Altered mRNA Expression of ATP-Sensitive and Inward Rectifier Potassium Channel Subunits in Streptozotocin-Induced Diabetic Rat Heart and Aorta

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Abstract. Cardiovascular diseases are the most frequent and costly complication of diabetes. Many previous studies showed that ATP-sensitive potassium channels (K\textsubscript{ATP}) and inward rectifier potassium channels (Kir) play important regulatory roles in functions of cardiovascular tissues. It’s still not very clear how these potassium channels are involved in cardiovascular complications of diabetes. We used the streptozotocin (STZ)-induced diabetic rats model to study the expressions of K\textsubscript{ATP} and Kir channel subtypes in diabetic cardiovascular tissues. The mRNA expression levels of Kir2.1, Kir3.1, Kir6.1, Kir6.2, and sulfonylurea receptor (SUR) 2A and 2B subunits in heart and aortal smooth muscles were determined by the reverse-transcription polymerase chain reaction. The results showed that in comparison with the control rats, mRNA expression of SUR 2A was reduced significantly in the diabetic heart (SUR 2A/GAPDH, 1.04 ± 0.16 vs 0.38 ± 0.09, \( P < 0.01, n = 3 \)); SUR 2B was reduced markedly in the aortal smooth muscle of diabetic rats (SUR 2B/GAPDH, 1.13 ± 0.14 vs 0.35 ± 0.07, \( P < 0.01, n = 3 \)). However, there are no significant expression changes of Kir2.1, Kir3.1, Kir6.1, and Kir6.2 in diabetic rats. These results suggested that expression of specific K\textsubscript{ATP} channel subunits were altered in the heart and aorta of diabetic rats.

Keywords: diabetes, mRNA expression, ATP-sensitive potassium channel, inward rectifier potassium channel

Introduction

Cardiovascular complications are the main causes of morbidity and mortality in patients with diabetes. Injection of streptozotocin (STZ) may induce type 1-like diabetes mellitus in rats and produce profound cardiovascular complications such as hypertension, bradycardia, depression of left ventricular developed pressure, and cardomyopathy (1). Inward rectifier potassium channels (Kir) and ATP-sensitive potassium channels (K\textsubscript{ATP}) are two important K\textsuperscript{+} channel superfamilies expressed in cardiovascular tissues. Kir channels may contribute to the resting membrane K\textsuperscript{+} conductance of the cells and also provide a mechanism for increases in extracellular K\textsuperscript{+} concentration. The Kir2.0 subfamily (strong inward rectifiers) and Kir3.0 subfamily (G-protein coupled K\textsuperscript{+} channels) are the two important subfamilies of inward rectifier K\textsuperscript{+} channels. Sometimes, K\textsubscript{ATP} channels may also contribute to the resting conductance and play a major role in controlling the membrane potential of \( \beta \)-cells and other tissue cells, because they can open under conditions of metabolic compromise. There are a few reports about K\textsuperscript{+} channel dysfunctions in cardiovascular tissues under diabetes (2, 3). However, the involvement of K\textsuperscript{+} channels in cardiovascular dysfunction in diabetes is not well understood at the molecular level. The aims of this study were therefore to determine the mRNA expression alterations of Kir and K\textsubscript{ATP} in heart and vascular smooth muscle of diabetic rats.

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Materials and Methods

Animal model
Male Wistar rats (190 – 210 g) were randomly divided into control and diabetic groups. Diabetic groups were acclimatized for 1 week before being fasted overnight and rendered diabetic by an intraperitoneal injection of STZ (70 mg/kg freshly dissolved in 150 mM citrate buffer, pH 4.5). Diabetes was defined as a nonfasting plasma glucose >250 mg/dl in tail vein blood 48 h after STZ injection. Animals were subsequently maintained for a time period of 3 months in individual air-filtered metabolic cages with free access to water and fed a standard rat diet. The protocol was approved by the Animal Care Committee of Chinese Academy of Medical Sciences & Peking Union Medical College.

