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Elevated plasma endothelin-1 level in streptozotocin-induced diabetic rats and responsiveness of the mesenteric arterial bed to endothelin-1

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1 Both the plasma endothelin-1 (ET-1) levels and the plasma glucose levels were markedly elevated in streptozotocin (STZ)-induced diabetic rats.

2 The maximum contractile response of the mesenteric arterial bed to ET-1 was significantly reduced, and the vasodilatation induced by the ET_B-receptor agonist IRL-1620 in the mesenteric arterial bed was significantly reduced in STZ-induced diabetic rats.

3 ET-1 (10^{-8} M) caused a transient vasodilatation followed by a marked vasoconstriction in methoxamine-preconstricted mesenteric arterial beds. The ET-1-induced vasodilatation was significantly larger in beds from diabetic rats than in those from age-matched controls. By contrast, the ET-1-induced vasoconstriction was significantly smaller in STZ-induced diabetic rats than in the controls.

4 Both removal of the endothelium with Triton X-100 and preincubation with BQ-788 (10^{-6} M) (ET_B-receptor antagonist) abolished the ET-1-induced vasodilatation. Preincubation with BQ-485 (10^{-6} M) or BQ-123 (3×10^{-6}) (ET_A-receptor antagonist) significantly augmented the ET-1-induced vasodilatation in control mesenteric arterial beds, but not that in beds from diabetic rats.

5 These results demonstrate that marked increases not only in plasma glucose, but also in plasma ET-1 occur in STZ-induced diabetic rats. We suggest that the decreased contractile response and the increased vasodilator response of the mesenteric arterial bed to ET-1 may both be due to desensitization of ET_A receptors, though ET_B receptors may also be desensitized. This desensitization may result from the elevation of the plasma ET-1 levels seen in STZ-induced diabetic rats.

Keywords: Endothelin-1; diabetes; mesenteric arterial bed; endothelium; ET_A receptor; ET_B receptor

Introduction

Endothelin-1 (ET-1), a vasoconstrictor peptide secreted from endothelial cells, is thought to play a role in a number of vascular diseases (Goto *et al*., 1996). The endothelin family consists of three isoforms, namely ET-1, ET-2 and ET-3, and these have been shown to exert a wide variety of biological actions, all of which are thought to be mediated by ET receptors (Inoue *et al*., 1989). Two distinct ET receptor subtypes, named ET_A and ET_B exist in mammalian tissues (Arai *et al*., 1990; Sakurai *et al*., 1990). ET_A and ET_B receptors are located on the smooth muscle and the endothelium, respectively (Goto *et al*., 1996). ET-1 constricts smooth muscle via the activation of ET_A receptors (D'Orleans-Juste *et al*., 1993) and also leads to the release of endothelium-derived relaxing factor (EDRF) via the activation of the ET_B receptors located on the endothelium (Warner, 1990).

Plasma ET-1 levels in the diabetic state are increased (Takahashi *et al*., 1990; Collier *et al*., 1992) and the plasma concentration of big endothelin-1, the precursor of ET-1, was found to be elevated in patients with diabetes mellitus (Tsunoda *et al*., 1991). In contrast to these findings, no changes in plasma ET-1 levels and decreased levels of renal tissue ET-1 is found in the diabetic state (Shin *et al*., 1995). In studies of an experimental model of diabetes, it has been found that the density of ET-1 receptors in cardiac tissue is reduced (Nayler *et al*., 1989) and the amount of ET-1 released from mesenteric arteries is increased (Takeda *et al*., 1991). Furthermore, the contractile response of the aorta to ET-1 was attenuated in rats with streptozotocin (STZ)-induced diabetes (Fulton *et al*., 1991; Hodgson & King, 1992; Lieu & Reid, 1994; Tada *et al*., 1994). The diabetic microcirculation is impaired responses to ET-1 and ET-3 (Lawrence & Brain, 1992), and although the initial hindquarters vasodilatation induced with ET-1 are not different in STZ-treated and control rats, the subsequent renal and mesenteric vasoconstrictions are greater in the former (Kiff *et al*., 1991). In addition, exposure of endothelial cells to a high glucose concentration has been found to enhance ET-1 secretion (Yamauchi *et al*., 1990; Hattori *et al*., 1991).

