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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

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Abstract

Troglitazone, a thiazolidinedione derivative, is an oral antidiabetic agent that enhances insulin sensitivity in insulin-resistant states. K_{ATP} channels, on the other hand, have important roles protecting cardiovascular system in ischemic and /or hypoxic states. They are also important in the control of vascular tone, and therefore of blood pressure. We tested whether troglitazone can directly affect vascular K_{ATP} channel opener-induced relaxations in vitro. 1, 10 or 100 μM troglitazone incubations for 30 min did not alter cromakalim (a K_{ATP} channel opener) - induced relaxations in endothelium-denuded aortas from rat, saphenous veins from type 2 diabetic and nondiabetic patients. In addition, we compared the sensitivity to cromakalim in diabetic saphenous veins with that of nondiabetic veins. The concentration-response curve for cromakalim was shifted to the right in diabetic vein. pD_2 values for cromakalim were 6.85 ± 0.08 vs. 6.61 ± 0.04 ($p<0.05$) in nondiabetic (n:10) and diabetic (n:7) veins respectively. % maximum response of cromakalim was also significantly decreased by 24 ± 3% in diabetic veins. However, responsiveness of veins to phenylephrine or sodium nitroprusside were similar in both groups. The results obtained may be clinically useful 1. suggesting that in ischaemic and / or hypoxic insults troglitazone may not worsen vascular dilatation, through K_{ATP} channel, in diabetic patients who are more prone to these conditions than healthy people, 2. providing an evidence that diabetes causes an impaired dilatation of human saphenous vein through K_{ATP} channels. This may partly be related with diabetes-induced vascular complications, such as vasospasm and even hypertension. Accordingly, since saphenous veins are used as conduit vessels in coronary by-pass graft surgery, the results also suggest that the defective dilatation through K_{ATP} channels may play a role on the performance of saphenous vein grafts in type 2 diabetes. © 2000 Elsevier Science Inc. All rights reserved.

Keywords: Troglitazone; K_{ATP} channels; Rat aorta; Human saphenous vein; Type 2 diabetes

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Introduction

Type 2 diabetes mellitus accounts for about 85 % of all cases of diabetes mellitus, and has been reported to be an important risk factor for cardiovascular morbidity and mortality (1). The ATP-sensitive K^+ (K_{ATP}) channels, on the other hand, represent a target for the hypoglycemic sulfonylureas a group of compounds that has been used in the treatment of type 2 diabetes for several decades. These drugs exert their insulinotropic effect by closing K_{ATP} channels in β-cells of the pancreas (2,3). K_{ATP} channels have been found in a wide variety of tissues including cardiovascular system (4,5). They are opened in response to cellular metabolic stress like hypoxia and / or ischaemia to protect myocardium and vascular smooth muscle (1, 6–8). These channels are also important in the control of vascular smooth muscle tone, and therefore of blood pressure (8). Drugs that inhibit vascular K_{ATP} channels have the potential to cause undesired side effects. Sulphonylureas, has been demonstrated to attenuate the vasodilator response to K_{ATP} channel activation (9) or to ischaemic stimulus (10). The question of whether sulfonylureas cause adverse cardiovascular side effects under clinical conditions has been debated, and conflicting results have been reported from different studies (1,9, 11–13). In addition, treatment with sulfonylureas is associated with a number of problems including hypoglycemic episodes, aggravation of pre-existing hyperinsulinemia and acceleration of β-cell exhaustion (13–15). In attempts to overcome these problems and since mainly improving the action of insulin is a new concept in type 2 diabetes therapy, several novel antidiabetic compounds are currently in development. Among them, troglitazone improves insulin sensitivity in skeletal muscle, liver and adipose tissue in insulin resistance syndromes (16–17). The drug decreases hyperglycemia, hyperinsulinemia and hyperglyceridemia associated with insulin resistance reversing many of the clinical manifestations of type 2 diabetes (16–18). The main target for the insulin sensitizing effect of thiazolidinediones is the peroxisome proliferator-activated receptor gamma (PPAR gamma) which thiazolidinediones bind and activate it (19). On the other hand, troglitazone has no effect on insulin secretion during therapy (14, 16–18). However, it was suggested in a radioligand binding study that troglitazone has a putative non-competitive binding site at the sulphonylurea receptor on rat pancreatic islets and hamster β-cell line (HIT cell) (20). Lee et al. (21), by patch-clamp method, reported that troglitazone inhibits K_{ATP} channel activity in rat insulinoma-GI (CRI-GI) insulin-secreting cells, suggesting a possible contribution to the stimulation of insulin secretion. It was also shown that troglitazone inhibits cardiac type K_{ATP} channel activity (22). Hence, it was concluded that the drug could adversely affect patients during cardiac ischaemia. Troglitazone also inhibits K_{ATP} channel activity in rat hypothalamic neurons (23).