Sample collection and total RNA extraction
Control and diabetic rats were quickly sacrificed by decapitation; and aortae were cleaned from adjacent tissues, rinsed in cold DEPC water, snap-frozen, and stored in liquid nitrogen until use. Total RNA was extracted from aorta homogenate by TRizol agents (Promega, Madison, WI, USA) according to the manufacturer’s instructions. The final RNA concentrations were determined by absorbance using a spectrophotometer (UV-260; Shimadzu, Kyoto).

Reverse transcription-polymerase chain reaction (RT-PCR)
Inward rectifier (Kir2.1 and Kir3.1) and K\textsubscript{ATP} channels mRNA levels were assessed by the RT-PCR. We did 3 independent experiments, using 8 animals from each group. DNA was generated by the reverse transcription (RT) reaction by incubating 2 μg of total RNA and random hexamer oligodeoxynucleotides (0.5 μg) 25-μl reaction mixture containing 20 U RNasin, 1.5 mM dNTPs, and 10 U reverse transcriptase AMV. The reaction was carried out in GeneAmp PCR System 2400 (Perkin-Elmer, Norwalk, CA, USA) at 42°C for 60 min and terminated with 5 min incubation at 95°C. The PCR reaction was performed in a 50-μl reaction mixture consisting of 2 μl of RT reaction product as template DNA, 1 x PCR buffer, 500 pmol specific primers (see Table 1 for primer sequences), 2 mM dNTPs, and 2 U Taq polymerase. The expression of the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) served as the internal control. Each PCR reaction of Kir channels and GAPDH was performed with the following cycling protocol: an initial denaturation at 94°C for 4 min, 30 cycles at 94°C for 1 min, 57°C for 30 s, 72°C for 1 min, with a final extension step at 72°C for 8 min. PCR reaction in the presence of sulfonylurea receptor (SUR) 2A/2B primer was performed as follows: an initial denaturation at 94°C for 4 min, 30 cycles at 94°C for 45 s, 55°C for 60 s, 72°C for 2 min, with a final extension step at 72°C for 8 min. PCR cycle numbers were chosen to ensure that the amplification of PCR products was in the exponential range. Sequences of the PCR primers and PCR products sizes are shown in Table 1.

Analytic methods
Aliquots of PCR reaction products (20 μl) equalized to give equivalent signals from the internal control (GAPDH) were electrophoresed through 1.7% agarose gels containing 0.2 μg/ml ethidium bromide. Gels were visualized under UV light and analyzed by computerized

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer sequences (5' to 3')</th>
<th>Size of product (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kir2.1</td>
<td>sense TGCCCGATTGCTGTTTTC</td>
<td>373</td>
</tr>
<tr>
<td></td>
<td>antisense GGCTGTCTTGTCTATTT</td>
<td></td>
</tr>
<tr>
<td>Kir3.1</td>
<td>sense GCA CCA GCC ATA ACACAC</td>
<td>221</td>
</tr>
<tr>
<td></td>
<td>antisense TTGCCAGGAACCGAACTT</td>
<td></td>
</tr>
<tr>
<td>Kir6.1</td>
<td>sense GAGTGAACTGTCCGCACCGAGA</td>
<td>247</td>
</tr>
<tr>
<td></td>
<td>antisense CGATCACGAGAATCGCAGCA</td>
<td></td>
</tr>
<tr>
<td>Kir6.2</td>
<td>sense TCCAACAGCGCGCTTAC</td>
<td>167</td>
</tr>
<tr>
<td></td>
<td>antisense GATGGGACAAAGGCGCTG</td>
<td></td>
</tr>
<tr>
<td>SUR 2</td>
<td>sense ACAGGCTCCGCTCCAGGCTG</td>
<td>2A: 407</td>
</tr>
<tr>
<td></td>
<td>antisense GCCAGGGACGAGCAGCTG</td>
<td>2B: 231</td>
</tr>
<tr>
<td>GAPDH</td>
<td>sense CGACGCAAAGAAGATCGCGCT</td>
<td>540</td>
</tr>
<tr>
<td></td>
<td>antisense ATGGATGCCGCGGTCCAGGGTGT</td>
<td></td>
</tr>
</tbody>
</table>

bp: base pair, Kir: inward rectifier potassium channel, SUR: sulfonylurea receptor, GAPDH: glyceraldehyde-3-phosphate dehydrogenase.
Fig. 1. RT-PCR amplification of Kir channel subunits and GAPDH (540 bp, served as internal control) mRNA from heart and aortic smooth muscle in diabetic and control rats. The marker lane (M) is 100-bp ladder DNA. Lane 1: control group and lane 2: diabetic group.

