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Predominant contribution of L-type Cav1.2 channel stimulation to impaired intracellular calcium and cerebral artery vasoconstriction in diabetic hyperglycemia

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ABSTRACT

Enhanced L-type Ca^{2+} channel (LTCC) activity in arterial myocytes contributes to vascular dysfunction during diabetes. Modulation of LTCC activity under hyperglycemic conditions could result from membrane potential-dependent and independent mechanisms. We have demonstrated that elevations in extracellular glucose (HG), similar to hyperglycemic conditions during diabetes, stimulate LTCC activity through phosphorylation of $Ca_V1.2$ at serine 1928. Prior studies have also shown that HG can suppress the activity of K^+ channels in arterial myocytes, which may contribute to vasoconstriction via membrane depolarization. Here, we used a mathematical model of membrane and Ca^{2+} dynamics in arterial myocytes to predict the relative roles of LTCC and K^+ channel activity in modulating global Ca^{2+} in response to HG. Our data revealed that abolishing LTCC potentiation normalizes $[Ca^{2+}]_i$, despite the concomitant reduction in K^+ currents in response to HG. These results suggest that LTCC stimulation may be the primary mechanism underlying vasoconstriction during hyperglycemia.

Introduction

Diabetes is a devastating disease that affects millions worldwide, and is a major risk factor for stroke, hypertension, heart disease, and other pathological conditions.[1,2] Hyperactivity of L-type Ca^{2+} channels (LTCCs) in arterial myocytes of small resistance vessels contributes to vascular complications during diabetes.[3-7] This is in part attributed to the effects of hyperglycemia (elevated blood glucose). We recently gained novel mechanistic insights into this phenomenon and demonstrated that stimulation of vascular LTCCs in response to elevated glucose (HG) is dependent upon phosphorylation of the LTCC pore forming subunit $Ca_V1.2$ at serine 1928 (S1928) via anchoring of the cAMP-dependent protein kinase (PKA) by the scaffolding protein AKAP150.[7] Glucose-mediated potentiation of LTCCs led to a significant increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) and constriction in cells and arteries from wild-type (WT) mice.

Conversely, in arterial myocytes and arteries from knock-in mice expressing a $Ca_V1.2$ with S1928 mutated to alanine (S1928A) to prevent phosphorylation at this site,[8] glucose-mediated changes in LTCC activity, $[Ca^{2+}]_i$ and excessive vasoconstriction were prevented.[7]

An increase in extracellular glucose also impacts the function of other ion channels in arterial myocytes. Indeed, acute and chronic exposure to HG has been shown to suppress the expression and/or activity of K^+ channels in coronary,[9] mesenteric,[10] and cerebral arterial myocytes.[11-13] K^+ channels are key regulators of arterial myocyte excitability and vascular reactivity due to their influence on the membrane potential (E_m).[14] Inhibition of K^+ channel function depolarizes arterial myocyte E_m, which in turn increases LTCC activity and Ca^{2+} influx, leading to global raises in $[Ca^{2+}]_i$ that induces arterial myocyte contraction and vasoconstriction. Thus, it is conceivable that reduced

K^+ channel activity contributes to enhanced myogenic tone in the presence of HG. However, observations of HG-induced K^+ channel suppression contrast with our finding that HG failed to evoke changes in $[Ca^{2+}]_i$ in arterial myocytes or vasoconstriction in arteries from S1928A mice.[7] The lack of a glucose effect in myocytes and arteries from S1928A mice is unlikely due to differences in basal K^+ channel density, as protein abundance for voltage-gated K^+ (K_V) $K_V 2.1$ subunit, and large-conductance Ca^{2+}-activated BK_{Ca} α and $\beta 1$ subunits, which regulate arterial myocyte excitability and are glucose-sensitive,[12-15] was similar between WT and S1928A arteries.[7] Changes in $Ca_V 1.2$ expression are also unlikely, as the S1928A point mutation itself did not alter the abundance of $Ca_V 1.2$.[8] Consistent with this, pressure-induced constriction, which is highly dependent on LTCC and K^+ channel function,[14,16] resulted in a vascular tone of equal magnitude in WT and S1928A arteries under normal extracellular glucose concentrations (i.e. 10 mM D-glucose).[7] Overall, our previous data suggest a

