Effects of Streptozotocin-Induced Diabetes on Kv Channels in Rat Small Coronary Smooth Muscle Cells

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Abstract

Diabetes impairs endothelium dependent vasodilation, but the mechanism of endothelium independent dilation is not well understood. In the present study, we examined the effect of streptozotocin (STZ)-induced diabetes on the vasomotor of small coronary artery and the activity of voltage-dependent K^+ channel of vascular smooth muscle cells in STZ rat using the videomicroscopy and patch clamp method. STZ-induced diabetes appeared reduce the vasodilation induced by β-adrenoceptor agonist, isoproterenol (10^{-9}-10^{-5} mol/l), and adenylyl cyclase activator forskolin (10^{-9}-10^{-5} mol/l) respectively (isoproterenol: 44.2±6.7% *vs.* 82.5±4.8%, and forskolin: 54.4±4.5% *vs.* 94.3±2.4%). 4-AP, a Kv channel blocker of VSMC, further decreased dilation to isoproterenol (44.2±6.7% *vs.* 10.2±3.5%) and forskolin (54.4±4.5% *vs.* 13.8±11.0%) significantly. Whole cell K^+ current recording demonstrated that STZ-induced diabetes decreased isoproterenol and forskolin-induced K^+ current (ISO: 55.6±7.8 pA/pF *vs.* 28.4±3.4 pA/pF, forskolin: 61.3±9.8 pA/pF *vs.* 32.4±3.4 pA/pF). 4-AP further reduced the decreased K^+ current (ISO: 28.4±3.4 pA/pF *vs.* 14.3±2.1 pA/pF, forskolin: 32.4±3.4 pA/pF *vs.* 14.8±2.9 pA/pF). These results indicated that STZ-induced diabetes impaired cAMP mediated dilation of small coronary artery and suppressed the Kv channel activity of vascular smooth muscle cells. Kv channel of VSMC was shown to play a determinate role reducing dilation of small coronary artery in STZ rats.

Key Words: streptozotocin induced diabetes of rat, small coronary artery, voltage K^+ channel, isoproterenol, forskolin

Introduction

The impairment of tissue perfusion induced by vascular alternations in diabetes mellitus (DM) is a serious complication associated with the disease. To the coronary circulation nitric oxide (NO), prostacylin (PGI_2), and endothelium derived hyperpolarizing factor (EDHF) are produced by endothelial cells and contribute to the local regulation of vascular tone (2, 7, 11). Much less is known about the endothelium independent vasodilation in small coronary artery. The alternations of ionic channel in the activity of vascular smooth muscle cells (VSMCs), especially the K_{Ca} and Kv channel, play an important role in the control of vascular tone and vasodilation (20, 22, 25). We have previously demonstrated that hyperglycemia impaired cAMP mediated and endothelium independent vasodilation of rat small coronary artery (RSCA), and we speculated that the impairment of cAMP mediated dilation of high glucose was primarily due to a reduction in Kv channel activity (5, 16). In the present study, we explored the effects of STZ-induced diabetes on the cAMP mediated dilation of small coronary artery and the activity of voltage dependent K^+ channel (Kv channel) of VSMCs. We hypothesized that the DM-reduced cAMP mediated

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dilation in RSCA was primary due to the suppress of Kv channel function of VSMCs.

Materials and Methods

Animal Preparation

The diabetes model of rat was used following a method described else where (23). Wistar rats aged 4-6 week, both males and females, weighing 180±10g, were provided by the Animal Center, School of Medicine, Shandong University. Diabetes was induced by tail vein injection of streptozotocin (STZ, 65 mg/kg body weight) dissolved in sodium citrate buffer 0.1 mol/l (pH 4.5). Age matched control rats were injected with an equal volume of citrate buffer solution. Blood glucose levels were measured on the third day, one week after injection by glucosmeter (One Touch Basic Plus, Johnson-Johnson). STZ-injected rats with blood glucose levels 15 mmol/l were considered to be diabetes (STZ rats). STZ rats and matched control rats were housed for 6 weeks before experiments. STZ-induced diabetes group did not accept exogenous insulin injection in the housing.

