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RESEARCH PAPER

The calcium ionophore A23187 induces endothelium-dependent contractions in femoral arteries from rats with streptozotocin-induced diabetes

Y Shi[1], M Feletou[2], DD Ku[3], RYK Man[1] and PM Vanhoutte[1]

[1]*Department of Pharmacology, The University of Hong Kong, Hong Kong, China;* [2]*Department of Angiology, Institut de Recherches Servier, Suresnes, France and* [3]*Department of Pharmacology and Toxicology, University of Alabama at Birmingham, AL, USA*

Background and purpose: To study the importance of endothelium-derived contracting factors (EDCFs) in arteries of rats with type I diabetes.
Experimental approach: Rat femoral arteries were collected four or twelve weeks after induction of diabetes with streptozotocin. Rings, with or without endothelium, were suspended in organ chambers for isometric tension measurement. COX protein levels were determined by Western blotting.
Key results: Four weeks after the injection of streptozotocin, the endothelium-dependent relaxations (during contractions to phenylephrine) to A23817 were attenuated, but the endothelium-dependent contractions (quiescent preparations) to the ionophore were augmented. Indomethacin and S18886 prevented the endothelium-dependent contractions, while dazoxiben reduced them in rings from streptozotocin-treated rats, suggesting that thromboxane A_2, activating TP- receptors, is involved. Twelve weeks after the injection of streptozotocin, the changes in endothelium-dependent relaxations and contractions to A23187 were even more noticeable. The protein expression of COX-1 was increased in femoral arteries of the diabetic rats. Valeryl salicylate and SC560 inhibited the contractions, suggesting that the EDCFs are produced by COX-1. At that time, a combination of S18886 with EP1-blockers was required to abolish the contractions, suggesting that the EDCFs involved act at both TP- and EP-receptors. Rings without endothelium from streptozotocin-treated rats exhibited a reduced maximal contraction to potassium chloride and U46619, combined with hyper-responsiveness to the latter, suggesting that more prolonged diabetes also alters the responsiveness of vascular smooth muscle.
Conclusion and Implications: The production of EDCFs is progressively increased in the course of type I diabetes. Eventually, the disease also damages vascular smooth muscle.
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Keywords: cyclooxygenase; endothelium; endothelium-derived contracting factor(s); endothelium-dependent contractions; smooth muscle; streptozotocin-induced diabetes; thromboxane-prostanoid receptor

Abbreviations: EP receptor, prostaglandin receptors E; TP receptor, thromboxane-prostanoid receptor

Introduction

Endothelial dysfunction is characterized by a reduced release and/or bioavailability of nitric oxide (NO) as well as by an enhanced production of endothelium-derived contracting factor (s; EDCF) (Vanhoutte *et al.*, 2005). Endothelium-dependent contractions were observed first in isolated veins of the dog (De Mey and Vanhoutte, 1982) and then in various arteries including the rat aorta (Lüscher and Vanhoutte, 1986, Auch-Schwelk *et al.*, 1992; Yang *et al.*, 2004a, b; Tang *et al.*, 2005a), the canine basilar artery (Katusic *et al.*, 1988; Shirahase *et al.*, 1988), the rabbit pulmonary artery (Russell and Rohrbach 1989) and the mouse aorta (Tang *et al.*, 2005b). In the main, they are due to a product(s) of endothelial cyclooxygenase, which activates thromboxane-prostanoid receptor (TP receptors) on vascular smooth muscle (Lüscher and Vanhoutte, 1986; Auch-Schwelk *et al.*, 1990; Yang *et al.*, 2003; Tang *et al.*, 2005b). The production of EDCF(s) is potentiated by aging (Koga *et al.*, 1993) and during pathophysiological conditions, such as hypertension (Lüscher and Vanhoutte, 1986; Koga *et al.*, 1989) and hyperglycemia (Tesfamariam *et al.*, 1990).

Correspondence: Professor PM Vanhoutte, Department of Pharmacology, The University of Hong Kong, Level 2, Laboratory Block, Faculty of Medcine Building, 21 Sassoon Road, Hong Kong, China.
E-mail: vanhoutte.hku@hku.hk
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Endothelial dysfunction has been reported in diabetic people and experimental animals with diabetes (De Vriese *et al*., 2000; Schalkwijk and Stehouwer, 2005). The purpose of the present study was to assess whether or not endothelium-dependent contractions occur in isolated arteries of streptozotocin-treated rats, an experimental model resembling type I diabetes in humans and, if so, to determine the mechanisms underlying this phenomenon.

