Piezo1-Nitric Oxide Signaling in a Population-based Model of Arterial Myocytes in Acute Hyperglycemia

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Abstract

Acute hyperglycemia (HG) plays a key role in the development of arterial complications and upregulation of Piezo1. Piezo1 appears to mediate shear stress-induced activation of cytosolic endothelial nitric oxide synthase (NO), through incompletely elucidated pathways. Here, we hypothesize that Piezo1 contributes to the organ-specific detrimental effects of HG on arterial myocytes vasoreactivity and excitability by activating NO signaling. We integrated our in silico model of Piezo1 channel into a model of rat arterial myocytes and introduced Piezo1-NO signaling consistent with in vitro data. We explored the influence of individual ionic currents and transporters on global cytosolic $[Ca^{2+}]_i$ (Cai) and membrane potential (Em) in a sensitivity analysis on 1000 virtual cells. Our results suggest the intensified contribution of L-type Ca^{2+} channel (LTCC) on Cai in HG. Similarly, the impact of large conductance Ca^{2+}-activated K^+ channel (BKCa) on Em and Cai increased in HG, in accord with reported BKCa channel activity and sensitivity to Ca^{2+} in vitro. Remarkably, Piezo1 perturbations instigated opposing effects on Em and Cai in control vs. HG-induced conditions, implying a possible role of Piezo1-NO signaling in the heterogeneity of organ-specific vasoactive response to HG in arterial myocytes and thus a promising therapeutic avenue.

1. Introduction

Transient increases in blood glucose levels are a predictor of mortality in diabetic patients [1]. Chronic hyperglycemia is associated with endothelial dysfunction and reduced nitric oxide (NO) bioavailability. Conversely, acute hyperglycemia (HG) paradoxically elicits endothelium-dependent vasodilation in specific vascular beds. Mechanosensitive Piezo1 channels, implicated in cardiovascular mechanotransduction and arterial remodeling processes in hypertension, are expressed in endothelial cells and are essential for maintaining normal arterial blood pressure [2]. These channels mediate shear stress-induced endothelial NO synthase (eNOS) activation through calcium-dependent pathways. Notably, hyperglycemia upregulates Piezo1 expression and intracellular calcium in microglia, suggesting a potential analogous mechanism in endothelial cells. Piezo1 contribution to hyperglycemia-induced arterial vasoreactivity through eNOS activation has been reported previously [2]. Interestingly, the vascular beds of various organs exhibit distinct responses to acute hyperglycemia, demonstrating both enhanced and diminished endothelial function [2]. We aimed to investigate the possible role of Piezo1-NO signaling in the heterogenous, e.g. organ-specific, detrimental effects of HG on arterial myocytes vasoreactivity and excitability by activating NO signaling.

2. Methods

We integrated our recent *in silico* model of Piezo1 channel [3] into a mathematical model of HG-induced arterial myocytes [4], [5], as shown in Figure 1. Piezo 1 model has a stretch-sensitive activation and voltage-dependent inactivation detailed in [3].

To simulate Piezo1-NO signaling in HG-induced simulations, we set:

$$[NO] = Piezo1\ Ca^{2+}\ flux \times c \qquad (1)$$

Where coefficient c was calculated so that the model yields ~2 fold increase in $[Ca^{2+}]_i$, in agreement with *in vitro* data [6]. We set 10 mM and 20 mM of glucose concentrations for WT and HG, respectively.

We explored the influence of individual ionic currents and transporters on global cytosolic Ca^{2+} concentration (Cai) and membrane potential (Em) in a sensitivity analysis leveraging a well-established population-based approach.

We simulated a virtual population of 1000 model variants. These variants were generated by introducing random perturbations to the conductances or maximal transport rates of all ion channels and transporters within the model. Specifically, each parameter in the baseline model was independently varied using a log-normal distribution with a standard deviation of 0.1, allowing for

changes between -30% and +50% of the original value [5]. For each model variant, the resulting values of Em and Cai were recorded. Subsequently, a non-linear iterative partial least squares regression analysis was performed on the log-transformed values to identify correlations between the variations in individual parameters and their effects on both Cai and Em. All simulations were done in MATLAB. We used ode15s integrator, and the steady-state results were obtained at the end of a 480 second timespan.