Body weight in STZ-induced diabetes group increased from 185±4g to196±13g, while that of the control group increased from 183±3g to 331±18g on the day of experiment. Blood glucose levels in STZ-induced diabetes group significantly increased from 3.1±0.2 mmol/l to 25.1±2.9 mmol/l. Blood glucose levels in control group had no obvious change on the day of experiment.

Preparation of Rat Small Coronary Arteries

Rats were anesthetized with pentobarbital sodium (60 mg/kg, ip). The heart was taken out quickly and put in 4°C Hepes solution containing (in mol/l) NaCl 138.0, KCl 4.0, $MgSO_4$ 1.2, $CaCl_2$ 1.6, KH_2PO_4 1.2, EDTA 0.0026, Glucose 6.0, HEPES acid 10.0, pH= 7.4. RSCA (100-150 mm ID) which was dissected in the ventricle and put in Hepes buffer for experiment.

Videomicroscopy Measurement (18)

RSCAs were placed into an organ chamber filled with physiological salt solution (PSS) containing (in mol/l) NaCl 123.0, KCl 4.7, $MgSO_4$ 1.2, $CaCl_2$ 2.5, $NaCO_2$ 16.0, KH_2PO_4 1.2, EDTA 0.0026, Glucose 11.0, pH=7.4, at 37°C. PSS in the organ chamber was bubbled with 95% O_2 and 5% CO_2, and was circulated by pump. RSCAs were mounted on the tip of glass micropipette and filled with PSS at an intraluminal pressure of 60 mmHg. The vessel image was conducted into the videomicroscopy measurement system (VIA-100, Boeckler Instruments, Inc., Tucson, AZ, USA) through phase contrast microscopy (MT-2, Olympus, Japan). This system was connected to monitor. The inside diameter of vessel was measured directly. RSCAs were equilibrated in the organ chamber for 60 min. When the spontaneous tone of vessels exceeded 40% of the passive diameter, the experiment will be started. On the contrary, the vessel would be preconstricted with U-46619 (10^{-8} mol/l) by 40% passive diameter. At the end of each experiment, vessels were maximally dilated with Ca^{2+}-free solution and the percentage of dilation to agonists was normalized to this maximum diameter.

Patch Clamp Recording of K^+ Current

Single VSMC was obtained by enzymatic isolation according to the methods for dissociation of rat microvessels (13). Whole-cell recordings of voltage-dependent Kv current were obtained by using standard pulse protocols (17). Families of K^+ currents were generated by stepwise 10 mV depolarizing pulses (400-ms duration, 5-second intervals) from a holding potential of -60 mV to 60 mV in cells dialyzed with 10^{-8} mol/l ionized Ca^{2+}. Seal resistance was 2 to 10 GΩ. Peak current elicited at a single membrane potential was defined as the average of 500 sample points encompassing the maximum current point. Trials were performed in triplicate, and peak current amplitudes were divided by membrane capacitance to obtain K^+ current density. Whole-cell tail currents were elicited in symmetrical 145 mmol/l K^+ by depolarizing cells from a constant holding potential of -60 mV in 10 mV increments to 60 mV, followed by an immediate repolarizing step back to -60 mV to generate families of tail currents.

Drugs

Streptozotocin, isoproterenol (ISO), forskolin, 4-aminopyrimide (4-AP), U 46619 were purchased from Sigma (St. Louis, MO, USA).

Statistical Analysis

The data were expressed as mean±SE. Statistical analysis of group difference was performed using *t*-test. A value of $P < 0.05$ was considered to be statistically significant.

