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AKAP150 Contributes to Enhanced Vascular Tone by Facilitating BK_{Ca} Channel Remodeling in Hyperglycemia and Diabetes

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Abstract

Rationale—Increased contractility of arterial myocytes and enhanced vascular tone during hyperglycemia and diabetes may arise from impaired large conductance Ca^{2+}-activated K^+ (BK_{Ca}) channel function. The scaffolding protein AKAP150 is a key regulator of calcineurin (CaN), a phosphatase known to modulate expression of the regulatory BK_{Ca} β1 subunit. Whether AKAP150 mediates BK_{Ca} channel suppression during hyperglycemia and diabetes is unknown.

Objective—To test the hypothesis that AKAP150-dependent CaN signaling mediates BK_{Ca} β1 downregulation and impaired vascular BK_{Ca} channel function during hyperglycemia and diabetes.

Methods and Results—We found that AKAP150 is an important determinant of BK_{Ca} channel remodeling, CaN/NFATc3 activation, and resistance artery constriction in hyperglycemic animals on high fat diet (HFD). Genetic ablation of AKAP150 protected against these alterations, including augmented vasoconstriction. D-glucose-dependent suppression of BK_{Ca} channel β1 subunits required Ca^{2+} influx via voltage-gated L-type Ca^{2+} channels and mobilization of a CaN/NFATc3 signaling pathway. Remarkably, HFD mice expressing a mutant AKAP150 unable to anchor CaN resisted activation of NFATc3 and downregulation of BK_{Ca} β1 subunits, and attenuated HFD-induced elevation in arterial blood pressure.

Conclusions—Our results support a model whereby subcellular anchoring of CaN by AKAP150 is a key molecular determinant of vascular BK_{Ca} channel remodeling, which contributes to vasoconstriction during diabetes.

Keywords

Smooth muscle cell; intracellular calcium; NFAT; ion channels; high fat mice; hypertension; high blood pressure; potassium channels; hyperglycemia; calcineurin

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DISCLOSURES

None.

INTRODUCTION

Vascular complications associated with non-insulin dependent (type 2) diabetes contribute to hypertension, heart disease, stroke, and retinal degeneration[1]. Although the cellular mechanisms of vascular dysfunction in diabetic patients are complex and poorly understood, elevated intracellular Ca^{2+} and enhanced contractility of smooth muscle lining the resistance vasculature represent a major contributing factor[2, 3].

Arterial myocyte contractility is predominantly controlled by membrane potential (V_M) and Ca^{2+} entry via voltage-gated L-type Ca^{2+} channels (LTCCs). The opening of a single or small cluster of these channels produces a localized elevation in intracellular Ca^{2+}, or "sparklet", near the plasma membrane[4]. Ca^{2+} sparklet activity increases in arterial myocytes during acute hyperglycemia and diabetes[5]. While this increase in LTCC-mediated Ca^{2+} influx directly enhances myocyte contractility, sustained activity could also potentially drive Ca^{2+}-dependent changes in gene expression during diabetes via activation of the Ca^{2+}/calmodulin-dependent phosphatase calcineurin (CaN) and subsequent dephosphorylation and nuclear translocation of the transcription factor NFATc3[6, 7]. CaN is anchored at the plasma membrane in close proximity to LTCCs by A-kinase anchoring protein 150 (AKAP150, murine ortholog of human AKAP79)[8], which is required for activation of CaN-NFAT signaling[9]. Disruption of the interaction between CaN and AKAP150 precludes subplasmalemmal CaN localization, and CaN-dependent NFAT transcriptional activation in rat hippocampal neurons[10]. However, the importance of AKAP150 in modulation of vascular gene expression and vascular tone during diabetes is unknown.

Activation of CaN/NFATc3 in arterial myocytes is linked to expression of BK_{Ca} channels[11, 12], which provide tonic feedback opposition to membrane depolarization and LTCC activation in arterial myocytes[13]. In these cells, BK_{Ca} channels are composed of pore-forming alpha (α) subunits in association with accessory beta1 (β1) subunits[14]. Loss of the β1 subunit results in decreased Ca^{2+} sensitivity, reduced BK_{Ca} activation and increased vascular tone[15].

The objective of the present study was to examine the role of AKAP150-dependent signaling in BK_{Ca} channel remodeling and vascular dysfunction during hyperglycemia and diabetes. Our hypotheses were tested in high fat diet-fed mice, a well-suited mouse model for the study of pathophysiology associated with induction of type 2 diabetes[16, 18]. We found that BK_{Ca} β1 subunit expression is suppressed, leading to reduced BK_{Ca} channel Ca^{2+} sensitivity and enhanced vasoconstriction in wild type (WT), but not AKAP150-null ($AKAP150^{-/-}$) high fat mice. This effect was dependent upon LTCC-mediated Ca^{2+} influx and CaN/NFATc3 activation. Moreover, we discovered that disruption of the interaction between AKAP150 and CaN was equally effective in preventing β1 suppression and NFATc3 activation, and attenuated increases in blood pressure in HFD mice. These results implicate AKAP150 as an essential component of BK_{Ca} suppression, thus contributing to enhance vascular tone during type II diabetes.

METHODS

WT (C57Bl/6J, BalbC), $AKAP150^{-/-}$ (C57Bl/6J), $NFATc3^{-/-}$ (BalbC), and knock-in mice expressing AKAP150 lacking its CaN binding site (ΔPIX)[16] were euthanized by intraperitoneal injection of sodium pentobarbital, as approved by the University of California, Davis Institutional Animal Care and Use Committee. Mice were placed on either a low fat (10% kcal; control) or high fat (60% kcal) diet (Research Diets) at 5 weeks of age and were sustained for 24–26 weeks. The composition of these diets and the propensity of mice maintained on this HFD to develop type 2 diabetes has been described previously[17].

For some experiments, cerebral and mesenteric arteries were acutely isolated from ct animals (5 weeks of age) and organ cultured by placing arteries in serum-free DMEM-F12 culture media (Thermo Scientific) with varying concentrations of D-glucose and incubating at 37°C and 5% CO_2 for 48 hours. Arterial myocytes were dissociated from cerebral and mesenteric arteries using enzymatic digestion techniques described previously[11]. Vascular tone was measured using an IonOptix Vessel Diameter system. Currents were recorded using an Axopatch 200B amplifier. Images were obtained using a confocal microscope. Data are presented as mean ± SEM. $P<0.05$ was considered statistically significant, which is denoted by an asterisk in the figures. An expanded Methods section is available in the Online Supplemental Material.

RESULTS

Our hypotheses were tested using freshly isolated arteries and arterial myocytes from age-matched WT and AKAP150$^{-/-}$ mice fed an ad libitum supply of either a low fat (10% kcal) or high fat (60% kcal) diet (see Methods for details)[17]. We used this model because it closely recapitulates features of clinically relevant human pathology in type 2 diabetes[17, 18]. Furthermore, it does not depend on genetic manipulation or chemical destruction of pancreatic β-cells. Non-fasting blood glucose and body mass were significantly higher in HFD mice compared with low fat fed (ct) mice (Online Table I). Genetic ablation of AKAP150 did not impact non-fasting blood glucose levels in ct or HFD mice, as compared with corresponding WT ct (Online Table I).

Impaired arterial tone and Ibtx sensitivity in WT, but not in AKAP150$^{-/-}$ HFD mice

At the physiological intravascular pressure of 60 mmHg[19], WT HFD arteries were consistently more constricted than WT ct (29 ± 4% vs 17 ± 2% vascular tone, respectively; Figure 1A–B). To evaluate the contribution of BK_{Ca} channels to regulation of arterial tone, the selective BK_{Ca} inhibitor iberiotoxin (IbTx; 100 nmol/L) was applied to the bath solution. Whereas application of IbTx caused marked constriction in WT ct arteries (13 ± 4% decrease in diameter), this agent had little effect on WT HFD vessels (2 ± 2% decrease in diameter; Figure 1A–C).