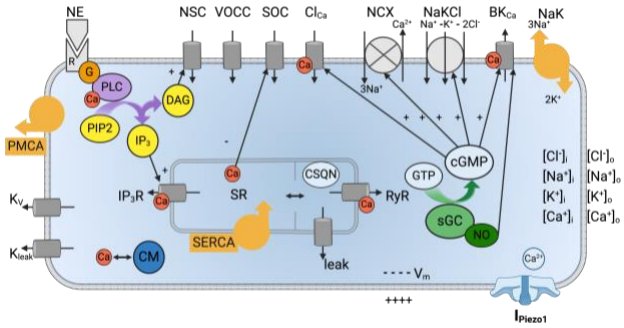

Figure 1. The schematic of the *in silico* arterial myocyte model components (created with Biorender.com) [4]. BKCa: large conductance calcium-activated potassium channel; Kleak: leak potassium channel; Kv: voltage-gated potassium channel; ClCa: calcium-activated chloride channel; NSC: non-selective cation channel; SOC: store-operated calcium-permeable non-selective cation channel; VOCC: L-type voltage-operated calcium channel; NaK: sodium-potassium ATPase; PMCA: plasma membrane calcium ATPase; NaKCl: sodium-potassium-chloride cotransporter; NCX: sodium-calcium exchanger; SR: sarcoplasmic reticulum; IP3R: IP3 receptor; RyR: ryanodine receptor; SERCA: sarco/endoplasmic reticulum calcium ATPase pump; CSQN: calsequestrin; CM: calmodulin; R: α1-adrenoceptor; G: G protein; PLC: phospholipase C; sGC: soluble guanylate cyclase; cGMP: cyclic guanosine monophosphate; NO: nitric oxide.

3. Results

Our results suggest the intensified contribution of L-type Ca^{2+} channel (LTCC) on Cai in HG (Figure 2A), indicating aggravation in the vasoconstriction mechanism. Similarly, the impact of large conductance Ca^{2+}-activated K^+ channel (BKCa) on Em and Cai increased in HG (Figure 2B), in accord with reported BKCa channel activity and sensitivity to Ca^{2+} in rat coronary artery smooth muscle cells [7]. The predominant role of Kv2.1, in comparison with BKCa, in the Em depolarization in control (WT) is consistent with the previous analysis [5]. However, the model predicts a reversal behavior in HG-induced condition, i.e., BKCa taking an upper hand (Figure 2A).

We also learned that Em is intensely influenced by the activity of mechanosensitive channel Piezo1, promoting depolarization in control condition and strongly repolarization in HG. Of note, Piezo1 perturbations elicited contrasting effects on Em and Cai in control vs HG-induced conditions (Figure 2C&D).