Results

Effects of STZ-induced Diabetes on cAMP-Mediated Dilation of RSCA

The effects of ISO, β-adrenoceptor agonist, and forskolin, adenylyl cyclase activator, on the dilation of RSCA were investigated in STZ rats. The results illustrated that ISO (10^{-9}-10^{-5} mol/l) and forskolin

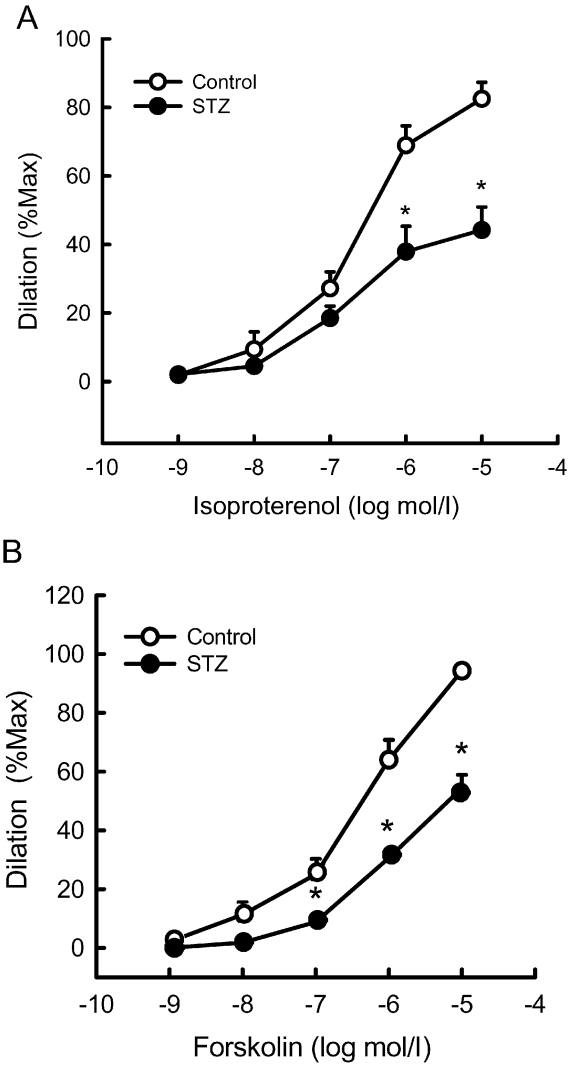

Fig. 1. Effects of streptozotocin-induced diabetes on ISO- and forskolin-induced dilation in rat small coronary arteries. The dilation caused by either ISO (A) or forskolin (B) was reduced significantly by streptozotoxin-induced diabetes (*$P < 0.01$ *vs.* control, $n = 6$).

