

# Impaired Vascular BK Channel Function in Type 2 Diabetes Mellitus

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## 1. Introduction

Diabetes mellitus has become a global epidemic. According to the World Health Organization estimate, about 285 millions people worldwide, corresponding to 6.4% of the world's population, have diabetes in 2010. By 2030, this figure will be more than doubled (<http://www.worlddiabetesday.org/media/press-materials/diabetes-data>).

Diabetes mellitus is a major cause of morbidity and mortality and is associated with increased risks of cardiovascular diseases, stroke, nephropathy, neuropathy, retinopathy and other microvascular complications. Type 2 diabetes mellitus is characterized by obesity, glucose intolerance, insulin resistance, hyperinsulinemia, hyperglycemia, dyslipidemia and hypertension, and accounts for 90% of the total cases of diabetes mellitus. Although the clinical course of type 2 diabetes is usually less aggressive compared to its type 1 counterpart, the end results are equally devastating even with intensive glycemic control.

The causes of diabetic vascular dysfunction are multifactorial, and involve endothelial-dependent and -independent mechanisms. The role of endothelial-dependent vascular dysfunction in diabetes is well-known, and it is related to increased activity/bioavailability of vasoconstrictors such as reactive oxygen species (ROS), reactive nitrogen species (RNS), endothelin-1 (ET-1), angiotensin II (Ang II) and thromboxane A<sub>2</sub> (TXA<sub>2</sub>), and reduced activity/bioavailability of endothelium-derived relaxing factors (EDRFs) such as nitric oxide (NO), carbon monoxide (CO), prostacyclin (PGI<sub>2</sub>) and endothelium-derived hyperpolarizing factors (EDHFs) (Avogaro et al., 2006; De Vriese et al., 2000; Xu & Zou, 2009). The role of endothelial-independent vascular dysfunction in diabetes mellitus, however, has received less attention, and it is by no means less important, because vascular smooth muscle physiology is profoundly modulated by diabetes mellitus.

A major ionic mechanism that facilitates vascular smooth muscle relaxation is the activation of the large conductance Ca<sup>2+</sup>-activated K<sup>+</sup> (BK) channels. Because of their large conductance and high density in vascular smooth muscle cells, BK channels are a key determinant of vascular tone, regulating tissue perfusion in response to changes in membrane potential and intracellular Ca<sup>2+</sup> homeostasis (Ledoux et al., 2006). Substantial experimental and clinical evidence exists indicating that vascular BK channel function is impaired in type 2 diabetes (Feng et al., 2008; Liu et al., 2008). Multiple mechanisms are known to produce BK channel dysfunction in diabetes mellitus. In this article, we will describe the cellular and molecular mechanisms that underlie vascular BK channel dysfunction in type 2 diabetes. We will also provide a detailed treatise on the altered BK channel gating associated with type 2 diabetes.

## 2. Vascular BK channel structure and function

The BK channel  $\alpha$  subunit is encoded by the Slo1 gene ( $K_{Ca1.1}$ , KCNMA1) and the functional channel has a homotetrameric assembly. The BK- $\alpha$  subunit shares homology with all voltage-gated  $K^+$  channels containing a backbone of six transmembrane domains (S1 to S6) in which the S1-S4 constitute the voltage-sensing unit and the S5-P loop-S6 form the ion permeation domain which encompasses the conserved  $K^+$  selectivity filter (TVGYG) (Cui et al., 2009; Ma et al., 2006). In addition, it has unique structural features. It has an additional transmembrane domain, S0, so the N-terminus is extracellular, and the C-terminus has 4 hydrophobic segments (S7 to S10) that contain two regulators of conductance for potassium (RCK1 and RCK2) (Fig. 1) (Jiang et al., 2002). Functionally, two high-affinity  $Ca^{2+}$  sensing regions with  $Ca^{2+}$  concentration at half-maximal effect ( $EC_{50}$ ) in the  $10^{-6}$  M range have been proposed. One is the  $Ca^{2+}$  bowl (889-QFLDQDDDD-897) in RCK2 (Bao et al., 2004; Schreiber et al., 1999; Xia et al., 2002) and the other (D362/D367) is located in RCK1 (Xia et al., 2002; Zeng et al., 2005). The RCK1s and RCK2s form the homotetrameric channel form an octameric gating ring which regulates  $K^+$  efflux through allosteric control by the  $Ca^{2+}$ -bowl and the voltage sensor (Yuan et al., 2010). The extracellular N-terminus of BK- $\alpha$  subunit is important for functional coupling with BK- $\beta$  subunit (Meera et al., 1997). In fact, the BK- $\alpha$  subunit S0, S1, S2, S3, and S6 are all implicated for functional and physical interaction with BK- $\beta$  subunits (Lee & Cui, 2010; Morrow et al., 2006; Orio et al., 2006).

The BK- $\beta_1$  subunit is the predominant subtype in vascular smooth muscle cells. It contains two transmembrane (TM1 and TM2) domains connected by a relatively large extracellular loop which can reach the inner mouth of the channel central pore, and can modulate scorpion toxin and tetraethylammonium (TEA) binding and regulate channel permeability (Hanner et al., 1997; Meera et al., 2000; Shen et al., 1994). The TM1 is thought to interact with the S2 of an adjacent BK- $\alpha$  subunit and the TM2 with S0 of another adjacent BK- $\alpha$  subunit (Fig. 1) (Liu et al.,). BK- $\beta_1$  subunits are abundantly expressed in vascular smooth muscle cells. BK channel activity is profoundly regulated by BK- $\beta_1$  which significantly enhances the channel voltage- and  $Ca^{2+}$ -sensitivity (Cox & Aldrich, 2000; McManus et al., 1995; Meera et al., 1996; Xia et al., 1999), modulates channel kinetics (Nimigeon & Magleby, 1999; Tanaka et al., 1997; Zeng et al., 2003) and stabilizes BK- $\alpha$  expression (Toro et al., 2006). The importance of BK- $\beta_1$  subunits in the regulation of vascular physiology is underscored by the  $\beta_1$  subunit knockout mice, in which  $Ca^{2+}$  sparks are uncoupled to BK channels in the vascular smooth muscle cells, and these animals are hypertensive (Brenner et al., 2000; Plugger et al., 2000). In addition, there is a compensatory increase in vascular BK- $\beta_1$  expression in spontaneously hypertensive rats (Chang et al., 2006), while a gain-of-function mutation in BK- $\beta_1$  (E65K) is associated with low prevalence of diastolic hypertension in humans (Fernandez-Fernandez et al., 2004; Kelley-Hedgpeeth et al., 2008; Nielsen et al., 2008) and with reduced risk of myocardial infarction and stroke, particularly in elderly women (Senti et al., 2005).

