Comparative Effect of Type 1 and Type 2 Diabetes Mellitus on Vascular Responses of Rat Thoracic Aorta to Potassium Ion Channel Openers

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ABSTRACT

Background: Diabetes mellitus is associated with many cardiovascular dysfunction and impairment of potassium channel function.

Aim: We compared the vascular reactivity in aorta from streptozotocin-induced and Goto-Kakizaki (GK) diabetic rats to potassium channel openers.

Methodology: Diabetes mellitus (DM) was induced in Sprague Dawley rats by intraperitoneal injection of streptozotocin (STZ) at 65 mg/kg body weight. After four weeks of DM, vascular reactivity of the aortic rings from STZ-induced Sprague Dawley and age-matched GK and control rats to phenylephrine, acetylcholine, levromakalim and naringenin were studied using standard organ bath procedure.

Results: The phenylephrine-induced contraction was significantly (P<0.05) increased in STZ-diabetic aortic rings [2.03 ±0.07 g] when compared with GK rats [1.47±0.14 g] and STZ-control [1.42±0.21 g]. Relaxation responses to acetylcholine, levromakalim and naringenin were significantly (P<0.05) attenuated in STZ-diabetic aorta when compared with GK rats. Maximal relaxation and potency of aorta to acetylcholine, levromakalim and (+/-)naringenin were significantly (P<0.05) decreased in STZ-diabetic aorta when compared with GK-diabetic and control groups.

Conclusion: The phenylephrine-induced contraction, endothelium-dependent relaxation, K<sub>ATP</sub>-and (+/-)-naringenin-induced vasorelaxation are not altered adversely in the early stages of Type 2 diabetes whereas there is exaggerated contractile response and a relaxant dysfunction involving the endothelium, K<sub>ATP</sub> in Type 1 diabetes mellitus.

Keywords: acetylcholine, aorta, diabetes, endothelium, naringenin, potassium ion channel, vasoconstriction

1. INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder that represents a serious public health concern. It is characterized by defective insulin secretion or deficiencies in the action of insulin. Diabetes mellitus is associated with a wide range of circulatory manifestations such as alterations in endothelial function and cardiovascular disease (Hermans, 2007; Kar and Holt, 2008). Most of the complications in diabetes are due to hyperglycaemia and increased generation of oxygen-derived free radicals, which may lead to vascular dysfunction (Singh et al., 2009; Kaneto et al., 2010).

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Vascular dysfunction affects various membrane ion channels and evidence of vascular potassium channel dysfunction due to oxidative stress in diabetes mellitus has been reported (Matsumoto et al., 2004; Bubolz et al., 2007). These include attenuation of ATP-dependent potassium channel (KATP) -mediated vasorelaxation and increased vascular tone (Irat et al., 2006). Potassium channels activation under normal conditions produces hyperpolarization of the cell membrane, closure of voltage-dependent calcium ion channels and vascular relaxation of smooth muscle cells (Nichols et al., 1991; Faraci and Heistad, 1998). Potassium channel openers especially the K_ATP and BK_Ca-channel openers have been useful in significantly improving diabetic vasculopathy (Matsumoto et al., 2004; Liu and Gutterman, 2002).

In DM studies, a number of animal models have been used. Streptozotocin (STZ) and alloxan are widely used to induce Type 1 diabetes mellitus. The Goto-Kakizaki (GK) rats is a widely accepted, non-obese and normotensive model of Type 2 DM that have elevated fasting blood glucose and impaired response to insulin (Goto et al., 1976; Yasuda et al., 2002).

Vascular dysfunction is associated with both Type 1 and Type 2 diabetes. For instance, increased contractile responses to adrenergic agonist and normal endothelium-dependent relaxation have been reported in diabetic GK rats (Brondum et al., 2005) whereas attenuated responses to acetylcholine (ACh) have been reported in arteries of Zucker diabetic rats (Phillips et al., 2005). While some studies had reported enhanced responsiveness of STZ- and GK-diabetic aorta to alpha adrenergic agonists (Orie et al., 1993; Schulingkamp et al., 2005), other studies have shown decreased aortic responses to alpha adrenergic agonist in GK-diabetic condition (Kobayashi et al., 2004).