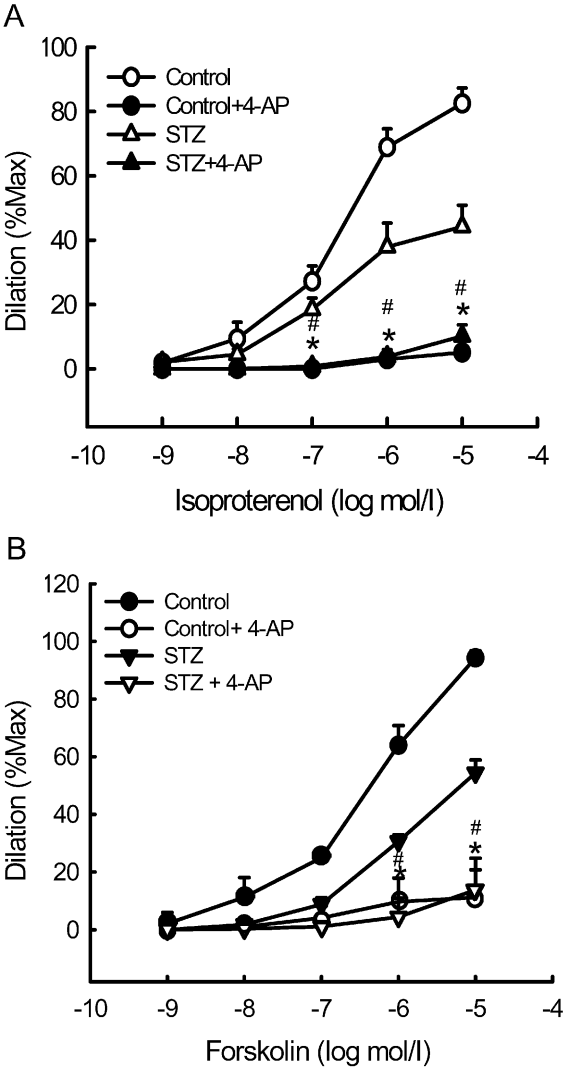

Fig. 2. Effect of 4-AP (3 mmol/l) on ISO- and forskolin-induced dilation in rat small coronary arteries. The dilation caused by either ISO (A) or forskolin (B) was inhibited in both control and streptozotocin-induced diabetes. (*#$P < 0.01$ *vs.* without 4-AP, $n = 6$)

(10^{-9}-10^{-5} mol/l) caused vasodilation of RSCA respectively in dose-dependent manner (Fig. 1), but the maximum dilation response in STZ rats was decreased significantly to ISO (82.5±4.8% *vs.* 44.2±6.7%, $n = 6$, $P < 0.01$) and forskolin (94.3±2.4% *vs.* 54.4±4.5%, $n = 6$, $P < 0.01$). The ISO-induced dilation is mediated by cAMP, and forskolin-induced dilation by increasing intracellular cAMP level. So these results demonstrated that STZ-induced diabetes impaired the cAMP-mediated vosodilation of RSCA.

Effects of STZ-induced Diabetes on Kv Channels of VSMC of RSCA

After application of 4-AP (3 mmol/l), a Kv channel blocker (12, 21), for 20 min, the ISO and forskolin-induced vasodilation were inhibited significantly both in control and STZ rat (Fig. 2). In the application of 4-AP, the maximum dilation to ISO decreased from 82.5±4.8% to 6.6±4.2% in control rats ($n = 6$, $P < 0.01$), and from 44.2±6.7% to 10.2± 3.5% in STZ rat ($n = 6$, $P < 0.01$), respectively. The maximum dilation to forskolin also decreased from 94.3±2.4% to 11.2±9.6% in control rats ($n = 6$, $P < 0.01$) and from 54.4±4.5% to13.8±11.0% in STZ rats ($n = 6$, $P < 0.01$), respectively in the application of 4-AP. These results demonstrated that decreased cAMP-mediated vasodilation was resulted from reduced Kv channel activity that can be inhibited by 4-AP.