Since diabetic patients are more prone to cardiovascular ischaemic insults than healthy people (1,9), it is tempting to know whether troglitazone has any effect on K_{ATP} channel opener-induced relaxation in vasculature. To our knowledge, however, there is no study examining the effect of troglitazone on the dilator responses through K_{ATP} channels in vascular smooth muscle. Therefore in this in vitro study we investigated if there was any ability of troglitazone to reduce the vasodilator response of cromakalim in rat thoracic aorta, saphenous veins from type 2 diabetic and from nondiabetic patients. In addition, we compared cromakalim-induced relaxations in diabetic saphenous veins with that of nondiabetic veins. Saphenous veins are widely used as coronary bypass grafts. Vasospasm of the grafts is a feared complication of perioperative and / or postoperative period. Graft closure due to

spasm and / or intimal proliferation limits the graft longevity and spasm of graft material may contribute to myocardial ischaemia and early postoperative morbidity (24). The effect of diabetes on K_{ATP} channel-mediated relaxation has not been evaluated in a bypass graft.

Methods

Preparation of isolated vessels

Male adult Wistar rats (150–200 g), were anesthetized with sodium pentobarbital (30 mg/kg, i.p.), and were killed by servical dislocation. The thoracic aorta was removed quickly and placed in standart bicarbonate-buffered physiological salt solution (PSS) with the 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), gassed with 5% CO_2 in O_2. Saphenous vein grafts were supplied from patients undergoing coronary artery revascularisation surgery. The study had approval by the Instutional Review Boards of Baskent University Hospital. Clinical characteristics of patients are summarized in Table 1. Diabetic patients had been treated with oral sulfonylurea (glipizide), biguanides or alpha-glucosidase inhibitors. Some of them had also received ACE inhibitors, or nitrovasodilators. Nondiabetic patients, on the other hand, had received calcium antagonists, ACE inhibitors, or nitrovasodilators. Saphenous veins were obtained before the dilatation procedure and then were placed immediately into cold (4°C) PSS, transported in a short time to the laboratory. Aortas and veins were cleaned of adherent connective tissues. The endothelium was denuded by a gentl rubbing the intimal surface of the vessels with curved forceps, cut into rings 3–4 mm in length and then the rings were mounted in 20-ml organ baths containing in PPS for isometric tension recordings connected to a force transducers. The baths were maintained at 37 °C and aerated with 5% CO_2 in O_2. Optimal resting tensions were maintained at 2 g for aortas, 5–6 g for veins (to determine optimal tensions, the rings were stretched step by step until optimal and reproducible contraction to KCL (60 mM) was achived). Then, aortas and veins were allowed to equilibrate 1 h or 2.5 h respectively (a prolonged period for veins was choosen to washout the drugs used by patients before operation). During these periods the rings were washed in every 15 min. Changes in

Table 1
The general characteristics of patients undergoing coronary arter revascularisation surgery