We determined whether the anchoring protein AKAP150 mediates decreased IbTx sensitivity in HFD arteries. To do this, we examined tone development and IbTx-induced constriction in AKAP150$^{-/-}$ ct and HFD isolated arteries. In contrast to WT arteries, levels of arterial tone and IbTx-induced constriction were similar in AKAP150$^{-/-}$ ct and HFD vessels (Figure 1A–C). Arteries from all groups responded with robust constriction to phenylephrine (Online Figure I), suggesting that altered IbTx responses between groups were not due to differences in the magnitude of baseline tone development. Constriction was significantly greater in the presence of phenylephrine in WT HFD, but not AKAP150$^{-/-}$ HFD arteries (compared with respective ct). Enhanced vascular tone was not due to increased expression of L-type Ca^{2+} channels, as basal expression of the pore-forming subunit $Ca_V1.2$ was similar between WT and AKAP150$^{-/-}$ ct and HFD arteries (Online Figure II). These data suggest impaired BK_{Ca} channel function and support the hypothesis that enhanced vasoconstriction in HFD mice requires AKAP150.

AKAP150 is required for down-regulation of BK_{Ca} β1 subunits during diabetes

We tested the possibility that reduced BK_{Ca}-mediated regulation of vascular tone in WT HFD results from altered Ca^{2+} sparks, which are the physiological activators of BK_{Ca} channels in arterial myocytes[20]. To do this, we optically measured Ca^{2+} sparks in freshly isolated cerebral arterial myocytes loaded with the Ca^{2+}-sensitive dye fluo-4 using spinning disk confocal microscopy. As shown in Online Figure III, the frequency and amplitude Ca^{2+}

sparks were similar in WT ct, WT HFD and AKAP150$^{-/-}$ HFD myocytes, suggesting that changes in Ca^{2+} spark activity do not underlie impaired BK_{Ca} function in WT HFD mice.

Next, we recorded single BK_{Ca} channel currents from WT and AKAP150$^{-/-}$ arterial myocytes using the inside-out configuration of the patch clamp technique to determine whether AKAP150 mediates impairment of BK_{Ca} channel function during diabetes. Currents were recorded at physiological V_M (−40 mV) in the presence of 1 and 10 μM free Ca^{2+}. While the open probability (P_o) of BK_{Ca} channels increased when Ca^{2+} was elevated from 1 μM to 10 μM in myocytes from both groups, BK_{Ca} channel P_o from WT HFD was significantly lower than that for WT ct at the Ca^{2+} concentrations tested (Figure 2A–B). Consistent with arterial diameter data above, P_o for BK_{Ca} channels was similar in AKAP150$^{-/-}$ ct and HFD cells (Figure 2A–B). Open time histograms for BK_{Ca} channels from WT and AKAP150$^{-/-}$ cells are shown in Figure 2C. Histograms were fit with a sum of two Gaussians function (see Methods for details), and revealed a shift towards shorter open times in WT HFD when compared to WT ct. Open times were not different for BK_{Ca} channels from AKAP150$^{-/-}$ HFD and ct cells. These results indicate that AKAP150 is required for a reduction in Ca^{2+} sensitivity and dwell open time of BK_{Ca} channels during diabetes.

Reduced Ca^{2+} sensitivity and open time for BK_{Ca} channels in WT HFD cells are consistent with down-regulation of the β1 subunit[15]. Accordingly, we found that application of 1 μmol/L tamoxifen, which increases BK_{Ca} channel P_o through the regulatory β1 subunit[21] (see Online Figure IV), significantly increased the P_o of BK_{Ca} channels from WT ct (5-fold), but had minimal effect in WT HFD cells (Figure 3A–B). In contrast, tamoxifen increased BK_{Ca} channel P_o from AKAP150$^{-/-}$ ct and HFD myocytes, which suggests restored β1 function (Figure 3A–B). Consistent with data above, Western blot analysis showed ~65% reduction in BK_{Ca} β1 protein in lysates from WT HFD, but not from WT and AKAP150$^{-/-}$ ct and AKAP150$^{-/-}$ HFD (Figure 3C–D). Expression of the pore-forming BK_{Ca} α subunit was unchanged between groups (Figure 3C–D). Note that ablation of AKAP150 did not alter basal expression of BK_{Ca} subunits (Online Figure V-A). Moreover, no change in channel density was observed, as the number of functional channels in membrane patches (HFD: 4.0 ± 1.0, control: 4.0 ± 0.4) and current-voltage relationship of whole-cell, IbTx-sensitive potassium currents (i.e. I_{BK}; Online Figure VI) was not different between WT or AKAP150$^{-/-}$ groups. Together, these data indicate that AKAP150 mediates downregulation of BK_{Ca} β1 subunits and decreased channel function in diabetic mice.

Elevation of extracellular glucose recapitulates AKAP150-dependent suppression of BK_{Ca} channel function and β1 subunit expression

To isolate the effects of glucose from confounding conditions that may be present in HFD mice (e.g. circulating agents, hypercholesterolemia, intravascular pressure), arteries were isolated and pre-incubated for 48 hours in media containing 5, 10 or 20 mmol/L D-glucose. These extracellular D-glucose concentrations are within the range of observed non-fasting blood glucose levels reported for HFD (20 mmol/L) and control mice (5–10 mmol/L; see Online Table I). Basal expression of BK_{Ca} channel subunits in vessels maintained in normoglycemic conditions (e.g. 5 and 10 mmol/L D-glucose) was similar to that in freshly isolated WT ct arteries (Online Figure V-B).

BK_{Ca} channel P_o in myocytes from arteries incubated in 5 or 10 mmol/L D-glucose were similar in bath solutions containing 1 and 10 μmol/L Ca^{2+} (Figure 4). BK_{Ca} channel P_o observed under these conditions was similar to that in myocytes from non-diabetic control mice (see Figure 2B). In contrast, the P_o of BK_{Ca} channels in myocytes from WT arteries maintained in 20 mmol/L D-glucose were significantly reduced (Figure 4). This reduction in BK_{Ca} channel P_o was not observed in cells from arteries incubated in 20 mmol/L D-glucose

when the LTCC antagonists nifedipine (1 μmol/L) or diltiazem (50 μmol/L) were present in the incubation media, or when mannitol (15 mmol/L; a stable and non-permeable monosaccharide) or the non-metabolized L-glucose (15 mmol/L) was substituted for D-glucose (Figure 4 and Online Figure VII). BK_{Ca} channel P_o was similar between AKAP150$^{-/-}$ myocytes incubated in 10 (chosen as the normoglycemic control) or 20 mmol/L D-glucose at both Ca^{2+} concentrations examined (Figure 4). Consistent with functional data, no difference in β1 transcript and/or protein levels were observed in WT arteries incubated in 5 or 10 mmol/L D-glucose (Online Figure VIII). Raising D-glucose to 20 mmol/L did not change α subunit protein levels, but caused >50% reduction in β1 transcript and protein in WT arteries, which was prevented by nifedipine and ablation of AKAP150$^{-/-}$(Online Figure VIII). Together, these data suggest that hyperglycemic conditions recapitulate downregulation of BK_{Ca} channel activity and β1 subunit expression observed in HFD mice. Furthermore, D-glucose-induced suppression of vascular β1 subunit expression and BK_{Ca} channel function are dependent upon Ca^{2+} influx via LTCCs and AKAP150.

Anchoring of calcineurin by AKAP150 is required for BK_{Ca} channel impairment during hyperglycemia and diabetes

Expression of BK_{Ca} channel β1 subunits in smooth muscle is modulated by Ca^{2+}/calmodulin-dependent activation of the CaN/NFATc3 pathway[12]. Supporting a role for this pathway in BK_{Ca} β1 suppression during hyperglycemia and diabetes, cellular CaN activity was significantly higher in arteries isolated from WT HFD mice (Figure 5A) and in arteries incubated in 20 mmol/L D-glucose (Online Figure IX-A) as compared with arteries from WT ct and incubated in 10 mmol/L D-glucose, respectively. To determine whether CaN activation plays a role in BK_{Ca} suppression, we measured single BK_{Ca} channel currents in myocytes from arteries pre-incubated *ex vivo* for 48 hrs in 10 or 20 mmol/L D-glucose in the absence and presence of the CaN inhibitor cyclosporine A (CsA; 1 μmol/L), which selectively inhibits CaN activity (see Online Figure IX-B–C). Whereas arterial myocytes exhibited reduced BK_{Ca} channel P_o and β1 subunit protein expression after incubation in elevated D-glucose, channel activity and β1 expression in cells maintained in 20 mmol/L D-glucose + CsA were similar to low D-glucose ct cells from arteries incubated in the presence or absence of CsA (Figure 5B–C). These data indicate that CaN activation is required for suppression of BK_{Ca} channel activity and β1 subunit expression in response to elevated extracellular D-glucose.

