Vascular smooth muscle membrane potential is a key determinant of vessel tone primarily through regulation of voltage-dependent calcium channels (VDCCs). The opening probability of VDCCs is increased by membrane depolarization, facilitating influx of calcium and producing contraction of vascular smooth muscle cells (VSMCs). Control of membrane potential and VDCC activity is orchestrated by a complex series of signals that occur just below the sarcolemmal membrane.

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Large-conductance calcium-activated potassium channels (also known as maxi-K or BK channels) play a pivotal role in modulating vasomotor tone in both health and disease. BK channels, expressed on VSMCs, act as breaks for the increase in vascular tone that occurs after membrane depolarization and elevation of cytosolic calcium. An intracellular rise in cytosolic calcium through VDCC leads to activation of ryanodine receptors that release quanta of calcium from the sarcoplasmic reticulum (calcium sparks). It is these intracellularly generated sparks that elevate local submembrane concentrations of calcium and activate BK channels which increase potassium conductance and lower membrane potential. This reduces calcium influx, thus attenuating the myogenic contraction.

A classic example of BK channel modulation of vascular tone is the attenuation of myogenic vasoconstriction. An increase in intraluminal pressure depolarizes the VSMC membrane eliciting calcium influx and myogenic contraction. The calcium influx triggers opening of previously quiescent BK channels resulting in increased potassium conductance, hyperpolarization of the smooth muscle membrane, and a reduction in calcium influx, thus attenuating the myogenic contraction.

BK channels are comprised of homotetrameric α subunits with a potassium-selective pore region. The channel complex is flanked by β subunits that enhance calcium sensitivity. Gain of function mutations of the β1 subunit in humans are associated with a low prevalence of hypertension and coronary disease, whereas genetic loss of function mutations in the α subunit or reductions in the β1 subunit produce increases in blood pressure and cardiovascular disease. Conversely, gain of function polymorphisms of the β subunit reduce diastolic blood pressure and may reduce the risk of cardiovascular events.

In diabetes mellitus, the action of BK channels is reduced, in part by decreased calcium sensitivity from a reduction in β1 subunit expression. Interestingly, the nature of the reduced activity changes over time. Early in the Zucker high-fat diet–induced model of diabetes mellitus (8 weeks), vascular dysfunction and reduced BK currents are observed in VSMCs. By 4 to 6 months, BK channels still open to voltage changes but calcium sensitivity is reduced. At this more advanced stage, the Zucker high-fat diet–induced model of diabetes mellitus rat also demonstrates abnormal biophysical properties of the BK channel, not observed at earlier time points, including impaired calcium sensitivity, more prolonged closed times, and shortened opening times.

It is easy to see how BK channels play a central role in vasodilation and development of vascular disease. However, little is known about how these channels are regulated. Which intracellular pathways are responsible for modulating BK channel activity and expression? Nystoriak et al20 in the current issue provide new findings that link the calcineurin–nuclear factor of activated T cells, c3 isoform (NFATc3) pathway to BK channel expression in an animal model of diabetes mellitus. The scaffolding protein A-kinase anchoring protein 150 is critical in linking calcineurin with L-type calcium channels (Figure). Calcineurin, the calmodulin-dependent serine/threonine phosphatase, is activated by elevations in intracellular calcium leading to dephosphorylation of NFATc3 which enhances nuclear translocation. There, NFATc3 blocks expression of the BK channel β1 subunit. Strong evidence is provided that in diabetes mellitus, activation of calcineurin dephosphorylates NFATc3, allowing it to move into the nucleus, reduce transcription of the BK channel β1 subunit, thereby decreasing calcium sensitivity of the BK channel. As a result, BK opening probability is impaired at any given level of calcium spark activity, leading to larger intracellular calcium levels and enhanced vasomotor tone. This was convincingly demonstrated by an elevation in intracellular calcium, reduced opening probability of BK channels in vascular myocytes, an elevation in blood pressure in animals with a constitutively active form of NFATc3, and normal calcium sensitivity in VSMCs from diabetic animals with reduced levels of NFATc3. Calcium spark activity did not change.

The NFATc3 pathway’s influence on vascular ion channels is not unique to the BK channel because nuclear localization of NFATc3 in response to angiotensin II downregulates Kᵥ2.1 channel expression. Thus, NFATc3 may engage multiple complementary pathways of vasodilation in disease. Indeed, like BK, Kᵥ channel activity is reduced and expression is...
Diabetes mellitus reduces BK channel activity by ≥3 mechanisms. First, as demonstrated by Nystoriak et al., through activation of calcineurin, dephosphorylation of activated T cells, c3 isoform (NFATc3) enters the nucleus where it inhibits transcription of the β1 subunit of BK, thereby reducing BK sensitivity to calcium. Second, diabetes mellitus–induced elevations in reactive oxygen species (ROS), particularly hydrogen peroxide, directly oxidize cysteine residues in the bowl region of the α subunit of BK to reduce opening. Finally, reduced insulin activation of phosphoinositol-3-kinase (PI3) kinase in diabetes mellitus reduces phosphorylation of forkhead box O family transcription factor (FOXO)-3a, allowing nuclear localization and proteosomal degradation of FOXO-3a, thereby reducing transcription of f-box only protein (FBXO) which then activates transcription factor (FOXO)-3a, allowing nuclear localization and reduced formation of the β1 subunit of BK. AKAP indicates A-kinase anchoring protein 150; Akt, protein kinase B; CaN, calcineurin; FA, fatty acid; LITCC, L-type calcium channel; STOC, spontaneous transient outward current; and U, ubiquitin.