Methods

Experimental animals

Type I diabetes was induced in 16-week-old male Sprague–Dawley rats (450–600 g) by a single administration of streptozotocin (55 mg kg^{-1}, given intravenously) dissolved in citric acid-trisodium citrate (0.2 mM) buffer (pH 4.0–4.5). After 72 h, blood samples were obtained from the tail and the glucose concentration measured using a one-touch glucometer (LifeScan Inc., Milpitas, CA, USA). Induction of diabetes was considered successful when the glucose level was higher than 300 mg dl^{-1}. Another group of rats was injected with vehicle alone and kept under identical conditions as non-diabetic controls. The animals were housed in the laboratory animal unit of the University of Hong Kong, fed with regular chow and given free access to water. Rats were studied either 4 or 12 weeks after induction of type I diabetes. They were anaesthetized with sodium pentobarbitone (70 mg kg^{-1}, i.p.), administered the anticoagulant heparin (0.5 U kg^{-1}) and exsanguinated. The non-fasting glucose level was measured again on the day of death (Table 1). Control rats with a glucose level higher than 200 mg dl^{-1} were excluded from the study. All procedures and protocols were approved by the institutional animal care committee.

Preparation of arteries

Both femoral arteries were dissected free, excised and placed into ice-cold modified Krebs–Ringer solution of the following composition (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 25.0, KH_2PO_4 1.18 and calcium disodium ethylenediaminetetraacetic acid 0.026, glucose 11.1 (control solution). The blood vessels were cut into rings (1.5–2 mm in length). In some preparations, the endothelial cell layer was removed by the injection of 1 ml of saponin solution (1 mg ml^{-1}; diluted with Krebs–Ringer solution; Samata *et al*., 1986) in the artery before cutting the rings. The preparations were suspended in organ chambers containing control solution (37°C) aerated with 95% of O_2 and 5% of CO_2. They were connected to a force transducer (Powerlab Model ML785 and ML119). Changes in isometric tension were recorded. The rings were stretched progressively to 1.0 g of optimal resting tension (determined in preliminary experiment, data not shown) and were allowed to equilibrate for 90 min.

The incubation time with drugs was 30 min and concentration–response curves were obtained in a cumulative manner. Increases in tension were expressed as a percentage of the reference contraction to 60 mM KCl before drug incubation. To study endothelium-dependent relaxations, the preparations were exposed to phenylephrine (10 nM–1 μM; in order to obtain 50–70% of response to KCl (60 mM)). Sodium nitroprusside (10 μM) was added at the end of the experiments and the relaxations were expressed as a percentage of the maximal relaxation induced by the nitrovasodilator. To study endothelium-dependent contractions, all experiments were performed in the presence of N^{ω}-nitro-L-arginine methyl ester hydrochloride (L-NAME) (0.3 mM; Auch-Schwelk *et al*., 1992).

Protein preparation and Western blot analysis

Femoral arteries, harvested 4 or 12 weeks after the injection of streptozotocin, were dissected in ice-cold modified Krebs–Ringer solution. The tissue samples were homogenized at 4°C in lysis buffer (Tris HCl 20 mM, NaCl 150 mM, ethylenediaminetetraacetic acid (EDTA) 1 mM, sodium pyrophosphate 25 mM, β-glycophosphate 1 mM, sodium orthovanadate 1 mM, leupeptin 2.1 μM, aprotinin 1 mg ml^{-1} and phenylmethylsulphonyl fluoride 1 mM, Triton X-100 1%) and incubated on ice for ten minutes. Samples were then centrifuged at 3000 *g* for 10 min at 4°C, and the supernatant was collected. Protein concentrations were determined using the Bradford assay. Lysates were used immediately or stored at −70°C. In all immunoblot experiments, 30 μg of total protein were loaded in each lane of 7.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis gel. Molecular masses of immunoreactive bands were determined by loading a biotinylated molecular mass standard (Bio-Rad Laboratories, Hercules, CA, USA). After electrophoresis, protein was transferred to nitrocellulose membranes (Bio-Rad Laboratories) and blocked with 5% non-fat dry milk in Tris-buffered saline (TBS) (pH 7.4, 20 mM Tris base, 137 mM NaCl). Membranes were then incubated with the

Table 1 Body weight, glucose level in plasma and response to 60 mM potassium chloride in rings of femoral arteries from control and STZ rats

	Before treatment (16 weeks)	*4 weeks after treatment*		*12 weeks after treatment*	
		Control	*STZ-treated*	*Control*	*STZ-treated*
Body weight (g)	542.9±4.4	684.5±23.6*	456.1±12.5*#	694.4±9.6*	416.5±4.0*#
Glucose (mg dl^{-1})	183.0±5.6	182.7±7.2	526.9±12.6#	183.0±4.8	524.7±4.7#
60 mM KCl (g)	1.94±0.15	2.04±0.13	1.75±0.08	1.79±0.11	1.15±0.06#