Considering that AKAP150 targets CaN near the membrane, we tested whether anchoring of CaN by AKAP150 is necessary for BK_{Ca} suppression in arterial myocytes during hyperglycemic conditions. We took advantage of a knock-in mouse expressing a mutant AKAP150 lacking amino acid residues 655–661 of the atypical PxIxIT motif (ΔPIX), which are responsible for tethering CaN (see references [16, 22] and Online Figure IX-D). No differences in basal BK_{Ca} α and β1 protein expression levels were observed between WT and ΔPIX arteries (Online Figure V-A). Similar to CsA inhibition of CaN, disrupting the AKAP150/CaN interaction completely abolished the reduction in BK_{Ca} P_o and β1 subunit protein expression (Figure 5B–C) in response to elevated D-glucose.

To further test whether AKAP150-anchored CaN regulates BK_{Ca} channel expression during diabetes, we fed ΔPIX mice with either a control or high fat diet (Online Table I). Consistent with our hypothesis, BK_{Ca} channel P_o, and α and β1 subunit protein expression were similar in ΔPIX ct and HFD arterial myocytes and arteries (Online Figure IX-E–F). Furthermore, vascular tone and IbTx-induced constriction were similar in ΔPIX ct and HFD vessels (Figure 5D). These data indicate that subcellular anchoring of CaN by AKAP150 is a major

determinant of signaling events regulating BK_{Ca} β1 subunit suppression and function that contributes to enhanced vasoconstriction during hyperglycemia and diabetes.

AKAP150-dependent anchoring of CaN is required for activation of NFATc3 in arterial myocytes during diabetes

CaN dephosphorylates the transcription factor NFATc3. Once dephosphorylated, NFATc3 translocates into the nucleus of arterial myocytes and alters expression of several genes, including BK_{Ca} β1[12]. We investigated NFATc3 localization in WT ct and HFD mesenteric artery myocytes transfected *in vivo* with EGFP-tagged NFATc3. While WT ct demonstrates mostly cytosolic NFATc3-EGFP fluorescence, WT HFD cells exhibited NFATc3-EGFP signal localized to the nucleus (Figure 6A–B). However, NFATc3-EGFP nuclear translocation in $AKAP150^{-/-}$ HFD myocytes was significantly attenuated (Figure 6A–B). Note that WT ct and HFD myocytes expressing a construct containing only EGFP exhibited mostly cytosolic fluorescence (Online Figure X). We also examined dephosphorylation of NFATc3 serine 265, which is required for unmasking a nuclear localization signal[23], in WT, $AKAP150^{-/-}$ and ΔPIX ct and HFD myocytes (Online Figure XI). Consistent with activation and nuclear localization of this transcription factor in WT myocytes during hyperglycemia, we found a ~75% reduction in (p)Ser^{265} signal in WT HFD arteries as compared to ct. However, differences in (p)Ser^{265} signal were not observed in either $AKAP150^{-/-}$ HFD or ΔPIX HFD arteries as compared to respective ct. These findings suggest that NFATc3 is activated in WT HFD arterial myocytes and anchoring of CaN by AKAP150 is a molecular prerequisite of NFATc3 activation during hyperglycemic conditions and diabetes.

Based on these findings, we investigated BK_{Ca} channel P_o in arterial myocytes isolated from WT and NFATc3-null ($NFATc3^{-/-}$) mice maintained in normal (10 mmol/L) and elevated (20 mmol/L) D-glucose. While a reduction in channel P_o was observed in WT myocytes maintained in elevated D-glucose, BK_{Ca} P_o, and α and β1 subunit expression was similar between $NFATc3^{-/-}$ arteries maintained in low and elevated glucose (Figure 6C–D). Together, these results suggest that the AKAP150/CaN signaling complex is required for NFATc3 activation leading to BK_{Ca} impairment during hyperglycemia and diabetes.

Loss of AKAP150-anchored CaN attenuates HFD-induced increases in blood pressure

We performed telemetric blood pressure measurements in WT, $AKAP150^{-/-}$ and ΔPIX ct and HFD animals. Figure 7A shows representative blood pressure waveforms for WT ct and HFD mice. Consistent with previous studies[24, 25], WT HFD mice exhibited a significant increase in mean arterial pressure when compared to ct (Online Table I). However, increases in blood pressure associated with HFD were significantly attenuated in both $AKAP150^{-/-}$ (~60%) and ΔPIX (~40%) mice as compared with WT mice (Figure 7B; $P<0.05$). These data are consistent with the concept that AKAP150-anchored CaN contributes to impaired regulation of blood pressure during diabetes.

DISCUSSION

In this study, we define a signaling pathway for the down-regulation of BK_{Ca} channel function leading to enhanced vascular tone during non-insulin dependent type II diabetes. In this pathway, anchoring of the Ca^{2+}/calmodulin-dependent phosphatase CaN by AKAP150 is a central mediator of glucose-induced NFATc3 activation and transcriptional suppression of regulatory BK_{Ca} β1 subunits during diabetes. This ultimately produces a reduction in Ca^{2+} sensitivity of BK_{Ca} channel activation and promotes enhanced vascular tone during hyperglycemic conditions and diabetes (Figure 7C). Our findings demonstrate that genetic ablation of AKAP150 or selective perturbation of AKAP150-CaN interaction prevents

suppression of BK_{Ca} channel function and β1 subunit expression, and attenuates increases in blood pressure in diabetic animals.

The most intriguing observation in this study is the contribution of AKAP150-anchored CaN in the modulation of molecular signaling events that promote vascular remodeling associated with suppression of BK_{Ca} β1 subunits during hyperglycemic conditions and diabetes. The significance of localized phosphatase signaling in physiology has also been underscored by recent findings of an important role for AKAP150-targeted CaN signaling in insulin secretion by pancreatic beta cells[26] and synaptic incorporation of AMPA receptors in hippocampal neurons[16]. Compartmentalization of CaN through its association with AKAP150 is considered to have two effects on local phosphatase activity. Phosphatase anchoring can concentrate signals at precise subcellular locations to facilitate the selective dephosphorylation of substrates or alternatively, precise sequestering of the phosphatase provides a means to segregate the action of this multifunctional enzyme[27]. Consistent with a crucial role for AKAP150-tethered CaN, disruption of the interaction between these two proteins was sufficient to prevent NFATc3 dephosphorylation and nuclear translocation, suppression of BK_{Ca} β1 subunit expression and channel function, and enhanced vascular tone in diabetic mice. Whether targeting of NFATc3 or other CaN substrates to specific subcellular compartments is part of this process is unclear. Regardless, the aforementioned data correlate with attenuation of an elevation in blood pressure in $AKAP150^{-/-}$ and ΔPIX HFD mice, presumably via a reduction in peripheral vascular resistance. However, while heart rate was not different between groups (Online Table I), we speculate that changes in cardiac output in WT HFD animals may also participate in modulation of blood pressure. Future echocardiographic experiments will be important to address potential changes in heart function in WT, $AKAP150^{-/-}$ and ΔPIX HFD mice during diabetes.

The effects of disrupting AKAP150-CaN interaction on BK_{Ca} suppression were similar to pharmacological inhibition of CaN with cyclosporine A (CsA). Note that application of CsA is known to increase basal LTCC activity in arterial myocytes by preventing CaN-mediated feedback of PKCα activation of L-type Ca^{2+} channels[28]. Yet, in the presence of CsA, an increase in LTCC activity fails to promote NFATc3 activation. Accordingly, we have recently demonstrated that CsA prevents nuclear accumulation of NFATc3, but not the LTCC-dependent rise in $[Ca^{2+}]_i$ upon PKCα activation[6]. Together, these findings suggest that inhibition of BK_{Ca} channel downregulation in the presence of CsA is due to inhibition of calcineurin/NFAT signaling, rather than a reduction in Ca^{2+} influx. Given that AKAP150 also interacts with the C-termini of LTCCs[9], this scaffolding protein may function to position CaN near Ca^{2+} microdomain regions formed by high activity LTCCs (Figure 7C). Consistent with this idea, LTCC-mediated NFATc3 translocation in arterial myocytes is insensitive to buffering bulk cytoplasmic Ca^{2+}, suggesting that the CaN/NFATc3 pathway is preferentially activated in arterial myocytes by microdomain Ca^{2+} gradients, rather than global elevation of cytosolic Ca^{2+} [6].