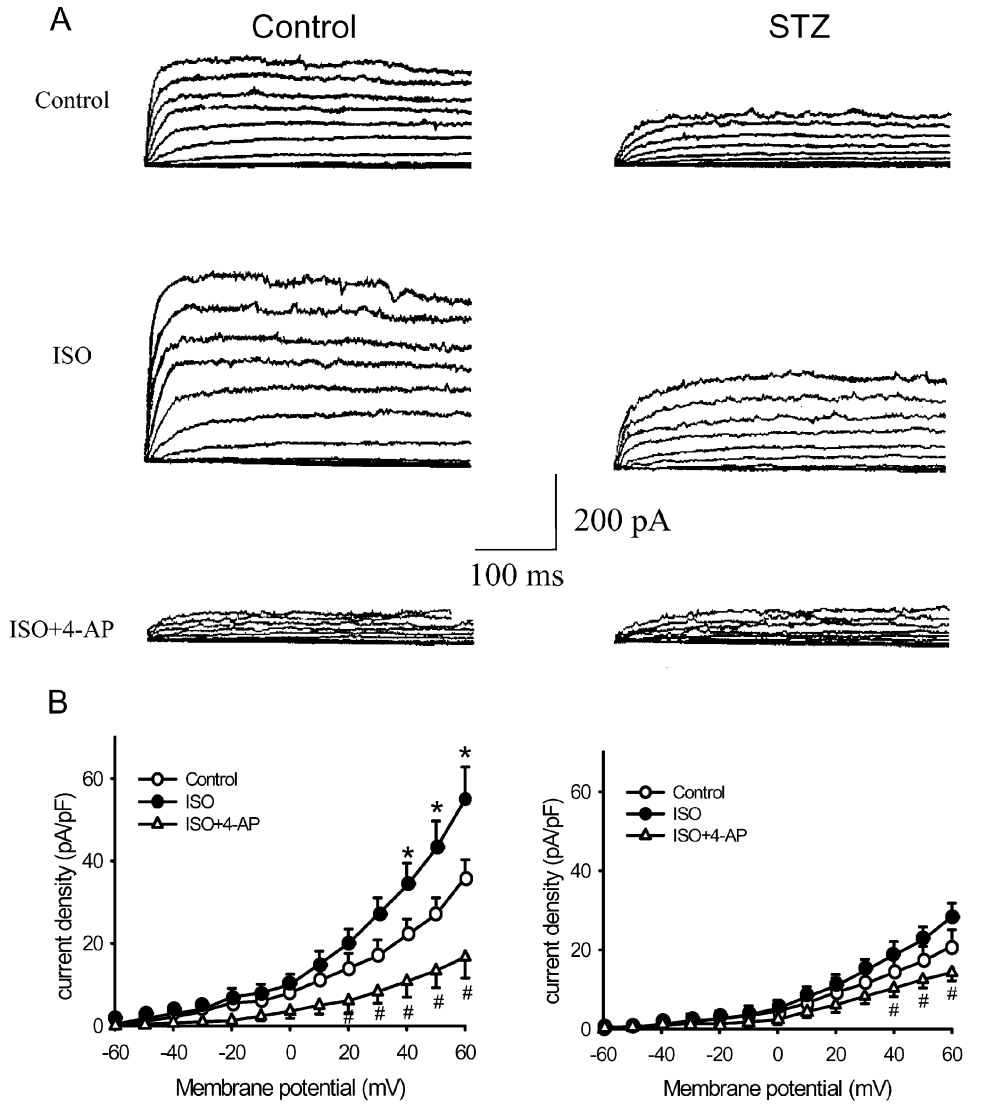

Fig. 3. A: Sample traces of whole cell K^+ currents in RSCA induced by 10^{-5} mol/l ISO. Whole cell K^+ currents were increased by 10 mV depolarizing steps of holding potential from -60 to +60 mV in physiological K^+ gradients. ISO increased K^+ current more in VSMC of STZ rats than in that of the control rats. 4-AP (3 mmol/l) inhibited the effect of ISO in both control and STZ rats. B: ISO increased peak K^+ current densities in VSMC of STZ rats, but had little effects on K^+ current density in VSMC of control rats (*P < 0.01 *vs.* control, n = 6,). 4-AP inhibited the effect of ISO in both control and STZ rats (#P < 0.01 *vs.* ISO, n = 6).

Effects of STZ-Induced Diabetes on ISO-Induced K^+ Current of VSMC of RSCA

Fig. 3 showed the simple trace of whole-cell K^+ current at holding potential from -60 mV to +60 mV by 10 mV incremental depolarizing in physiological K^+ gradients and the curve of current density of VSMC of RSCA in control and STZ rats. These results illustrated that STZ-induced diabetes inhibited K^+ current of VSMC significantly, as compared to the control rats (21.3±4.13 pA/pF *vs.* 36.3±4 pA/pF, n = 6, P < 0.01). In both control rats and STZ rats, the ISO could increase K^+ current (55.6±7.8 pA/pF in control rats and 28.4±3.4 pA/pF in STZ rats, n = 6, P < 0.01), but the increase of K^+ current in STZ rats was less than that in the control rats. The 4-AP could inhibit K^+ current in control and STZ rats (16.7±5.1 pA/pF in control rats and 14.3±2.1 pA/pF in STZ rats, n = 6, P < 0.01).