Abbreviation: STZ, streptozotocin-treated.
Data shown as means±s.e.m.; $n=6$–12.
*Statistically significant difference with control at the induction day ($P<0.05$).
#Statistically significant difference with control rat at the same stage ($P<0.05$).

corresponding primary antibodies (anti-COX-1 monoclonal antibody (1:300), anti-COX-2 polyclonal antibody (1:500) and anti-β-actin monoclonal antibody (1:6000)) overnight at room temperature, followed by three 10 min washes in 0.1% Tween-20-TBS (T-TBS). Blots were then incubated in secondary antibody at 1:1000 dilution for 2 h at room temperature and were then washed three times for 10 min in T-TBS. Membranes were then incubated with an enhanced chemiluminescence reagent (Amersham Biosciences, Buchinghamshire, UK) for min and exposed to X-ray films (Fuji Super RX medical X-ray films; Fuji Photo Film, Dusseldorf, Germany) for optimal time, depending on signal strength. Software for electrophoresis analysis (Multi-analyst (Bio-rad Laboratories) was used for the densitometric analysis of immunoreactive bands. The presence of protein was normalized to β-actin. All protein concentrations are presented as a percentage of control.

Data analysis

Data are presented as means $\pm$ s.e.m.; n refers to the number of rats. Statistical analysis was carried out by one-way analysis of variance (ANOVA) followed by *post hoc* comparison using Bonferroni's test or two-way ANOVA, as appropriate (Prism version 3a, GraphPad Software, San Diego, CA, USA). Differences were considered to be statistically significant when $P>0.05$.

Drugs

A23187, AH 6809 (6-isopropoxy-9-oxoxanthene-2-carboxylic acid), acetylcholine; anti-β-actin monoclonal antibody indomethacin, L-NAME, phenylephrine, saponin, sodium nitroprusside, SC 19220 (2-acetylhydrazide 10(11*H*)-carboxylic acid 8-chloro-dibenz [*b*,*f*][1,4]oxazepine-10(11*H*)-carboxylic acid) and SC560 (5-(4-chlorophenyl)-1-(4-methoxyphenyl)-3-trifluoromethyl pyrazole) were purchased from Sigma Chemical Company (St Louis, MO, USA). U46619 (15(*S*)-hydroxy-11,9-(epoxymethano)prostadienoic acid), NS-398 (*N*[-2-(cyclohexyloxy)-4-nitrophenyl]-methanesulphonamide) and valeryl salicylate (2-[(1-oxopenytyl)oxy]-benzoic acid), COX-1 monoclonal antibody and COX-2 polyclonal antibody were purchased from Cayman Chemical Company (Ann Arbor, MI, USA). Heparin was purchased from LEO Pharm (Ballerup, Denmark). Secondary antibodies (enhanced chemilumiscence (ECL) anti-rabbit IgG and ECL anti-mouse IgG) were bought from Amersham Biosciences. S18886 (3-[(6-amino-(4-chlorobenzensulphonyl)-2-methyl-5,6,7,8-tetrahydronapht]-1-yl)propionic acid) and dazoxiben were kind gifts of the Institut de Recherches Servier (Paris, France). Drugs concentrations are given as final molar concentrations in the bath solution.

Results

General conditions

At the age of 16 weeks, the body weight of the rats averaged 542.9 ± 4.4 g. Four weeks later, the control rats had gained body weight whereas streptozotocin-treated rats had lost weight significantly. The streptozotocin-treated group had a significantly higher glucose level than the controls. Similar results were obtained after 12 weeks (Table 1).

Vascular responsiveness

Contracted preparations

Four weeks after streptozotocin. Four weeks after the injection of vehicle or streptozotocin, during contractions to phenylephrine, the calcium ionophore A23187 (0.1 nM–0.1 μM) induced a concentration- and endothelium-dependent relaxation in arteries from both control and streptozotocin-treated rats. Higher concentrations of A23187 (0.3–1 μM) induced a secondary increase in tension which was inhibited by both indomethacin (inhibitor of cyclooxygenase; 5 μM) and S18886 (selective blocker of TP-receptors; 0.1 μM; Figure 1a and b). In the rings without endothelium, the calcium ionophore A23187 (0.1–1 μM) induced similar contractions in femoral arteries from streptozotocin-treated rats to those from control rats, but not relaxations (data not shown). The streptozotocin-treated group exhibited significantly higher secondary contractions than the controls in the rings with endothelium. In the presence of indomethacin, the endothelium-dependent relaxations to A23187, in the control group were comparable to those in the streptozotocin-treated groups (Figure 1a and b).