Although previous data suggest that activation of an AKAP-dependent pathway is necessary for increased LTCC activity during sustained hyperglycemic stimulation and diabetes[5], the molecular identity of the AKAP involved in this process is currently unknown. Thus, it is possible that AKAP150-dependent post-translational modifications (e.g. channel phosphorylation) together with suppression of BK_{Ca} channel function could concomitantly upregulate LTCC activity to contribute to enhanced vasoconstriction and vascular dysfunction during diabetes. Whether AKAP150 can also directly influence V_M of arterial myocytes in this process remains unclear. Previous studies have demonstrated that genetic ablation of AKAP150 reduces basal persistent LTCC activity under voltage-clamp conditions[29], suggesting that this scaffold could influence arterial myocyte $[Ca^{2+}]_i$ independently of changes in V_M[19]. Thus, the finding that IbTx inhibition of BK_{Ca} channels,

which are less active at hyperpolarized V_M, induces a robust constriction in AKAP150$^{-/-}$ arteries suggests that AKAP150$^{-/-}$ arterial myocyte V_M is within the range in which BK_{Ca} channels act to oppose vasoconstriction.

The CaN/NFATc3 pathway of transcriptional regulation has been proposed as a metabolic sensor in vascular smooth muscle, via detection of elevated extracellular glucose[30]. Taking into consideration that activation of NFATc3 is an absolute requirement for BK_{Ca} channel suppression during diabetes, the time-course of β1 downregulation may closely follow that for the relationship between extracellular glucose and NFATc3 nuclear translocation. A previous report has found that significant NFATc3 nuclear accumulation begins as early as 8 minutes following exposure to elevated glucose in smooth muscle cells of intact cerebral arteries[30]. Thus, it is conceivable that downregulation of the BK_{Ca} β1 subunit may be initiated at this time point during sustained hyperglycemia. However, this process may occur more slowly *in vivo*, given the highly dynamic nature of NFATc3 nuclear import/export rate[31] and non-fasting plasma glucose levels in diabetic animals. Moreover, the AKAP150/CaN/NFATc3 axis may be critical for pathophysiological induction or suppression of a number of other genes in smooth muscle. For instance, NFATc3 has been linked to downregulation of the gene encoding the voltage-dependent potassium channel $K_V2.1$[11], increased expression of the contractile protein α-actin, vascular smooth muscle cell proliferation and increased arterial wall thickness[32]. Thus, our data render plausible the concept that AKAP150-anchored CaN may be a key molecular event underlying NFATc3-dependent transcriptional regulation in smooth muscle, thus contributing to pathological vascular complications in the diabetic population.

Suppression of smooth muscle β1 subunits in response to elevated glucose in WT HFD mice is unlikely to be mediated by differential inflammatory response, as it has been previously shown that systemic inflammation occurs at later stages (>35 weeks) of diet-induced obesity[17, 33]. Yet, despite this, additional signals could also contribute to NFATc3 activation[30, 34] and β1 down-regulation in response to elevated glucose in smooth muscle. For instance, $G_{q/11}$-coupled vasoactive compounds such as uridine triphosphate (UTP) are released in response to high glucose. Indeed, nuclear translocation of NFATc3 in native arterial myocytes exposed to elevated extracellular glucose is sensitive to the ectonucleotidase apyrase and P2Y6 receptor antagonist MRS2578[30], consistent with an additional role for nucleotide signaling in NFAT activation. In addition, recent reports have revealed a role for post-translational modifications, such as phosphorylation and oxidation, leading to decreased β1 protein levels and impaired BK_{Ca} channel activity following streptozotocin-induced type 1 diabetes[35–37]. In this model of type 1 diabetes, downregulation of coronary arterial smooth muscle BK_{Ca} β1 subunit has been linked to oxidative stress causing an increase in ubiquitin ligase-dependent protein degradation. Although this mechanism has not yet been tested in experimental type 2 diabetes, it is conceivable that decreased mRNA/protein production and increased protein degradation may act in concert to reduce β1 function during hyperglycemia and diabetes. Note however, that activation of additional pathways may vary between vascular beds and experimental models of diabetes. Future studies should determine whether transcriptional and post-translational mechanisms cooperatively act to impair BK_{Ca} channel function and expression during type 2 diabetes.

The proposed model of enhanced vascular tone in this study integrates systemic blood pressure measurements, biochemical data from small mesenteric arteries, as well as biophysical and functional studies in cerebral arterial myocytes. Therefore, our findings suggest a common mechanism (i.e. anchored CaN-driven BK_{Ca} β1 subunit downregulation) of vascular dysfunction in the cerebral and mesenteric vasculature. We caution that while changes in BK_{Ca} channel function in cerebral arteries could alter cerebral blood flow and

increase the probability of stroke during diabetes, they will have a limited impact on systemic blood pressure. Instead, down-regulation of β1 in mesenteric arteries will likely have a larger influence on systemic blood pressure. In addition, while inhibition of CaN in the vasculature with CsA may prevent BK_{Ca} channel suppression during diabetes, this agent does not represent a beneficial therapeutic strategy, given that a well-known and seemingly paradoxical side effect of this drug is hypertension[38, 39]. Although the causes of CsA-induced hypertension are multifactorial, renal sympathetic overactivity is a major contributing factor[40, 41]. To circumvent this, our current study suggests that novel agents that selectively target vascular AKAP150-CaN interactions may be advantageous in preventing vascular K^+ channel remodeling, while avoiding widespread effects of cell-wide calcineurin inhibition in the periphery.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Nonstandard Abbreviations and Acronyms

BK_{Ca}	large conductance Ca^{2+}-activated potassium channel
BK_{Ca} β1	BK_{Ca} beta 1 subunit
LTCCs	voltage-gated L-type Ca^{2+} channels
AKAP150	A-kinase anchoring protein 150
WT	wild type
HFD	high fat diet
ct	control
EGFP	enhanced green fluorescent protein
CaN	calcineurin
CsA	cyclosporine A
nifed	nifedipine
NFATc3	nuclear factor of activated T cells, c3 isoform
ΔPIX	AKAP150 lacking binding site for calcineurin
IbTx	iberiotoxin
V_M	membrane potential
P_o	open probability

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Novelty and Significance

What Is Known?

- In the resistance vasculature, activation of large conductance Ca^{2+}-activated K^+ (BK_{Ca}) channels opposes vasoconstriction; however their activity is suppressed during diabetes, leading to enhanced vascular tone.
- Expression of BK_{Ca} β1 subunits, which confer Ca^{2+} and voltage sensitivity to the channel, is modulated by activation of the Ca^{2+}/calmodulin-dependent phosphatase calcineurin (CaN) and the transcription factor nuclear factor of activated T cells (NFATc3) in arterial myocytes.
- The scaffolding protein A kinase anchoring protein 150 (AKAP150) is a key regulator of CaN.

What New Information Does This Article Contribute?

- During hyperglycemic conditions and in an experimental animal model of type 2 diabetes, activation of the CaN/NFATc3 pathway leads to transcriptional suppression of BK_{Ca} β1 subunits, which results in reduced BK_{Ca} channel function, and enhanced vascular tone.
- AKAP150 is required for activation of the CaN/NFATc3 pathway and suppression of BK_{Ca} channel function and β1 expression in arterial myocytes of diabetic animals.
- Selective disruption of the interaction between AKAP150 and CaN prevents activation of NFATc3, BK_{Ca} β1 suppression and enhanced vascular tone, and attenuates increases in mean arterial blood pressure in diabetic animals.