Effects of STZ-Induced Diabetes on Forskolin-Induced K^+ Current of VSMC of RSCA

Fig. 4 showed the sample trace of whole-cell K^+ current at holding potential from -60 mV to +60 mV by 10 mV incremental depolarizing in physiological

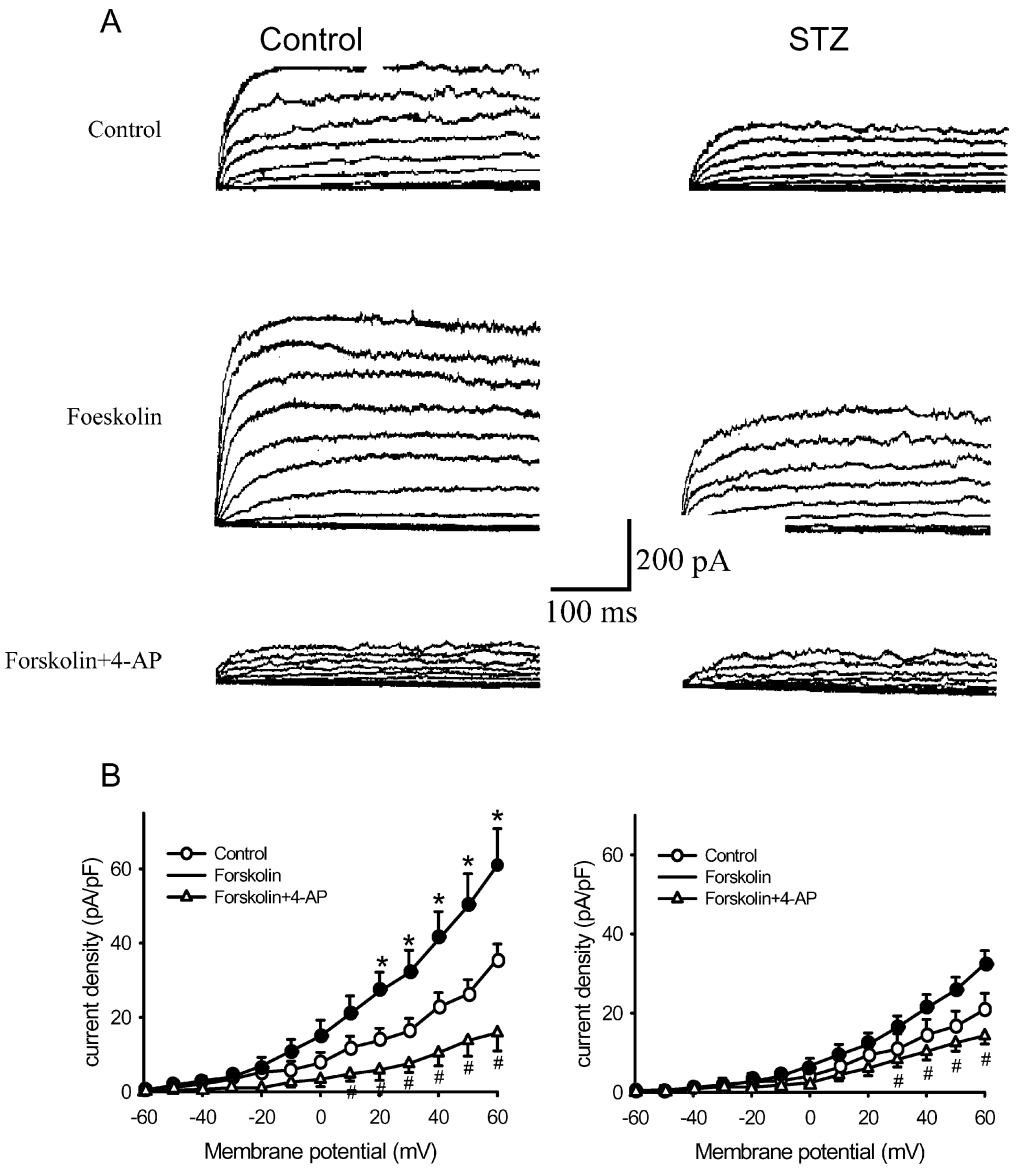

Fig. 4. A: Sample traces of whole cell K^+ currents in RSCA induced by 10^{-5} mol/l forskolin. Whole cell K^+ currents were increased by 10 mV depolarizing steps of holding potential from -60 to +60 mV in physiological K^+ gradients. Forskolin increased K^+ current more in VSMC of STZ rats than in that of the control rats. 4-AP (3 mmol/l) inhibited the effect of forskolin in both control and STZ rats. B: Forskolin increased peak K^+ current densities in VSMC of STZ rats, but had little effects on K^+ current density in VSMC of control rats (*P < 0.01 *vs.* control, n = 6). 4-AP inhibited the effect of forskolin in both control and STZ rats (#P < 0.01 *vs.* forskolin, n = 6).

