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

Daniel U. Owu[1*], Nelson N. Orie[1], Chukwuemeka R. Nwokocha[2], Lucie H. Clapp[1] and Eme E. Osim[3]

[1]BHF Laboratories, Rayne Building, Department of Medicine, University College London, UK.
[2]Department of Basic Medical Sciences, University of West Indies, Mona Campus, Kingston, Jamaica, West Indies.
[3]Department of Physiology, College of Medical Sciences, University of Calabar, Calabar, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author DUO performed the experiment and author NNO designed the study and managed the analyses of the study, author CRN wrote the second draft, authors LHC and EEO reviewed the manuscript. All authors read and approved the final manuscript.

Research Article

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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, levcromakalim and naringenin was studied using standard organ bath procedure.
Results: The phenylephrine-induced contraction was significantly ($P<0.05$) increased in

**Corresponding author: Email: danielowu@unical.edu.ng, d_owu@yahoo.com;*

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]. Maximal relaxation and potency to acetylcholine, levcromakalim 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_{ATP} - and (+/-)-naringenin-induced vasorelaxation are not altered in the early stages of Type 2 diabetes whereas there is exaggerated contractile response and a relaxant dysfunction involving the endothelium, K_{ATP} in Type 1 diabetes mellitus.

Keywords: Acetylcholine; aorta; Gotto Kakizaki rat; naringenin; potassium channel openers; streptozotocin; 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 [1-2]. Most of the complications in diabetes are due to hyperglycaemia and increased generation of oxygen-derived free radicals, which may lead to vascular dysfunction [3,4].

Vascular dysfunction affect various membrane ion channels and evidence of vascular potassium channel dysfunction due to oxidative stress in DM has been reported [5,6]. These include attenuation of ATP-dependent potassium channel (K_{ATP}) - mediated vaso relaxation and increased vascular tone [7]. Potassium channels activation under normal condition produces hyperpolarization of the cell membrane, closure of voltage-dependent calcium channels and vascular relaxation of smooth muscle cells [8,9]. Potassium channel openers especially the K_{ATP} and BK_{Ca}-channel openers have been useful in significantly improving diabetic vasculopathy [5,10].

In DM studies, several animal models have been used. Streptozotocin (STZ) and alloxan are widely used to induce Type 1 diabetes mellitus. The Goto-Kakizaki (GK) rat is a widely accepted, non-obese and normotensive model of Type 2 DM that has elevated fasting blood glucose and impaired response to insulin [11,12].

Vascular dysfunction is associated with both Type 1 and Type 2 DM. For instance, increased contractile responses to adrenergic agonist and normal endothelium-dependent relaxation have been reported in diabetic GK rats [13] whereas attenuated responses to acetylcholine (ACh) have been reported in arteries of Zucker diabetic rats [14]. While some studies had reported enhanced response to adrenergic agonists [15,16], another study has shown decreased aortic responses to the same agent in GK-diabetic animals [17].

The involvement of potassium 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 Type 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 DM on vascular responses of rat thoracic aorta to potassium channel openers.

2. MATERIALS 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. The latter 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.

2.2 Experimental Animals

Male Sprague-Dawley, Wistar and Goto Kakizaki (GK) rats (8-10 weeks old) weighing between 200-220 g were obtained from the Biological Services Unit of the University College London (UK). 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 rat chow and tap water ad libitum, and were kept under controlled temperature condition of 19-21ºC. All studies were conducted in accordance with the Guide for the Care and Use of Laboratory Animals of University College London (UK) 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 [18]. The diabetic animals were experimented upon after four weeks of diabetes mellitus induction.

2.4 Aortic Rings 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; $CaCl_2$ 1.8; $MgCl_2$ 1, $NaHCO_3$ 25; KH_2PO_4 0.5; NaH_2PO_4 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% O_2 and 5% CO_2. 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. Rings were equilibrated for 90 min while being rinsed every 15 min. During the equilibration period, 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 PE (1 µMol/L).

After 90 min equilibration period, PE (10^{-9}-10^{-5}Mol/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 following concentration. 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^{-5}Mol/L), K_{ATP} channel opener, levcromakalim (10^{-9}-10^{-5}Mol/L), or a flavonoid, (+/-)-naringenin (10^{-8}-10^{-4}Mol/L). The subsequent concentration was added to the organ bath after the previous one had reached its steady state. Each aortic ring was used for one drug protocol.