The aim of the present study was to examine whether the vasoactive actions of ET-1 are altered in resistance arteries, such as the mesenteric arterial bed, in STZ-induced diabetic rats. Hence, we investigated the effects of ET-1 or its analogue on isolated perfused mesenteric arterial beds taken from STZ-induced diabetic rats.

Methods

Animals and experimental design

Male Wistar rats, 8 weeks old and 220–250 g in weight, received a single injection via the tail vein of STZ 60 mg kg^{-1}, dissolved in citrate buffer. Age-matched control rats were injected with the buffer alone. Food and water were available *ad libitum* to all animals. The concentration of glucose in the plasma was determined by the *O*-toluidine method (Dubowski, 1962). This study was conducted in accordance with the Guide for the Care and Use of Laboratory Animals adopted by the Committee on the Care and Use of Laboratory Animals of

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Hoshi University, which is accredited by the Ministry of Education, Science, Sports and Culture, Japan.

Radioimmunoassay of ET-1

Plasma samples taken 10 weeks after injection of STZ or buffer were extracted with Amprep C2 columns (Amersham International plc., Buckinghamshire, U.K.) following Amprep activation by 2 ml of 100% methanol and then 2 ml water. One millilitre of each plasma sample was acidified with 0.25 ml 2 M HCl, centrifuged at 10000 *g* for 5 min at room temperature and loaded onto the column. The column was washed with 5 ml of 0.1% trifluoroacetic acid (TFA). Immunoreactive-endothelin was eluted with 2 ml of 80% acetonitrile/water containing 0.1% TFA. Then, the eluate was dried down under nitrogen and the resulting pellet reconstituted in assay buffer (0.02 M borate buffer pH 7.4 containing 0.1% sodium azide).

The concentration of ET-1 in the eluate of plasma samples was determined by radioimmunoassay by use of commercially available kits (endothelin 1-21 specific^{125} assay system, Amersham International plc., Buckinghamshire, U.K.).