Patients	Nondiabetic	Diabetic
Number (f / m)	26 (6/20)	19 (5/14)
Age (years)	58 ± 3	60 ± 2
Body-mass index (kg/m^2)	35 ± 6	34 ± 6
Serum glucose (mg/dl)	93 ± 5	181 ± 9*
Total cholesterol (mg/dl)	199 ± 17	202 ± 26
Serum triglyceride (mg/dl)	216 ± 20	239 ± 22
HDL cholesterol (mg/dl)	44 ± 6	38 ± 5
LDL cholesterol (mg/dl)	128 ± 22	121 ± 16
Systolic blood pressure (mm Hg)	110 ± 10	120 ± 12
Diastolic blood pressure (mm Hg)	80 ± 4	80 ± 8

Values are mean ± S.E.M. * significance $p<0.05$

isometric tensions were recorded on a computer assisted data acquisition system with force-displacement transducers (Commat Ltd., Turkey). During the experiments, indomethacin (10 μM) was in the bath to prevent local prostaglandin release since troglitazone acutly stimulates production of vasodilator prostaglandins (25).

Experimental protocol

After equilibration periods, each vessel was contracted twice with 60 mM KCl for 3 min to assess their viability. Any vessel failing to reach its predetermined target tension was discarded. After 20-min resting period, the rings were sub-maximally precontracted with phenylephrine (3 μM in aortas, 10 μM in veins; the concentrations were determined to be the sub-maximal concentrations), and the contractions assessed for stability over a period of at least 15 min. Then the tissues were serially washed and reequilibrated to baseline. After 30-min rest period, precontraction was repeated and the absence of endothelium was checked by confirming no relaxation to acetylcholine (10 μM). Each vessel was washed and was rested for 30 min and then they were incubated for 30 min with vehicle dimethyl sulphoxide (DMSO) or troglitazone (10 μM) in aortic rings and diabetic saphenous veins; 1, 10 and 100 μM in nondiabetic saphenous veins) (the drug was kindly supplied from Parke-Davis (USA) and Sankyo (Japan)), or 10 μM glibenclamide, a K_{ATP} channel blocker sulphonylurea, in parallel studies. At the end of incubation periods, phenylephrine exposure was repeated, but since troglitazone has a L-type calcium channel blocker action in vascular smooth muscle (26), the rings were precontracted with an equieffective concentrations of phenylephrine (10 μM or 30 μM for aortas and 30 μM or 100 μM for veins). At the plateau tension, cromakalim, a K_{ATP} channel opener, was added to the baths, in increasing cumulative concentrations to obtain relaxation curves. Experiments were performed in parallel rings from the same vessel. Only one concentration-response curve was performed in a single ring. In some experiments in human vessels, cumulative concentration-response curves of phenylephrine were also obtained to compare of the reactivities of diabetic and nondiabetic vessels. At the and of these set of experiments, cumulative concentration-response curves of sodium nitroprusside (SNP) were also obtained in rings sub-maximally precontracted with phenylephrine. Agonist pD_2 values (apparent agonist affinity constant: -log EC_{50} : the negative log of the molar concentration of the drug giving 50% of the maximal response) were calculated by linear regression analysis of the concentration-response curves and taken as a measure of the sensitivity of the vessels to the agonists. Troglitazone, cromakalim and glibenclamide were dissolved in DMSO. Maximum concentration of DMSO ($\leq$0.2%) (v/v) in the bath had no significant effect on the reactivity of the vessels. Indomethacin was dissolved in $NaHCO_4$. Other chemicals were dissolved in distilled water. All were prepared daily from their stock solutions.

Statistical analysis

The degree of cromakalim- or SNP-induced relaxations were expressed as a percentage of the initial tension induced by phenylephrine. The contractile responses to phenylephrine were expressed as gram tension. All data are presented as mean $\pm$ SEM; n represents the number of vessels. A level of probability less than 0.05 obtained from unpaired Student's t test or ANOVA (followed by Neuman-Keul's test), as appropriate, was regarded as significant.

