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Alteration of vascular reactivity in diabetic human mammary artery and the effects of thiazolidinediones

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Abstract

Vascular reactivity was investigated in endothelium-denuded human internal mammary artery (IMA) rings from type 2 diabetic patients. It was also investigated whether insulin sensitizer thiazolidinedione drugs, pioglitazone and rosiglitazone, can directly affect the reactivity of IMA. Using organ bath techniques, cumulative concentration–response curves to phenylephrine (PE), KCl, cromakalim (CRO) and sodium nitroprusside (SNP) were constructed in diabetic and non-diabetic IMA rings. Means of maximal responses (% Emax) and pEC50 values (sensitivity) were compared. Emax values and the sensitivity to PE and KCl were increased while K_{ATP}-channel-mediated relaxations were reduced significantly in diabetic rings compared with non-diabetic rings ($n = 5–12$, $P < 0.05$). No changes were observed for SNP responses ($n = 5$, $P > 0.05$). Incubations with pioglitazone (1 and 10 μM) and rosiglitazone (1 and 20 μM), for 30 min, did not affect K_{ATP}-channel-mediated relaxations ($n = 5$ each, $P > 0.05$). Pioglitazone partly inhibited pre-contractions of PE and KCl at 10 μM, rosiglitazone did not. Vascular dysfunction observed in diabetic IMA may be of specific importance since they are widely used as coronary bypass material. Thiazolidinedione drugs may not worsen arterial dilatation through K_{ATP} channels in ischaemic or hypoxic insults in diabetic patients who are prone to such conditions. Pioglitazone has vasorelaxant property in the grafts.

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Introduction

Abnormal vascular function is one of the most complicated features of diabetes (Harris 2000). While impaired endothelium-dependent relaxation has been investigated in a variety of human diabetic vasculature (McVeigh et al 1992; Karasu et al 1995; Bijlstra et al 1996; Cipolla et al 1996; Rizzoni et al 2001; Okon et al 2005), considerably less attention has been devoted to the changes in vascular smooth muscle reactivity. In fact, the smooth muscles of blood vessels are affected in the diabetic state (McVeigh et al 1992; Karasu et al 1995; Okon et al 2005; Fleischhacker et al 1999; Yöntem et al 2000). Internal mammary arteries (IMA) are widely used as coronary bypass grafts (Grondin et al 1984). Although abnormal reactivity to vasoconstrictor agents has been reported (Karasu et al 1995; Okon et al 2005), the effect of diabetes on ATP-sensitive K^+ (K_{ATP}) channel-mediated relaxations has not been evaluated in human IMA grafts. The K_{ATP} channels are present in a variety of tissues. They participate in vasodilation during hypoxia and ischaemia in blood vessels (Ashcroft & Gribble 1999; Sobey 2001). A diminished relaxation response to K_{ATP}-channel openers has been reported in the saphenous vein and in coronary arterioles from diabetic patients (Yöntem et al 2000; Miura et al 2003).

Pioglitazone and rosiglitazone are novel insulin sensitizer thiazolidinediones (TZDs) that represent a new class of oral agents for treatment of type 2 diabetes. Their efficacy in diabetes is mainly due to activation of peroxisome proliferator-activated receptor γ (Hauner 2002). In addition, TZDs have direct beneficial effects on vascular wall structure, inflammation and endothelial function (Hetzel et al 2005; Campbell 2005; Martens et al 2005; Pfützner & Forst 2006). On the other hand, many studies have shown that TZDs directly modify ion channels (Buchanan et al 1995; Kotchen et al 1996; Lee et al 1996; Song et al 1997; Nakamura et al 1998; Knock et al 1999; Mishra & Aaronson 1999; Sunaga et al 1999; Eto et al 2001; Majithiya et al 2005). For example, pioglitazone directly dilates blood vessels by blocking Ca^{2+} channels (Zhang et al 1994; Buchanan et al 1995; Walker et al 1998; Majithiya et al 2005). Rosiglitazone enhances Ca^{2+}-activated K^+ currents in rat aortic cells

and inhibits delayed rectifier K^+ currents in rat pulmonary artery smooth muscle cells (Knock et al 1999). Interestingly, rosiglitazone blocks the K_{ATP} channels in rat aorta myocytes (Mishra & Aaronson 1999). In contrast, pioglitazone has no effect on cloned cardiac type K_{ATP} channel activity (Sunaga et al 1999). It seems likely that each TZD differs in its ability to influence ion channel function. If these drugs can affect human arterial reactivity, they could affect tissue perfusion during therapy. The first goal of this study was to determine whether vascular smooth muscle reactivity might be altered in IMA from type 2 diabetic patients. Our second goal was to test whether TZD drugs can affect vascular reactivity. Endothelium-denuded IMA rings obtained from type 2 diabetic patients undergoing coronary bypass operation were used in organ bath experiments.