Twelve weeks after streptozotocin. Twelve weeks after the injection of vehicle or streptozotocin, rings from the control rats exhibited relaxations and secondary contractions comparable to those observed in the 4 weeks control rats. Contracted rings from the streptozotocin-treated group did not exhibit a significant relaxation to A23187 but a significantly greater secondary contraction. Indomethacin reversed the secondary contractile response to a relaxation. The relaxant responses restored by indomethacin were greater in arteries from control rats than in those from streptozotocin-treated animals (Figure 1c and d). S18886 partially restored relaxations and reduced the secondary contraction (Figure 1c and d). The rings without endothelium exhibited similar responses to those observed in the 4-week treated rats (data not shown).

Quiescent preparations

Four weeks after streptozotocin. In rings from the control group, A23187 induced a concentration-dependent contraction that was not observed in rings without endothelium. Indomethacin and S18886 inhibited this response completely. In the rings from the streptozotocin-treated group, A23187 induced a significantly greater endothelium-dependent contraction that was abolished by either indomethacin or S18886 (Figure 2a and b). Dazoxiben (inhibitor of thromboxane A_2 synthase; 10 μM) significantly reduced the endothelium-dependent contraction (Figure 3a).

Twelve weeks after streptozotocin. The rings from the control rats exhibited a comparable endothelium- and concentration-dependent contraction to A23187 as those from the 4 weeks control rats. Indomethacin and S18886 abolished the response. The rings from the streptozotocin-treated group exhibited a significantly greater contraction to A23187. The

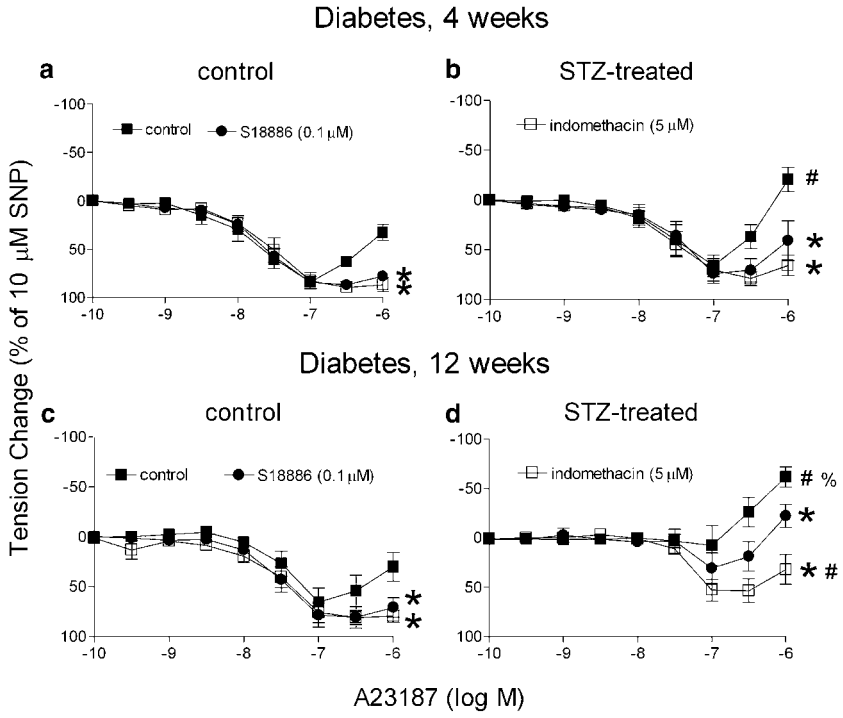

Figure 1 Relaxations to A23187 in femoral arteries from control and streptozotocin-treated rats after 4 (**a** and **b**) and 12 (**c** and **d**) weeks. Data shown are means ± s.e.m. (vertical lines); $n = 7$. *Statistically significant difference from control rings ($P < 0.05$). #Statistically significant difference from rings of control rats under identical conditions ($P < 0.05$). % indicates a statistically significant difference from rings of 4 weeks streptozotocin-treated rats under identical conditions ($P < 0.05$).