Preparation of the perfused mesenteric arterial bed

Ten weeks after treatment with STZ or buffer, rats were anaesthetized with ether and then given an intravenous injection of 1000 units kg^{-1} heparin. Following decapitation under anaesthesia, a midline incision was made and the mesenteric arterial bed rapidly dissected out and placed in a bath of modified Krebs-Henseleit solution (KHS, composition in mM: NaCl 118.0, KCl 4.7, $NaHCO_3$ 25.0, $CaCl_2$ 1.8, NaH_2PO_4 1.2, $MgSO_4$ 1.2, dextrose 11.0 and 0.25% bovine serum albumin). The mesenteric artery and vein were tied off near the caecum and the remaining intestinal wall. The mesenteric arterial bed was perfused by the method described by McGregor (1965), with various modifications previously described by us (Kamata *et al.*, 1989c; Kamata & Makino, 1997; Abiru *et al.*, 1993a). Briefly, the method used was as follows: warm (37°C), oxygenated (95% O_2-5% CO_2) KHS was pumped into the mesenteric arterial bed, by a peristaltic pump operating at a rate of 5 ml min^{-1}, through a cannula inserted into the superior mesenteric artery. Vascular responses were detected as changes in perfusion pressure, which was monitored continuously via a pressure transducer (Nihon Kohden, Model AP2001, Tokyo, Japan) and recorded on a pen recorder. Following a 60 min equilibration period, the perfusion circuit was transformed into a closed system by collecting the perfusate in a second bath and from there recirculating it through the mesenteric arterial bed. The total volume of the closed system was 50 ml and agents were administered via the second bath. The drug doses quoted represent the final concentration in this system. After equilibration, the mesentery preparation was constricted by perfusion with a solution containing 4×10^{-6} to 3×10^{-5} M methoxamine, which resulted in a perfusion pressure of between 120–140 mmHg. It was then maximally relaxed with a perfusion solution containing 10^{-6} M acetylcholine (ACh), a response which confirmed the integrity of the endothelium in our preparation. The mesenteric arterial bed was completely relaxed after exposure to 10^{-6} M ACh. Drug-induced relaxation was expressed as a percentage of the increase in perfusion pressure induced by an equieffective concentration of methoxamine (4×10^{-6} to 3×10^{-5} M). When the methoxamine-induced contraction had reached a plateau, the vasodilator and vasoconstrictor responses to ET-1 were examined by use of a single concentration of this agent. Dose-response curves for ET-1 (10^{-10} to 10^{-6} M) were obtained by cumulative administration. To investigate the influence of 10^{-6} M BQ-788, 10^{-6} M BQ-485, 3×10^{-6} M BQ-123, 10^{-4} M N^G-nitro-L-arginine (L-NOARG), isotonic high K^+ (60 mM) and 10^{-5} M indomethacin on the agonist-induced responses in the mesenteric arterial bed, the mesentery was incubated in the appropriate solution for 30 min before the addition of ET-1. To exclude the involvement of endothelium-derived hyperpolarizing factor (EDHF), some experiments were performed in which the mesenteric preparation was depolarized with isotonic high K^+ (60 mM) in the presence of nicardipine (10^{-7} M) before being constricted. Each preparation was used to test only one antagonist or isotonic high K^+ medium. In some experiments, the mesenteric preparation was perfused with Triton X-100 for 1 min to remove functionally the endothelial cells lining the resistance vessels. This treatment reduced the ACh (10^{-6} M)-induced vasodilatation by more than 90% without reducing the contractile effects of methoxamine.

Drugs

Streptozotocin, methoxamine hydrochloride, N^G-nitro-L-arginine (L-NOARG), indomethacin, nicardipine, papaverine hydrochloride, tetraethylammonium, bovine serum albumin (Fraction V) and Triton X-100 were purchased from Sigma Chemicals Co. (St. Louis, MO, U.S.A.). Acetylcholine chloride was purchased from Daiichi Pharmaceutical Co. Ltd. (Tokyo, Japan). Endothelin-1 and Suc-[Glu^9, Ala^{11,15}]-endothelin-1 (8-21) (IRL1620) were purchased from Peptide Institute, Inc. (Osaka, Japan). Cyclo (D-α-aspartyl-L-propyl-D-valyl-L-leucyl-D-tryptophyl) (BQ-123) and N-[N-[N-[2,6-dimethyl-1-piperidinyl)carbonyl]-4-methyl-L-leucyl]-1-(methoxycarbonyl)-D-tryptophyl]-D-norleucine monosodium (BQ-788) were purchased from Research Biochemicals International (Natick, MA, U.S.A.). Perhydroazepin-1-yl-L-Leucyl-D-tryptophanyl-D-tryptophan (BQ-485) was purchased from Banyu Pharmaceutical Co. Ltd. (Tsukuba, Japan). Isotonic high K^+ (60 mM) solution was prepared by replacing the NaCl with KCl.

Statistics

Data are presented as the mean ± s.e.mean. In some experiments, statistical differences were determined by Dunnet's test for multiple comparison, after a one-way analysis of variance, and a probability level of $P<0.05$ was regarded as significant. Statistical comparison between concentration-response curves was determined by two-way ANOVA with Bonferroni correction performed *post-hoc* to correct multiple comparison. A $P<0.05$ was considered significant.