2.5 Statistics

All results are reported as mean ± SEM and n represents the number of animals tested per group. pEC_{50} or pIC_{50} values (defined as the negative log of drug concentration 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 analysis (Graph Pad Prism software version 5.0, Graph Pad Software, San Diego, CA, USA). Statistical analysis of the data was performed by one way analysis of variance (ANOVA) followed by Bonferroni's post 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 and Table 2). There was a notable shift of the phenylephrine concentration-response curve of STZ-diabetic rats 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 pEC_{50} or pIC_{50} values for various drugs applied to the aorta from control and diabetic rats

Parameter	STZ-control	STZ-DM	Wistar control	GK diabetes
Phenylephrine pEC_{50}	7.39±0.10	7.42±0.13	7.43± 0.11	7.41 ±0.13
Phenylephrine Max (g)	1.42±0.21	2.03 ±0.07*†	2.07±0.12	1.47±0.14‡
Acetylcholine pIC_{50}	7.73±0.11	7.31±0.07*	7.41 ± 0.07	7.69 ± 0.13‡
Acetylcholine Max (%)	95.14±2.9	90.04±2.01*	92.67 ± 3.2	92.30 ± 2.22
Levcromakalim pIC_{50}	7.41±0.12	7.16±0.06†	7.39 ± 0.08	7.53±0.08
Levcromakalim Max (%)	97.63±1.15	92.45±1.27*†	93.17 ± 1.5	97.6 ±0.88
NaringeninpIC_{50}	4.6±0.21	3.7±0.14*†	3.39 ± 0.05*	4.93±0.35‡
Naringenin Max (%)	86.49±3.0	77.77±2.24*†	87.17 ± 3.8	90.98 ±2.23

*Values are expressed as the mean ± SEM; * = P < 0.05 vs. STZ-control rats; †= P < 0.05 vs. GK diabetic rat;*

‡ = P < 0.05 vs. Wistar Control; *pEC_{50} or pIC_{50} = 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.*

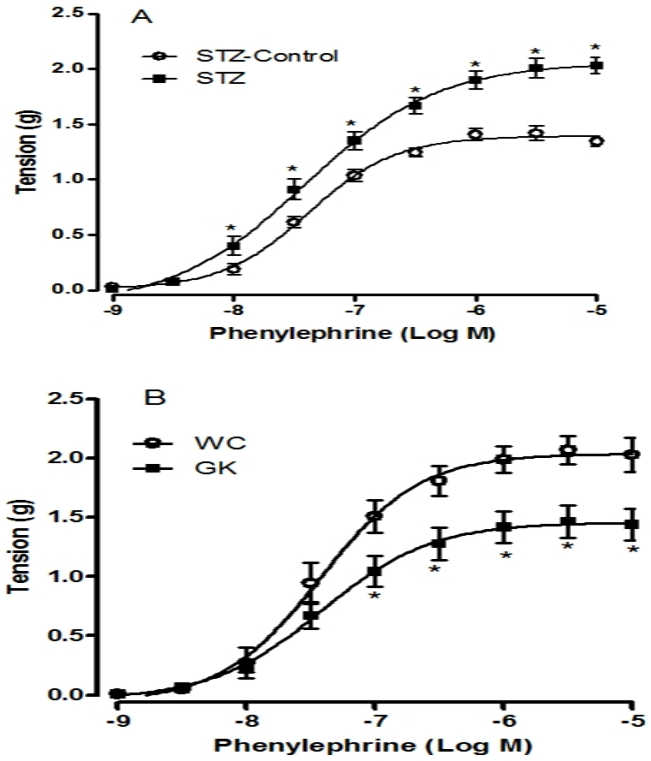

Fig.1. Concentration-response curves for PE in aortic rings from (A) STZ-diabetic vs. STZ-control rats and (B) GK diabetic vs. Wistar control (WC) rats

** = P< 0.01 vs. corresponding control; n = 6*

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.