Materials and Methods

Materials

Cromakalim was purchased from Tocris Cookson Ltd (UK). Rosiglitazone was kindly supplied by GlaxoSmithKline (UK) and pioglitazone from Takeda Chem. Ind. Ltd (Osaka, Japan). Other chemicals were purchased from Sigma Chemical Co. (St Louis, MO). Cromakalim, glibenclamide, pioglitazone and rosiglitazone were dissolved in dimethyl sulfoxide (DMSO). The maximum concentration of DMSO ($\leq 0.2\%$) (v/v) in the bath had no significant effect on the reactivity of the rings. Other chemicals were prepared in distilled water daily from their stock solutions.

Preparation of IMA rings

IMA segments were supplied from patients undergoing coronary artery revascularization surgery in Baskent University Hospital in Ankara, Turkey. Approval to use discarded IMA tissues was given by the Institutional Review Boards of Baskent University Hospital. The discarded IMA segments were placed in a container with oxygenated physiological solution (Krebs bicarbonate) maintained at 4°C and transferred to the laboratory in a short time (10–15 min). On arrival, the vessels were transferred to fresh aerated Krebs bicarbonate solution (composition (in mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$. $7H_2O$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, EDTA 0.026 and glucose 11.1), pH 7.4. Vessels were cleaned of adherent connective tissues, endothelium was denuded by gently rubbing the intimal surface with curved forceps, cut into rings 3–4 mm in length and then the rings were mounted in 20-mL organ baths. Change in arterial tension was recorded isometrically by a force-displacement transducer (model FDT10-A; Commat Ltd, Ankara, Turkey) connected to a computer-assisted data acquisition system (Commat Ltd, Ankara, Turkey). The baths were maintained at 37 °C and aerated with 5% CO_2 in O_2. Optimal resting tensions were maintained at 2 g.

Diabetic patients were treated with biguanide metformin, and or alpha-glucosidase inhibitor acarbose and or insulin. None of them used oral sulphonylurea or TZD drugs. Some of them had also received ACE inhibitors or nitrovasodilators. Non-diabetic patients, on the other hand, had received ACE inhibitors or nitrovasodilators. There was no information on their background (long-term) therapy, but the average diabetes duration was 11 ± 4 years.

Experimental protocol

After equilibration periods, each ring was contracted twice with 40 mM KCl to assess their viability. The rings were submaximally pre-contracted with phenylephrine (PE) (10 μM), the absence of endothelium was checked by confirming no relaxation to acetylcholine (Ach) (10 μM). Each ring was washed and rested for 20 min. Cumulative concentration–response curves to PE or KCl were performed in diabetic or non-diabetic rings. In separate experiments, cumulative concentration–response curves to cromakalim (CRO; a K_{ATP}-channel opener) or sodium nitroprusside (SNP; an endothelium-independent vasodilator) were obtained in pre-contracted rings with PE. Since diabetic rings were more responsive to PE, they were pre-contracted with an equieffective concentration of PE (usually 0.3 μM).

In separate and parallel experiments, the rings were incubated for 30 min with vehicle (DMSO) or pioglitazone (1 μM or 10 μM) or rosiglitazone (1 μM or 20 μM) or glibenclamide (10 μM) (a K_{ATP}-channel blocker sulphonylurea). At the end of incubation periods, pre-contraction with PE was repeated, and cumulative concentration–response curves to CRO were performed. Since pioglitazone has a vasodepressive effect at 10 μM concentration, the rings were pre-contracted with an equieffective concentration of PE after 10 μM pioglitazone incubations.

Separately, in some diabetic rings, concentration-dependent vasodepressive effects of pioglitazone (10^{-7} to 10^{-5} M) or rosiglitazone (10^{-7} to 10^{-5} M) were tested cumulatively in the rings pre-contracted with PE or KCl.

Only one drug was tested and one concentration–response curve was performed in a single ring.

Statistical analysis

The contractile responses to PE were expressed as the percentage of maximal contraction of KCl. The degree of CRO- or SNP-induced relaxation was expressed as the percentage of the initial tension induced by PE. Agonist pEC50 (pD_2 value; apparent agonist affinity constant; −log EC50; the negative log of the molar concentration of the drug giving 50% of the maximal response) was calculated by linear regression analysis of the concentration–response curves and taken as a measure of the sensitivity of the arteries to the agonists. All data are presented as mean ± s.e., with n representing the number of arteries. When more than one vessel segment was used from the same patient, the results were pooled. $P < 0.05$ obtained from unpaired Student's t-test or analysis of variance (followed by Neuman–Keul's test), as appropriate, was regarded as significant.

Results and Discussion

Some clinical characteristics of patients are summarized in Table 1.

Table 1 Some characteristics of patients

Patients	Non-diabetic	Diabetic
Number (female/male)	46 (14/32)	19 (5/14)
Age (years)	64 ± 2	66 ± 2
Serum glucose (mg dL^{-1})	85 ± 3	196 ± 9*
HbA1c (%)	—	8.3 ± 1.1
Total cholesterol (mg dL^{-1})	187 ± 14	191 ± 14
Serum triglyceride (mg dL^{-1})	205 ± 15	211 ± 17
HDL cholesterol (mg dL^{-1})	42 ± 2	38 ± 2
LDL cholesterol (mg dL^{-1})	133 ± 16	135 ± 14
Systolic BP (mmHg)	110 ± 3	123 ± 4*
Diastolic BP (mmHg)	73 ± 3	76 ± 2

Values are mean ± s.e.m. *$P < 0.001$, compared with non-diabetic group.