BK channels maintain smooth muscle cell  $Ca^{2+}$  homeostasis and regulate vascular tone through a negative feedback mechanism. Activation of the voltage-gated  $Ca^{2+}$  channels in vascular smooth muscle cells triggers  $Ca^{2+}$  release from the sarcoplasmic reticulum ( $Ca^{2+}$  sparks) which activates the BK channels in its vicinity and gives rise to the spontaneous transient outward currents (STOCs). STOCs hyperpolarize the cellular membrane potential, which in turn inactivates the voltage-gated  $Ca^{2+}$  channels, thereby relaxes the vascular smooth muscles (Brenner et al., 2000; Lohn et al., 2001; Plugger et al., 2000). In addition, the presence of splice variants of BK- $\alpha$  subunits (Xie & McCobb, 1998) contributes to the diversity of BK channel function in the body.

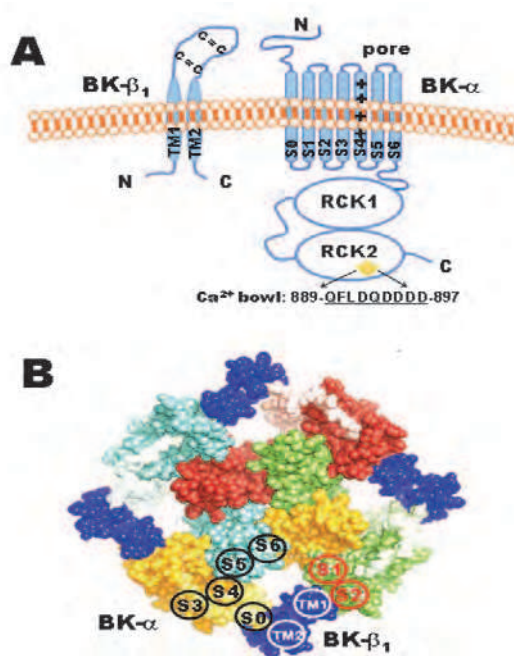


Fig. 1. Vascular BK channel structure. A: Membrane topology of BK- $\alpha$  and BK- $\beta_1$  subunits. Domain boundaries and the Ca<sup>2+</sup> bowl are indicated. S0-S6 corresponds to the seven transmembrane domains of BK- $\alpha$  subunit; TM1 and TM2 represent the transmembrane domains of BK- $\beta_1$  subunit. B: Orientation of BK- $\alpha$  and BK- $\beta_1$  subunits in tetrameric BK channels, where the TM1 interacts with the S0 of adjacent BK- $\alpha$  and the TM2 interacts with the S2 of another adjacent BK- $\alpha$ . (Fig. 1B was adapted from Liu et al., 2010).

### 3. Regulation of vascular BK channel activity by signaling molecules

BK channels are targets of many signaling molecules and biological vasoactive mediators, which include protein kinases (Barman et al., 2004; Chae et al., 2005; Schopf et al., 1999; Tian et al., 2004), protein tyrosine kinases (Alioua et al., 2002; Lu et al., 2010), phospholipids (Vaithianathan et al., 2008), polyunsaturated fatty acid metabolites of the cytochrome P-450 epoxygenase (Campbell et al., 1996; Lauterbach et al., 2002; Lu et al., 2001; Wang et al. 2011; Zhang et al., 2001), the lipoxygenase (Obara et al., 2002; Zink et al., 2001) and the cyclooxygenase pathways (Burnette and White, 2006; Tanaka et al., 2004; Yamaki et al., 2001), reactive oxygen species (ROS) (Lu et al., 2006; Tang et al., 2004), reactive nitrogen species (RNS) (Liu et al., 2002; Lu et al., 2006), nitric oxide (NO) (Mandala et al., 2007; Wu et al., 2002), carbon monoxide (CO) (Dong et al., 2007; Wu et al., 2002), heme (Jaggar et al., 2005; Tang et al., 2003), angiotensin II (Ang II) (Minami et al., 1995; Zhang et al., 2010), endothelin -1 (ET-1) (Minami et al., 1995) and steroid hormones (Han et al., 2008; Lovell et al., 2004). It is worthwhile to point out that the regulation of BK channels by these signaling molecules is frequently complicated by the exhibition of signal cross-talk, with species and tissue specificity.

#### 4. Impaired vascular BK channel function in the early stages of type 2 diabetes – Deficiency in the bioavailability of BK channel activating vasodilators

A commonly used animal model for the study of type 2 diabetes is the Zucker Diabetic Fatty (ZDF) rats, which are derived from selective inbreeding of Zucker Obese rats with the highest blood glucose levels (Shafir, 1992). These animals exhibit many features found in patients with non-insulin dependent diabetes mellitus, including obesity, insulin resistance, hyperglycemia, hypertriglyceridemia, hypercholesterolemia (Corsetti et al., 2000; Shafir, 1992), and microvascular pathology (Oltman et al., 2009; Oltman et al., 2008; Yang et al., 2000). ZDF rats have been used for studying insulin resistance (Kuhlmann et al., 2003; Srinivasan and Ramarao, 2007; Zhou et al., 1999), and vascular dysfunction (Oltman et al., 2009; Oltman et al., 2008; Zhou et al., 2005). We found that vascular BK channel function is impaired in ZDF rats and that the culprits change with progression of the disease.