NFATc3 can also directly increase L-type calcium channel influx by connecting the channel with the activator protein kinase Cα. Reciprocally, protein kinase Cα, calcineurin, and subsarcolemmal persistent calcium sparklets collude to alter vascular gene expression via NFATc3.20

The calcineurin–NFATc3 pathway may have an ally in reducing BK channel function in disease. Increased proteosomal degradation of BK channel β1 subunits occurs in diabetes mellitus, reducing channel activity.21 Thus, enhanced destruction and reduced formation of the β1 subunit may synergistically contribute to reduced BK sensitivity in diabetes mellitus. Expression of the pore-forming α subunit is not affected.22

Findings of the current study extend well beyond diabetes mellitus. Some forms of hypertension are characterized by reduced β1 subunit expression and decreased BK function.9 Heart failure is also associated with elevated peripheral vascular resistance, in part attributable to the accompanying reduction in BK channel sensitivity and increase in nuclear NFATc3.3 It is plausible that downregulation of BK channel activity is a common pathophysiological feature of cardiovascular maladaptive conditions, suggesting a common therapeutic target.

Several questions raised by the present study remain unanswered. To what extent can the roles of NFATc3 and calcineurin be generalized to other models of diabetes mellitus? Vascular changes in the high-fat diet mouse model may not be representative, even though BK β1 subunit expression is reduced in other forms of diabetes mellitus.11 Furthermore, contribution of the excessive fat intake versus glucose dysregulation cannot be distinguished from this study. Assessment of vascular function was made in conduit arteries (aorta, mesenteric artery) and not arterioles, the primary source of vascular resistance in hypertension; however, the changes in blood pressure in genetically modified mice suggest a more global effect of altered calcium signaling across the arterial bed. NFATc3 transcriptionally regulates other proteins that might contribute to altered vasomotor tone in diabetes mellitus including cyclooxygenase.23

Another unanswered question is how disease activates the NFATc3 pathway. What is the proximate signal that triggers dephosphorylation and nuclear translocation of NFATc3? A common denominator in many cardiovascular risk factors, including diabetes mellitus, hypertension, and heart failure, is an elevation in oxidants resulting in impaired endothelial and VSMC function. Lu et al.24 showed that downregulation of BK channels in a rat model of type 1 diabetes mellitus (streptozotocin) was attributable to elevated oxidative stress. Diabetes mellitus in vivo or elevated glucose in vitro stimulate reactive oxygen species production through nicotinamide adenine dinucleotide phosphate oxidase, attenuating Akt and activating forkhead box O family transcription factor-3a/f-box only protein–dependent BK β1 degradation (Figure). This is consistent with a study by Friedman et al.25 showing that vascular NFATc3 upregulation via endothelin-1 is prevented by Tempol, a superoxide scavenger. The situation is even more complex in that hydrogen peroxide, thought to be the primary redox signaling derivative of superoxide, is a potent inhibitor of calcineurin.26,27 which would be expected to prevent BK β1 downregulation via NFATc3.

In summary, BK channels are an integral part of the subsarcolemmal calcium signaling complex that regulates vascular tone. Understanding how this modulating influence on vasoconstriction is impaired in disease may open new pathways for treating cardiovascular complications of diabetes mellitus, hypertension, and heart failure. The present study takes the first step by establishing that the calcineurin–NFATc3 pathway contributes to hypertension in diabetes mellitus by downregulating expression of the calcium-sensitizing β1 subunit of the BK channel.

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Disclosures
None.

References

Figure. Pathways for activation and modulation of vascular large-conductance calcium-activated potassium (BK) channels. Diabetes mellitus reduces BK channel activity by ≥3 mechanisms. First, as demonstrated by Nystoriak et al., through activation of calcineurin, dephosphorylated nuclear factor of activated T cells, c3 isoform (NFATc3) enters the nucleus where it inhibits transcription of the β1 subunit of BK, thereby reducing BK sensitivity to calcium. Second, diabetes mellitus–induced elevations in reactive oxygen species (ROS), particularly hydrogen peroxide, directly oxidize cysteine residues in the bowl region of the α subunit of BK to reduce opening. Finally, reduced insulin activation of phosphoinositol-3-kinase (PI3) kinase in diabetes mellitus reduces phosphorylation of forkhead box O family transcription factor (FOXO)-3a, allowing nuclear localization and transcription of f-box only protein (FBXO) which then activates proteosomal degradation of β1 subunits of BK. AKAP indicates A-kinase anchoring protein 150; Akt, protein kinase B; CaN, calcineurin; FA, fatty acid; LITCC, L-type calcium channel; STOC, spontaneous transient outward current; and U, ubiquitin.


Key Words: Editorials ■ calcineurin ■ membrane potentials ■ potassium channels ■ vasodilation
Vascular Dysfunction in Diabetes Mellitus: Large Conductance Calcium-Activated Potassium Channels as Part of a Subsarcolemmal Signaling Soiree

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