The involvement of potassium ion channels in diabetic disease has been controversial probably due to the stage of the disease and experimental models used for the study. Comparative study on the vascular responses of aorta from Type 1 and 2 DM to adrenergic, nitric oxide and potassium ion dependent channel activity in both types of diabetic models is lacking. The aim of this study was to compare the effect of type 1 and type 2 diabetes mellitus on vascular responses of rat thoracic aorta to potassium channel openers.

2. MATERIAL AND METHODS

2.1 Chemicals

Levcromakalim, phenylephrine, acetylcholine and (+/-)-naringenin were obtained from Sigma Chemical Company (Poole, UK). Levcromakalim and (+/-)-naringenin were dissolved in dimethyl sulphoxide (DMSO) before subsequent dilutions were made in water to ensure that tissues were not exposed to more than 0.1% of DMSO which had no effect on tissue response. Other drugs were dissolved in distilled water. All drugs were added directly into the organ baths and the concentrations given are the final bath concentration in the bath solution.

2.2 Experimental Animals

Male Sprague-Dawley, Wistar and GotoKakizaki (GK) rats (8-10 weeks old) weighing between 200-220 g were obtained from the Biological Services Unit of the University College London for the study. The rats were placed randomly into four groups of six rats each namely STZ-control, STZ-induced diabetic, GK diabetic and Wistar Control groups. All animals were fed a standard ratchow and tap water ad libitum, and were kept in room temperature controlled at 19-21 °C. All studies were conducted in accordance with the Guide for the Care and Use of Laboratory Animals of the University and conformed to the UK Animal Scientific Procedures Act of 1986.

2.3 Induction of Diabetes Mellitus

DM was induced in Sprague Dawley rats by a single intraperitoneal injection of 65 mg/kg body weight streptozotocin (STZ) dissolved in citrate buffer (pH 4.5). Weight and age-matched Sprague Dawley control rats were injected with the citrate buffer vehicle alone. Body weight and basal blood glucose levels were measured just prior to STZ injection using animal balance and an automated glucose
analyzer (glucometer Acucheck mini plus, Roche, Germany) respectively. After 48 h following STZ administration, blood samples were taken from the tail vein and hyperglycaemia was confirmed in animals by blood glucose above 10 mmol/L (Clark et al., 2004). The diabetic animals were experimented upon after four weeks of diabetes mellitus induction.

2.4 Aortic Ring Preparation

After the rats were sacrificed by cervical dislocation, the aorta was rapidly removed and placed in cold (4ºC) physiological salt solution of the following composition (mmol/L): NaCl, 112; KCl 5; CaCl2 1.8; MgCl2 1, NaHCO3 25; KH2PO4 0.5; NaH2PO4 0.5; Glucose 10; pH 7.4. Each aorta was cleaned of connective tissues under the dissecting microscope and cut into rings (~3 mm long) and mounted in 20 ml organ baths at 37ºC containing physiological salt solution gassed with 95% O2 and 5% CO2. The aortic rings were connected to an isometric force transducer (Grass FT03), connected to a preamplifier Powerlab (AD Instruments Ltd, Australia) data acquisition unit and isometric contraction was recorded in a computer using AD Chart Software version 4.2.4 (AD Instruments Ltd). A passive tension of 1 g was applied to the aortic rings using a movable device and was equilibrated for 90 min while being rinsed every 15 min. During the equilibration period, the rings were challenged with 1 µMol/L phenylephrine (PE) and the aorta was relaxed with 10 µMol/L acetylcholine to test the endothelial integrity, the classical method of testing for functional endothelium. The presence of functional endothelium was verified by the ability of acetylcholine (10 µMol/L) to induce more than 80% relaxation in rings preconstricted with phenylephrine (1 µMol/L).