In the early stages of diabetes development (2 to 4 weeks with blood glucose >300 mg/dl), BK channel-mediated vasodilatation in ZDF rats was impaired. Fig. 2A shows that arachidonic acid (AA) produced 50% less dilatation in the isolated coronary arteries from ZDF rats, compared to those from Lean control rats. The AA effects were significantly inhibited by preincubation with indomethacin (the cyclooxygenase inhibitor) in Lean rat vessels but not in ZDF rat vessels. Exposure of freshly isolated coronary smooth muscle cells to 1  $\mu\text{M}$  AA produced a 4-fold increase in whole-cell  $\text{K}^+$  currents in Lean rats, while these effects were significantly blunted in those from ZDF rats (Fig. 2B). The effects of AA on  $\text{K}^+$  current activation were inhibited by preincubation with indomethacin, suggesting that the vasoactive molecules were cyclooxygenase products of AA (Lu et al., 2005).

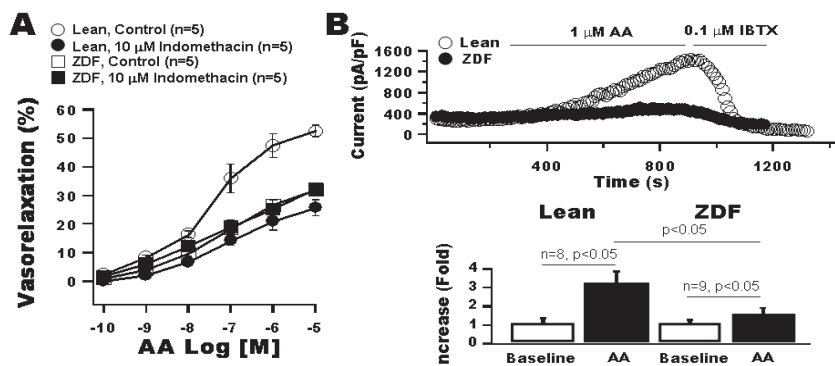


Fig. 2. Reduced arachidonic acid (AA)-mediated dilatation of coronary arteries and BK channel activation of coronary arterial smooth muscle cells from ZDF rats with 8 weeks of diabetes. A: Effects of AA on coronary arterial relaxation in Lean and ZDF rats with and without a 30-min incubation with indomethacin (10  $\mu\text{M}$ ). Compared to Lean rats, AA-mediated vasorelaxation was diminished in ZDF rats and the AA effects were abolished by preincubation with indomethacin. B: Time course of the effect of 1  $\mu\text{M}$  AA on coronary smooth muscle  $\text{K}^+$  currents in Lean control rats and ZDF rats. Group results on the increase in BK current density (iberiotoxin sensitive component) before and after exposure to AA are represented by the bar graphs. (adapted from Lu et al., 2005)

The reduced AA-induced vasodilatation and diminished BK channel activation resulted from deficient PGI<sub>2</sub> bioavailability in the ZDF vasculature (Lu et al., 2005). Protein expression of PGI<sub>2</sub> synthase (PGIS) was down-regulated by 65% in the coronary arteries of ZDF rats (Fig. 3A), leading to a 6.8-fold reduction in the conversion of AA to 6-keto-PGF<sub>1 $\alpha$</sub> , the stable product of PGI<sub>2</sub> metabolism, in ZDF vessels (Fig. 3B). Exposure to the stable PGI<sub>2</sub> analog, iloprost (1  $\mu$ M), produced similar BK channel activation in coronary smooth muscle cells from Lean control rats and ZDF rats, indicating that the ability of BK channels to respond to agonist activation was intact.

The biophysical properties of BK channel were intact during the early stages of diabetes in ZDF rats. Whole-cell BK current density and current-voltage relationships were not different between coronary smooth muscle cells from Lean control and ZDF rats (Fig. 4A and 4B). Determination of BK channel sensitivity to voltage- and Ca<sup>2+</sup>-mediated activation of single channels in inside-out excised membrane patches also showed similar opening probability (P<sub>o</sub>)-voltage and P<sub>o</sub>-Ca<sup>2+</sup> relationships between Lean and ZDF rats (Fig. 4C and 4D). There was no significant difference in the voltage at half maximal activation (V<sub>0.5</sub>) or in the equivalent charge movement (z) value between Lean and ZDF rats. The Ca<sup>2+</sup> EC<sub>50</sub> and the Hill coefficient (which reflects the cooperativity of Ca<sup>2+</sup> binding) for the P<sub>o</sub>-Ca<sup>2+</sup> curves were likewise similar between the two groups, suggesting that in the early stage of type 2 diabetes, the voltage- and Ca<sup>2+</sup>-dependent activation of BK channels were intact in ZDF rats.