Vascular complications are a major cause of death and disability in the diabetic population. Elevated blood pressure and reduced blood flow during diabetes results in large part from enhanced contractility of arterial myocytes in the resistance vasculature, yet the contributing mechanisms are not well understood. Here, we establish the scaffolding protein AKAP150 as a critical mediator of transcriptional remodeling in arterial myocytes leading to enhanced vascular tone during diabetes. Our findings demonstrate that AKAP150-dependent anchoring of the phosphatase CaN is a key molecular determinant of NFATc3 activation and downstream BK_{Ca} β1 suppression during hyperglycemic conditions and diabetes. Disrupting the interaction between AKAP150 and CaN is as sufficient in preventing BK_{Ca} channel remodeling in hyperglycemic conditions as cell-wide inhibition of CaN with cyclosporine A or genetic ablation of NFATc3. Our current study highlights the significance of compartmentalized phosphatase signaling in cardiovascular biology. Furthermore, it suggests broad importance of AKAP150-CaN in the pathophysiological induction of chronic remodeling during diabetic vascular dysfunction and as a novel target for therapeutic intervention.

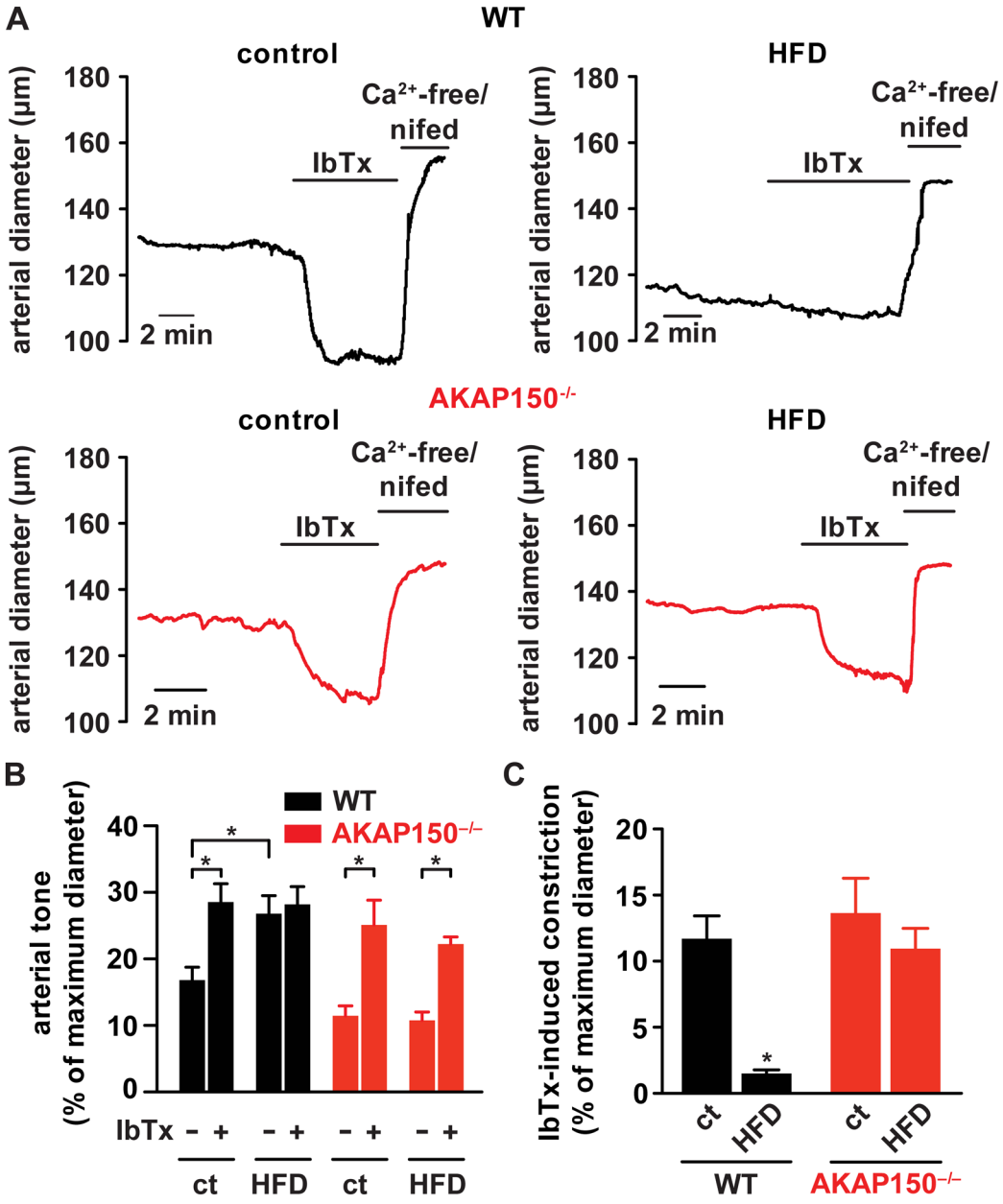

Figure 1. AKAP150 is necessary for enhanced arterial tone and diminished iberiotoxin-sensitive constriction in HFD arteries

A Representative diameter recordings from pressurized (60 mmHg) WT and AKAP150$^{-/-}$ ct and HFD cerebral arteries before and after IbTx (100 nmol/L). ***B, C*** Bar plot of vascular tone (in absence (−) and presence (+) of IbTx; *B*) and IbTx-induced constriction (*C*) at 60 mmHg in WT ct (n=8 from 6 animals), WT HFD (n=6 from 5 animals), AKAP150$^{-/-}$ ct (n=7 from 5 animals) and AKAP150$^{-/-}$ HFD (n=8 from 5 animals) arteries. *$P < 0.05$.

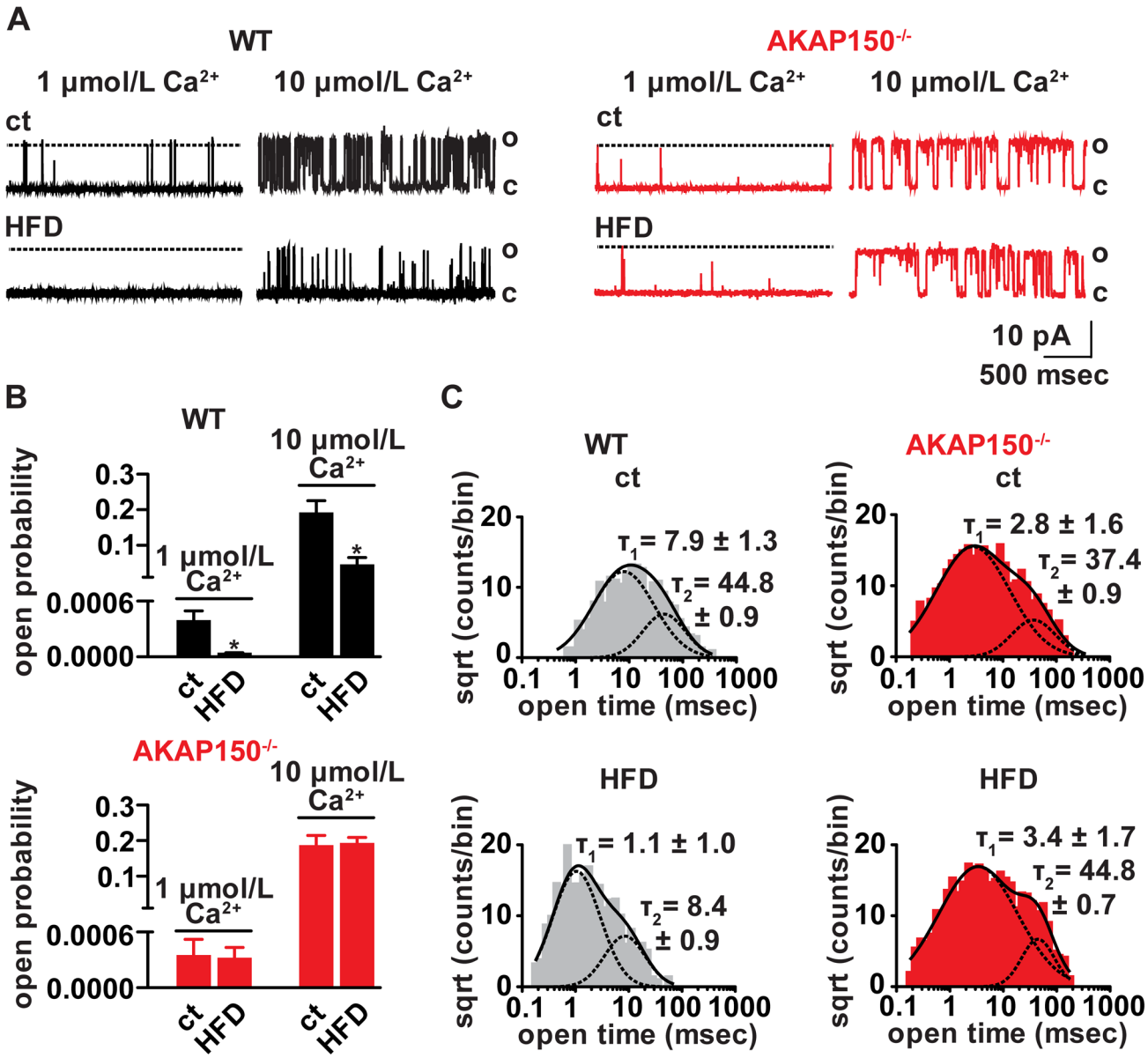

Figure 2. AKAP150 is necessary for reduced BK_{Ca} channel Ca^{2+} sensitivity and dwell open time in HFD arterial myocytes