Results

Plasma immunoreactive ET-1 concentration in controls and diabetic animals

The plasma glucose level in diabetic animals was approximately four times that seen in the controls. The plasma

immunoreactive ET-1 level was also significantly elevated in the diabetic rats (controls: 3.43 ± 0.33 fmol 100 μl^{-1}; diabetes: 5.82 ± 0.77 fmol 100 μl^{-1} (Figure 1).

Vasoconstrictor responses to ET-1

Cumulative concentration-response curves for the vasoconstriction induced by ET-1 (10^{-10} to 10^{-6} M) were obtained in perfused mesenteric arterial beds from both controls and diabetic rats (Figure 2). The maximum response of the mesentery to ET-1 was significantly reduced in STZ-induced diabetic rats. pEC_{50} (-log EC_{50}) values for ET-1 were 8.8 ± 0.1 M ($n=11$) and 7.2 ± 0.2 M ($n=12$, $P<0.001$), in controls and diabetes, respectively. When similar experiments were performed in the presence of the nitric oxide synthase (NOS) inhibitor, L-NOARG (10^{-4} M), ET-1-induced vasoconstrictions were slightly increased in diabetic or control rats, respectively (Figure 2). pEC_{50} values for ET-1 in the presence of L-NOARG (10^{-4} M) were 9.2 ± 0.1 M ($n=9$) and 7.0 ± 0.1 M ($n=7$, $P<0.001$), in controls and diabetes.

As shown in Figure 3a, vasoconstriction induced by ET-1 (10^{-11} to 10^{-7} M) was significantly enhanced by L-NOARG (10^{-4} M) plus tetraethylammonium (TEA) (10 mM), whereas TEA (10 mM) or indomethacin (10^{-5} M) had no effects on ET-1-induced vasoconstriction. BQ-788 (10^{-6} M) (ET_B-receptor antagonist) shifted the dose-response curve for ET-1-induced

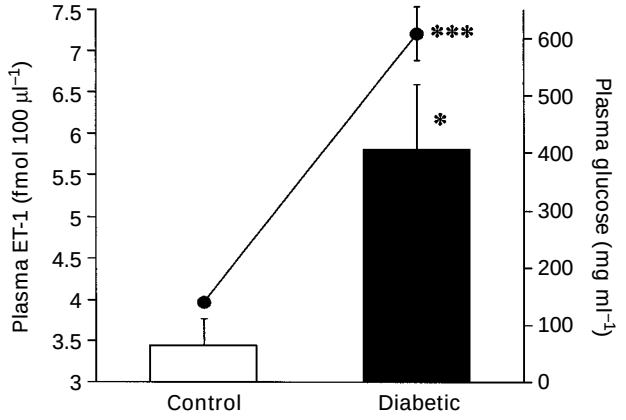

Figure 1 Changes in the levels of plasma glucose and plasma ET-1 in STZ-induced diabetic rats. Plasma glucose (controls and diabetic rats, $n=5$); ET-1 (controls, $n=5$; diabetic rats, $n=7$). Each data point represents the mean and vertical lines show s.e.mean. $^*P<0.05$, $^{***}P<0.001$.

