

Impairment of TRPC1-BK complex in diabetic rat coronary artery

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To the Editor: Diabetes mellitus (DM) is a chronic disease that endangers human health and can lead to different complications.^[1] Coronary artery disease is one of the most serious complications in DM patients. Recently, increasing evidence has shown that the pathophysiology of diabetic coronary diseases is related to ion channels dysfunction in coronary artery smooth muscle cells (SMCs).^[2] Therefore, a better understanding of the ionic mechanism underlying diabetic coronary artery dysfunction is of clinical importance.

The transient receptor potential C (TRPC) channels and the large-conductance calcium-activated potassium (BK) channels are the two important ion channels in coronary artery SMCs.^[3,4] The TRPC1 channel is a member of the classical TRP cation channels family and is widely distributed in vascular SMCs. Calcium depletion in the sarcoplasmic reticulum induces TRPC1 channel opening, leading to calcium influx, cells depolarization, and vasoconstriction. The BK channel is also widely distributed in vascular SMCs. Cell membranes depolarization or cytosolic calcium concentrations increase induced by BK channel opening, further lead to membrane hyperpolarization in vascular SMCs and the blood vessels relaxation.

It has been reported that TRPC1 channels are physically associated with BK channels in rat aorta SMCs.^[5] In the present study, we confirmed that the TRPC1 channel and BK channel form a complex in normal rats' coronary artery SMCs. Additionally, this increased calcium entry and decreased BK channel activity lead to vascular reactivity changes in coronary arteries. These results suggested that TRPC1-BK complex dysfunctions could be involved in this process. These findings may provide new

insights into ionic mechanisms that lead to coronary diseases and cardiac adverse events in diabetes.

In this study, streptozotocin-induced diabetic rats were sacrificed 8 weeks after hyperglycemia development. First, we evaluated the proteins' co-immunolocalization between TRPC1 channels and BK channels in coronary arteries by co-immunoprecipitation. Our immunoblot experiments confirmed a single BK- α protein expression band in the anti-TRPC1 antibodies pull-down from coronary artery lysates. Similarly, TRPC1 proteins were detected in anti-BK- α antibodies precipitates [Figure 1A]. Also, immunoprecipitation fraction intensity quantifications were similar in control and DM groups [Supplementary Figure 1, <http://links.lww.com/CM9/A859>]. Furthermore, double-labeling immunofluorescence experiments suggested that TRPC1 channels and BK channels are co-immunolocalized on diabetic coronary artery SMCs [Supplementary Figure 2, <http://links.lww.com/CM9/A859>]. The results indicated that there is a physical association of TRPC1 channels and BK channels in both control and diabetic coronary artery SMCs.

Then we detected the TRPC1 channel and BK channel expressions in diabetic coronary arteries. Protein [Figure 1B, C] and mRNA [Figure 1F] expressions of TRPC1 channels were significantly increased in the diabetic group. Also, we examined protein and mRNA expressions of α and β 1 subunits of BK channel. Protein [Figure 1D] and mRNA [Figure 1G] expressions of BK- α subunits were similar between control and diabetic rats [Figure 1E]. Meanwhile, BK- β 1 subunits expressions were significantly downregulated in diabetes [Figure 1H].