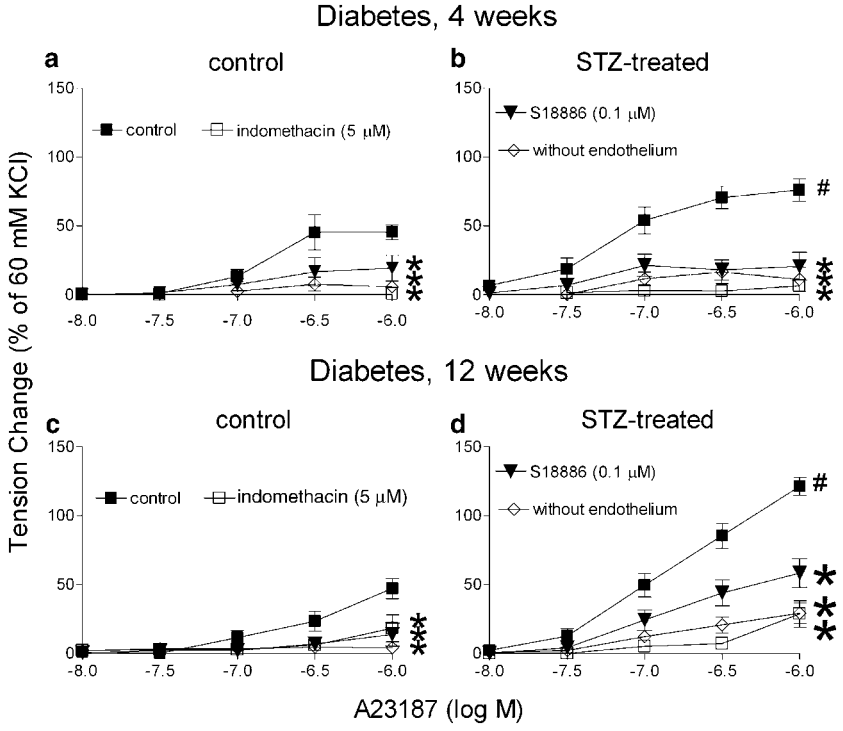

Figure 2 Contractions to A23187 in femoral arteries from control and streptozotocin-treated rats in the presence of L-NAME (0.3 mM) after 4 (**a** and **b**) and 12 (**c** and **d**) weeks. Data shown are means ± s.e.m. (vertical lines); $n = 7$. *Statistically significant difference from control rings ($P < 0.05$). #Statistically significant difference from rings of control rats under identical conditions ($P < 0.05$).

response of the rings without endothelium was comparable to that from controls (Figure 2c and d). Indomethacin abolished the endothelium-dependent contraction (Figure 2d). Valeryl salicylate as well as SC 560 prevented the contraction whereas NS-398 did not (Figure 4). S18886 only partially reduced the endothelium-dependent contraction (Figure 2d). AH 6809 (nonspecific blocker of prostaglandin E receptors; 30 μM) and SC 19220 (specific blocker of the subtype 1 of prostaglandin E receptors; 0.1 mM) alone reduced the endothelium-dependent contraction to A23187 significantly (Table 2). The combination of these blockers plus S18886 abolished this contraction (Figure 5; Table 2). Dazoxiben no longer significantly inhibited the endothelium-dependent contraction (Figure 3b).

Smooth muscle. Four weeks after the injection of streptozotocin, rings from both the control and treated group exhibited comparable contractions to 60 mM potassium chloride, which were not significantly different from those obtained in rings from 16-week-old untreated rats. After 12 weeks of diabetes, the rings from the streptozotocin-treated group had a significantly reduced contraction to 60 mM potassium chloride (Table 1).

Four weeks after the injection of streptozotocin, the rings without endothelium from both the control and treated group showed comparable responses to U46619. In rings without endothelium from the 12-week streptozotocin-treated group, the maximal contraction to U46619 was reduced to a similar extent as that to 60 mM potassium chloride, but the concentration–response curve to the TP-agonist was shifted significantly to the left (Figure 6a and b).

Cyclooxygenases

Four weeks after the injection of streptozotocin, the protein level of COX-1 was significantly greater in arteries from the streptozotocin-treated group than those from the control rats. Twelve weeks after the injection of streptozotocin, it was enhanced further. The level of COX-2 was similar after 12 weeks treatment to that after 4 weeks of treatment (Figure 7).

Discussion

In the present experiments, the rats treated with streptozotocin had a lower body weight and a higher glucose level, suggesting that diabetes was successfully induced. The diabetes was maintained for at least 12 weeks, without further changes in weight and glucose level.