A Representative single BK_{Ca} channel records at −40 mV obtained from excised membrane patches of isolated WT and AKAP150$^{-/-}$ ct and HFD arterial myocytes in the presence of 1 and 10 μmol/L free Ca^{2+} bath solution (C: closed; O: open). ***B*** Bar plot summarizing BK_{Ca} channel P_o at indicated free Ca^{2+} concentrations from WT ct (n=11 from 5 mice) and HFD (n=11 from 6 mice) and AKAP150$^{-/-}$ ct (n=17 from 6 mice) and HFD (n=10 from 6 mice) cells. ***C*** Open dwell time histograms for BK_{Ca} channels in WT and AKAP150$^{-/-}$ ct and HFD arterial myocytes. Black lines represent best fit to data with a two component Gaussian function with centers at indicated values (τ; msec; $R^2 > 0.90$). *$P < 0.05$.

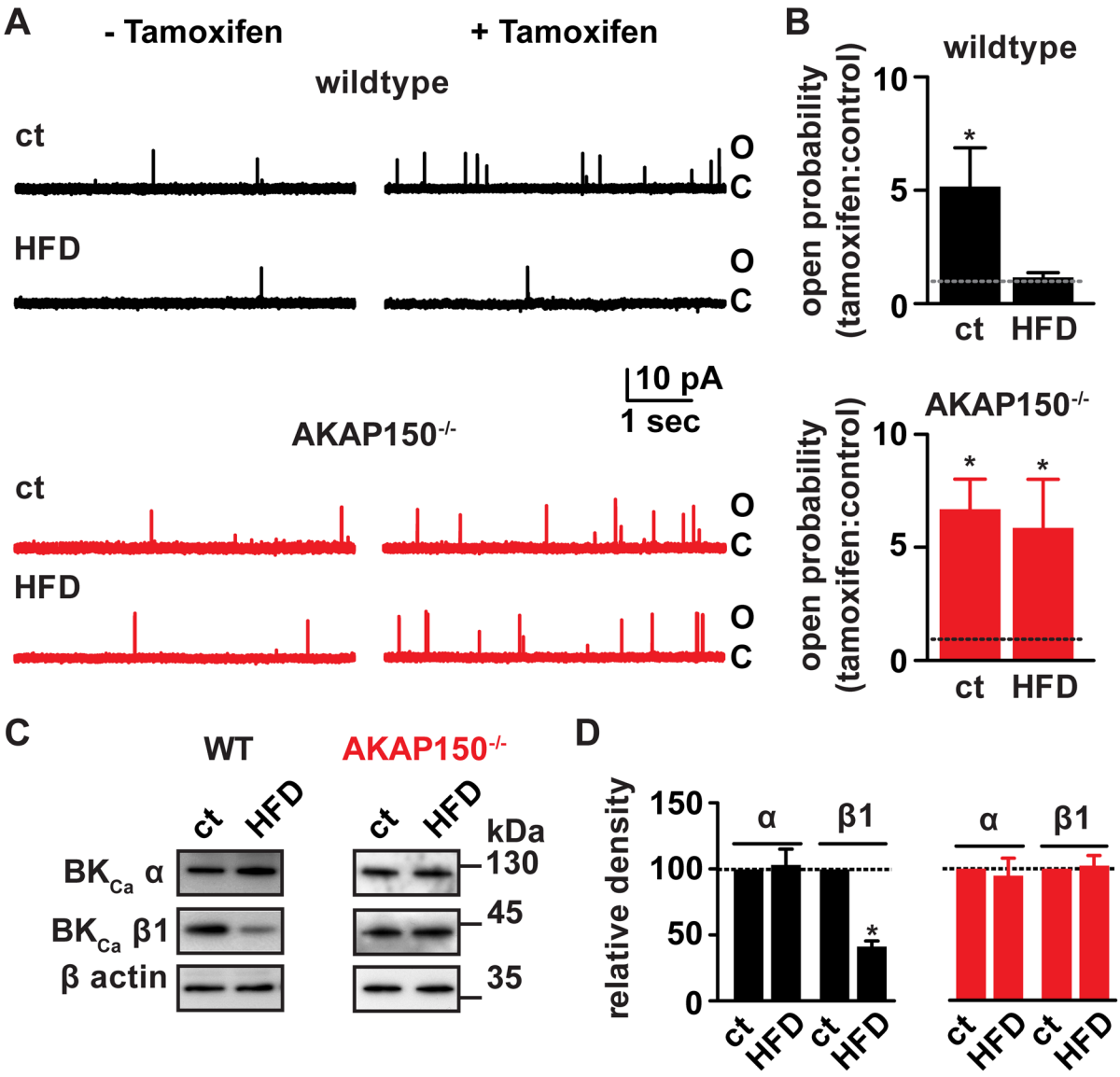

Figure 3. AKAP150 is necessary for impaired BK_{Ca} β1 subunit function and down-regulation of β1 subunit expression in HFD arterial myocytes

A Exemplar single BK_{Ca} channel records at −40 mV and 1 μmol/L Ca^{2+} obtained from excised membrane patches of isolated WT and $AKAP150^{-/-}$ ct and HFD myocytes in presence and absence of tamoxifen (1 μmol/L). ***B*** Bar plots summarizing P_o (tamoxifen:control) from WT ct (n=9 from 4 mice), WT HFD (n=9 from 3 mice), $AKAP150^{-/-}$ ct (n=9 from 4 mice) and $AKAP150^{-/-}$ HFD (n=8 from 4 mice) myocytes. *P < 0.05 (one sample t-test using hypothetical value = 1.0). ***C and D*** Representative Western blots (*C*) and corresponding densitometric summary data (*D*) for BK_{Ca} α and β1 subunits in WT ct (n=6) and HFD (n=8) and $AKAP150^{-/-}$ ct (n=5) and HFD (n=5) arteries.