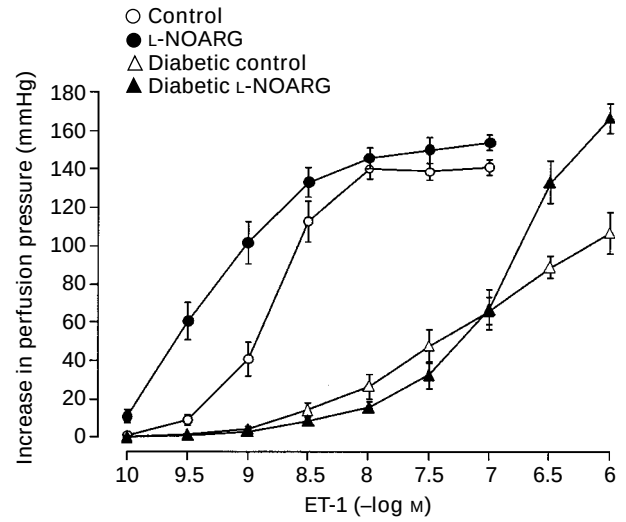

Figure 2 Concentration-response curves for ET-1-induced vasoconstriction of mesenteric arterial beds taken from age-matched controls and STZ-induced diabetic rats. Concentration-response curves in the absence (control; $n=11$) or presence ($n=9$) of 10^{-4} M L-NOARG in age-matched controls, and absence (control diabetic; $n=12$) or presence ($n=7$) of 10^{-4} M L-NOARG in diabetic rats. The ordinate scale shows the increase in perfusion pressure measured at the peak of the response. Each data point represents the mean and vertical lines show s.e.mean. Control vs STZ-induced diabetic groups ($P<0.001$); control and 10^{-4} M L-NOARG-treated groups ($P<0.05$).

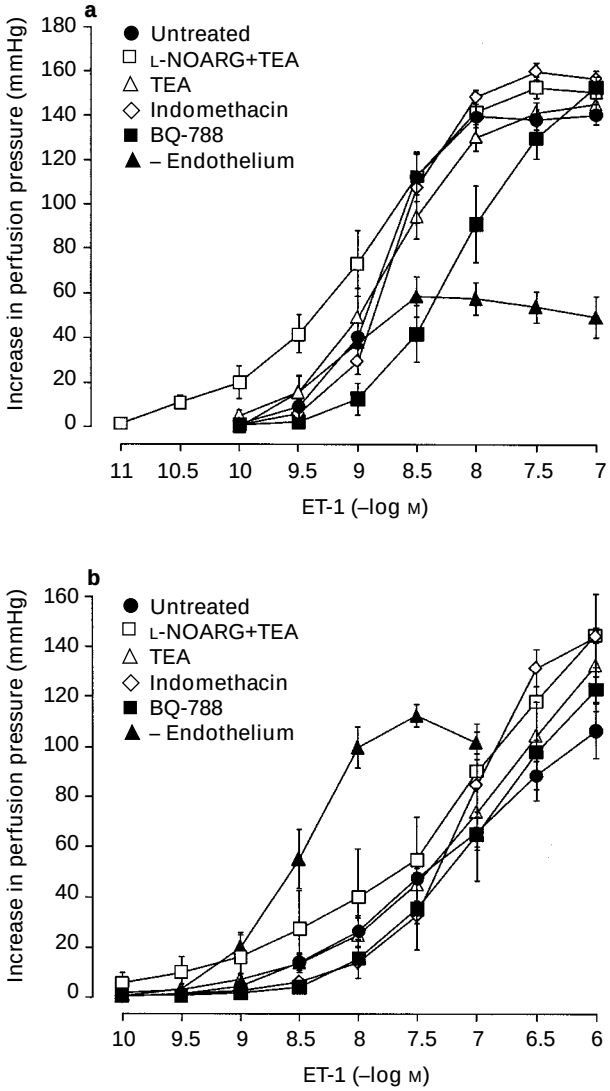

Figure 3 Effects of various agents on the concentration-response curves for ET-1-induced vasoconstriction of mesenteric arterial beds taken from age-matched controls and STZ-induced diabetic rats. (a) Control groups: untreated groups ($n=11$) 10^{-4} M L-NOARG plus 10 mM TEA treated groups ($n=7$, $P<0.05$), 10 mM TEA-treated groups ($n=7$), 10^{-5} M indomethacin treated groups ($n=6$), 10^{-6} M BQ-788 treated groups ($n=7$, $P<0.01$), and without endothelium groups ($n=7$, $P<0.001$ vs untreated). (b) Diabetic groups: untreated group ($n=12$), 10^{-4} M L-NOARG plus 10 mM TEA treated groups ($n=5$, $P<0.05$), 10 mM TEA-treated groups ($n=7$), 10^{-5} M indomethacin-treated groups ($n=6$), 10^{-6} M BQ-788-treated groups ($n=7$) and without endothelium groups ($n=6$, $P<0.001$ vs untreated). The ordinate scales show the increase in perfusion pressure measured at the peak of the response. Each data point represents the mean and vertical lines show s.e.mean.