A23187 induced a concentration-dependent relaxation in arteries from both control and streptozotocin-treated groups that was not observed in the rings without endothelium, confirming that the calcium ionophore A23187 is an endothelium-dependent vasodilator (Singer and Peach,

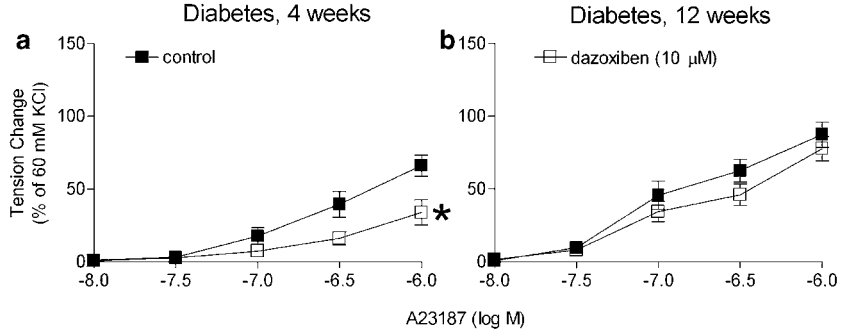

Figure 3 Effects of the inhibitor of thromboxane synthase dazoxiben (10 μM) on the response to A23187, in the presence of L-NAME (0.3 mM), in femoral arteries with endothelium of rats 4 weeks (**a**) and 12 weeks (**b**) after injection of streptozotocin. Data shown are means $\pm$ s.e.m. (vertical lines); $n = 7$. *Statistically significant difference from rings under control conditions ($P < 0.05$).

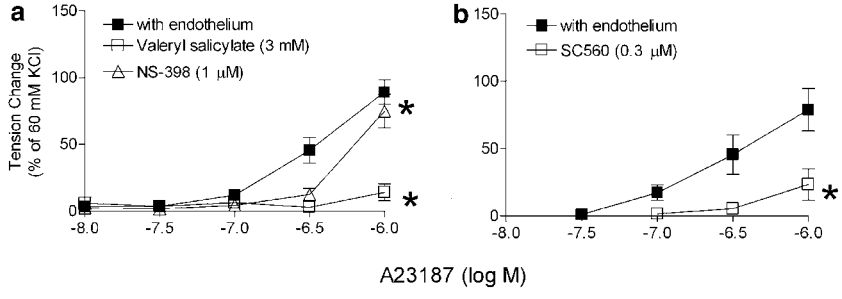

Figure 4 Effects of inhibitors of cyclooxygenase on the response to A23187 in quiescent femoral arteries of rats 12 weeks after the injection of streptozotocin. The experiments were performed in the presence of L-NAME (0.3 mM). Data shown as means $\pm$ s.e.m. (vertical lines); $n = 7$–8. *Statistically significant difference from rings (with endothelium) under control conditions (COX-1 inhibitors: valeryl salicylate (**a**) and SC560 (**b**); COX-2 inhibitor: NS-398 (**a**)).

Table 2 Effects of antagonists at TP and EP1 receptors on the response to A23187 in quiescent femoral arteries 12 weeks after the injection of streptozotocin

	Control	*S18886 (0.1 μM)*	*AH6809 (30 μM)*	*SC19220 (0.1 mM)*	*SC18886 + AH6809 + SC19220*
E_{max} (% of 60 mM KCl)	112.77 ± 4.95	70.70 ± 13.60	69.08 ± 9.95	52.74 ± 13.54*	37.00 ± 11.54*
pEC_{50} (−log M)	6.84 ± 0.12	6.66 ± 0.12	6.56 ± 0.12	6.57 ± 0.12	6.44 ± 0.12

Abbreviations: EP-receptor, prostaglandin receptors E; TP, thromboxane-prostanoid.
Data shown as means ± s.e.m.; $n = 6–9$.
*Statistically significant differences with control rings in the presence of L-NAME (one-way ANOVA; $P < 0.05$).

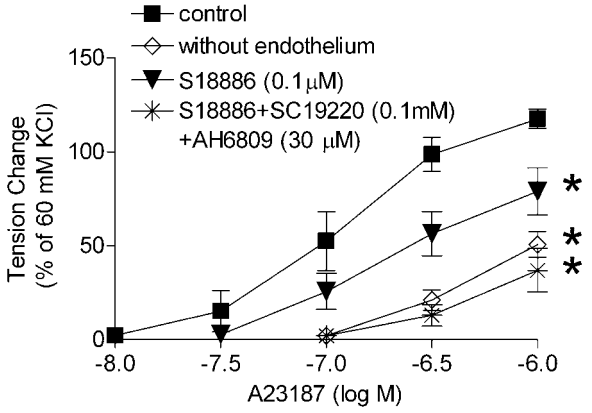

Figure 5 Effects of antagonists of TP and EP_1 receptors on the response to A23187 in quiescent femoral arteries 12 weeks after the injection of streptozotocin. The experiments were performed in the presence of L-NAME (0.3 mM). Data shown are means ± s.e.m. (vertical lines); $n = 6–9$. *Statistically significant difference from rings under control conditions ($P < 0.05$).