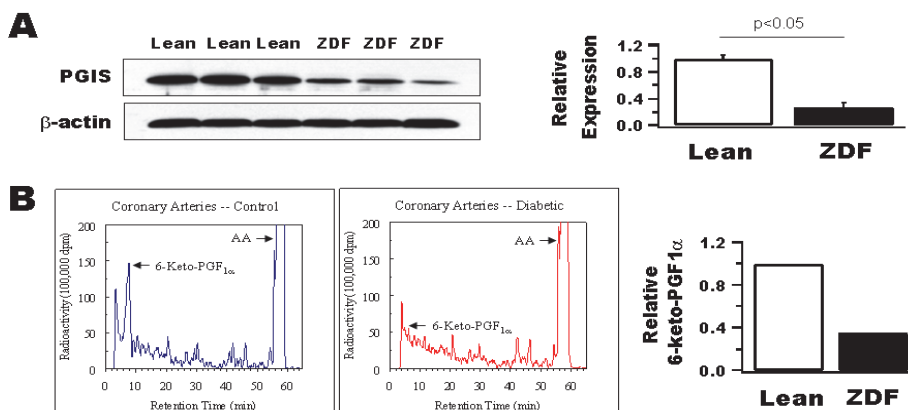


Fig. 3. Decreased PGI<sub>2</sub> synthase (PGIS) expression and PGI<sub>2</sub> production in coronary arteries from ZDF rats. A: Immunoblot with statistical analysis of PGIS expression in arteries of Lean and ZDF rats. B: Analysis of AA metabolism in coronary arteries from Lean rats and ZDF rats. Isolated vessels from 3 pairs of Lean and ZDF rats were incubated with 5  $\mu$ M [<sup>3</sup>H] AA (specific activity 1  $\mu$ Ci/nM) for 1 h at 37°C. Lipids were extracted and analyzed by HPLC. The major peak at 7.5 min has the same retention time as a 6-keto-PGF<sub>1 $\alpha$</sub>  standard, the stable product of PGI<sub>2</sub> that was significant decreased in ZDF rat vessels. (adapted from Lu et al., 2005).

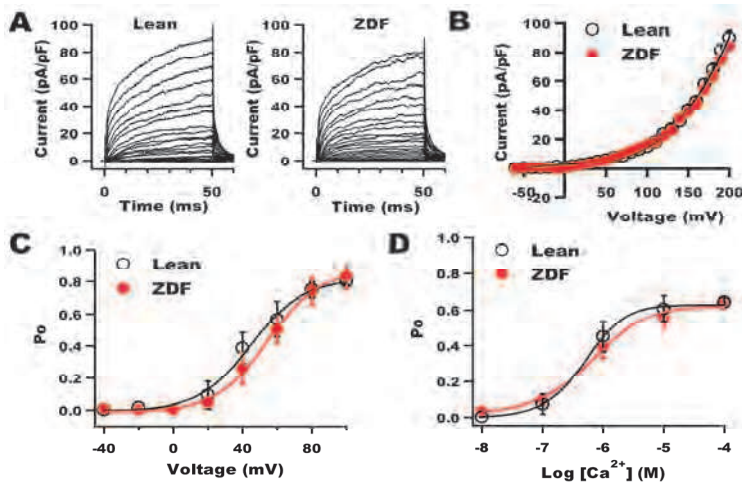


Fig. 4. Normal BK channel activity from the coronary smooth muscle cells of ZDF rats with 8-week development of hyperglycemia. A: Representative tracings of whole-cell BK currents (iberiotoxin-sensitive components) from freshly isolated coronary smooth muscle cells of Lean and ZDF rats. BK currents were elicited with 10 mV increments from  $-40$  mV to  $+200$  mV with a holding potential of  $-60$  mV in the presence of  $0.2 \mu\text{M}$  free  $\text{Ca}^{2+}$  in the pipette solution. B: The current-voltage (I-V) relationships of BK channels from Lean and ZDF rats. C:  $\text{Ca}^{2+}$  dose-dependent curves obtained from inside-out single BK channel currents recorded at  $+60$  mV from coronary smooth muscle cells of Lean and ZDF rats. D: The open probability-voltage (Po-V) relationship obtained from inside-out single BK channel currents of Lean and ZDF rats in the presence of  $1 \mu\text{M}$  free  $\text{Ca}^{2+}$  in the bath solution. There were no significant differences in current density,  $\text{Ca}^{2+}$ -sensitivity and voltage-sensitivity of BK channels between ZDF rats and age-matched Lean rats.

We also found that AA-induced dilatation was impaired in the small mesenteric arteries of ZDF rats at 4 weeks after the development of diabetes. The effects of AA were dependent on lipoxygenase activity and ZDF vessels showed an 81% downregulation in 12-lipoxygenases protein expression accompanied by a 54% reduction in AA conversion to its vasoactive product, 12-hydroxyeicosatetraenoic acid (Zhou et al., 2005). Moreover, AA-mediated vasodilatation in Lean rats was partially abolished by iberiotoxin, while exogenous application of 12-hydroxyeicosatetraenoic acid produced similar vasodilatation in Lean control and ZDF rat vessels, suggesting that the impaired AA-induced dilatation in mesentery arteries of ZDF rats is due to the deficiency of 12-lipoxygenase generated vasodilating metabolites (Zhou et al., 2005). Hence, during the early stages of type 2 diabetes, a common feature that impairs BK channel-mediated vasodilatation is the reduced bioavailability of BK channel activating vasodilators.

## 5. Impaired vascular BK channel function in the advanced stages of type 2 diabetes – Altered channel intrinsic biophysical properties

### 5.1 Reduced $\text{Ca}^{2+}$ -dependent BK channel activation in ZDF rats with advanced diabetes

With further progression in type 2 diabetes, the biophysical properties of BK channel were altered, giving rise to BK channelopathy. Fig. 5A illustrates the normalized BK channel Po-V