Table 2 pEC50 values and % maximum responses (Emax) for phenylephrine (PE), sodium nitroprusside (SNP) and KCl in non-diabetic and diabetic human internal mammary arteries (IMAs)

	Non-diabetic IMAs		Diabetic IMAs	
	pEC50	Emax (%)	pEC50	Emax (%)
PE	5.49 ± 0.1	204 ± 10	6.52 ± 0.1*	270 ± 14*
SNP	7.88 ± 0.04	98 ± 5	8.02 ± 0.05	105 ± 6
KCl	EC50 (mM)		EC50 (mM)	
	21.2 ± 0.02	100 ± 12	18.3 ± 0.04*	156 ± 8*

*$P < 0.001$ compared with non-diabetic IMA group. Values are mean ± s.e.m., n = 5–12. Phenylephrine responses are given as % contraction of 40 mM KCl.

Emaxs of PE and KCl were increased by 33 ± 12%, and by 43 ± 10% in diabetic rings, respectively. The pEC50 values were also changed significantly (Figure 1, Table 2). The Emax of CRO was decreased significantly by 36 ± 4% (n = 5, $P < 0.05$) in diabetic rings. Sensitivity to CRO was also changed significantly (Figure 2, Table 3). Glibenclamide, a

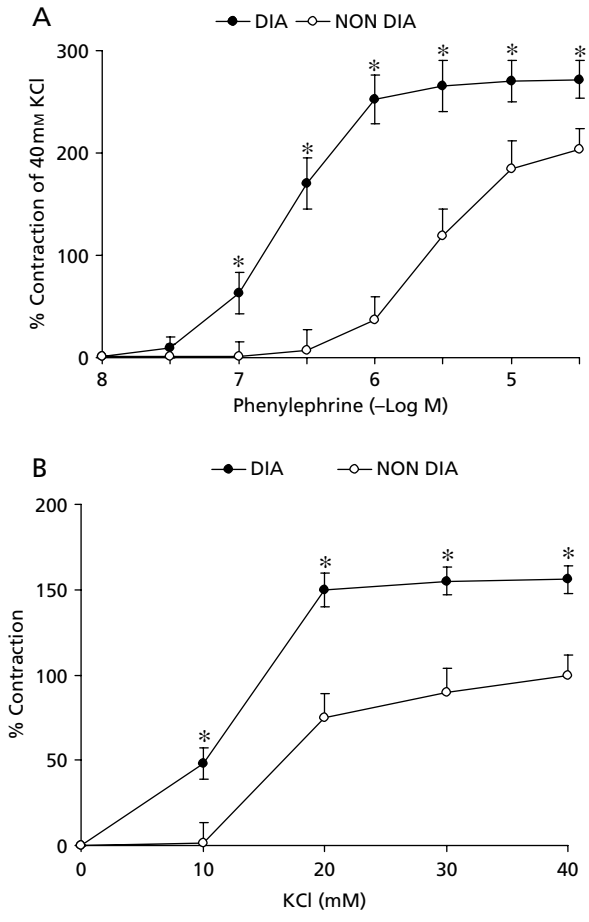

Figure 1 Concentration–response curves to phenylephrine (A) (n = 12) and KCl (B) (n = 5) in IMA rings from non-diabetic and type 2 diabetic patients. Values are mean ± s.e.m. *$P < 0.05$ compared with non-diabetic group.