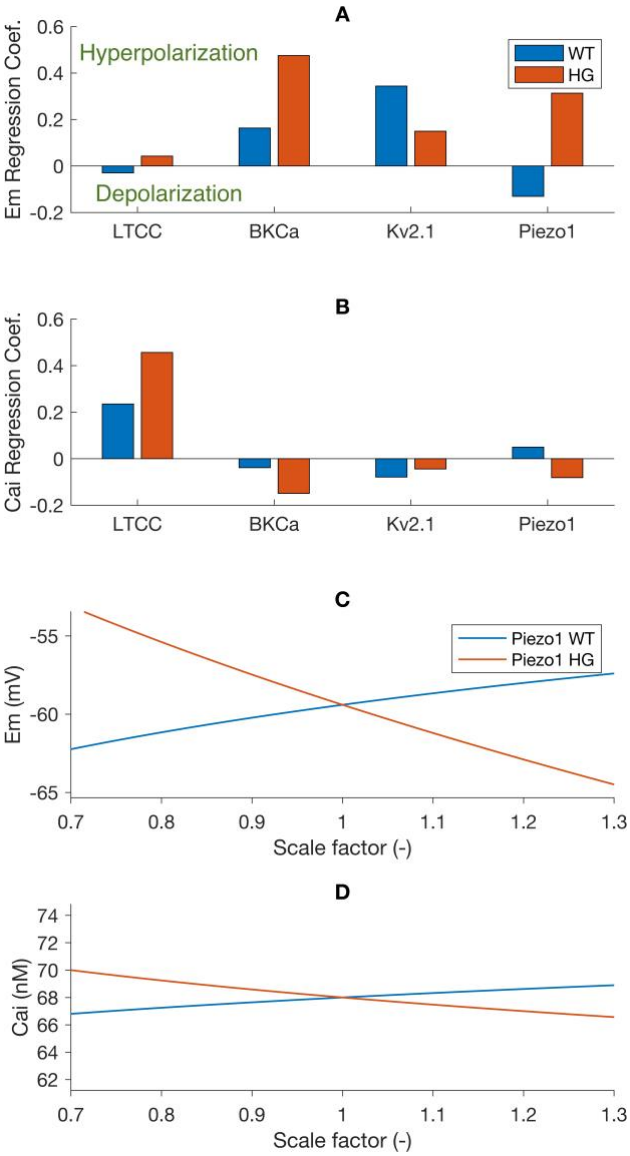

Figure 2. Model response to perturbations in the sensitivity analysis targets. Bar graphs illustrate the regression coefficients quantifying the influence of conductance alterations in L-type calcium (LTCC), BKCa, and Kv2.1 potassium channels on intracellular calcium concentration (Cai) (A) and membrane potential (Em) (B). Panels C and D illustrate the isolated impact of modulating a single conductance by ±30% on the respective outputs as predicted by the regression model.

4. Discussion

Acute hyperglycemia induces a complex interplay of signaling pathways within vascular smooth muscle cells. While the precise mechanisms are still under investigation, emerging evidence suggests that BKCa channels might be involved in the hyperglycemic response [8]. BKCa channels play a critical role in regulating vascular tone by repolarizing the smooth muscle cell membrane and limiting calcium influx. Under normal conditions, BKCa channels contribute to vasodilation and maintain vascular homeostasis [8]. Our work contributes to the exploration of BKCa involvement in HG by suggesting exacerbation of Cai and Em alterations due to BKCa perturbations. Understanding of this effect may be refined through modeling the role of reactive oxygen species in BKCa associated pathways and performing sensitivity tests that consider interaction between BKCa and LTCC in HG.

A prior study demonstrated that acute hyperglycemia significantly elevates Cai in cerebral arterial myocytes and constricts cerebral arteries derived from wild-type (WT) mice [6]. These effects were contingent on augmented LTCC activity, as confirmed in our simulations (Figure 2B).

The impact of acute or short-term hyperglycemia on myogenic tone exhibits significant variability across species, vascular beds, and glucose concentrations [8]. The predicted HG-induced pathological vasodilation mediated by Piezo1 and potential of its inhibition as a therapeutic strategy (Figure 2B) are consistent with *in vitro* results obtained from mouse mesenteric arteries in HG [2]. Remarkably, Piezo1 perturbations instigated opposing effects on Em and Cai in control vs. HG-induced conditions (Figure 2C&D). This would imply a possible role of Piezo1-NO signaling in the heterogeneity of organ-specific vasoactive response to HG in arterial myocytes and thus a promising therapeutic avenue.

The present *in silico* framework can benefit from integration of a contractile element encompassing Calcium-Calmodulin kinetics, and a force generation mechanism. This can further help elucidation of Piezo1-NO-CaMKII (Ca^{2+}/Calmodulin-dependent protein kinase type II) pathway. Furthermore, while the influence of sex on these glucose-induced vascular responses in smooth muscle has been understudied, predominantly male experimental models highlight the need for further investigation incorporating sex as a biological variable [8]. Finally, our mathematical model enables quantification of HG-induced effects communicated by cGMP as a downstream factor in Piezo1-NO signaling which could be an interesting future research direction.

Demonstrating the power of computational population-based statistical simulations, our model enables quantification of contribution of major currents, including Piezo1, to vasoreactivity in HG. Our results offer insights upon the underlying factors at play in the heterogenic response to HG while corroborating the impact of therapeutic strategies based on mechanotransduction.

Acknowledgments

AF and NA were supported by SMASH-HCM EU Horizon project grant.

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