curves in the coronary smooth muscle cells from ZDF rats with 8 months of hyperglycemia and from age-matched Lean control rats. Inside-out BK currents were elicited from freshly isolated coronary smooth muscle cells in the absence of  $\text{Ca}^{2+}$  and in the presence of  $1 \mu\text{M}$  free  $\text{Ca}^{2+}$  in the bath solution. Without  $\text{Ca}^{2+}$ , the Po-V relationships from Lean and ZDF rats were identical, indicating that the intrinsic voltage-dependent activation of BK channels remained unchanged. In the presence of  $1 \mu\text{M}$  free  $\text{Ca}^{2+}$ , the Po-V relationships were leftward shifted in both Lean and ZDF rats, but there was a significant lag in the effects of  $\text{Ca}^{2+}$  on the shift in the Po-V relationship in ZDF rats, suggesting a decreased  $\text{Ca}^{2+}$ -dependent BK channel activation in these animals. Changes in the intrinsic free energy of  $\text{Ca}^{2+}$ -binding ( $\Delta\Delta\text{Ca}^{2+}$ ) that contributes to BK channel activation can be estimated, based on the shift of Po-V relationship from 0 to  $1 \mu\text{M}$  free  $\text{Ca}^{2+}$  in Lean and ZDF rats, using the equation:  $\Delta\Delta\text{Ca}^{2+} = -\Delta(z e V_{0.5})$ , where  $z$  is the number of equivalence charge movement,  $e$  is the elementary charge (Shi et al., 2002). There was a 62.3% reduction in the change in free energy for  $\text{Ca}^{2+}$ -binding to BK channels in ZDF rats. Any decrease in the free energy for  $\text{Ca}^{2+}$ -binding must be associated with reduced  $\text{Ca}^{2+}$ -sensitivity and/or  $\text{Ca}^{2+}$  cooperativity in BK channel function. These results indicated that  $\text{Ca}^{2+}$ -dependent activation was less favorable in ZDF rats at an advanced stage of type 2 diabetes.

Since the intrinsic voltage-sensitivity of BK channel was not significantly changed in ZDF rats 1 to 8 months after developing hyperglycemia, according to our experimental results (unpublished observations), the  $\text{Ca}^{2+}$   $\text{EC}_{50}$  value can be used to evaluate BK channel  $\text{Ca}^{2+}$ -sensitivity. Fig. 5B shows the  $\text{Ca}^{2+}$  dose-dependent curves of coronary arterial BK channel activation from Lean and ZDF rats with 6 months of diabetes. In ZDF rats, there was reduced maximal channel Po, a rightward shifted Po-V relationship with a smaller value of the Hill coefficient, compared to those in Lean rats. Hence, impaired BK channel function in ZDF rats at advanced stages of type 2 diabetes was due to reduced free energy for  $\text{Ca}^{2+}$  binding to the channel with reduced  $\text{Ca}^{2+}$  cooperativity and reduced sensitivity to  $\text{Ca}^{2+}$ -mediated activation.

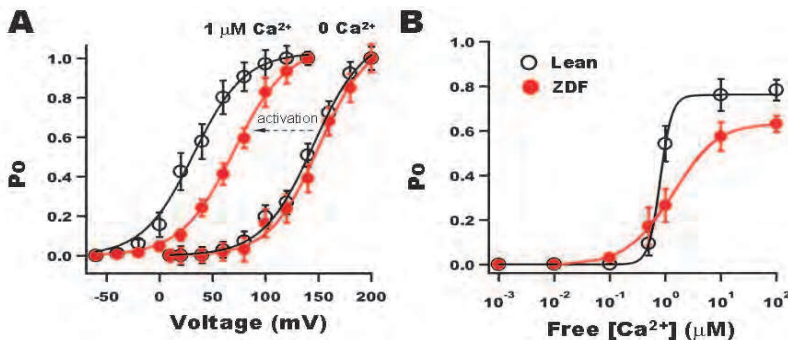


Fig. 5. Impaired  $\text{Ca}^{2+}$ -dependent BK channel activation in the coronary smooth muscle cells from ZDF rats 8 months after development of diabetes. A: The Po-V relationships of BK channels from ZDF rats and age-matched Lean rats in the absence of and in the presence of  $1 \mu\text{M}$  free  $\text{Ca}^{2+}$  in the bath solution. Less  $\text{Ca}^{2+}$ -dependent leftward shift was observed in ZDF rats, compared with Lean rats. B:  $\text{Ca}^{2+}$  dose-dependent curve of BK channel activation from Lean and ZDF rats. Reduced maximal channel Po, increased  $\text{Ca}^{2+}$   $\text{EC}_{50}$  and decreased slope steepness were found in ZDF rats, indicating that BK channel  $\text{Ca}^{2+}$ -sensitivity and  $\text{Ca}^{2+}$ -cooperativity were impaired in ZDF rats. (adapted from Lu et al., 2008).

## 5.2 Altered vascular BK channel kinetics in ZDF rats with advanced diabetes

To better understand the altered  $\text{Ca}^{2+}$ -dependent BK channel activation in ZDF rats, we examined the  $\text{Ca}^{2+}$ -dependent gating properties in ZDF rats and age-matched Lean rats. We compared single channel gating between Lean and ZDF rats at various  $\text{Ca}^{2+}$  concentrations from 1  $\mu\text{M}$  to 100  $\mu\text{M}$  with a testing potential of +60 mV. Fig. 6 illustrates typical tracings of inside-out single-channel BK currents in Lean and ZDF rats with expanded details. In the presence of 1  $\mu\text{M}$   $\text{Ca}^{2+}$ , BK channel  $P_o$  was much higher in Lean rats than in ZDF rats. An increase of  $\text{Ca}^{2+}$  to 10 and 100  $\mu\text{M}$  markedly increased the channel  $P_o$  in both Lean (Fig. 6A) and ZDF rats (Fig. 6B). However, increased cytoplasmic  $\text{Ca}^{2+}$  enhanced  $P_o$  in Lean rats by significantly prolonging the mean channel open durations without altering the channel mean closed durations. In contrast, increased cytoplasmic  $\text{Ca}^{2+}$  augmented  $P_o$  in ZDF rats by significantly abbreviating channel mean closed durations without a marked increase in channel mean open durations (Fig. 6C and 6D). These results indicated that there was an altered gating response to activation by  $\text{Ca}^{2+}$  in vascular BK channels in ZDF rats. Because normal intracellular  $\text{Ca}^{2+}$  concentration can reach >10  $\mu\text{M}$ , especially in the vicinity of the microdomains where calcium sparks are elicited, these fundamental changes in BK channel properties are physiologically relevant.