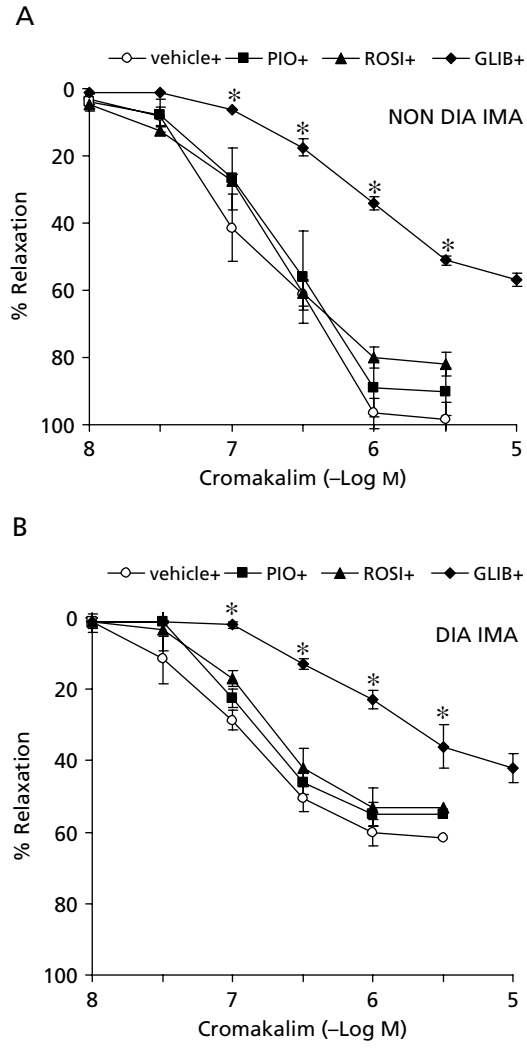

Figure 2 Concentration–response curves to cromakalim after 30-min incubations in IMA rings, from non-diabetic (A) and type 2 diabetic (B) patients, pre-contracted with phenylephrine. Vehicle, DMSO; PIO, pioglitazone (10 μM); ROSI, rosiglitazone (20 μM); GLIB, glibenclamide (10 μM). Relaxation responses are expressed as a percentage of the initial tension induced by phenylephrine. Values are mean ± s.e.m., n = 5 each. *$P < 0.05$, compared with control.

Table 3 pEC50 values and % maximum relaxations (Emax) for cromakalim in non-diabetic and diabetic human internal mammary arteries (IMAs) after 30-min incubations

Incubations	Non-diabetic IMAs		Diabetic IMAs	
	pEC50	Emax (%)	pEC50	Emax (%)
Vehicle (control)	6.93 ± 0.02	98 ± 6	6.82 ± 0.04*	63 ± 4*
Pioglitazone 1 μM	n.t.	n.t.	6.80 ± 0.04	58 ± 8
Pioglitazone 10 μM	6.86 ± 0.02	90 ± 11	6.77 ± 0.15	52 ± 6
Rosiglitazone 1 μM	n.t.	n.t.	6.80 ± 0.03	60 ± 7
Rosiglitazone 20 μM	6.93 ± 0.07	94 ± 6	6.82 ± 0.03	53 ± 5
Glibenclamide 10 μM	6.17 ± 0.06†	51 ± 2†	6.32 ± 0.05†	36 ± 6†

*$P < 0.05$, compared with non-diabetic IMA group; †$P < 0.001$, compared with control group. Vehicle, DMSO; n.t., not tested. For the comparison, Emaxs were also given at 3 μM concentration of cromakalim after glibenclamide incubations (see Figure 2). Values are mean ± s.e.m., n = 5 for each group.

specific K_{ATP}-channel blocker, inhibited the concentration–response curves of CRO in a competitive manner in both groups of arteries. Percentage inhibition of Emax values of CRO (at 3 μM concentration) was 48 ± 4% in non-diabetic and 43 ± 5% in diabetic rings (n = 5 each, $P > 0.05$) after glibenclamide incubation (Figure 2, Table 3). Pioglitazone (1 and 10 μM) and rosiglitazone (1 and 20 μM) incubations did not alter significantly the relaxations induced by CRO in either group of arteries (Figure 2, Table 3).

Relaxations to SNP were similar in both groups of arteries (n = 5 each, $P > 0.05$) (Table 2).

Addition of pioglitazone (10^{-7} to10^{-5} M) cumulatively in diabetic rings pre-contracted with PE or KCl did not produce any significant relaxation less than 10 μM. The drug depressed pre-contractions by 14 ± 4% and by 24 ± 9%, respectively, at 10 μM concentration (n = 5 each, $P < 0.05$) (Figure 3). Rosiglitazone (10^{-7} to 10^{-5} M) did not attenuate pre-contractions induced by PE or KCl significantly (data not shown).

Our results demonstrate that endothelium-free IMA from type 2 diabetic patients is more responsive to vasoconstrictor agents and less responsive to K_{ATP}-channel openers than IMA from non-diabetic patients. In fact, though endothelium dysfunction has a major role in diabetic vascular complications there is limited data on the effects of diabetes on vascular smooth muscle reactivity. So far, impaired endothelium-dependent relaxation has been consistently demonstrated in diabetic human vessels (McVeigh et al 1992; Karasu et al 1995; Cipolla et al 1996; Rizzoni et al 2001; Okon et al 2005). Increased vessel contractility was found for receptor-dependent and receptor-independent mechanisms in diabetes (White & Carrier 1990; Taylor et al 1994; Tam et al 1997; Xavier et al 2003). Higher alpha-adrenergic responsiveness has been reported in endothelium-free human IMA from type 2 diabetic patients (Karasu et al 1995). Augmented vasoconstriction in the presence of NO synthase inhibitor in diabetic IMA suggests that NO-independent mechanisms also contribute to vascular dysfunction in diabetes (Okon et al 2005). In rings obtained from diabetic uterine arteries, smooth muscle sensitivity to noradrenaline and PE was found to be enhanced because of the changes in subcellular calcium distribution. Relaxation caused by activation of Ca^{2+}-activated potassium channels (K_{Ca}) was found to be reduced in diabetic uterine arteries (Fleischhacker et al 1999). Recent studies strongly

