ on the shear-induced polymerization of cytoskeleton was proposed by the flow chamber experiments and the mathematical modeling of cytosolic calcium oscillations to access the correlation of signal transduction and the mechanotransduction of endothelial cells. From the results of flow chamber experiments of endothelial cells adhered on the fibronectin, the opening characteristic of membrane-bound calcium channel was assumed to biphasic function to the exposure time of shear stress. With this modification, the model for IP$_3$-induced calcium oscillation shows a good agreement with the waveforms of shear-induced cytosolic calcium oscillations measured with digital fluorescence imaging of fura-2-loaded endothelial cells. This result shows the polymerization of cytoskeleton may closely correlate with the periodic formation of IP$_3$ and cytosolic calcium oscillations by a shear stress.

1 Introduction

Shear stress is one of the agonist to increase in the concentration of the cytosolic calcium ([Ca$^{2+}$]$_i$) of vascular endothelial cells, and this shear-induced calcium transient seems to serve a role in the response of endothelial cell to flow [1, 2]. The detail mechanism of shear-induced calcium transient is not known yet, but the importance of increased [Ca$^{2+}$]$_i$ in the mechanotransduction of endothelial cell should be
emphasized for the potent role of Ca\(^{2+}\) in the intracellular signal transduction.

There are two sources of agonist-induced increases in \([\text{Ca}^{2+}]_i\). These are the endoplasmic reticulum (sarcoplasmic reticulum) and the extracellular fluid. Both excitable and inexcitable cells utilize calcium sequestered in cytoplasmic storage compartments for signaling, but excitable cells (e.g. nerve and muscle) rely on a “calcium-induced calcium release” mechanism [3, 4] while, for inexcitable cells (e.g., epithelial or endothelial cells) the predominant mechanism for release is triggered by a diffusible messenger, inositol 1,4,5-triphosphate (IP\(_3\)) [5, 6].

In this paper, the inherent role of \([\text{Ca}^{2+}]_i\) on the shear-induced polymerization of cytoskeleton was proposed by the flow chamber experiments and the mathematical modeling of cytosolic calcium oscillations to access the correlation of signal transduction and the mechanotransduction of endothelial cells.

2 Materials and Methods

**Flow Chamber and Experimental Setup**

The flow chamber and image analysis system were developed to investigate the role of \([\text{Ca}^{2+}]_i\) of endothelial cells, which satisfy following requirements, i) accurate temperature control of circulating culture medium to 37 °C, ii) pH control of circulating culture medium to 7.4, iii) shear stress of physiological levels which driven by the hydrostatic pressure head to eliminate the pulsatility of the recirculating roller pump, and iv) convenient sterilization of whole system to prevent the contamination of cells during flow exposure. Figure 1 shows the schematic side view of laminar flow chamber and Figure 2 shows the block diagram of experimental setups.

Shear stress with physiological range (20 dyne/cm\(^2\)) was applied on the apical membrane of endothelial cells cultured on the 5 \(\mu\)g/cm\(^2\) fibronectin-coated glass during the flow experiments for 1-2 hours. Endothelial cells were incubated with 0.1 \(\mu\)g/ml cytochalasin D (Sigma Chemical Co., MO) for 1 hour before the experiments to elucidate the initial role of \([\text{Ca}^{2+}]_i\) in the cytoskeletal organization.

**Calcium-related Drug Treatments**

The calcium chelating culture medium containing ethylene glycol-bis(\(\beta\)-amino-ethyl ether) N,N,N',N'-tetraacetic acid (EGTA) and cytosolic calcium antagonist, 8-diethylamino-octyl-3,4,5-trimethoxybenzoate hydrochloride (TMB-8), and histamine were used for clarifying the role of external calcium and cytosolic calcium from the internal calcium pools to the mechanotransduction of endothelial cells.
To study the role of extracellular calcium concentration $[\text{Ca}^{2+}]_0$ calcium-free culture medium containing 10 mM EGTA (Sigma Chemical Co., MO) was used for a perfusate of flow experiments. TMB-8 (100 μM, Molecular Probe, Inc., OR) was used as an inhibitor of agonist-induced mobilization of calcium from the putative intracellular calcium pools by the inhibition of the release of Ca$^{2+}$ from the endoplasmic reticulum and sarcoplasmic reticulum to investigate the role of intracellular calcium mobilization [7]. In addition, 100 μM histamine (Sigma Chemical Co., MO) was added to the culture medium to increase the calcium influx of endothelial cell from the extracellular space and decreases actin-gelsolin bindings by changes in polyphosphoinositides. Figure 3 shows the schematic illustrations of drug actions in calcium signaling motifs and drug actions.