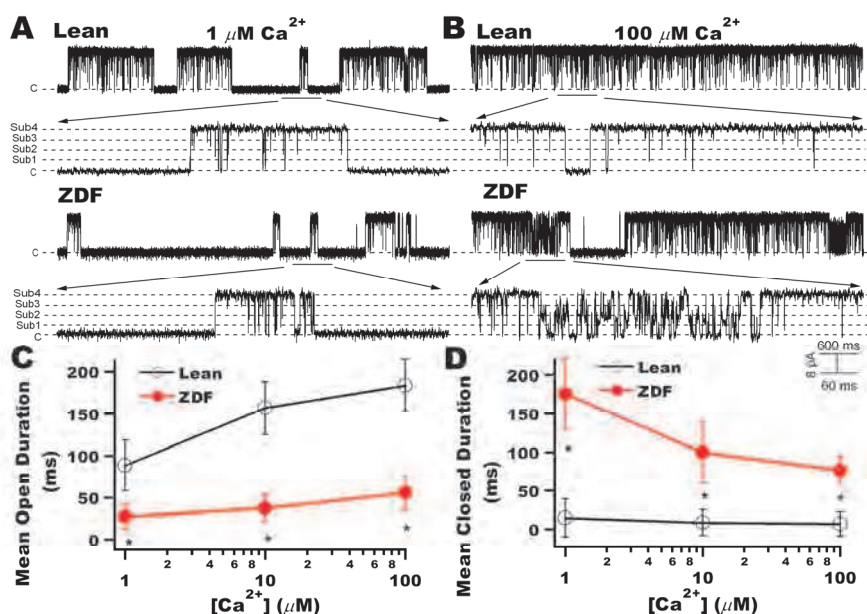


Fig. 6. Altered single BK channel openings in ZDF rats 6 months after development of diabetes. Representative inside-out single BK channel currents were recorded at +60 mV from freshly isolated coronary smooth muscle cells of ZDF rats and age-matched Lean rats in the presence of 1  $\mu\text{M}$   $\text{Ca}^{2+}$  (A) and 100  $\mu\text{M}$   $\text{Ca}^{2+}$  (B). Plots of relationships between  $\text{Ca}^{2+}$  concentrations and mean burst durations (C) and mean closed times (D) of BK channels in Lean and ZDF rats are shown. Compared with Lean rats, ZDF rats had shorter mean burst open durations and longer mean closed durations. Data are presented as mean  $\pm$  SE. \* $p < 0.05$  vs. Lean ( $n = 6$ ). (adapted from Lu et al., 2008).



BK channel gating kinetics is known to contain multiple components of open and closed dwell-times. Based on single BK channel kinetic analysis from our group and other laboratories, the best fit of the open dwell-time distribution histograms showed three components: fast ( $\tau_{o1}$ ), intermediate ( $\tau_{o2}$ ) and slow ( $\tau_{o3}$ ); the closed dwell-time distribution histograms showed four components: fast ( $\tau_{c1}$ ), intermediate ( $\tau_{c2}$ ), slow ( $\tau_{c3}$ ) and very slow ( $\tau_{c4}$ ) (Fig. 7) (Lu et al., 2001; Lu et al., 2008; McManus & Magleby, 1988; McManus & Magleby, 1991). Compared to Lean rats, BK channels from the coronary arterial smooth muscle cells of ZDF rats had shorter open dwell-times and longer closed dwell-times, in agreement with the lower channel opening probability observed in ZDF rats (Fig. 5). These changes in BK channel gating were consistent with reduced free energy for  $\text{Ca}^{2+}$ -dependent channel activation, favoring BK channel closure in ZDF rats.

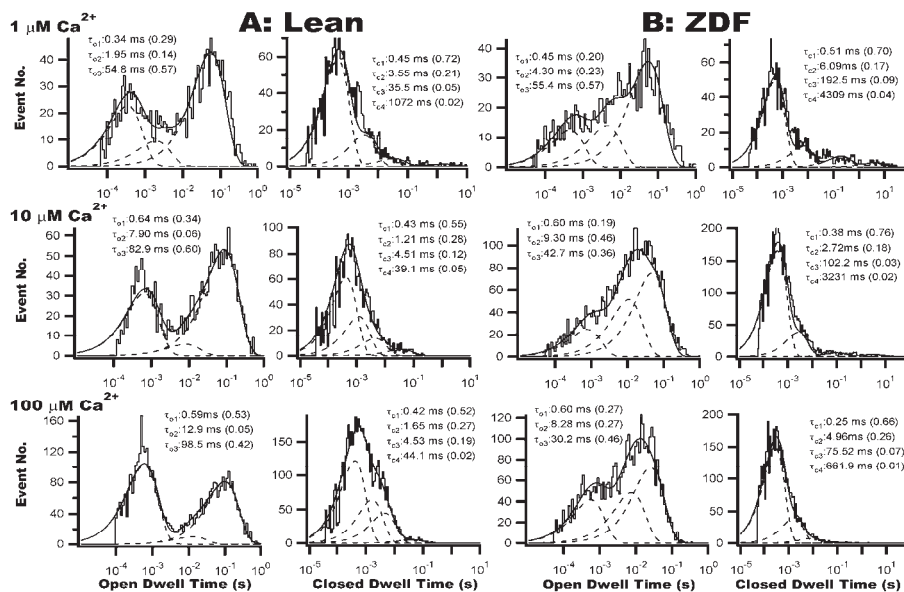


Fig. 7. Altered  $\text{Ca}^{2+}$ -dependent kinetics of BK channel from ZDF rats after 8 months of diabetes. Representative histograms of single BK channel open and closed dwell-time durations in the presence of  $1 \mu\text{M}$ ,  $10 \mu\text{M}$  and  $100 \mu\text{M}$  free  $\text{Ca}^{2+}$  in the bath solution. Dwell-time distributions were best fitted with three open dwell-time components ( $\tau_o$ ) and four closed dwell-time components ( $\tau_c$ ). The values of each time constants and its relative weight (in parentheses) are shown in each histogram. Dashed lines represent distribution of exponential components, determined by the logarithm likelihood ratio test.