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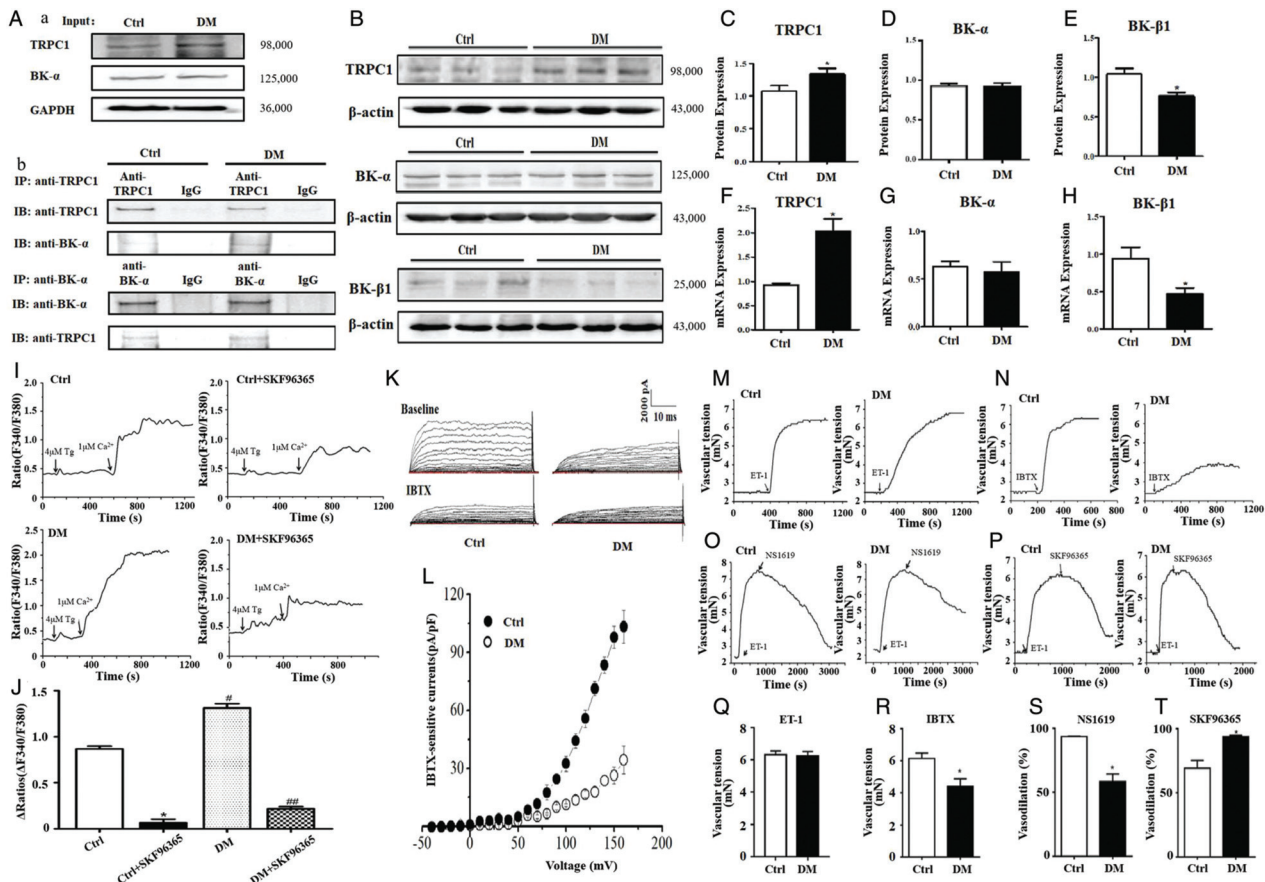


Figure 1: Impairment of TRPC1-BK complex in diabetic rat coronary artery SMCs. (A) representative images showing BK- α subunits and TRPC1 Input immunoblots. The amount of protein used in the input lane was 20% of the IP lane. b: representative images showing BK- α subunits immunoblots combined with anti-TRPC1 antibody; and representative images showing immunoblots of TRPC1 channels were combined with anti-BK- α antibody. (B) Representative blots of TRPC1 channels protein and BK channels protein in control and diabetic rat coronary arteries. (C-H) Graph data of TRPC1 channels, BK- α subunits, and BK- β 1 subunits proteins, and mRNAs expressions in control and diabetic rat coronary arteries. (I) Representative tracings of $[Ca^{2+}]_i$ changes in response to Tg and extracellular Ca^{2+} . (J) Group data of Δ ratios showing $[Ca^{2+}]_i$ changes in four groups. (K) Representative potassium currents of control and diabetic coronary artery SMCs in baseline and after application of 100 nmol/L IBTX. Whole-cell potassium currents were recorded from a holding potential (HP) of -60 mV with pulses of 100 ms at TPs from -40 to $+160$ mV in 10 mV increments. (L) IBTX-sensitive BK current density-voltage relationship of control and diabetic coronary artery SMCs. (M) No significant difference between control and diabetic groups regarding vascular tension increases after ET-1 treatment ($P > 0.05$, $n = 5$). (N) Vascular tensions in control and diabetic groups increased after IBTX treatment, but the maximal vascular tension of the diabetic group was lower compare to controls ($P < 0.05$, $n = 5$). (O) Decreased vasodilatation induced by NS1619 in the diabetic group arteries pre-contracted by ET-1 ($P < 0.05$, $n = 5$). (P) Increased vasodilatation induced by SKF96365 in the diabetic group arteries pre-contracted by ET-1 ($P < 0.05$, $n = 5$). (Q-T) Graph data of vascular tensions in control and diabetic groups. ET-1: Endothelin-1; IB: Immunoblotting; IP: Immunoprecipitation; SMCs: Smooth muscle cells; TRPC: Transient receptor potential C; Tg: Thapsigargin; TPs: Testing potentials.