K^+ gradients, and the curve of current density of VSMC of RSCA in control and STZ rats. These results illustrated that STZ-induced diabetes inhibited K^+ current of VSMC significantly, as compared to control rats (20.9±4.5 pA/pF *vs.* 35.7±4.4 pA/pF, n = 6, P < 0.01). In both control rats and STZ rats, Forskolin could increase K^+ current (61.3±9.8 pA/pF in control rat and 32.4±3.4 pA/pF in STZ rat, n = 6, P < 0.01), but the increase of K^+ current in STZ rats was less than that in the control rats. The 4-AP could inhibit K^+ current in control and STZ rats (15.9±4.9 pA/pF in control rats and 14.8±2.9 pA/pF *vs.* in STZ rats, n = 6, P < 0.01).

Discussion

Hyperglycemia or diabetes has been reported to impair endothelium-dependent dilation (9, 10, 26, 27). In previous study, we have demonstrated that STZ-induced diabetes reduced endothelium independent dilation of RSCA (6). This study showed that STZ-induced diabetes reduced the dilation of RSCA to ISO and forskolin. The vasodilation to β-adrenoceptor agonist ISO or adenylyl cyclase activator

forskolin has been found to mediate by cAMP signal system (1, 25). Hence, reduced dilation to ISO and forskolin in STZ rats indicated suppression of cAMP activity. 4-AP-induced further reduction of dilation to ISO and forskolin obviously. This indicated that the further reduction of dilation to 4-AP is due to inhibition of Kv channel activity.

The Kv channels are highly expressed in vascular smooth muscle cells and contribute to resting membrane potential and to repolarization in graded membrane potential changes (14, 20). Since Kv channels play an important role in vascular dilation, the Kv channel blocker 4-AP was able to induce further reduction of dilation.

Fig. 3 and Fig. 4 show that ISO and forskolin enhanced whole cell K^+ current and that 4-AP could inhibit this K^+ current in the control rat. This indicated that K^+ currents to ISO or forskolin were enhanced *via* the cAMP system and were dependent on Kv channel activity. These results correlated to our previous findings (5, 16) and the report on the portal vein of rabbits (1). In the coronary circulation, the vasomotor effects of angiotension II (8) and change of pH (4) are also mediated in part by Kv channel activity. Several reports deal with the K_{Ca} channel activity of guinea pig basilary artery or porcine coronary artery in ISO, forskolin, and analog of cAMP-induced dilating response (19, 24). In the present study, 4-AP suppressed the Kv current significantly. We did not exam the effects of K_{Ca} channel in this study, but it has been shown that if the K_{Ca} channel took part in this process, its effect is very little (16).

In STZ rats the enhancement of K^+ current induced by ISO and forskolin was decreased obviously. The 4-AP suppressed the K^+ current to ISO and forskolin evidently. It is suggested that STZ-induced diabetes suppressed Kv channel activity of VSMCs and inhibited-cAMP mediated K^+ current to ISO and forskolin. Some reports imply that in the impaired dilator responses to ATP-sensitive K^+ channel openers in aorta and in mesenteric and cerebral arteries of streptozotocin-induced diabetic rats (3, 15, 28). The effect of diabetes on different potassium channel of different vessels indicated the vascular heterogeneity.

Disturbance of tissue perfusion induced by vascular alternations in DM is a serious clinical problem. The pathophysiological mechanism of this complication is very complex. We now showed that the suppression of Kv channel activity of VSMCs of small coronary artery in STZ rats may induce reduction of dilation and malperfusion. The present study may have provided a new direction for the improvement of treatment.

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