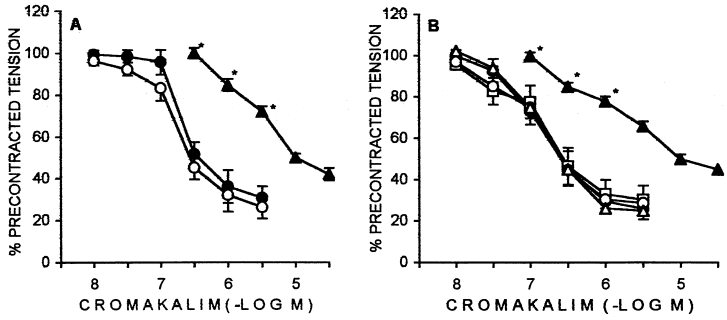

Fig. 1. A: Rat aorta. B. Human sapheneous vein from nondiabetic patients Concentration-response curves to cromakalim after 30-min incubations with vehicle (DMSO) (●, n:7 and n:10), troglitazone :1μM (□, n:5); 10 μM (○, n:7 and n:6); 100 μM (△, n:5) and glibenclamide (10 μM) (▲, n:5) precontracted with phenylephrine. Relaxation responses are expressed as a percentage of the initial tension induced by phenylephrine. Values are mean ± S.E.M. * significance with other groups, $p<0.001$.

Results

Cromakalim, a spesific K_{ATP} channel opener, produced concentration-dependent relaxations in rings pre-contracted with phenylephrine. Troglitazone incubations for 30 min did not change significantly cromakalim-induced responses in all vessels. Neither the pD_2 values nor the maximum relaxations (Emax) of cromakalim were altered significantly (Figs. 1 and 2, Table 2). Glibenclamide (10 μM), a specific K_{ATP} channel blocker, produced a significant shift to the right of the concentration-response curves of cromakalim in a competitive manner in each group of vessel (Figs. 1 and 2 and Table 2). The pD_2 and Emax values of cromakalim after incubations with vehicle, troglitazone or glibenclamide were listed in Table 2. The concentration - response curve of cromakalim was also significantly shifted to the right in dia-

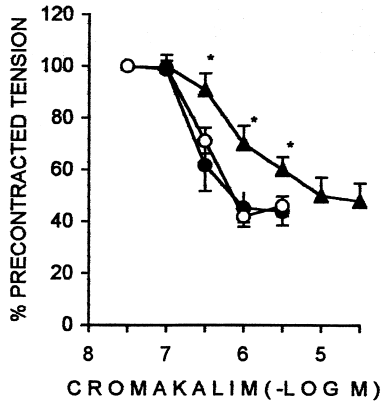

Fig. 2. Human sapheneous vein from type 2 diabetic patients. Concentration-response curves to cromakalim after 30-min incubations with vehicle (DMSO) (●, n:7), troglitazone (10 μM) (○, n:6) and glibenclamide (10 μM) (▲, n:5) precontracted with phenylephrine. Relaxation responses are expressed as a percentage of the initial tension induced by phenylephrine. Values are mean ± S.E.M. * significance with other groups, $p<0.001$.

Table 2
pD_2 values and % maximum relaxations (Emax) for cromakalim in the rat aorta, nondiabetic and diabetic human saphenous veins (SV) after 30-min incubations

	Rat aorta *(n: 5–7 for each)*		Nondiabetic human SV *(n: 5–10 for each)*		Diabetic human SV *(n: 5–7 for each)*	
Incubation	pD_2	Emax	pD_2	Emax	pD_2	Emax
DMSO(con)	6.48 ± 0.05	69 ± 9	6.85 ± 0.08	74 ± 3	6.61 ± 0.04†	56 ± 4†
TRO						
1μM	nt	nt	6.94 ± 0.13	70 ± 5	nt	nt
10 μM	6.60 ± 0.05	74 ± 5	6.89 ± 0.09	71 ± 7	6.50 ± 0.05	54 ± 5
100 μM	nt	nt	6.88 ± 0.08	75 ± 2	nt	nt
GLI 10 μM	5.55 ± 0.01*	28 ± 2*	5.82 ± 0.16*	34 ± 2*	6.03 ± 0.14*†	40 ± 5*†