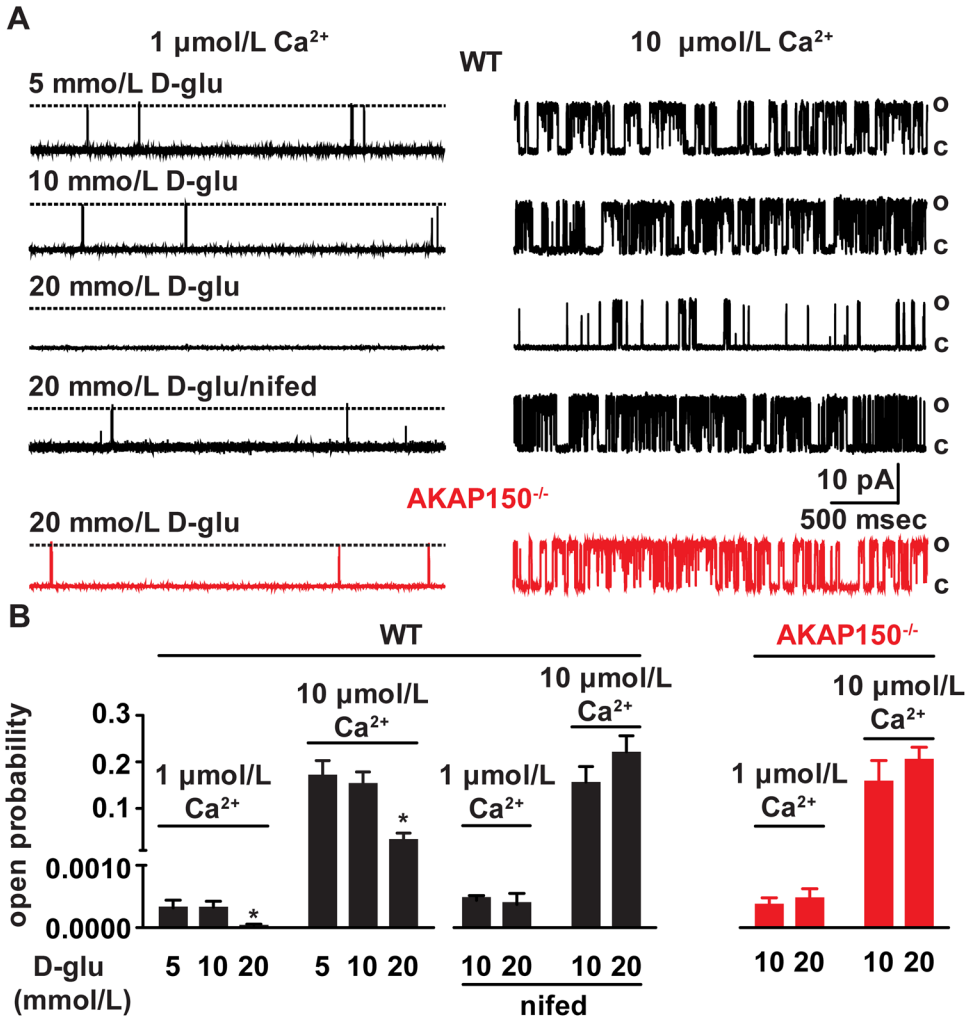

Figure 4. Nifedipine and ablation of AKAP150 prevent decreased BK_{Ca} channel function by elevated glucose

A Exemplar single BK_{Ca} currents recorded from excised membrane patches of myocytes isolated from WT and AKAP150$^{-/-}$ cerebral arteries incubated (48 hours) in specified D-glucose concentration in presence and absence of nifedipine (1 μmol/L) at −40 mV and in the presence of 1 and 10 μmol/L free Ca^{2+} bath solution, (C: closed; O: open). ***B*** Bar plot summarizing BK_{Ca} channel P_o in myocytes from WT arteries incubated in presence of 5 (n=8 cells), 10 (n=7 cells), and 20 mmol/L (n=8 cells) D-glucose, and 10 (n=11 cells) and 20 mmol/L (n=11 cells) D-glucose + nifedipine, and from AKAP150$^{-/-}$ arteries incubated in 10 (n=12 cells) and 20 mmol/L (n=11 cells) D-glucose at indicated Ca^{2+} concentrations. *P < 0.05.

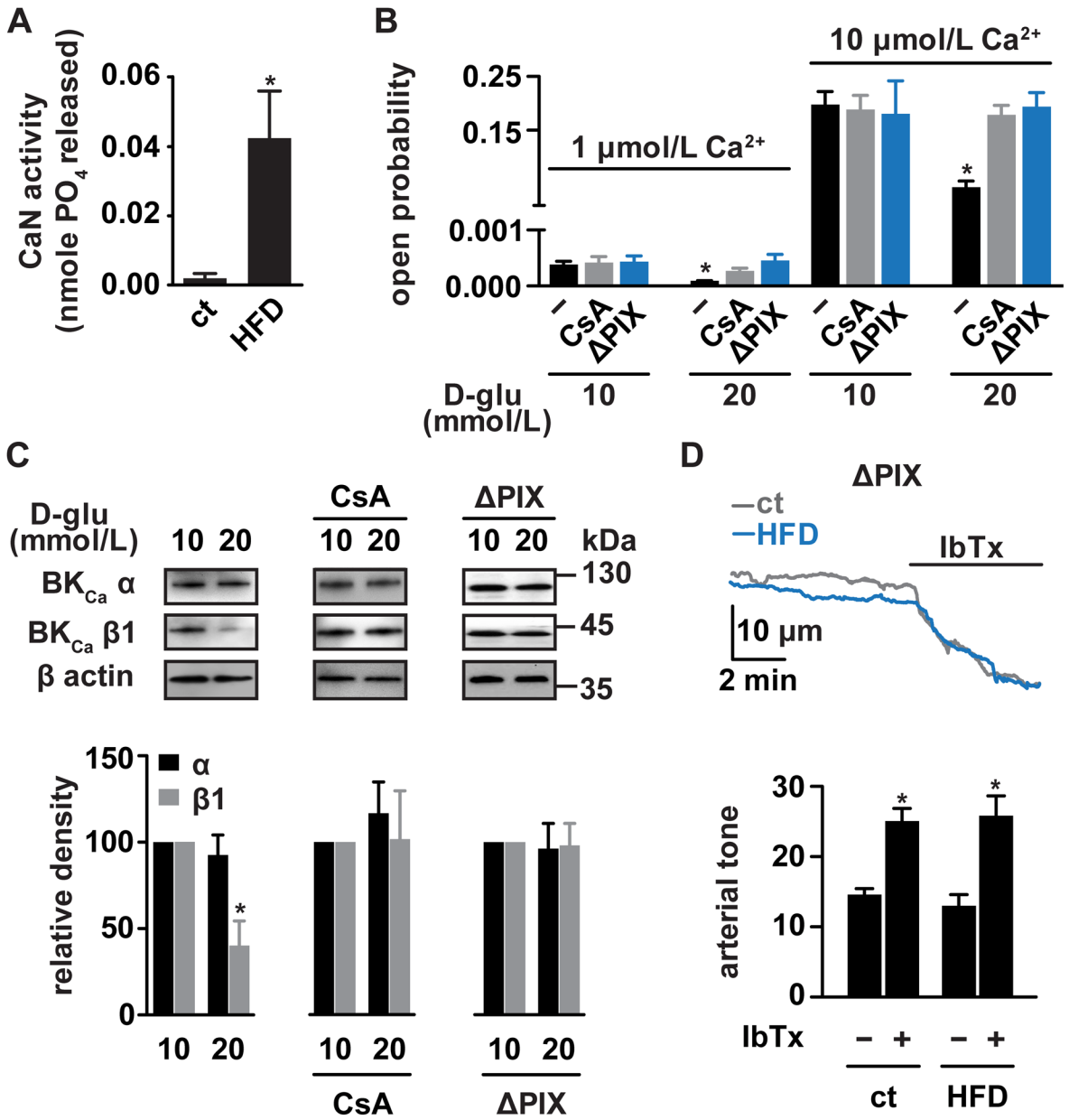

Figure 5. Anchoring of calcineurin by AKAP150 is necessary for BK_{Ca} channel suppression and impaired vasoconstriction in response to elevated glucose and HFD

A Cellular CaN activity in WT ct (n=4 from 4 animals) and HFD (n=4 from 4 animals) mesenteric arteries. ***B*** Bar plot summarizing BK_{Ca} channel P_o from WT cerebral arteries incubated in 10 and 20 mmol/L D-glucose in the absence (DMSO ct; n=11 and 10 cells, respectively) and presence of CsA (1 μM; n=10 and 6 cells, respectively). Also shown are summary data for ΔPIX arteries incubated in 10 (n=13 cells) and 20 mmol/L (n=11 cells) D-glucose. ***C*** Representative Western blots (*top*) and summary densitometric data (*bottom*) showing immunoreactive bands corresponding to BK_{Ca} α and β1 subunits and β actin for WT arteries maintained in 10 and 20 mmol/L D-glucose in the absence (n=4) and presence (n=4) of 1 μmol/L CsA and ΔPIX arteries (n=5) maintained in 10 and 20 mmol/L D-glucose. ***D*** Representative diameter recordings (*top*) and summary vascular tone data (*bottom*) from pressurized (60 mmHg) ΔPIX ct (n=9 from 6 animals) and HFD (n=8 from 5 animals)

cerebral arteries in the absence (−) and presence (+) of IbTx (100 nmol/L; passive diameters for representative recordings = 143 μm and 145 μm, respectively). *$P < 0.05$.