1982). However, at higher concentrations (0.3–1 μM), A23187 caused a secondary contraction that was not observed in the rings without endothelium. This endothelium-dependent contraction was abolished by indomethacin and S18886. Thus, A23187 releases an endothelium-dependent contracting factor produced by cyclooxygenase and activating TP-receptors (Lüscher and Vanhoutte, 1986; Auch-Schwelk *et al*., 1990, Yang *et al*., 2004a, b). The observation that the contraction to A23187 was greater in the arteries from streptozotocin-treated rats suggests that under comparable conditions, the endothelial cells from diabetic rats must produce more EDCF(s). At a later stage of streptozotocin-induced diabetes, A23187 failed to induce relaxation during contraction to phenylephrine, but evoked an augmented endothelium-dependent contraction instead. Indomethacin abolished the contraction, while S18886 reversed it only partially. This indicates that the endothelial dysfunction is augmented with continued exposure to diabetes. Reduced relaxant responses to A23187 have been observed after arterial grafting (Miller *et al*., 1987), in diabetes (Gebermedhin *et al*., 1988; Cameron and Cotter 1992) and in hypertension (Yang *et al*., 2004a, b). The present experiments support the hypothesis that in the case of diseases such as diabetes, the inhibition of the relaxation to endothelium-dependent dilators can be attributed, at least in part to the enhanced production of an EDCF, produced by cyclooxygenase, that then activates mainly, but not exclusively TP- receptors (Vanhoutte *et al*., 2005).

To study endothelium-dependent contractions directly, experiments were performed in quiescent arteries in the presence of L-NAME, which optimizes such responses (Auch-Schwelk *et al*., 1992; Yang *et al*., 2004a, b; Tang *et al*., 2005a; Zhou *et al*., 2005). The finding that the A23187-induced endothelium- and concentration-dependent contractions in the arteries from streptozotocin-treated rats were larger in the presence of the NOS-inhibitor demonstrates that streptozotocin-induced diabetes does indeed potentiate the production or the action of EDCFs.

In the present experiments, the selective inhibitors of COX-1, valeryl salicylate (Bhattacharyya *et al*., 1995) and SC560 (Smith *et al*., 1998), but not NS-398, a preferential inhibitor of COX-2 (Futaki *et al*., 1993), abolished the endothelium-dependent contraction to A23187 (Ge *et al*., 1999; Yang *et al*., 2002; Tang *et al*., 2005b; Gluais *et al*., 2006). Furthermore, the Western blot analysis revealed that the presence of COX-1 is significantly augmented in the arteries from the streptozotocin-treated group compared to control preparations, whereas that of COX-2 is unaffected. These data confirm the importance of COX-1, the constitutive isoform of the enzyme for the production of EDCFs (Ge *et al*., 1999; Yang *et al*., 2002; Tang *et al*., 2005b).

The exact nature of the endothelium-derived, cyclooxygenase-dependent contracting factor depends on the experimental model studied and the stimulating agents used. In the aorta of the spontaneously hypertensive rat, in response to acetylcholine, endoperoxides and prostacyclin are involved in the activation of TP receptors (Kato *et al*., 1990; Ge *et al*., 1995; Rapoport and Williams, 1996; Gluais *et al*., 2005), whereas in response to A23187, thromboxane A_2, along with prostacyclin and endoperoxides, is implicated (Gluais *et al*., 2006). Similarly in the aorta of diabetic rabbits, thromboxane A_2 or possibly its precursor, endoperoxide, is the EDCF involved (Tesfamariam *et al*., 1989). The present experiments support the hypothesis that thromboxane A_2 is responsible for the endothelium-dependent contractions (Ingerman-Wojenski *et al*., 1981; Gonzales *et al*., 2005), at least in femoral arteries from animals with an early stage of type I diabetes, as dazoxiben, an inhibitor of thromboxane A_2 synthase (Vermylen *et al*., 1981) reduced the contractions to the ionophore. However, dazoxiben is less effective at a later stage of the disease and this is also associated with a reduced efficacy of S18886 to prevent the endothelium-dependent contractions evoked by A23187. Antagonists at prostaglandin receptors E (EP-receptors) (SC 19220 (Armstrong *et al*., 1986) and AH 6809 (Sanner, 1969)) given alone partially inhibited the endothelium-dependent contractions, but when combined with S18886 abolished them. Accumu-