Mathematical Modeling of Cytosolic Calcium Oscillations

The basic consideration of the model was shown in figure 3, which includes the activation pathway for the IP$_3$ production as well as the individual contributions for changes in the intracellular calcium oscillations. The four differential equations are proposed based on the model of Eichwald and Kaiser [8].

\[
\frac{d\Gamma}{dt} = V_T \frac{\beta^i}{K_H^i + \beta^i} \frac{1-\Gamma}{K_T + 1-\Gamma} - \kappa_T \Gamma \quad (1)
\]

\[
\frac{dQ}{dt} = \sigma \Phi(\Gamma, Z) - \kappa_Q Q \quad (2)
\]

\[
\frac{dY}{dt} = v_2(Z) - v_3(Q, Y) - k_F Y \quad (3)
\]

\[
\frac{dZ}{dt} = -v_2(Z) + v_3(Q, Y) + k_F Y + v_0 + \alpha(t)v_1(Q) - kZ \quad (4)
\]

The variables are the fraction of activated G-protein, the intracellular concentrations of IP$_3$ and calcium and the concentration of calcium in an intracellular calcium store, abbreviated as $\Gamma$, $Q$, $Z$, and $Y$. The opening characteristics of membrane calcium channel to fluid shear stress were incorporated to Equation (4) as $\alpha(t)$.

3 Results

To verify the role of $[\text{Ca}^{2+}]_i$ in the mechanotransduction of endothelial cell with shear stress, calcium chelator, EGTA, and cytosolic calcium antagonist, TMB-8, were used for the experiments on the shear-induced actin microfilament polymerization of endothelial cells. The cytoskeletal and morphological recovery of endothelial cells with calcium-related drug treatment were expressed in Figure 4 as the dimensionless area.
When endothelial cells were exposed to the dynamic environment without extracellular calcium, morphology of cells was changes to the round form and cellular spreading area was diminished to 70% of original ones. Endothelial cells, when exposed to the fluid shear with EGTA-containing culture medium, were loose the activation of adhesion receptors and focal adhesions and no filamentous form of actin bundle was detected in the cytoplasm of endothelial cells. No directionality of cell to the direction of flow was detected in the endothelial cell with the flow of EGTA-containing culture medium. For the cytochalasin D-treated endothelial cell, the absence of extracellular calcium plays a more potent role in cell adhesions than normal endothelial cell case. Cytochalasin D-treated endothelial cell's adhesion was maintained only by the focal adhesion at the front ends of microspikes and these small adhesion sites cannot preserve the drag resistance of the total cell mass driven by the fluid flow. Thus, the extracellular calcium is very important to cell adhesion and spreading, especially the activation and the maintenance of integrin receptor and focal contact formation.

The calcium stored in the internal calcium store plays an important role in the recognition of shear stress, which was closed correlated with the cytosolic calcium oscillations. The efflux of the calcium from the internal calcium store was stimulated by the increased IP$_3$ production with the shear stress [9]. Endothelial cells, in the absence of extracellular calcium, show the biphasic decrease in cellular spreading area. For the first 15 minutes, the decrease of cellular area was not so large, but, after 15 minutes, profound decrease was evoked in the endothelial cell with exposed to the flow of the culture medium containing 10 mM EGTA. Another proof of the role of calcium in the internal calcium store was the delayed increasing pattern of cellular area in the cytochalasin D and TMB-8 treated endothelial cells. A slight increase of cellular area was detected for first 15 minutes of exposure of shear stress with the culture medium containing 100 μM TMB-8. The lack of the aligned actin microfilament to the direction of flow also indicates the importance of internal calcium on the shear-induced actin polymerization of endothelial cells.

Interestingly, no bust of cellular area during first 15 minutes was measured in cytochalasin D and histamine treated endothelial cells. The rapid recovery of cellular area was detected after 20 minutes of exposure with 100 μM histamine. It means that the extracellular calcium is needed for cellular adhesion and maintaining the mechanotransduction of endothelial cell, but the calcium in the internal calcium store controls the initial perception of shear stress on the mechanotransduction of endothelial cells. This assumption was reconfirmed with shear stress.
experiments of cytochalasin D and TMB-8 treated endothelial cell with the culture medium containing 100 µM histamine. The recovery of cellular area during first 15 minutes was depressed with the treatment of cytosolic calcium antagonist TMB-8. By summarizing the above findings, the opening characteristics of membrane-bound calcium channel, \( \alpha(t) \) in equation (4), was assumed to biphasic function to the exposure time of shear stress.

In conclusion, the model shows a good agreement with the waveforms of shear-induced cytosolic calcium oscillations measured with digital fluorescence imaging of fura-2-loaded human umbilical vein endothelial cells. This result shows the polymerization of cytoskeleton may closely correlate with the periodic formation of IP\(_3\) and cytosolic calcium oscillations by a shear stress.

5 References


Figure 1. Schematic diagram of laminar flow chamber

Figure 2. Experimental setups

Figure 3. Calcium signaling motifs and drug actions
Figure 4. Cytoskeletal Recovery of endothelial cells with calcium-related drug treatment