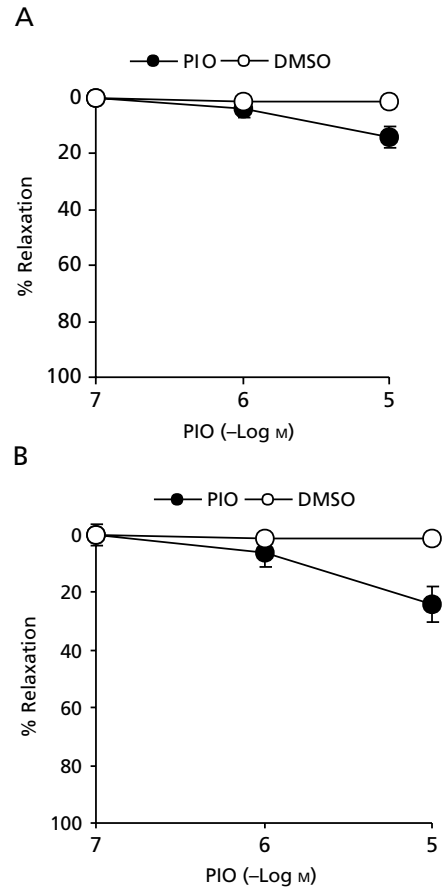

Figure 3 Vasodepressor effect of pioglitazone (PIO) in IMA rings from type 2 diabetic patients pre-contracted with phenylephrine (A) and KCl (B). DMSO, vehicle. Values are mean ± s.e.m., n = 5 each.

indicate that oxidative stress is increased abnormally in the diabetic state, having an important outcome at the cellular and subcellular levels affecting numerous mechanisms, including Ca^{2+} homoeostasis, and thus altering reactivity of the vessels (Fleischhacker et al 1999, Liu & Gutterman 2002; Irat et al 2003; Niedowicz & Daleke 2005). Some pathological processes that lead to endothelium dysfunction can also

affect smooth muscle function. Hypertension is a common complication of type 2 diabetes/insulin resistance (Osei 1999). Diabetic patients were hypertensive in our study. We can conclude that, in addition to endothelial dysfunction (McVeigh et al 1992; Karasu et al 1995; Bijlstra et al 1996; Cipolla et al 1996; Rizzoni et al 2001; Okon et al 2005), abnormal reactivity of arterial vascular smooth muscle may play a role in the development of hypertension in type 2 diabetes. Due to weaker production of endothelium-dependent relaxant factors from the endothelium, the smooth muscle contraction may be unmasked. Additionally, some pathological processes that lead to endothelium dysfunction can also affect smooth muscle function.

The new finding in this study is that diabetes impairs vasodilator responses to K_{ATP}-channel opening in IMA. Previously, a diminished relaxation response to K_{ATP}-channel openers has been observed in aortas (Kamata et al 1989), cerebral arteries (Mayhan & Faraci 1993; Mayhan 1994; Zimmermann et al 1997) and basilar arteries (Matsumoto et al 2004) from experimental-diabetic animals and in saphenous vein grafts (Yöntem et al 2000) and coronary arteries (Miura et al 2003) from diabetic patients. Chronic insulin treatment can reverse the impaired relaxation to cromakalim in basilar arteries from diabetic rats indicating this is specific for diabetes (Matsumoto et al 2004). Excess production of superoxide has been implicated in the impaired responses to K_{ATP}-channel openers in diabetic rats (Liu & Gutterman 2002). Impairment of coronary dilation to K_{ATP} channel opening leads to reduced dilation to hypoxia and this reduction could contribute to cardiovascular mortality and morbidity in diabetes (Miura et al 2003). Since IMA is used as coronary bypass material, our observation may have clinical significance. The relaxation by SNP was not altered in diabetic IMAs indicating that the reduced vasodilation in the diabetic state is unlikely to be a result of the defective response of vascular smooth muscle. On the other hand, pre-contractile values for PE were not changed in the presence of glibenclamide in our diabetic vessels suggesting that hyperresponsiveness to PE in our study is independent of the defective responses to K_{ATP}-channel function in diabetes.

TZD drugs, pioglitazone and rosiglitazone, at the range of therapeutic, even high, concentrations did not affect the relaxation induced by K_{ATP} channel opening in IMA. Electrophysiological studies have demonstrated that rosiglitazone is less potent ($IC50 = 20\,\mu M$) on ion channels than other TZDs (pioglitazone and troglitazone) in vascular myocytes (Knock et al 1999; Mishra & Aaronson 1999, Sunaga et al 1999). Besides low concentration, we examined a higher concentration of rosiglitazone ($20\,\mu M$) than PIO ($10\,\mu M$), although their clinical effective blood concentrations are about 0.6–1 μM and 0.8–8 μM, respectively. Hence, we can assume that TZDs may not adversely affect vascular haemodynamic adaptive changes via K_{ATP} channels during therapy.