* significance with TRO and control: con groups, $p<0.001$; † significance with nondiabetic SV $p<0.05$. DMSO: vehicle, TRO: troglitazone, GLI: glibenclamide, nt: not tested. (Emax were also given at 3 μM concentration of cromakalim after glibenclamide incubations for the comparison). Values are ± S.E.M.

betic saphenous veins when compared with the nondiabetic veins (Fig. 3 and Table 2). Maximum response of cromakalim was decreased significantly by 24 ± 3% ($p<0.05$) on diabetic veins. Considering pD_2 and Emax values of cromakalim, glibenclamide (10 μM) (only one concentration was tested) was found to be more effective in inhibiting of cromakalim-induced relaxations in nondiabetic veins when compared with the diabetic veins (Figs. 1B and 2, Table 2). % inhibition of Emax value of cromakalim (at 3 μM concentration) were 54 ± 3% in nondiabetic veins and 29 ± 5% ($p<0.05$) in diabetic veins by glibenclamide. The concentration-response curves to phenylephrine and to SNP were similar in veins obtained from both group of patients (Fig. 4, Table 3).

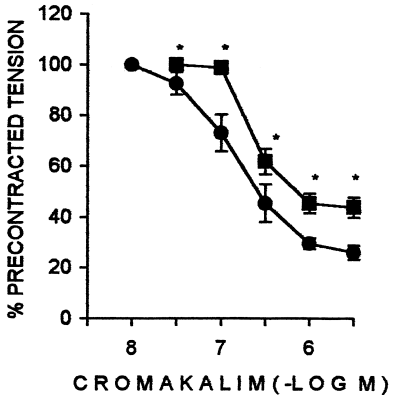

Fig. 3. Human sapheneous vein from nondiabetic and from type 2 diabetic patients. Concentration-response curves to cromakalim in human saphenous rings from nondiabetic (●, n:10) and from type 2 diabetic patients (■, n:7) precontracted with phenylephrine. Relaxation responses are expressed as a percentage of the initial tension induced by phenylephrine. Values are mean ± S.E.M. * significance, $p<0.05$.

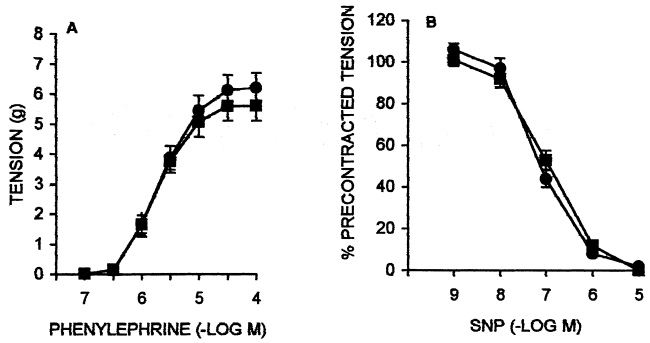

Fig. 4. Human saphenic vein rings from nondiabetic (●) and from type 2 diabetic (■) patients. A: Concentration-response curves to phenylephrine B: Concentration-response curves to sodium nitroprusside. Values are mean ± S.E.M. (n: 8 for each)

Discussion

In this study we have demonstrated that insulin sensitizing drug troglitazone, at the range of therapeutic concentrations, has no ability to inhibit K_{ATP} channel opener-induced relaxations in the rat aorta and in saphenous veins from type 2 diabetic and nondiabetic patients. However, Lee et al. (21) have demonstrated by patch-clamp technique that troglitazone reversibly inhibits K_{ATP} channel activity in CRI-C1 insulin secreting cells, suggesting a possible contribution to the stimulation of insulin secretion. They also reported that while tolbutamide, a K_{ATP} blocker, exerts rapid depolarization, effect of troglitazone develops slowly taking approximately 20 min. We also tested similar concentrations (1, 10 μM), and similar incubation time (30 min) with troglitazone. In a recent study, using electrophysiological techniques, it was shown that troglitazone (3, 10, 30 μM), but not pioglitazone, inhibits both β-cell and cardiac type K_{ATP} channels. Therefore, it was concluded that troglitazone could adversely affect patients during cardiac ischaemia or exercise (22). Our findings in vasculature do not support this conclusion. This may be expected since the electrophysiological changes do not always reflect the functional changes. It is also possible that vessel type of K_{ATP} channel is different from cardiac channel type. On the other hand, in a radioligand binding study, Masuda et al. (20) have demonstrated that specific binding of [^{3}H]-glibenclamide to rat pancre-