Fig. 2. RT-PCR amplification of K_{ATP} channel subunits and GAPDH (540 bp, served as internal control) mRNA from heart and aortal smooth muscle in diabetic and control rats. The marker lane (M) is 100-bp ladder DNA. Lane 1: control group and lane 2: diabetic group.
densitometric scanning of the images using a Bio-Rad GS-700 imaging densitometer. All data were expressed as means ± S.D. Student’s t-test was used to analyze the significant difference between the groups, and \( P<0.05 \) was taken as the criteria for significance.

**Results**

**Effects of STZ on body weight and plasma glucose**

After rats were injected with STZ for 3 months, the plasma level of glucose in the STZ-treated group was significantly higher than that in the control group (387 ± 79 vs 98 ± 20 mg/dl, \( P<0.05, n = 8 \)). However, the body weight of the STZ-induced diabetic group was markedly lower than that in the control group (230 ± 28 vs 393 ± 51 g, \( P<0.05, n = 8 \)).

**mRNA expression of Kir2.1 and Kir3.1 channels in STZ-treated rat heart and aortic smooth muscle**

The mRNA expression of Kir2.1 and Kir3.1 (Kir2.1/GAPDH, Kir3.1/GAPDH) in hearts and aortic smooth muscles were not significantly different between the control and STZ-induced diabetic rat (Figs. 1 and 3).

**mRNA expression of K\textsubscript{ATP} subunits in diabetic rat heart and aortic smooth muscle**

In comparison with the mRNA concentration of control rats, both relative expression levels of SUR 2A and SUR 2B were significantly down-regulated in STZ-induced diabetic rat heart (SUR 2A/GAPDH, 1.04 ± 0.16 vs 0.38 ± 0.09, \( P<0.01, 3 \) independent experiments from 8 animals) and aortic smooth muscle (SUR 2B/GAPDH, 1.13 ± 0.14 vs 0.35 ± 0.07, \( P<0.01, 3 \) independent experiments from 8 animals), respectively. The expression levels of Kir6.1 and Kir6.2 mRNA (Kir6.1/GAPDH, Kir6.2/GAPDH) had no obvious changes in STZ-induced diabetic rats (Figs. 2 and 3).

**Discussion**

Diabetes has profound adverse effects on cardiovascular and endothelial functions. Pial arterioles of diabetic rats lose their ability to dilate to the K\textsubscript{ATP} channel opener RP52891; this may not relate to nitric oxide or calcium-activated potassium channels (4). It was also found that the coronary microvascular responses to ischemia are impaired in diabetic dog and may relate to impaired K\textsubscript{ATP} channel responsiveness (2).