Pioglitazone, but not rosiglitazone, in our vessels, partly inhibited pre-contractions induced by KCl and PE. Walker at al (1998) has reported that rosiglitazone, at concentrations up to 100 μM, did not induce any relaxation on human subcutaneous small arteries. In a recent study conducted in type 1 model of diabetic rats, the blood-pressure-lowering effect of pioglitazone is attributed partly to its direct vasodepressive effect on the vessel wall (Majithiya et al 2005). The direct vasorelaxant effect of pioglitazone (and troglitazone) is due to its L-type Ca^{2+}-channel blocker property (Zhang et al 1994; Song et al 1997; Nakamura et al 1998; Walker et al 1998). The precise mechanism of the inhibitory action of TZDs on Ca^{+2} channels is not known, but is reported to be different from the known organic Ca^{2+}-channel antagonists (Nakamura et al 1998). The right half of the structure, which includes benzyl-TZD, is essential for the inhibition of Ca^{2+} channels and the other half of the structure may affect the ability to inhibit Ca^{2+} channels by TZDs (Eto et al 2001). On the other hand, studies in diabetic subjects as well in those with metabolic syndrome have shown that TZDs improve endothelium-dependent vasodilation (Pistrosch et al 2004; Forst et al 2005; Pfützner & Forst 2006) and reduce blood pressure (Fullert et al 2002) by improvement of insulin sensitivity. In addition, TZDs have direct effects, including decreased biomarkers of atherosclerosis and inhibition of vascular smooth muscle proliferation, promoting the concept that pioglitazone and rosiglitazone with their pleiotrophic effects may exhibit protective effects in the micro- and macrovascular vessel wall in both type 2 diabetic and insulin resistant non-diabetic patients (Campbell 2005, Pfützner & Forst 2006). Very recently, it has been reported that rosiglitazone can ameliorate arterial stiffness with augmented plasma adinopectin levels in patients with non-diabetic metabolic syndrome (Kim et al 2006). Morevor, Hetzel et al (2005) reported that short-term rosiglitazone treatment of healthy subjects, even within the first day of treatment, increases endothelium-dependent vasodilation with rapid reduction of some biomarkers on atherosclerosis. Again, short-term pioglitazone treatment ameliorates endothelial dysfunction in conduit arteries irrespective of beneficial metabolic changes in type 2 diabetic patients (Martens et al 2005). Taken together with these observations, pioglitazone also directly may help in preventing diabetes-induced intracellular Ca^{2+} overload and subsequent vascular smooth muscle changes or vasodilation.

Conclusion

Increased contractility of vascular smooth muscle in diabetic IMA may be important together with endothelial dysfunction in diabetes-induced vascular complications. Due to weaker production of endothelium-dependent relaxing factors in diabetes, abnormal smooth muscle contraction may be unmasked in diabetic IMA grafts. Defective dilation to K_{ATP} channel opening may play a role on the performance of diabetic IMA grafts in (patho)physiological conditions, such as ischaemic or hypoxic insults, to which diabetic patients are prone. Having no adverse effect of TZDs on K_{ATP} channel-mediated relaxations in IMA grafts may have clinical importance during treatment. Pioglitazone, with direct small vasorelaxant effect, may have additional benefits in vascular wall.

References

Ashcroft, F. M., Gribble, F. M. (1994) ATP-sensitive K^+ channels and insulin secretion: their role in health and disease. *Diabetologia* **42**: 903–919

Bijlstra, P. J, Lutterman, J. A, Russel, F. G., Thien, T., Smits, P. (1996) Interaction of sulphonylurea derivatives with vascular ATP-sensitive potassium channels in humans. *Diabetologia* **39**: 1083–1090

Buchanan, T. A., Meehan, W. P., Jeng, Y. Y., Yang, D., Chan, T. M., Nadler, J. L., Scott, S., Rude, R. K., Hsueh, W. A. (1995) Blood pressure lowering by pioglitazone. Evidence for a direct vascular effect. *J. Clin. Invest*. **96**: 354–360

Campbell, I. W (2005) The clinical significance of PPAR gamma agonism. *Curr. Mol. Med*. **5**: 349–363

Cipolla, M. J., Harker, C. T., Porter, J. M. (1996) Endothelial function and adrenergic reactivity in human Type II diabetic resistance arteries. *J. Vasc. Surg*. **23**: 940–949

Eto, K., Ohya, Y., Nakamura, Y., Abe, I., Fujishima, M. (2001) Comparative actions of insulin sensitizers on ion channels in vascular smooth muscle. *Eur. J. Pharmacol*. **423**: 1–7

Fleischhacker, E., Esenabhalu, V. E., Spitaler, M., Holzmann, S., Skrabal, F., Koidl, B., Kostner G. M., (1999) Human diabetes is associated with hyperreactivity of vascular smooth muscle cells due to altered subcellular Ca^{2+} distribution. *Diabetes* **48**: 1323–1330

Forst, T., Lubben, G., Hohberg, C., Kann, P., Sachara, C., Gottschall, V., Friedrigh, C., Rosskopf, R., Pfutzner, A. (2005) Influence of glucose control and improvement of insulin resistance on microvascular blood flow and endothelial function in patients with diabetes mellitus type 2. *Microcirculation* **12**: 543–550