Cytosolic calcium concentrations before and after incubation with SKF96365 (a commonly used and relatively specific TRPC1 blocker) in control and diabetic coronary artery SMCs were measured with Fura-2/AM, a cytoplasmic calcium indicator. Coronary artery SMCs were pre-treated with thapsigargin (4 μ mol/L) for depletion of endoplasmic reticulum calcium stores for 6 to 8 minutes in calcium-free D-Hanks buffer to deplete intracellular Ca^{2+} stores, causing a rise in $[Ca^{2+}]_i$. Then, extracellular Ca^{2+} (1 mmol/L) was applied to activate TRPC1 channels and led to another increase in $[Ca^{2+}]_i$ [Figure 1I, J]. Results showed that the Δ ratios were higher in diabetic coronary artery SMCs compared with controls. Additionally, Δ ratios significantly decreased in control and diabetic groups after incubation with SKF96365.

Total potassium currents at baseline and after exposure to 100 nmol/L iberiotoxin (IBTX, a BK channel blocker) were recorded in control and diabetic rats' coronary SMCs.

Current densities of IBTX-sensitive BK channels on the coronary artery SMCs decreased in diabetic rats compared with control. Also, we recorded the total potassium currents at baseline and after exposure to 30 μ mol/L NS1619 (a BK channel agonist) in the coronary SMCs of control and diabetic rats. Current densities of NS1619-activated BK channels on the coronary artery SMCs in diabetic rats were significantly higher compared with controls [Supplementary Figure 3, <http://links.lww.com/CM9/A859>].

To investigate the effects of TRPC1-BK complex imbalance on diabetic coronary artery function, vascular tension measurements were performed. Vascular tensions in the presence of vasoconstrictor, endothelin-1 (ET-1) (2 nmol/L), in both control and diabetic rats did not present significant differences [Figure 1Q]. The BK channel inhibitor, IBTX (100 nmol/L), induced coronary vessels contraction and was increased in the diabetic rats

compared with control rats [Figure 1R]. In the presence of the BK channel agonist, NS1619 (30 $\mu\text{mol/L}$), and the TRPC1 channel blocker, SKF96365 (10 $\mu\text{mol/L}$), coronary vessels that pre-contracted with ET-1 showed vasodilation. Induced coronary vasodilation percentages decreased with NS1619 [Figure 1S] and increased with SKF96365 in the diabetic rats than control rats ($P < 0.05$; $n = 5$) [Figure 1T]. Altogether, these data confirmed that diabetic coronary artery vasorelaxation and vasoconstriction dysfunction can be related to the TRPC1-BK complex imbalance.

Overall, we presented different novel findings in the ionic mechanism underlying diabetic coronary dysfunctions. First, we show that the TRPC1 channel was associated with the BK channel forming a complex on diabetic coronary artery SMCs, similar to normal ones. Second, TRPC1-BK complex expression was imbalanced in the diabetic coronary artery: TRPC1 channel expressions increased and BK channel decreased. Third, Ca^{2+} signaling and BK channel activation dysregulation by TRPC1-BK complex resulted in coronary arteries dysfunction in diabetic rats. These findings indicated that the TRPC1-BK complex are important for coronary artery function regulation. Also, the diabetic coronary artery dysfunction, triggered by TRPC1-BK complex imbalance, may lead to serious cardiovascular complications and cardiac adverse events.

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Conflicts of interest

None.

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