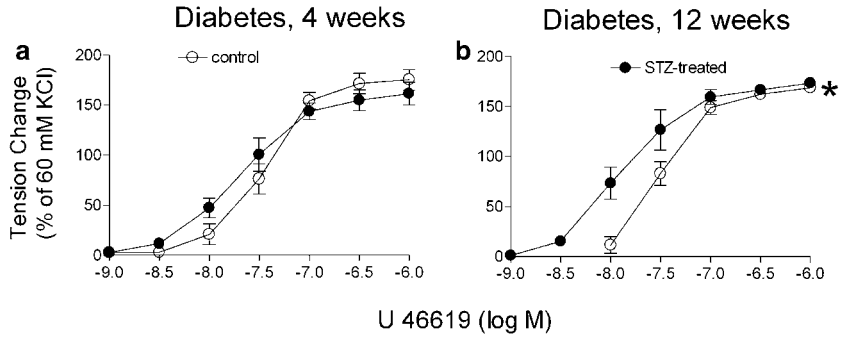

Figure 6 Response to U46619 in rings of femoral arteries without endothelium from control rats and those 4 (**a**) and 12 (**b**) weeks after an injection of streptozotocin. Data shown are means ± s.e.m. (vertical lines); $n = 7$. *Statistically significant difference from rings of control rats ($P < 0.05$).

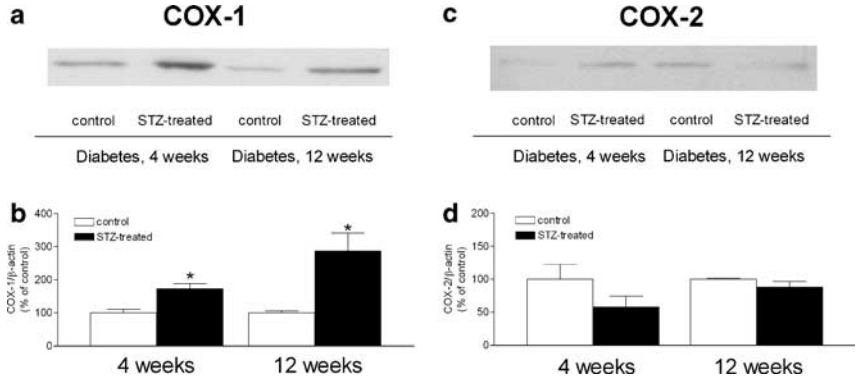

Figure 7 The presence of COX-1 (**a** and **b**) and COX-2 (**c** and **d**) in intact femoral artery from control and streptozotocin-treated rats, 4 weeks and 12 weeks after the injection of streptozotocin. Data presented are a percentage of control and shown as means ± s.e.m.; $n = 6$. *Statistically significant difference from control rats ($P < 0.05$).

lation of endoperoxides favours the metabolism to prostaglandin E_2 (Zou *et al.*, 1999). Prostaglandin E_2 is involved in the cyclooxygenase-dependent contractions of rat resistance arteries (Bolla *et al.*, 2004). Therefore, the present results suggest that after 12, but not 4 weeks of streptozotocin-induced diabetes, the endothelium-dependent contractions induced by A23187 are owing to the combined activation of TP- and EP-receptors.

With the progression of diabetes the maximal contractions to potassium chloride and U46619 were reduced to a comparable extent, but the concentration–contraction response curve to U46619 was shifted to the left, in agreement with previous findings demonstrating enhanced contractions in response to U46619 or endothelin 1 in arteries from streptozotocin-treated rats (Hattori *et al.*, 1999). These data indicate that after 12 weeks of diabetes the vascular smooth muscle exhibits both a reduced intrinsic ability to contract but at the same time a hypersensitivity to TP agonists. The present experiments do not allow us to conclude whether or not at the later stage of streptozotocin-induced diabetes, this hyperresponsiveness of the smooth muscle cells contributes to the exaggerated endothelium-dependent contractions.

Most studies of endothelium-dependent contractions have been performed using acetylcholine (Lüscher and Vanhoutte, 1986; Auch-Schwelk *et al.*, 1992; Yang *et al.*, 2004a, b; Tang *et al.*, 2005a, b; Zhou *et al.*, 2005). Acetylcholine activates endothelial muscarinic receptors which when expressed in blood vessels control both relaxation and contraction (Boulanger *et al.*, 1994; Ehlert 2003). However, in the femoral artery of the diabetic rat, although the acetylcholine-induced relaxation is impaired (Shi *et al.*, 2006), acetylcholine failed to induce contractions (data not shown). The calcium ionophore, A23187, is more potent than acetylcholine at inducing endothelium-dependent contractions (Yang *et al.*, 2004a, b; Tang *et al.*, 2005b; Gluais *et al.*, 2006). Therefore, A23187 was used as a tool to amplify EDCF-mediated responses and to allow a proper identification of the mediators involved.

Conclusion

The present study suggests a progressive change in the nature of the EDCF occurs during the course of type I diabetes. In addition, as it progresses, the disease also damages vascular smooth muscle.

Acknowledgements

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Conflict of Interest

The authors state no conflict of interest.

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