relatively small influence of glucose-induced K^+ channel inhibition and consequent E_m depolarization to arterial myocyte hypercontractility in response to HG. These findings point toward a key role for $Ca_V 1.2$ phosphorylation at S1928 in global Ca^{2+} increases and vasoconstriction in response to HG that is, at least partly, independent of changes in E_m. Here, we used an established mathematical model of arterial myocyte electrophysiology and Ca^{2+} dynamics to quantitatively compare the relative contribution of K^+ channels and LTCCs in modulating E_m and $[Ca^{2+}]_i$ changes in response to HG. Our analysis supports a predominant role of LTCC potentiation (vs. K^+ channel inhibition) in HG-mediated increase in $[Ca^{2+}]_i$ leading to vasoconstriction of cerebral arteries.

Results and discussion

We used the Kapela et al. model of an arterial myocyte [17] to assess changes in $[Ca^{2+}]_i$ when simulating an acute elevation in glucose. Based on previous experimental
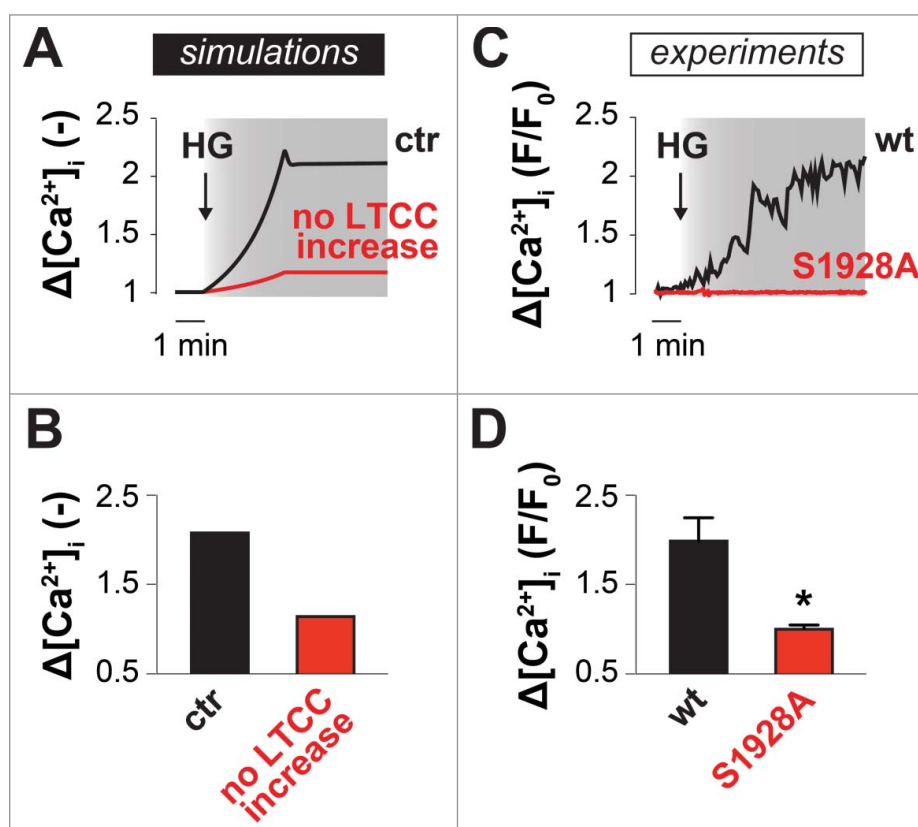

Figure 1. LTCCs play a key role in $[Ca^{2+}]_i$ regulation during acute hyperglycemic conditions. Simulated (A, B) and experimental (C, D) HG-mediated effects on $[Ca^{2+}]_i$ in WT and S1928A cells. To simulate gradual increase of glucose concentration over time, full modification of HG-dependent model parameters is reached after 180 s from the initial administration (indicated by the arrow). Fig. 1C and D are from our previously published study.[7] © AAAS. Reproduced by permission of AAAS. Permission to reuse must be obtained from the rightsholder.