After 90 min equilibration period, PE (10⁻⁹-10⁻⁵Mol/L) was added cumulatively to the bath until a maximal response was achieved. A plateau response was allowed to develop before the addition of the next concentration of the same agonist. For relaxation studies, aortic rings from both the diabetic and control rats were precontracted with 1 µMol/L PE. When the PE contraction had stabilized, relaxation responses were elicited in a cumulative manner using one of the following: acetylcholine (10⁻⁹-10⁻⁵Mol/L), K_ATP channel opener, levocromakalim (10⁻⁷-10⁻³Mol/L), or a flavonoid, (+/-)-naringenin (10⁻⁸-10⁻⁴Mol/L). A subsequent concentration was added to the organ bath after the previous concentration had reached its steady state. Each aortic ring was used once for each drug protocol.

2.5 Statistics

All results are reported as mean ± SEM and n represents the number of animals tested per group. Log EC₅₀ or IC₅₀ values (defined as the negative log of concentration of drug that induced 50% of the maximal contraction or relaxation) and E_max values (maximal contraction or relaxation) were derived from individual concentration-response curves fitted to a sigmoidal curve using nonlinear regression program of GraphPad Prism software (version 5.0, GraphPad Software, San Diego, CA, USA). Statistical analysis of the data was performed by one way analysis of variance (ANOVA) followed by Bonferroni’s test. Relaxation responses were given as percentages of the initial contraction induced by 1 µMol/L PE. In all comparisons, P<0.05 was considered significant.

3. RESULTS

3.1 Body Weight and Blood Glucose Level

As indicated in Table 1, blood glucose levels were significantly (P<0.01) elevated in STZ-induced diabetic rats when compared with STZ-control and GK-diabetic rats. The blood glucose level in the GK rats was significantly (P<0.01) higher than the Wistar control rats. In contrast, body weight was comparable between the age- matched control and GK rats whereas it was significantly (P<0.01) reduced in STZ-diabetic group compared with STZ-control.

Table 1. Body weight and blood glucose levels in control and diabetic rats
Parameter & STZ-Control & STZ-induced DM & Wistar Control & GK DM  
--- & --- & --- & --- & ---  
Body weight (g) & 430 ± 26.2 & 286 ±19.6** & 344± 5 & 350 ± 10.3  
Blood glucose (mmol/L) & 6.9 ± 0.38 & 32.9 ±0.57**† & 7.4 ± 0.51 & 12.9 ±1.6‡  

Values are expressed as the mean ± SEM; ** = P <0.01 vs. STZ-control rats; † = P<0.01 vs. GK DM rat; ‡= P<0.01 vs. Wistar control rats; n=6 in each group.

3.2 Contraction with Phenylephrine

Exposure of aortic rings to phenylephrine led to a concentration-dependent rise in tension in all experimental groups. Aortic rings from the STZ diabetic rats showed significant (P<0.05) increase in maximum contractile force compared with those of age-matched control and GK rats (Fig. 1). There was a notable shift of the phenylephrine concentration-response curve of STZ-diabetic to the left of control. In contrast, aortas from GK diabetic rats showed a significant (P<0.05) decrease in maximum contraction induced by phenylephrine compared with Wistar control rats (Table 2) although the potency was not different.