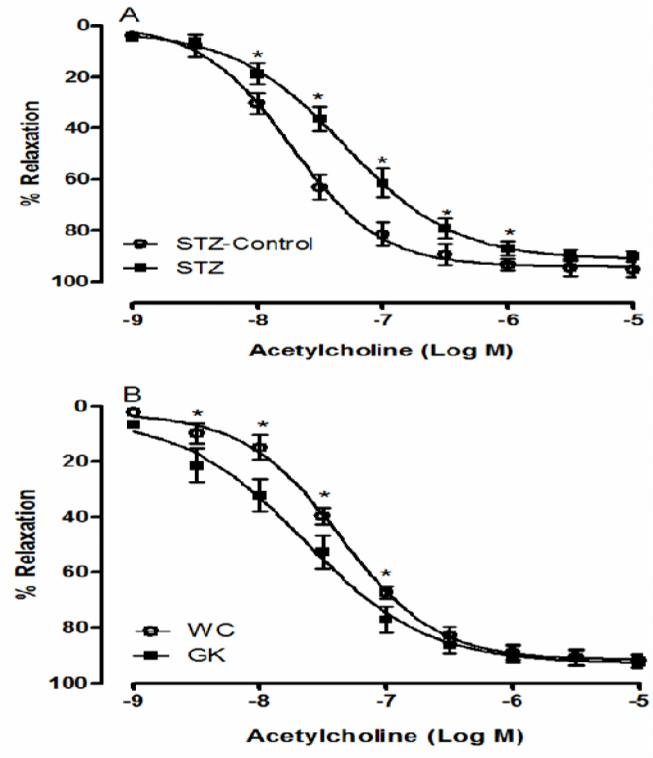

Fig. 2. Concentration-response curves for ACh in aortic rings from (A) STZ-diabetic vs. STZ-control rats and (B) GK diabetic v s. Wistar control (WC) rats

**=P< 0.05 vs. corresponding control n = 6.*

3.4 Relaxation to Levcromakalim

The cumulative concentration-response curves to the ATP-sensitive potassium channel opener, levcromakalim, 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 levcromakalim 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 levcromakalim (Fig. 3B). When the two diabetic groups were compared, both potency and maximum response to levcromakalim were significantly (P<0.05) increased in the GK diabetic group compared with the STZ-diabetic group.

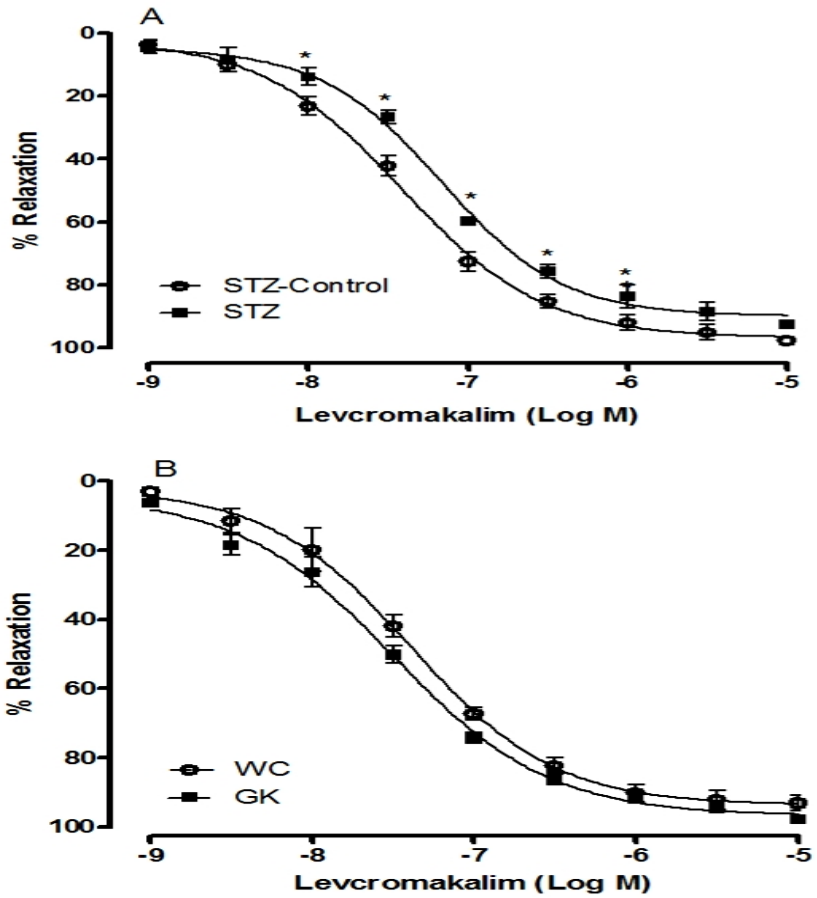

Fig. 3. Concentration-relaxation response curves for levcromakalim from STZ-diabetic aorta (A) and GK diabetic (B) rats

** = P< 0.05 vs. corresponding 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 was 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.