data,[3,7,9-13] HG targets both Ca^{2+} and K^+ channels to enhance LTCC currents and suppress K^+ currents. Under these conditions, our model predicted that HG would lead to a ~2-fold increase in $[Ca^{2+}]_i$ at steady-state in WT arterial myocytes (Fig. 1A and B). Indeed, this result is similar to our experimental data in isolated arterial myocytes from WT mice (Fig. 1C and D).[7] When potentiation of LTCC activity in response to HG is ablated to simulate lack of S1928 phosphorylation, as in S1928A cells, the predicted increase in $[Ca^{2+}]_i$ is limited to ~20% (Fig. 1A and B), which again is in agreement with our experimental observations (Fig. 1C and D). These results suggest that changes in $[Ca^{2+}]_i$ in arterial myocytes in response to HG are minimal in the absence of S1928 phosphorylation and LTCC

potentiation, despite a reduction in K^+ channel activity and subsequent E_m depolarization, which is expected to enhance Ca^{2+} influx.

To dissect the relative contribution of HG-induced modifications of LTCCs and K^+ channels to $[Ca^{2+}]_i$ and E_m, we compared the effects of HG on each target in isolation (Fig. 2). Under normal conditions (i.e., in the baseline model assuming 10 mM D-glucose), predicted $[Ca^{2+}]_i$ and E_m are 68 nM and −59.4 mV, respectively. When specific glucose-mediated alterations in K^+ channels are simulated E_m underwent a ~5 mV depolarization (Fig. 2B), due to the reduction in outward currents, whereas the increase in $[Ca^{2+}]_i$ was limited (~10 nM, Fig. 2A). This limited change in E_m is similar to that observed by Rainbow et al. in

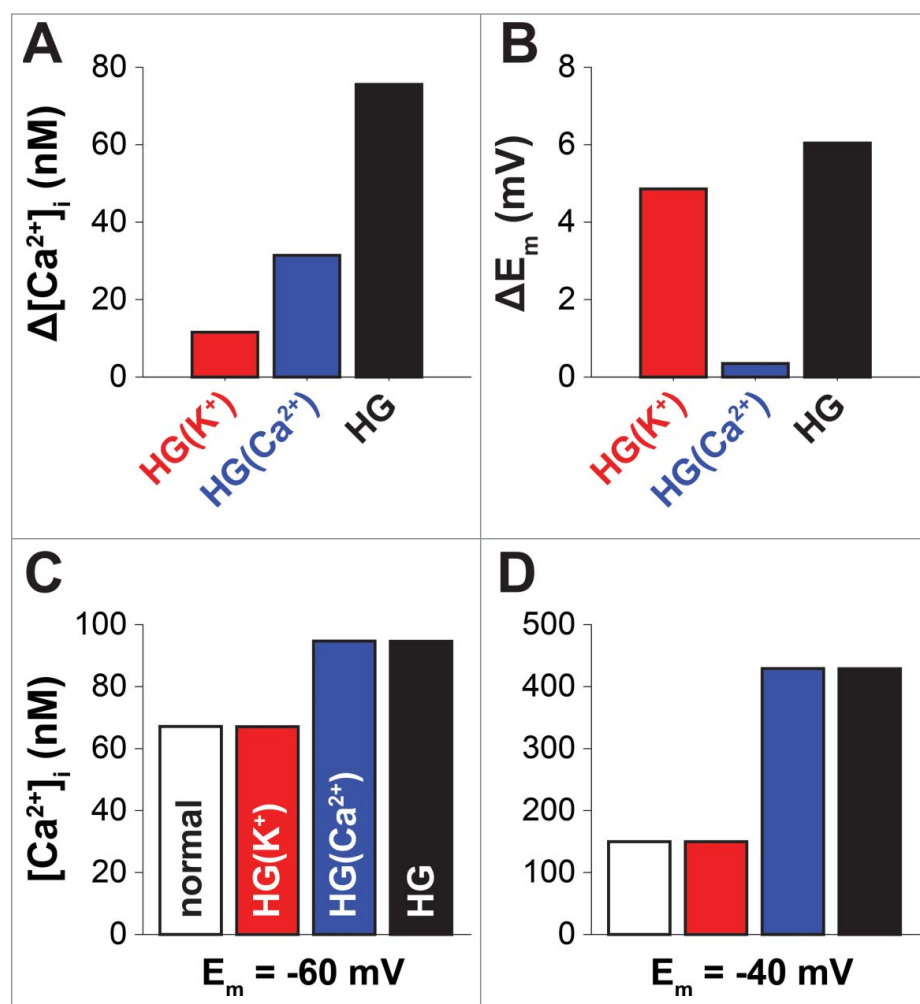**Figure 2.** Effects of glucose-induced modifications on $[Ca^{2+}]_i$ and E_m. Predicted variations of $[Ca^{2+}]_i$ (A) and E_m (B) (compared with control) obtained at rest (i.e., absence of external stimuli) when glucose-mediated modifications are included for K^+ channels (BK_{Ca} and $K_v2.1$) only (red), for LTCC only, or for both. When glucose concentration is normal resting $[Ca^{2+}]_i$ and E_m are 68 nM and −59.4 mV, respectively. (C, D) Steady-state $[Ca^{2+}]_i$ values obtained by clamping the virtual cell at the indicated E_m with normal glucose or when HG-dependent modifications are included as described in the text.