vasoconstriction to the right. Surprisingly, ET-1-induced vasoconstriction was significantly reduced in the endothelial-denuded preparation from control rats (Figure 3a). In contrast, the dose-response curve for the ET-1-induced vasoconstriction was shifted to the left in STZ-induced diabetic rats (Figure 3b). Treatment of the mesentery with TEA (10 mM), L-NOARG (10^{-4} M) plus TEA (10 mM), indomethacin (10^{-5} M) or BQ-788 (10^{-6} M) had no effect on ET-1-induced vasoconstriction in diabetes (Figure 3b).

Vasodilator responses to IRL-1620

Cumulative concentration-response curves for the vasodilatation induced by the ET_B-receptor agonist, IRL-1620 (10^{-10} to 10^{-6} M) were obtained in mesenteric arterial beds from controls and diabetic rats (Figure 4). The mesenteric preparation was constricted by perfusion with a solution containing 4×10^{-6} to 3×10^{-5} M methoxamine, which resulted in a perfusion pressure of approximately 120–140 mmHg (controls, 137.9 ± 7.3 mmHg; diabetes, 130.0 ± 5.5 mmHg, respectively). Under these conditions, IRL-1620 induced a concentration-dependent vasodilatation. The maximum response to IRL-1620 was significantly smaller in the diabetic rats than in controls (Figure 4). pEC_{50} values for IRL-1620 were 8.5 ± 0.1 M ($n=5$) and 8.2 ± 0.2 M ($n=4$), in controls and diabetes, respectively.

The IRL-1620-induced vasodilatation was significantly inhibited by L-NOARG (10^{-4} M) or isotonic high K^+ (60 mM) but not indomethacin (10^{-5} M) in control rats (Figure 5a). The vasodilatation induced by IRL-1620 was markedly reduced in diabetes and the decreased response was not affected by either L-NOARG (10^{-4} M), isotonic high K^+ (60 mM) or indomethacin (10^{-5} M) (Figure 5b).

Effects of various agents on the vasodilatation induced by ET-1

ET-1 (10^{-8} M) caused a transient vasodilatation followed by a marked vasoconstriction in the mesenteric arterial bed preconstricted with methoxamine (4×10^{-6} to 3×10^{-5} M) (Figure 6). The maximum vasodilatation evoked by ET-1 in the controls ($31.5\pm1.9\%$, $n=6$) was significantly smaller than that evoked in the STZ-induced diabetic rats ($62.0\pm6.4\%$, $n=5$, $P<0.001$). By contrast, the maximum vasoconstriction in the controls ($185.5\pm33.3\%$, $n=6$) was significantly larger than that seen in STZ-induced diabetic rats ($70.4\pm26.1\%$, $n=5$, $P<0.05$).

Removal of endothelial cells by perfusion with Triton X-100 for 60 s almost abolished the ET-1-induced vasodilatation in both age-matched controls and STZ-induced diabetic rats (data not shown). Preincubation with BQ-788 (10^{-6} M) (ET_B-receptor antagonist) also abolished the ET-1-induced vasodilatation in both age-matched controls and STZ-induced diabetic rats (Figure 7). Preincubation with BQ-485 (10^{-6} M) or BQ-123 (3×10^{-6} M), both antagonists of the ET_A receptor,