Table 3
pD_2 values and maximum responses (Emax) for phenylephrine and sodium nitroprusside (SNP) in nondiabetic and diabetic human saphenous veins (SV)

	Nondiabetic human SV *(n: 8 for each)*		Diabetic human SV *(n: 8 for each)*	
Agonist	pD_2	Emax	pD_2	Emax
Phenylephrine	5.79 ± 0.03	6.2 ± 0.5 g	5.84 ± 0.03	5.6 ± 0.5 g
SNP	7.14 ± 0.11	99 ± 4%	6.92 ± 0.06	100 ± 3%

Values are ± S.E.M.

atic and hamster β-cell membranes is replaced by 100 μM troglitazone in a non-competitive manner, but 1 μM troglitazone failed to eliminate K_{ATP} channel activity. Hence, they suggested that troglitazone has a putative binding site on sulphonylurea receptor in rat pancreatic islets and hamster β-cell line. In our experimental conditions, even at high concentration (100 μM) of troglitazone, we did not observe any inhibition on cromakalim-induced relaxations in the vessels. In patients treated with therapeutic doses of troglitazone, maximum plasma concentrations of the drug were reported to be 0.9–10 μM depending on dose-treatment (21). Therefore, it is unlikely that, at therapeutic plasma concentrations, troglitazone affects vascular smooth muscle dilatation through K_{ATP} channels during ischaemia and / or hypoxia. K_{ATP} channels are composed of two proteins: An inwardly rectifying subunit ($K_{IR}6.1$ or $K_{IR}6.2$) and the receptors for sulfonylureas (SUR) (27–29). SUR1 serves as the regulatory subunit of β-cell K_{ATP} channels, and SUR2A, acts as the cardiac and skeletal muscle and possibly SUR2B or SUR2C act as vascular smooth muscle SUR (12, 30–33). This site binds sulfonylureas, resulting in channel closure and insulin release. The SUR also binds K_{ATP} channel openers (34). While glibenclamide inhibits both β-cell and cardiac channels with high affinity, another sulfonylurea tolbutamide exerts high-affinity inhibition of β-cell but not cardiac-type K_{ATP} channels (12, 35). Also repaglinide, benzoic acid derivative, a novel insulin secretagogue, also binds and closes both β-cell and cardiac type K_{ATP} channels (36). In view of the data, it is also necessary to carry out binding studies with troglitazone on vascular SUR subunit.

Interestingly, the concentration-response curve of cromakalim was shifted significantly to the right in diabetic saphenous vein as compared with nondiabetic vein. This indicates that cromakalim is less potent in diabetic vein. A diminished relaxation response to cromakalim has also been reported in aortas from experimental-diabetic rats (37). It was proposed that conformational change on the sites for K_{ATP} channel activators may occur in diabetes, and this defect may be one of the reasons of diabetes-induced cardiovascular complications, such as hypertension (37). However, it is important to note that some of the diabetic patients, in our study, had been treated with sulfonyurea drug. Hence, it can be assumed that K_{ATP} channels had already been inhibited, at least partially, in saphenous veins obtained from diabetic patients and this may explain a diminished response to K_{ATP} channel opener observed in diabetic vessels. In previous studies such an interaction with sulfonylureas during therapy were reported (1,9,13). To elucidate this possibility we compared cromakalim-induced relaxations in saphenous veins from diabetic patients treated by sulfonylurea (n:6) with that of the patients treated by nonsulfonylurea drugs (n:5). We observed that cromakalim-induced relaxations were decreased by a similar extent in all of the diabetic veins (data not shown) suggesting that a 2.5 h-equlibration period in the bath is enough to washout the sulfonylurea drug from SUR site. Thus, in the present experimental conditions, it appears that sulfonylurea traetment has no influence on cromakalim-induced relaxations in veins from diabetic patients.