response to elevations in extracellular glucose.[10] When only glucose-induced LTCC potentiation was simulated E_m was unchanged (Fig. 2B), as expected.[16] However, LTCC potentiation caused a pronounced increase in $[Ca^{2+}]_i$ (~30 nM, Fig. 2A). When both effects were included (Figs 1 and 2), HG-induced elevations in $[Ca^{2+}]_i$ and E_m were further enhanced (~75 nM and ~6 mV, Fig. 2A-B). Notably, the effect of HG on $[Ca^{2+}]_i$ when alterations of all targets were simulated was larger than the sum of the effect at each individual target (Fig. 2B), suggesting potential synergy between LTCC activation and K^+ channel inhibition. When simulations were repeated in voltage-clamped cells at different E_m (e.g. −60 mV or −40 mV) to identify the effect of HG on $[Ca^{2+}]_i$ independently of E_m variations (Fig. 2C-D), K^+ channel inhibition did not affect global Ca^{2+}. However, holding E_m at a more depolarized voltage augmented the HG- (and LTCC-) mediated increase in $[Ca^{2+}]_i$.

We also analyzed how modulating the activity of all ion currents and transport mechanisms affect $[Ca^{2+}]_i$ and E_m in response to HG, following the methodology described by Sobie.[18] The results of this statistical process are summarized in the regression coefficients reported in Table 1, which can be used to estimate changes in $[Ca^{2+}]_i$ and E_m due to given variations in each model parameter. Importantly, our analysis on the HG targets (Fig. 3) showed that variations in $[Ca^{2+}]_i$ are positively correlated with LTCC activity, while E_m is insensitive to this model parameter. Inhibition of K^+ channels, which is weakly correlated with an increase in $[Ca^{2+}]_i$, has a strong depolarizing effect. Interestingly, modulation of K_V channel activity has a more prominent effect on $[Ca^{2+}]_i$ and E_m regulation compared with BK_{Ca} channels. We also found that $[Ca^{2+}]_i$ is strongly affected by the activity of non-selective cation channels (NSCs), which also strongly regulate E_m, causing depolarization,[19] and plasma membrane Ca^{2+}-ATPase (PMCA). Thus, this analysis revealed new mechanistic information that warrants further experimental studies to investigate the role of these fluxes in response to HG and their impact on arterial myocyte excitability and vascular reactivity. Overall, the sensitivity analysis confirmed our mechanistic interpretation of the predominant role of LTCC potentiation in HG-mediated increase in $[Ca^{2+}]_i$, and the secondary role of K^+ channels in this outcome.

Table 1. Regression coefficients. Sensitivity analysis was performed to assess the consequences of changes in all ion channels and transport mechanisms in the model: L-type Ca^{2+} channels (LTCC), large-conductance Ca^{2+}-activated K^+ channels (BK_{Ca}), voltage-gated K^+ channels ($K_V2.1$), unspecified K^+ leak channels (K_{leak}), Ca^{2+}-activated Cl^- channels (Cl_{Ca}), Na^+-K^+-Cl^- cotransporter (NaKCl), Na^+-K^+ ATPase (NaK), Na^+-Ca^{2+} exchanger (NCX), plasma membrane Ca^{2+}-ATPase (PMCA), store-operated Ca^{2+}-permeable non-selective cation channels (SOC), non-selective cation channels (NSC), IP_3 receptor (IP_3R), SR passive Ca^{2+} leak (SR_{leak}), ryanodine receptor (RyR), SR Ca^{2+}-ATPase pump (SERCA). A positive coefficient indicates positive correlation between changes in the observed outcome (Ca^{2+} or E_m) and a given parameter, and vice versa. Given these coefficients, one can quantify how a change in a certain parameter affects a certain output as explained in the caption of Fig. 3.