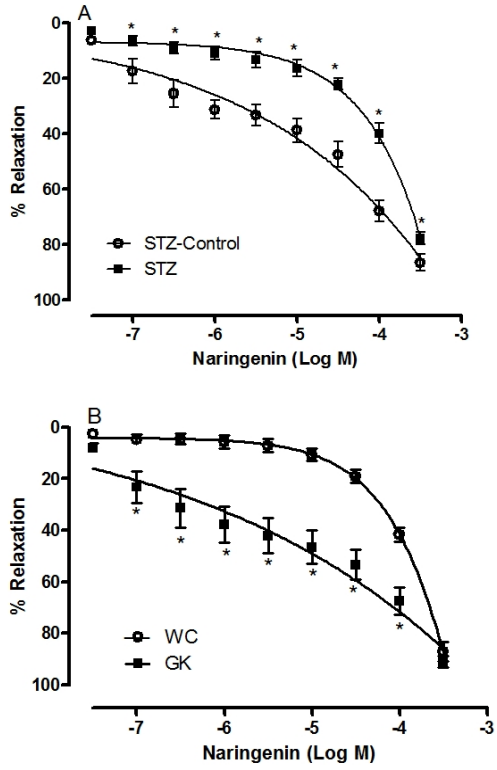

Fig. 4. Concentration-relaxation curves for (+/-)-naringenin in aortic rings obtained from STZ-induced (A) and GK diabetic (B) rats

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

4. DISCUSSION

The study compared the vascular reactivity to potassium channel openers and contraction to PE in Type 1 and Type 2 diabetic models. The main findings are that the K_{ATP} channel function and (+/-)-naringenin-induced relaxation were enhanced in GK model compared with STZ model. PE-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 DM both endothelium-dependent relaxation and potassium channel-mediated relaxation are preserved in Type 2 diabetic model whereas they are altered in Type 1 DM.

The result of decreased PE-induced contraction in Type 2 diabetes is in agreement with a previous study conducted on the aorta and mesenteric artery of STZ-induced DM, Zucker and GK rats that showed a decrease in contractile responses to alpha adrenergic agonist in early diabetic condition [17]. Various mechanisms for the decreased PE response include receptor desensitization, alteration in calcium potency/handling mechanisms and inhibition of contractile effects of alpha-adrenergic agonists in vascular smooth muscle by NO [17,19]. Therefore, the reduced contractility to PE 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.

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 DM [20], an increased alpha receptors density have been reported in STZ-diabetic rats [16]. 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 [17]. Taken together, the observed differences in contractile responses to PE 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 [13]. In contrast, attenuated relaxation response was observed in STZ-diabetic aorta which is in agreement with previous study [15]. In STZ-induced DM, the basal levels of NO production and expression of eNOS are reduced in diabetic arteries suggesting eNOS uncoupling [21]. On the other hand, there is enhanced NO production via over-expression of eNOS in GK diabetes [22]. It is therefore possible that the NO pathway is preserved through enhanced expression of eNOS in the early stage of Type 2 DM thereby 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 [23]. Studies have described differential responses of K_{ATP} channel in Type 1 and Type 2 DM [24-26]. Our result showed attenuation of the relaxations of the thoracic aorta induced by levcromakalim, a K_{ATP} channel opener in Type 1 DM which is in agreement with a previous study [27]. The mechanisms that underlie potassium channel dysfunction in diabetic vessels are dependent on the animal model, vessel bed, and stage of DM [28]. Therefore, K_{ATP} and BK_{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 ion [29,30]. Thus, the K_{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 flavonoid present in citrus fruits [31] is also a BK_{Ca} channel opener that induces concentration-dependent relaxation in aortic tissue from normal rats [32]. In this study, (+/-)-naringenin improved vasorelaxation in GK rats but not in STZ DM group. BK_{Ca} channel like the K_{ATP} channel mediates the vasorelaxant effect of K^+ channel openers [33]. A reduction in the potency of this opener as seen in STZ diabetic model could further explain the diabetic dysfunctions observed with potassium channels. (+/)-Naringenin could act both as an antioxidant and/or a BK_{Ca} opener. It is noteworthy that the pIC_{50} values for (+/-)-naringenin obtained in the two controls were 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 hyperglycaemia 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, hyperglycaemia may be a preconditioning stimulus that induces endothelial nitric oxide synthase and haemeoxegenase that exert both vasodilation as well as cardiovascular protection at early or late stage of type 2 DM [22]. 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 [34].

5. CONCLUSION

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

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

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COMPETING INTERESTS

Authors do not report any conflicts of interest.

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