Members of the Kir2.0 subfamily express channels that appear to correspond to the "classical" inward rectifier in heart. The first family member, Kir2.1 (IRK1), was cloned from a mouse macrophage cell line; and its mRNA was also expressed in heart and skeletal muscle and at lower abundance, in forebrain and cerebellum (5). Most work has focused on Kir2.1, which showed properties characteristic of native strong inward rectifiers. The Kir3.0 subfamily (GIRK) encodes potassium channels that are expressed heterologously and modulated by G-protein. In the heart, acetylcholine is released from the vagus nerve. It binds to the M\textsubscript{2} receptor and activates the GIRK channel through a G protein and finally induces the negative chronotropic effect. Functional inward rectifier channels are believed to be heterooligimers formed by GIRK1 with the other subunits (6). We found that the mRNA expression of Kir2.1 and Kir3.1 showed no significant differences in the heart and aortic smooth muscle between control and diabetic rats. Further research should determine whether the inward rectifier currents are affected in the cells of heart and vascular smooth muscle in diabetic rats.
Since the $K_{\text{ATP}}$ channels had been discovered in cardiomyocytes by Noma (7), $K_{\text{ATP}}$ channels were shown to exist in many tissues including pancreatic β-cell, skeletal muscle, smooth muscles, and the central nervous system. They have the general function of linking the membrane $K^+$ permeability of cells to their metabolic state and electrical activity (8). Generally, cardiac type $K_{\text{ATP}}$ is formed from Kir6.2/SUR 2A and vascular smooth muscle (VSM) type $K_{\text{ATP}}$ contains Kir6.1/SUR 2B. However, our laboratory showed that there is wide expression of Kir6.1 and Kir6.2 in rat tissues. Although Liss et al. (9) found that both SUR 2A and SUR 2B are expressed in mouse heart, most studies found that only SUR 2A is expressed in rat left ventricle. SUR 2A and SUR 2B are the two isomers of SUR 2 that were isolated from the mouse library and presumed to be splice variants (10). In the present study, we observed that the mRNA expression level of SUR 2A reduced dramatically in the left ventricle of STZ-induced diabetic rat compared with that in the control rats. We did not find any report about the changes in mRNA expression of $K_{\text{ATP}}$ subunits in STZ-induced diabetic rats before. In the model of STZ-induced diabetic rat, insulin therapy does not restore insulin-stimulated skeletal transport and transporter translocation to normal (11). We postulated that sustained high blood glucose and/or reduced insulin (or other factors such as elevated lipids?) might affect the mRNA expression of SUR in the rat heart and VSM.

What’s the physiological role of the reduced $K_{\text{ATP}}$ channel expression in diabetes? The $K_{\text{ATP}}$ channel does not function in isolation. It is closely associated with a binding site for the antidiabetic sulfonylurea compounds and requires the presence of this binding site for its function (12, 13). It is now evident that there are low (SUR 1) and high affinity (SUR 2) forms of SUR subunits (14, 15), and the high and low affinity sites were independently regulated by the propensity of rats to develop or resist the development of diet-induced obesity and hyperinsulinemia on high energy diets (16). Recent results from Levin and Dunn-Meynell (17) showed that rats with streptozotocin-induced diabetes are associated with up- or down-regulation of SUR-binding sites in different areas of the rat brain. It is unclear whether down-regulation of SUR-binding sites appeared in diabetic rat heart and VSM cells. A recent electrophysiological study (18) indicated that outward single-channel currents were significantly larger in the diabetic rat ventricular cells than that in control rat cells, while the inward $K_{\text{ATP}}$ currents were similar in amplitude in control and diabetic rat cells. Moreover, in diabetic rat ventricular myocyte $K_{\text{ATP}}$, the IC$_{50}$ for ATP inhibition was twice that in the control ($82 \pm 7$ vs $43 \pm 4 \mu M$) (19); this is consistent with our results that down-regulation of SUR 2A may be involved in the increment of IC$_{50}$ for ATP inhibition. It can be concluded that the major difference found between $K_{\text{ATP}}$ channels from control and diabetic hearts was the greater outward diabetic single-channel current, which may contribute to the enhanced sensitivity to hypoxia or ischemia in diabetic hearts.

Our results showed down-regulation of SUR 2A appeared in heart and SUR 2B appeared in vascular smooth muscle in diabetic rats. We conclude that diabetes alters gene expression of specific $K_{\text{ATP}}$ channel subunits that may result in the injury of heart from ischemia and may be involved in the impaired vascular functions observed in diabetes. Recently Liu and Gutterman observed the effects of reactive oxygen species (ROS) on $K^+$ channels in diabetic vascular tissues and proposed functional impairment is related to an excess production of superoxide ions (20). It appears that complex processes are involved in the mechanism of diabetic cardiovascular complications.

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References


