PKC and Calcium as Regulators of Shear-Stress Induced Nitric Oxide Production: A Compartmental Model

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Introduction: The production of nitric oxide (NO) by vascular endothelial cells in response to shear stress is a key regulator of blood vessel diameter and angiogenesis. Store-operated calcium entry (SOCE) is a key intermediate in the shear stress-induced NO production pathway. Endothelial nitric oxide synthase (eNOS) activity is regulated by Ca^{2+} and the phosphorylation by kinases at several different residues. Accumulating experimental evidence suggests two roles for PKC in eNOS regulation: an inhibitory influence at the Thr495 residue in the Ca^{2+}/Calmodulin binding site as well as a stimulatory influence at Ser1177. Notwithstanding, the manner in which PKC and Ca^{2+} interact to produce an increase in NO production in response to flow is poorly understood. Elucidation of the mechanisms of eNOS regulation may shed light on endothelial dysfunction, which is marked by reduced NO production and is a key cause of atherosclerotic diseases. As such, the purpose of this study, which is an element of the multiscale research effort of our group to understand the mechanisms of NO production and transport in the microcirculation, is to develop a cell-scale compartmental model to investigate the regulatory role of PKC and Ca^{2+} on caveolar eNOS in response to flow.

Materials and Methods: Shear-induced intracellular Ca^{2+} dynamics were modeled by a system of ordinary differential equations describing the exchange of Ca^{2+}, PKC and second messengers between four compartments. The compartments, which allow for signal segregation at low computational cost, are: (1) the endoplasmic reticulum calcium store, (2) the bulk cytosol, (3) the peri-caveolar microdomain and (4) the extracellular space. SOCE was represented as an increased flux through caveolar Ca^{2+} channels as they interact with an ER-derived diffusible second messenger (termed the calcium influx factor, CIF). We investigated the modulatory effects on eNOS activity and NO production of Ca^{2+} as well as Ca^{2+}-dependent and independent PKC isozymes (cPKC & nPKC respectively).
Parameter values were drawn from the literature where possible or were otherwise adapted (within physiological ranges when they were known) by optimization to account for the experimental results. Simulations were run from the same initial condition as in the experiments, which was usually the physiological equilibrium. Model simulations and parameter estimation were performed using MATLAB Version 8.1; Mathworks, Nattick, MA.

Results and Discussion: The model successfully reproduces the transient NO dynamics observed experimentally in response to flow. We took the sequential activation of PKC by cytosolic Ca^{2+}, its translocation to the plasma membrane and subsequent activation by DAG to be determinants of temporal and spatial cPKC dynamics. nPKC dynamics were largely governed by the concentration of DAG in the microdomain. Our simulations could reproduce translocation of PKC in response to flow and by optimizing model parameters to experimental data, we were able to quantify the contribution of PKC to NO production.The NO concentrations achieved when simulating phosphorylation by cPKC as opposed to nPKC showed some difference in kinetics but require experimental validation.
This model is more physiological than previous descriptions of shear-induced NO production and so is more dependent on experimental data to verify to what extent different PKC isotypes contribute to eNOS phosphorylation at the different eNOS residues. Further investigation is required to establish how eNOS outside caveolae contributes to this pathway and how this fits into an otherwise highly compartmentalized signaling pathway.

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