Fullert, S., Schneider, F., Haak, E., Rau, H., Badenhoop, K., Lubben, G., Usadel, K. H., Konrad, T. (2002) Effects of pioglitazone in nondiabetic patients with arterial hypertension: a double-blind, placebo-controlled study. *J. Clin. Endocrinol. Metab*. **12**: 5503–5506

Grondin, C. M., Campeau, L., Lesperance, J., Enjalbert, M., Bourassa, M. G. (1984) Comparison of late changes in internal mammary artery and saphenous vein grafts in two consecutive series of patients 10 years after operation. *Circulation* **70** (Suppl. 1): 208–212

Harris, M. I. (2000) Health care and health status and outcomes for patients with type 2 diabetes. *Diabetes Care* **23**: 754–758

Hauner, H. (2002) The mode of action of thiazolidinediones. *Diabetes Metab. Res. Rev*. **18**: S10–S15

Hetzel, J., Balletshofer, B., Rittig, K., Walcher, D., Kratzer, W., Hombach, V., Haring, H. U., Koening, W., Marx, N. (2005) Rapid effects of rosiglitazone treatment on endothelial function and inflammatory biomarkers. *Arterioscler. Thromb. Vasc. Biol*. **25**: 1804–1809

Irat, A. M., Aktan, F., Ozansoy, G. (2003) Effects of L-carnitine treatment on oxidant / antioxidant state and vascular reactivity of streptozotocin-diabetic rat aorta. *J. Pharm. Pharmacol*. **3**: 1389–1395

Kamata, K., Miyata, N., Kasuya, Y. (1989) Functional changes in potassium channels in aortas from rats with streptozotocin-induced diabetes. *Eur. J. Pharmacol*. **166**: 319–323

Karasu, C., Soncul, H., Altan, V. M. (1995) Effects of non-insulin dependent diabetes mellitus on the reactivity of human internal mammary artery and human saphenous vein. *Life Sci*. **57**: 103–112

Kim, S. G., Ryu, O. H., Kim, H. Y., Lee, K. W., Seo, J. A., Kim, N. H., Choi, K. M., Lee, J., Baik, S. H., Choi, D. S. (2006) Effect of rosiglitazone on plasma adinopectin levels and arterial stiffness in subjects with prediabetes or non-diabetic metabolic syndrome. *Eur. J. Endocrinol*. **154**: 433–440

Knock, G. A., Mishra, S. K., Aaranson, P. I. (1999) Differential effects of insulin-sensitizers troglitazone and rosiglitazone on ion currents in rat vascular myocytes. *Eur. J. Pharmacol*. **368**: 103–109

Kotchen, T. A., Zhang, H. Y., Reddy, S., Hoffmann, R. G. (1996) Effect of pioglitazone on vascular reactivity in vivo and in vitro. *Am. J. Physiol*. **270**: R660–666

Lee, K., Ibbotson, T., Richardson, P. J., Boden, P. R. (1996) Inhibition of K_{ATP} channel activity by troglitazone in CRI-GI insulin-secreting cells. *Eur. J. Pharmacol*. **313**: 163–167

Liu, Y., Gutterman, D. D. (2002) The coronary circulation in diabetes: influence of reactive oxygen species on K^+ channel-mediated vasodilation. *Vasc. Pharmacol*. **38**: 43–49

Majithiya, J. B., Paramar, A. N., Balaraman, R. (2005) Pioglitazone, a PPAR gamma agonist, restores endothelial function in aorta of STZ-induced diabetic rats. *Cardiovasc. Res*. **66**: 150–161

Martens, F., Visseran, F., de Koning, E., Rabelink, T. (2005) Short-term pioglitazone treatment improves vascular function irrespective of metabolic changes in patients with type 2 diabetes. *J. Cardiovasc. Pharmacol*. **46**: 773–778

Matsumoto, T., Yoshiyama, S., Wakabayashi, K., Kobayashi, T., Kamata, K. (2004) Effect of insulin on cromakalim-induced relaxations in established streptozotocin-diabetic rat basilar artery. *Eur. J. Pharmacol*. **504**: 129–137

Mayhan, W. G. (1994) Effect of diabetes mellitus on response of the basilar artery to activation of ATP-sensitive potassium channels. *Brain Res*. **636**: 35–39

Mayhan, W. G., Faraci, M. (1993) Responses of cerebral arterioles in diabetic rats to activation of ATP-sensitive potassium channels. *Am. J. Physiol*. **265**: H152–H157

McVeigh, G. E., Brennan, G. M., Johnston, G. D., Mc Dermott, B. J., McGrath, L. T., Henry, W. R., Andrews, J. W., Hayes, D. R. (1992) Impaired endothelium-dependent and independent vasodilation in patients with type 2 (non-insulin-dependent) diabetes mellitus. *Diabetologia* **35**: 771–776

Mishra, S. K., Aaronson, P. I. (1999) Differential block by troglitazone and rosiglitazone of glibenclamide-sensitive K^+ current in rat aorta myocytes. *Eur. J. Pharmacol*. **386**: 121–125