Table 2: Maximum responses and log EC<sub>50</sub> or IC<sub>50</sub> values for various drugs applied to the aorta from control and diabetic rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>STZ-DM</th>
<th>Wistar Control</th>
<th>GK diabetes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenylephrine –logEC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>7.42±0.13</td>
<td>7.43±0.11</td>
<td>7.41 ± 0.13</td>
</tr>
<tr>
<td>Phenylephrine Max (g)</td>
<td>2.03 ±0.07**†</td>
<td>2.07±0.12</td>
<td>1.47±0.14</td>
</tr>
<tr>
<td>Acetylcholine –log IC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>7.31±0.07*</td>
<td>7.41 ± 0.07</td>
<td>7.69 ± 0.13</td>
</tr>
<tr>
<td>Acetylcholine –Max (%)</td>
<td>92.67 ± 3.2</td>
<td>92.30 ± 2.22</td>
<td>92.30 ± 2.22</td>
</tr>
<tr>
<td>Levromakalim –log IC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>7.16±0.06†</td>
<td>7.39 ± 0.08</td>
<td>7.53±0.08</td>
</tr>
<tr>
<td>Levromakalim Max (%)</td>
<td>93.17 ± 1.5</td>
<td>97.6 ± 0.88</td>
<td>97.6 ± 0.88</td>
</tr>
<tr>
<td>Naringenin –log IC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>3.7±0.14**†</td>
<td>3.39 ± 0.05*</td>
<td>4.93±0.35</td>
</tr>
<tr>
<td>Naringenin Max (%)</td>
<td>88.01±3.0</td>
<td>77.77±2.24**†</td>
<td>87.17 ± 3.8</td>
</tr>
</tbody>
</table>

Values are expressed as the mean ± SEM; * = P < 0.05 vs. STZ-control rats; †= P < 0.05 vs. GK diabetic rat; –LogEC<sub>50</sub> or –LogIC<sub>50</sub> = negative logarithm of the molar concentration of each drug causing 50% of the maximal contraction or relaxation; max = maximal contraction or relaxation to each drug.
3.3 Relaxation to acetylcholine

The cumulative ACh concentration-response curves on PE-pre-constricted aortic rings in both diabetic and control aortas are presented in Fig 2. DM significantly reduced the potency to ACh in STZ rat aorta compared with control (Fig 2A). However in GK rat aorta, the potency, but not maximum relaxation was significantly (P<0.05) increased compared with Wistar control (Fig 2B). The potency to ACh was significantly (P<0.05) decreased in STZ-diabetic aorta when compared with GK diabetic rats.

Fig. 2. Concentration-response curves for acetylcholine in aortic rings from (A) streptozotocin (STZ)-diabetic vs STZ-control rats and (B) GK diabetic vs Wistar control (WC) rats. *=P< 0.05 vs. respective control n = 6.
3.4 Relaxation to Levocromakalim

The cumulative concentration-response curves to the ATP-sensitive potassium ion channel opener, levocromakalim, show that the aortic rings from all experimental groups relaxed to it. The relaxation curve for STZ-diabetic group was significantly (P<0.05) shifted to the right of STZ-control (Fig. 3A). Similarly, maximum relaxation to levocromakalim was significantly (P<0.05) reduced in STZ-diabetic group compared with STZ-control (Table 2). In contrast, the GK diabetic and Wistar control groups produced comparable maximum response to levocromakalim (Fig. 3B). When the two diabetic groups were compared, both potency and maximum response to levocromakalim were significantly (P<0.05) increased in the GK diabetic group compared with the STZ diabetic group.

Fig. 3. Concentration-relaxation response curves for levocromakalim from STZ-diabetic aorta (A) and GK diabetic (B) rats.

* = P< 0.05 vs. respective control, n = 6.

3.5 Relaxation to (+/-)-naringenin

The relaxation induced by the flavonoid, (+/-)-naringenin was significantly reduced in the aorta from STZ-diabetic rat when compared with age-matched control (Fig 4A). The maximal relaxation of aortic rings to (+/-)-naringenin was also significantly (P<0.05) reduced in STZ-diabetic group when compared with STZ-control groups. In contrast, relaxation of aortic rings from GK rats to (+/-)-naringenin were significantly (P<0.05) enhanced when compared with Wistar control, although maximum relaxation in both groups were similar. (Fig. 4B). The pIC$_{50}$ values for (+/-)-naringenin obtained in the two controls were significantly (P<0.05) different from each other.
**DISCUSSION**