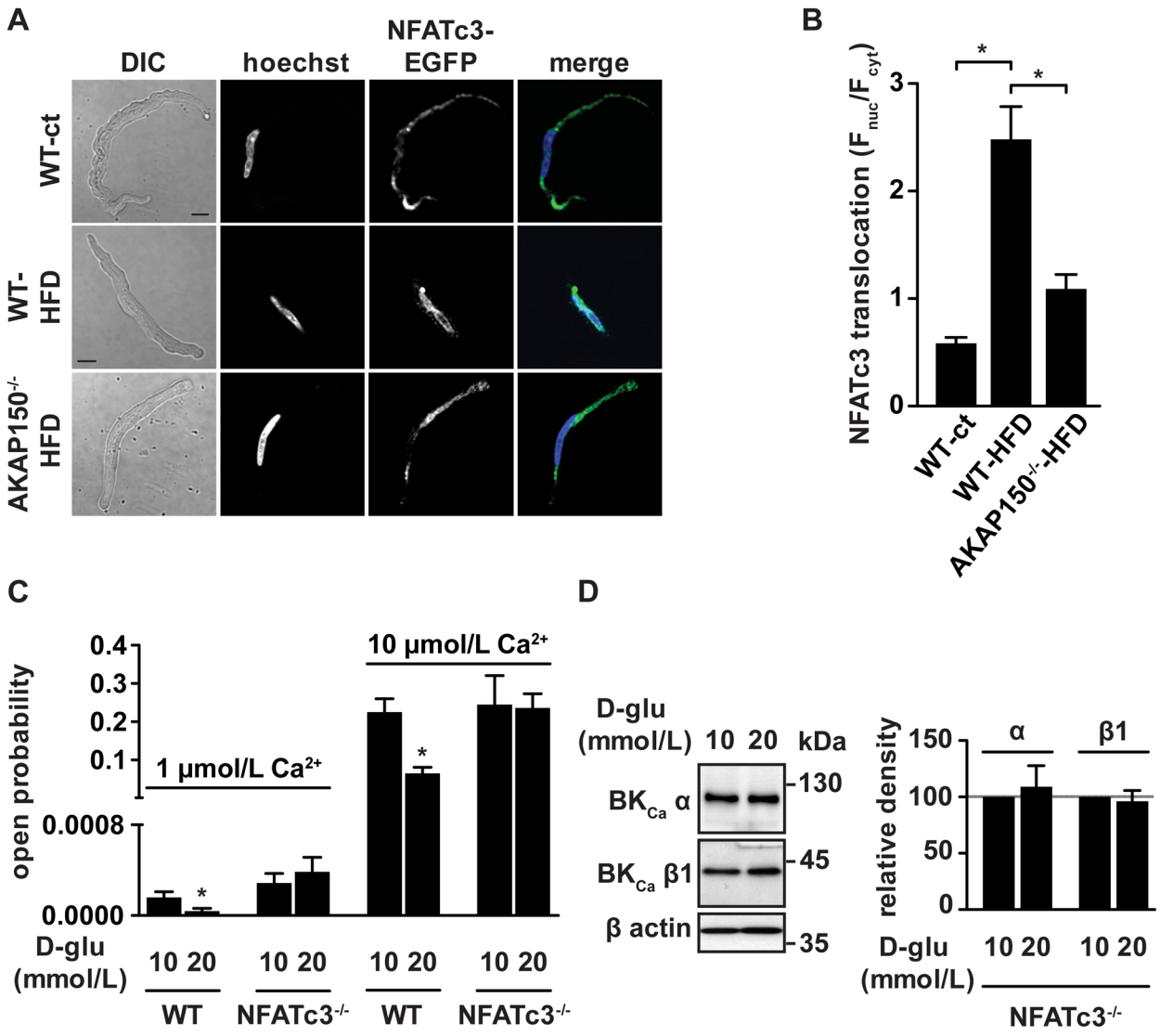

Figure 6. Activation of NFATc3 signaling is necessary for BK_{Ca} channel suppression in response to elevated glucose and HFD, and proceeds through AKAP150

A Transmitted light and confocal images of ct and HFD WT and AKAP150$^{-/-}$ mesenteric artery myocytes expressing NFATc3-EGFP. ***B*** Bar plot summarizing NFATc3-EGFP nuclear translocation ($F_{nuclear}/F_{cytosolic}$) in WT ct (n=44 from 3 animals) and HFD (n=49 from 3 animals) and AKAP150$^{-/-}$ HFD (n=51 from 3 animals) myocytes. ***C*** Bar plot of BK_{Ca} channel P_o in WT (BalbC) and NFATc3$^{-/-}$; myocytes maintained in 10 (WT=6 cells; NFATc3$^{-/-}$=9 cells) or 20 mmol/L (WT=10 cells; NFATc3$^{-/-}$=11 cells) D-glucose. ***D*** Representative Western blots (*top*) and corresponding densitometric summary data (*bottom*) for BK_{Ca} α and β1 subunits from NFATc3$^{-/-}$ arteries maintained in 10 and 20 mmol/L D-glucose (n=5 each). *$P < 0.05$.

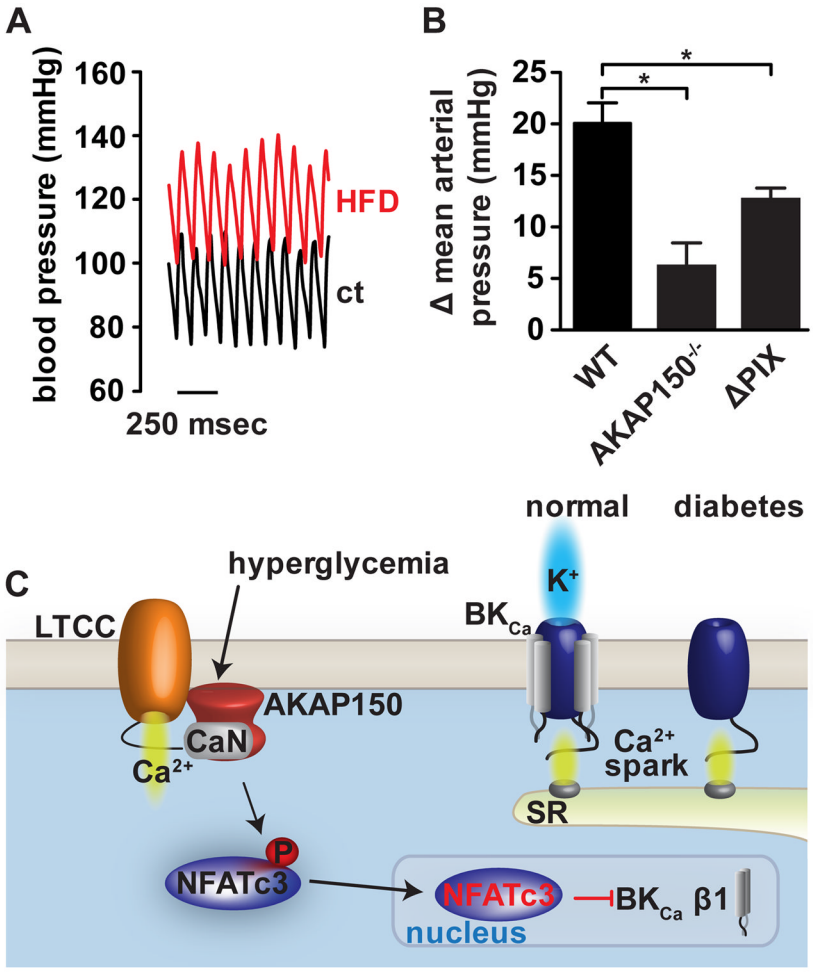

Figure 7. Ablation of AKAP150 or disruption of the interaction between CaN and AKAP150 attenuates HFD-induced elevation of blood pressure

A Representative telemetric blood pressure waveforms for WT ct and HFD mice. ***B*** Bar plot of the change in mean arterial pressure for HFD versus corresponding ct for WT, AKAP150$^{-/-}$ and ΔPIX mice (n=3 animals per condition). *$P < 0.05$. ***C*** Proposed model for AKAP150-dependent suppression of BK_{Ca} channels in arterial myocytes during hyperglycemia and diabetes.