Miura, H., Watchtel, R. E., Loberiza, F. R., Saito, T., Miura, M., Nicolosi, A. C., Gutterman, D. D. (2003) Diabetes mellitus impairs vasodilation to hypoxia in human coronary arterioles. Reduced activity of ATP-sensitive potassium channels. *Circ. Res*. **92**: 151–158

Nakamura, Y., Ohya, Y., Onaka, U., Fujii, K., Abe, I., Fujishima, M. (1998) Inhibitory action of insulin-sensitising agents on calcium channels in smooth muscle cells from resistance arteries of guinea-pig. *Br. J. Pharmacol*. **123**: 675–682

Niedowoicz, D. M., Daleke, D. L. (2005) The role of oxidative stress in diabetic complications. *Cell Biochem. Biophys*. **43**: 289–330

Okon, E. B., Chung, A. W., Rauniyar, P., Padilla, E., Tejenna, T., McManus, B. M., Luo, H., Van Breemen, C. (2005) Compromised arterial function in human Type 2 diabetic patients. *Diabetes* **54**: 2415–2423

Osei, K. (1999) Insulin resistance and systemic hypertension. *Am. J. Cardiol*. **84**: 33J–36J

Pfützner, A., Forst, T. (2006) Pioglitazone: an antidiabetic drug with potency to reduce cardiovascular mortality. *Exp. Opin. Pharmacother*. **7**: 463–476

Pistrosch, F., Passauer, J., Fischer, S., Fuecker, K., Hanefeld, M., Gross, P. (2004) In type 2 diabetes, rosiglitazone therapy for insulin resistance ameliorates endothelial dysfunction independent of glucose control. *Diabetes Care* **27**:484–490

Rizzoni, D., Porteri, E., Guelfi, D., Muiesan, M. L. (2001) Endothelial dysfunction in small resistance arteries of patients with non-insulin-dependent diabetes mellitus. *J. Hypertens*. **19**: 913–919

Sobey, C. G. (2001) Potassium channel function in vascular disease. *Arteriosc. Thromb. Vasc. Biol*. **21**: 28–38

Song, J., Walsh, M. F., Igwe, R., Ram, J., Barazi, M., Dominguez, L. J., Sowers, J. R. (1997) Troglitazone reduces contraction by inhibition of vascular smooth muscle cell calcium currents and not endothelial nitric oxide production. *Diabetes* **46**: 659–664

Sunaga, Y., Inagaki, N., Gonoi, T., Yamada, Y., Ishida, H., Seino, Y., Seino, S. (1999) Troglitazone but not pioglitazone affects ATP-sensitive K^+ channel activity. *Eur. J. Pharmacol*. **381**: 71–76

Tam, E. S., Ferguson, D. G., Bielefeld, D. R., Lorenz, J. N., Cohen, R. M., Pun, R. Y. (1997) Norepinephrine-mediated calcium signaling

is altered in vascular smooth muscle of diabetic rat. *Cell Calcium* **21**: 143–150

Taylor, P. D., Oon, B. B, Thomas, C. R., Poston, L. (1994) Prevention by insulin treatment of endothelial dysfunction but not enhanced noradrenaline-induced contractility in mesenteric resistance arteries from streptozotocin-induced diabetic rat. *Br. J. Pharmacol*. **111**: 35–41

Walker, A. B., Naderali, E. K., Chattington, P. D., Buckingham, R. E., Williams, G. (1998) Differential vasoactive effects of the insulin sensitizers rosiglitazone (BRL 49653) and troglitazone on human small arteries in vitro. *Diabetes* **47**: 810–814

White, R. E., Carrier, G. O. (1990) Vascular contraction induced by activation of membrane calcium ion channels is enhanced in streptozotocin-diabetes. *J. Pharmacol. Exp. Ther*. **253**: 1057–1062

Xavier, F. E., Davel, A. P., Rossoni, L. V., Vassalo, D. V. (2003) Time-dependent hyperreactivity to phenylephrine in aorta from untreated diabetic rats: role of prostanoids and calcium mobilisation. *Vasc. Pharmacol*. **40**: 67–76

Yöntem, Ö., Sahilli, M., Karasu, C., Ozcelikay, A. T., Altan, V. M., Ari, N. (2000) Troglitazone has no effect on K_{ATP} channel opener induced-relaxations in rat aorta and in human saphenous veins from patients with type 2 diabetes. *Life Sci*. **68**: 557–568

Zhang, F., Sowers, J., Ram, J. L., Standley, P. R., Peuler, J. D. (1994) Effects of pioglitazone on calcium channels in vascular smooth muscle. *Hypertension* **24**: 170–175

Zimmermann, P. A., Knot, H. J., Stevenson, A. S., Nelson, M. T. (1997) Increased myogenic tone and diminished responsiveness to ATP-sensitive potassium channel openers in cerebral arteries from diabetic rats. *Circ. Res*. **81**: 996–1004