An intriguing observation during single BK channel recordings in coronary smooth muscle cells from ZDF rats with 8 months of diabetes was the conspicuous increased encounter of subconductance openings (Fig. 8A). Amplitude histograms fitted with a Gaussian function clearly showed four levels of subconductance and channel state transition appeared to be slow with subconductance constituting  $\frac{3}{4}$  of full channel opening seen 20% of the time (Fig. 8B and 8C), while BK channel subconductance openings was less frequently observed in Lean rats. Although the underlying mechanism of BK channel subconductance openings is not fully

understood, this may be due to the conformational changes that each subunit of the tetrameric channel has to make from a closed state to an open state as the channel opens (Chapman & VanDongen, 2005). Normally, such transitions of conformational states are too transient to be discerned (Ferguson et al., 1993). However, conditions that cause slowing of the tetrameric conformational transitions would result in prolonged sojourn of intermediate state conformations and lead to discernible subconductance openings. The reduced  $\text{Ca}^{2+}$  cooperativity and  $\text{Ca}^{2+}$  sensitivity in BK- $\alpha$  subunits of ZDF rats with advanced diabetes could cause slowing of the conformational transitions of the heteromeric states. The cooperative conformational changes of the channel subunits can be estimated from the relationship between the number of subconductance states and their relative frequencies. As shown in Fig. 8D, the relative frequency was plotted against each subconductance state in Lean and ZDF rats, which were fitted by a single exponential function:  $y = \omega \exp(\psi x)$ , where  $\omega$  is the fitting constant and  $\psi$  is the coefficient of subunit conformational change. The coefficient of BK channel subunit conformational change was estimated to be 3.3 in Lean rats and 1.4 in ZDF rats, in agreement with the reduction of the Hill coefficient of the  $\text{Ca}^{2+}$  dose-dependent curve from 4.1 in Lean rats to 1.1 in ZDF rats (Fig. 5B). Hence, these observations suggested that changes in  $\text{Ca}^{2+}$ -cooperativity and in subunit conformations in BK channels could be coupled, but such coupling was impaired in ZDF rats with more frequent subconductance openings.

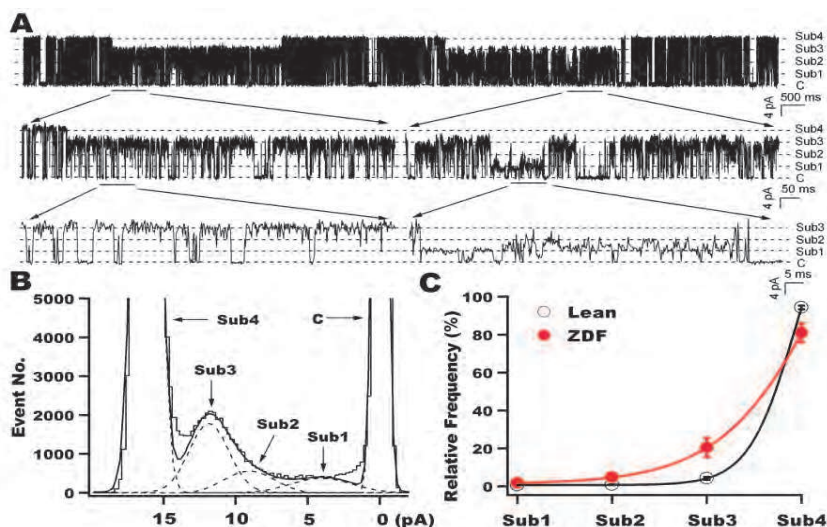


Fig. 8. Increased BK channel subconductance openings in ZDF rats with advanced diabetes. A: Representative single BK current recordings were obtained at +60 mV in the presence of 1  $\mu\text{M}$  free  $\text{Ca}^{2+}$ , with selected segments that showed expanded details, demonstrating the presence of 4 sublevels of openings. B: Amplitude histogram was fitted using a Gaussian function and showed four peaks with unitary amplitudes of 4 pA (Sub1), 8 pA (Sub2), 12 pA (Sub3) and 16 pA (Sub4 or fully open). The relative frequencies of each subconductance state were calculated by the area under each component of the Gaussian function. C: Relative frequencies were plotted against subconductance states, and the relationships were fitted using a single exponential function. The coefficient of subconductance conformational changes was estimated to be 3.3 in Lean rats ( $n=3$ ) and 1.4 in ZDF rats ( $n=3$ ). (adapted from Lu et al., 2008).

### 5.3 Downregulation of vascular BK- $\beta_1$ subunit expression in ZDF rats with advanced diabetes

BK- $\beta_1$  subunits play an important role in the regulation of channel  $\text{Ca}^{2+}$ - and voltage-sensitivity. Fig. 9 shows the loss of BK- $\beta_1$ -mediated channel activation in ZDF rats with 6 months of diabetes. Dehydrosoyasaponin-1 (DHS-1) is a cell-impermeable BK- $\beta_1$  subunit-specific activator, enhancing BK channel activity by acting on the cytoplasmic surface of the membrane. DHS-1 (0.1  $\mu\text{M}$ ) applied to the bath solution in inside-out excised membrane patches significantly increased the  $P_o$  of BK channels in Lean rats, but not those in ZDF rats (Fig. 9A and 9B). A 2.1-fold reduction in BK- $\beta_1$  protein expression was observed in ZDF rats while BK- $\alpha$  expression was unchanged (Fig. 9C and 9D). The downregulation of vascular BK- $\beta_1$  expression appears to be a common feature in BK channelopathy for both type 1 and type 2 diabetes (Dong et al., 2008; Lu et al., 2008; McGahon et al., 2007; Zhang et al., 2010). Since the BK- $\beta_1$  subunit is known to modulate the  $\text{Ca}^{2+}$ - and voltage-dependent activation of BK channels and the subconductance activity of BK channel is also thought to be regulated by BK- $\beta_1$  subunits (Nimigean & Magleby, 1999), we can conclude that the vascular BK channelopathy in type 2 diabetes is produced by the downregulation of BK- $\beta_1$  expression.