The study compared the vascular reactivity to potassium channel openers and contraction to phenylephrine in type 1 and type 2 diabetic models. The main findings are that the $K_{\text{ATP}}$ channel function and (+/-)-naringenin-induced relaxation were enhanced in GK model compared with STZ model. Phenylephrine-induced contraction was increased and endothelium-dependent relaxation was attenuated in STZ-induced DM when compared with the GK diabetic rat aorta. These results suggest that in the early stage of diabetes both endothelium-dependent relaxation and potassium channel-mediated relaxation are preserved in Type 2 diabetic model whereas they are altered in Type 1 diabetes.

The result of decreased phenylephrine-induced contraction in Type 2 diabetes is in agreement with a previous studies conducted on the aorta and mesenteric artery of streptozotocin-induced DM, Zucker and GK rats that showed a decrease in contractile responses to alpha adrenergic agonist in early diabetic condition (Kobayashi et al., 2004). Various mechanisms for the decreased phenylephrine response include receptor desensitization signalling pathways, alteration in calcium potency/handling mechanisms and inhibition of contractile effects of alpha-adrenergic agonists in vascular smooth muscle by NO (Vanhoutte and Miller, 1989, Kobayashi et al., 2004). Therefore, the reduced contractility to phenylephrine in the GK aorta may be due to exaggerated NO-dependent relaxation that inhibits the contractile mechanism of the alpha-adrenoceptor in the endothelium of the GK rats.

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Fig. 4. Concentration-relaxation curves for (+/-)-naringenin in aortic rings obtained from streptozotocin-induced (A) and GK diabetic (B) rats. 

* = $P<0.05$ vs. respective control, $n = 6$. 

4. DISCUSSION
Although a previous study had reported that down-regulation of the alpha-1 adrenoceptor is not likely to mediate the enhanced contractile responses in arteries from STZ diabetes (Weber and Macleod, 1997), an increased alpha receptors density have been reported in STZ-diabetic rats (Schulingkamp et al., 2005). Another study reported that there is a reduced adrenergic-induced contractile response seen at an early stage in the GK diabetic aorta due to NO-dependent relaxation mediated via an increased expression of the alpha 2D-adrenoceptor in the endothelium (Kobayashi et al., 2004). Taken together, the observed differences in contractile responses to phenylephrine found between the STZ-diabetic and GK diabetic models could probably be due to differences in the adrenergic receptor expressions and coupling mechanisms that mediate contractile responsiveness elicited by alpha-1 adrenoceptor stimulation.

Aortas from GK diabetic rats exhibited an enhanced endothelium-dependent relaxation to ACh in comparison with the age-matched STZ-diabetic rats suggesting that endothelium-dependent relaxation is preserved in early stage of Type 2 DM. The preserved ACh-mediated relaxation of aortic rings from the GK rats is in agreement with a previous study (Brondum et al., 2005). In contrast, attenuated relaxation response was observed in STZ-diabetic aorta which is in agreement with previous studies (Orie et al., 1993). In STZ-induced DM, the basal levels of NO production and expression of eNOS are reduced in diabetic arteries suggesting eNOS uncoupling (Leo et al., 2011). On the other hand, there is enhanced NO production via over-expression of eNOS in GK diabetes (Zhong et al., 2012). It is therefore possible that the nitric oxide pathway is preserved through enhanced expression of eNOS in the early stage in Type 2 diabetes therefore contributing to the enhanced endothelium-dependent relaxation to ACh.