Ion current or transport mechanism	Output	
	$[Ca^{2+}]_i$	E_m
LTCC	0.220	−0.021
BK_{Ca}	−0.025	0.135
$K_V2.1$	−0.054	0.298
K_{leak}	−0.087	0.481
Cl_{Ca}	9.00e-3	−0.038
NaKCl	1.44e-3	0.012
NaK	−0.190	−0.097
NCX	−0.048	−0.044
PMCA	−0.566	0.060
SOC	0.100e-3	0.51e-3
NSC	0.753	−0.795
IP_3R	2.00e-3	0.35e-3
SR_{leak}	1.45e-3	2.96e-3
RyR	−0.76e-3	1.65e-3
SERCA	1.31e-3	1.52e-3

Our recent study demonstrated that acute elevations in extracellular glucose causes a significant increase in $[Ca^{2+}]_i$ and constriction in cerebral arterial myocytes and arteries from WT mice.[7] These changes were dependent on increased LTCC activity mediated by phosphorylation of $Ca_V1.2$ at S1928. The computational analysis performed here indicates that glucose-induced inhibition of K^+ channels in the absence of LTCC hyperactivation (i.e., as it may occur in S1928A myocytes) does not cause appreciable changes in $[Ca^{2+}]_i$. Yet, LTCC potentiation in response to HG drives significant $[Ca^{2+}]_i$ enhancement, as it seems to occur in WT myocytes. Note that during chronic elevations in extracellular glucose, as in diabetes, both E_m-independent (e.g., PKA-mediated upregulation of LTCC activity[7]) as well as E_m-dependent (e.g., downregulation of K^+ channel activity leading to depolarization and further LTCC activation[9,12,13]) mechanisms synergize to contribute to modulate $[Ca^{2+}]_i$ in arterial myocytes. Indeed, our simulation studies showed that the increase in $[Ca^{2+}]_i$ seen with concomitant inhibition of K^+ currents and LTCC potentiation is
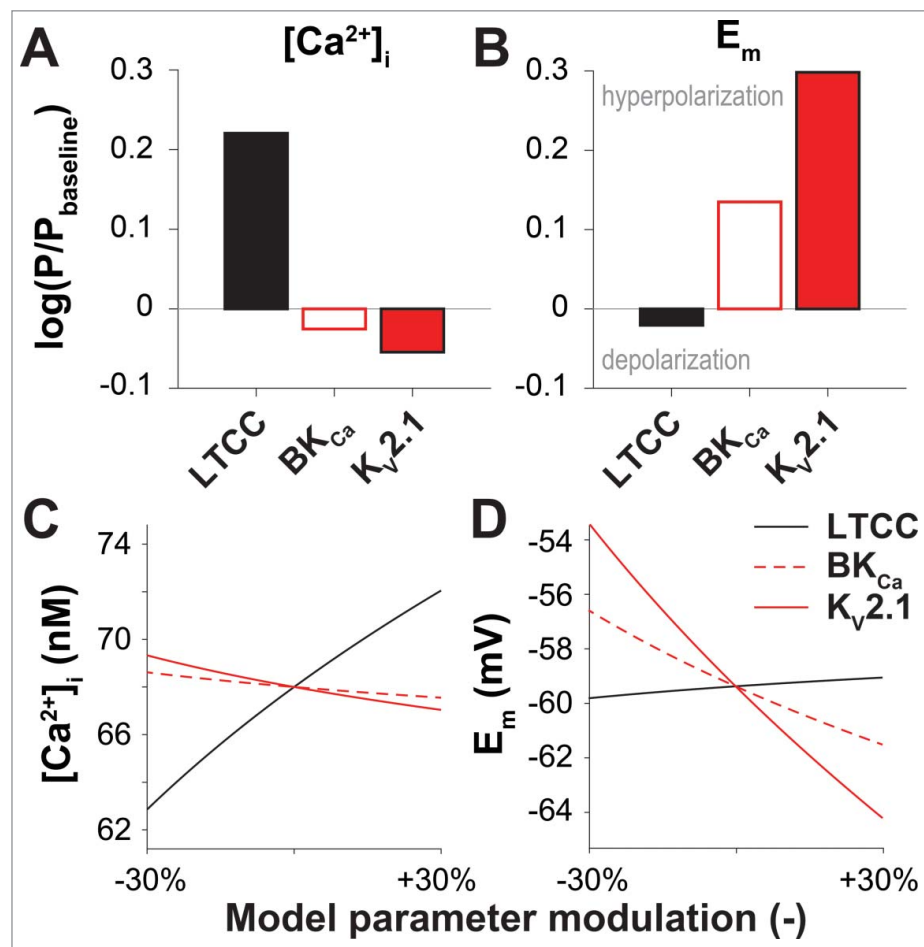
Figure 3. Effects of perturbations in HG-sensitive target parameters on $[Ca^{2+}]_i$ and E_m. Bar graphs show the regression coefficients indicating how perturbations in the conductance of LTCCs, BK_{Ca} and $K_V2.1$ channels affect $[Ca^{2+}]_i$ (A) and E_m (B). (C) and (D) show the variations induced in these outputs when modulating ($\pm30\%$) only one conductance at a time, as predicted by the regression model. Given the coefficient B_{P-O} (correlating the parameter P to the output O), one can quantify the variation of O (with respect to the baseline value $O_{baseline}$) induced by a change in P (with respect to the baseline value $P_{baseline}$) using the following formulation: $O/O_{baseline} = (P/P_{baseline})^{\wedge}B_{P-O}$.