In addition to changes in the BK- $\beta_1$ -mediated channel regulation, the BK- $\alpha$  subunit may also undergo alterations in intrinsic properties as a result of prolonged diabetes. For example, hyperglycemia is known to enhance production of ROS, and  $\text{H}_2\text{O}_2$  has been shown to directly inhibit BK channel function through redox modulation of the BK- $\alpha$  C911 residue

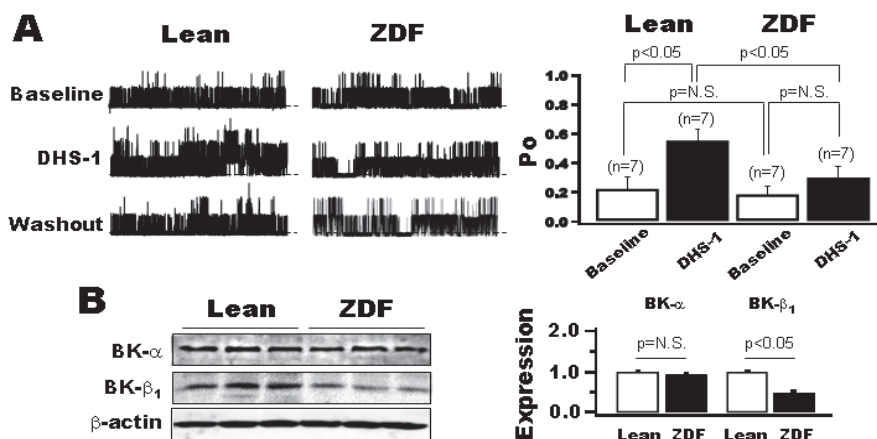


Fig. 9. Impaired the  $\beta_1$ -mediated channel activation and reduced BK- $\beta_1$  expression in the arteries of ZDF rats 8 weeks after the development of diabetes. A: Inside-out single BK currents recorded in the coronary smooth muscle cells from ZDF rats and age-matched Lean rats at +60 mV in the presence of 0.5  $\mu\text{M}$  free  $\text{Ca}^{2+}$  at baseline, with application of 0.1  $\mu\text{M}$  DHS-1, followed by drug wash out. Bar graphs show a significant DHS-1-induced increase in BK channel  $P_o$  in Lean rats, but not in ZDF rats. B: Immunoblot analysis shows significant decrease in BK- $\beta_1$  expression but not that of BK- $\alpha$  expression in the aortas from ZDF rats, compared to those from Lean rats. (adapted from Lu et al., 2008)

(Lu et al., 2006; Tang et al., 2004). Also, the molecular mechanism that underlies the downregulation of BK- $\beta_1$  in type 2 diabetes is unknown. However, we have recently reported that in type 1 diabetes and in human coronary smooth muscle cells cultured with high glucose, BK- $\beta_1$  protein degradation was significantly accelerated through upregulated ubiquitin-proteasomal pathway (Zhang et al., 2010). Taken together, it is most likely that the above mechanisms could contribute to vascular BK channel dysfunction in type 2 diabetes, although direct confirmation will be necessary using appropriate tissues from human and animal models with type 2 diabetes mellitus.

## 6. Summary

Vascular BK channel function is impaired in type 2 diabetic animals. During the early stages of diabetic development, abnormal BK channel function is likely due to reduced activity and bioavailability of vasodilators (e.g., PGI<sub>2</sub>, 12-hydroxyeicosatetraenoic acid) or increased activity and bioavailability of vasoconstrictors (e.g., Ang II, ROS). However, the BK channel biophysical properties remain intact. During advanced stages of type 2 diabetes, vascular BK channel gating properties, especially those pertaining to Ca<sup>2+</sup>-dependent kinetics, are altered. These changes in BK channel gating are associated with reduced BK- $\beta_1$  subunit expression and increased BK- $\alpha$  subunit post-translational modification, contributing to BK channelopathy and vascular complications in type 2 diabetes. These results suggest that the potential therapeutic targets for restoring BK channel function are dependent on progression of the disease. Hence, a better understanding on the fundamental mechanisms of BK channel dysfunction in association with type 2 diabetes may help us provide better approaches for the treatment of diabetic vascular complications and improve the quality of life in these patients.

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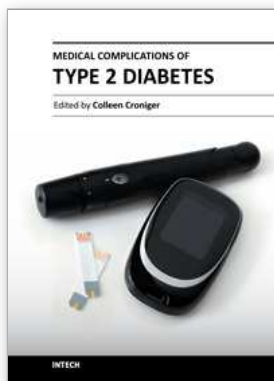
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## **Medical Complications of Type 2 Diabetes**

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Obesity and type 2 diabetes are increasing worldwide problems. In this book we reviewed insulin secretion in both healthy individuals and in patients with type 2 diabetes. Because of the risk associated with progression from insulin resistance to diabetes and cardiovascular complications increases along a continuum, we included several chapters on the damage of endothelial cells in type 2 diabetes and genetic influences on endothelial cell dysfunction. Cardiovascular complications occur at a much lower glucose levels, thus a review on the oral glucose tolerance test compared to other methods was included. The medical conditions associated with type 2 diabetes such as pancreatic cancer, sarcopenia and sleep disordered breathing with diabetes were also discussed. The book concludes with several chapters on the treatments for this disease offering us hope in prevention and successful alleviation of the co-morbidities associated with obesity and type 2 diabetes.

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