Vascular potassium channels play a major role in the modulation of vascular tone and local blood flow (Orie et al., 2009). Studies have described differential responses of $K_{\text{ATP}}$ channel in Type 1 and 2 DM (Zimmermann et al., 1997; Miura et al., 2003; Erdos et al., 2004). Our result showed attenuation of the relaxations of the thoracic aorta induced by levocromakalim, a $K_{\text{ATP}}$ channel opener in Type 1 DM which is in agreement with a previous study (Kamata et al. 1989). The mechanisms that underlie potassium channel dysfunction in diabetic vessels are dependent on the animal model, vessel bed, and stage of diabetes (Lu et al., 2005). Therefore, $K_{\text{ATP}}$ and $BK_{\text{Ca}}$ channels may provide a compensatory mechanism for preserving the dilator responses attributed to the dysfunction of the aorta at an early stage in this model of Type 2 diabetes. However, other regulatory mechanisms may contribute to the observed preservation of potassium channel in Type 2 DM such as G-protein coupled receptor-evoked release of NO which is influenced by endothelial calcium-activated potassium channels and an influx of calcium ions (Misurski and Gopalakrishnan, 2002; Simonsen et al., 2009). Thus, the $K_{\text{ATP}}$ channels may contribute to vasodilator mechanisms in the early stage of Type 2 DM disease where the level of reactive oxygen species is at a minimal level.

Naringenin, a naturally occurring flavanone present in citrus fruits (Cavia-Saiz et al., 2010) is also a $BK_{\text{Ca}}$ channel opener that induces concentration-dependent relaxation in aortic tissue from normal rats (Saponara et al., 2006). In this study, (+/-)-naringenin improved vasorelaxation in GK rats but not in STZ DM group. $BK_{\text{Ca}}$ channel like the $K_{\text{ATP}}$ channel mediates the vasorelaxant effect of K+ channel openers (Balwierzczak et al., 1995). A reduction in the potency of this opener as seen in STZ diabetic model could further explain the diabetic dysfunctions observed with potassium channels.(+/-)-Naringenincould act both as an antioxidant and/or a $BK_{\text{Ca}}$ opener. It is noteworthy that the pIC50 values for ( +/-)-naringenin obtained in the two controls were extremely significantly different. The discrepancy could not be easily explained but one can speculate it to be due to species and genetic differences of the animals used in the study.

The differences in responses between type 1 and type 2 DM may not be attributed to hyperglycaemia alone. It was observed in this study that the maximum contraction to PE and relaxation to ACh in the STZ-DM was similar to Wistar control despite a great difference in their blood glucose levels. A recent study also reported marked hyperglycemia in lean GK rats with well-preserved endothelium function at early and later stages of DM suggesting that hyperglycemia may not cause vessel dysfunction in this model. On the other hand, hyperglycemia maybe a preconditioning stimulus that induces endothelial nitric oxide synthase and haemoxegogenase that exert both vasodilation as well as cardiovascular protection at early or late stage of type 2 diabetes (Zhong et al., 2012). The strength of antioxidant defence at this early stage of DM could probably contribute to the differences in vascular reactivity in both types of DM. A
study suggested that in the early stage of type 2 DM, the antioxidant defense system counters the effects of increased free radicals, but by the advanced stage the balance between generation of free radicals and antioxidant defense is impaired as a result of decreased antioxidant levels or activity (Pasaoglu et al, 2004).

5. CONCLUSION

In conclusion, the present study confirms earlier report that the phenylephrine-induced contraction, endothelium-dependent relaxation, \( K_{\text{ATP}} \) and (+/-)-naringenin-induced vasorelaxation are preserved in the early stages of Type 2 diabetes whereas there is exaggerated contractile response and a relaxant dysfunction involving the endothelium and \( K_{\text{ATP}} \) channel in Type 1 diabetes mellitus. The citrus antioxidant (+/-)-naringenin, could prevent the functional changes in vascular reactivity in Type 2 diabetic rats. The effect of (+/-)-naringenin and other potassium channel on vascular reactivity in a stable normoglycaemic condition need further investigation.

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COMPETING INTERESTS

Authors do not report any conflicts of interest.

AUTHORS’ CONTRIBUTIONS

DUO performed the experiment, NNO designed the study and managed the analyses of the study, CRN wrote the second draft, LHC and EEO reviewed the manuscript. All authors read and approved the final manuscript.

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