SNP-induced relaxations were not changed in all human vessels. Since SNP increases cGMP levels by stimulating guanylate cyclase in vascular smooth muscle, the reduced cromakalim-induced vasodilatation in diabetic vessels is unlikely to be a result of the defective response of vascular smooth muscle. Interestingly, phenylephrine-induced contractions were also not changed. Abnormal vascular reactivity to various vasoactive agents has been reported extensively on experimental animal models of diabetes mellitus. The increased vessel contractility in diabetes was found for receptor-dependent and receptor-independent mechanisms (38–40).

Several evidence exists for impaired endothelium-dependent relaxations (41–43). On the other hand, it has been reported that the contractile responses and sensitivity to noradrenaline were found to be increased in endothelium-denuded human internal mammary artery from type 2 diabetic patients while no change was observed in human saphenous veins, either in the absence or in the presence of endothelium, as compared with nondiabetic veins. SNP-induced relaxations were also not influenced by type 2 diabetes (42). Our findings are in agreement with these observations. Contrary, in rings from uterine arteries, smooth muscle sensitivity to norepinephrine and phenylephrine was found to be enhanced because of change in subcellular calcium distribution in diabetes. In addition, endothelium-dependent relaxation was found to be decreased in diabetic uterine arteries (44). Nevertheless, there is a very limited data in human vasculature for the effects of diabetes on smooth muscle reactivity. So far, most studies are focused on the mechanisms of attenuated endothelium-dependent relaxation. Therefore, besides to endothelium, alterations of human vascular smooth muscle dysfunction in diabetes needs to be clarified. On the other hand, it has been reported that vasodilatory profiles to cromakalim were not affected by endothelium in rat arteries (45). In our study, since we used endothelium-denuded vessels, any possible contribution of endothelium is not involved on cromakalim-induced relaxations. The mechanism (s) that the diabetes affects K_{ATP} channel opener-induced relaxation in human saphenous vein also deserves further investigation.

Referring to Fig. 1B, Fig. 2 and Table 2, inhibitory effect of single concentration of glibenclamide has been observed to be less in diabetic vein by a comparison of pD_2 and Emax values of cromakalim concentration-response curves. This result may indicate that binding characters of K_{ATP} channels may be altered in diabetes. Based on the present data, it appears that an impaired response to K_{ATP} channel opener is specific for type 2 diabetes. From the metabolic data (Table 1), it is inferred that diabetic patients are in good metabolic control, but there is no detailed information of their backround (long-term) therapy. It is well known that type 2 diabetes is commonly accompanied by hypertension (46). However, diabetic patients are not hypertensive in our study. This result can be explained by the fact that all patients had chronic stable angina and were taking oral antianginal and antihypertensive medication.

Conclusion

In conclusion, based on our findings, an insulin sensitizing drug troglitazone has no effect on K_{ATP} channel opener-induced relaxations in rat and diabetic or nondiabetic human vascular smooth muscles at therapeutic, even high, concentrations in vitro. In an anology with glimepiride which is the newly developed pancreas specific sulphonylurea (9, 47), lacking of unwanted cardiovascular effects, it may be expected that troglitazone may not worsen the vascular reactivity through K_{ATP} channels during therapy.

The present results provide an evidence that diabetes causes an impaired dilatation of human saphenous vein through K_{ATP} channels. This may partly be related with diabetes-induced vascular complications such as vasospasm and even hypertension. Accordingly, since saphenous veins are widely used as conduit vessels in coronary bypass graft surgery, the results also suggest that the defective dilatation through K_{ATP} channel may play a role on the performance of saphenous vein grafts in type 2 diabetes.

Acknowledgments

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