attenuated when E_m depolarization is prevented. Our results also suggested a predominant role of $K_V2.1$ (vs. BK_{Ca}) in the HG-induced E_m depolarization. To summarize, our findings confirmed an essential role for $Ca_V1.2$ phosphorylation at S1928 in stimulating LTCC activity and vasoconstriction in response to HG. We also demonstrated the power of computational models and population-based statistical approaches in quantifying the relative roles of many elements that interact non-linearly to regulate such complex processes in arterial myocytes.

Methods

The Kapela et al. mathematical model of electrophysiology and Ca^{2+} dynamics in arterial myocytes[17] served as the basis for our simulations. This

model is a system of non-linear ordinary differential equations describing the main transmembrane fluxes of Ca^{2+}, K^+, Na^+ and Cl^- involved in E_m regulation and the mechanisms controlling intracellular (cytosol and sarcoplasmic reticulum, SR) Ca^{2+} homeostasis. We modified the baseline model to include glucose-dependent effects on LTCCs, BK_{Ca} and $K_V2.1$ conductances that recapitulate previous experimental observations.[3,12,13] Specifically, elevated glucose caused a 2-fold increase in LTCC conductance, whereas BK_{Ca} and $K_V2.1$ currents were reduced by 58 and 63%, respectively.

We performed sensitivity analysis to investigate the influence of the various currents and transporters in the regulation of global Ca^{2+} and E_m using an established population-based approach.[18] The baseline model (i.e., with normal glucose at 10 mM) was used

to simulate a family of 1000 model variants, generated by randomly varying conductances or maximal transport rates of all ion currents and transport mechanisms (Table 1). Specifically, the baseline value of each parameter was independently varied with a log-normal distribution (standard deviation of 0.1), that approximately allows changes within the range −30% to +50%. We assessed the resulting values of E_m and $[Ca^{2+}]_i$ for each model variant, and performed multivariable regression (non-linear iterative partial least squares method) on log-transformed values to correlate the variation in each parameter to the consequent effect on these 2 outputs.[18]

All simulations were performed in MATLAB (The MathWorks, Natick, MA, USA) using the stiff ordinary differential equation solver ode15s. Our codes are freely available for download at:

somapp.ucdmc.ucdavis.edu/Pharmacology/bers
or
elegrandi.wixsite.com/grandilab/downloads.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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