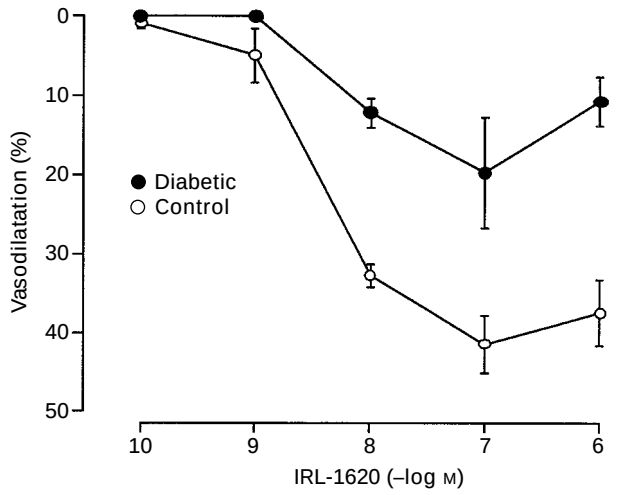

Figure 4 Concentration-response curves for IRL-1620-induced vasodilatation of mesenteric arterial beds taken from age-matched controls and STZ-induced diabetic rats. Concentration-response curves: age-matched controls ($n=5$); diabetic rats ($n=4$). The dose-response curves between controls and diabetes were significantly different ($P<0.01$). The ordinate scale shows the percentage decrease in perfusion pressure measured at the peak of the response. Each data point represents the mean and vertical lines show s.e.mean.

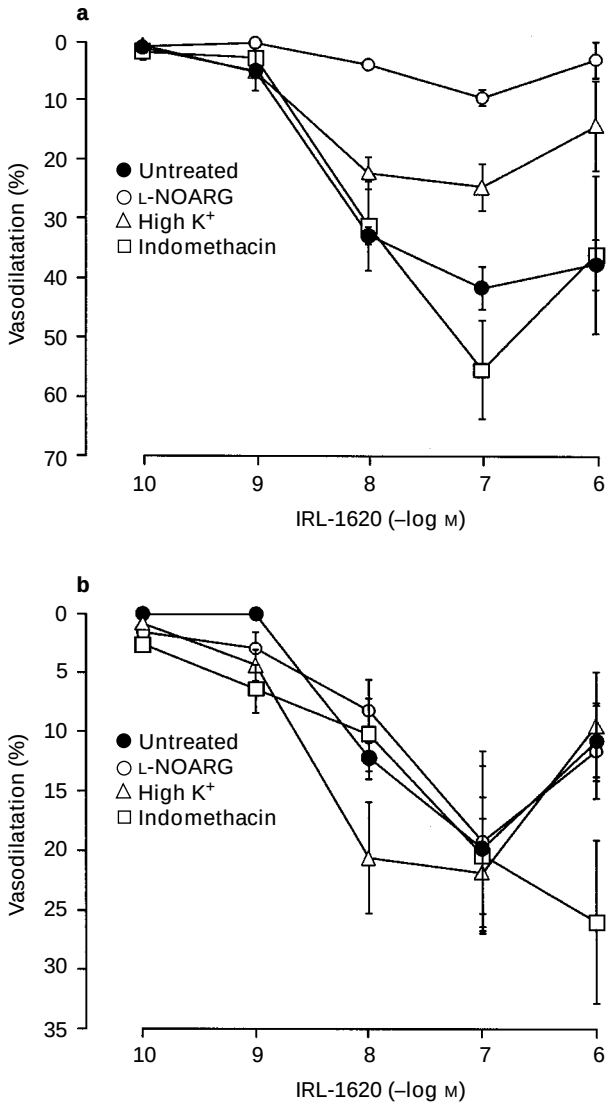

Figure 5 Concentration-response curves for IRL-1620-induced vasodilatation of mesenteric arterial beds taken from age-matched controls and STZ-induced diabetic rats. (a) Control groups: untreated group ($n=5$), 10^{-4} M L-NOARG-treated group ($n=3$, $P<0.001$), isotonic high K^+ group ($n=5$) 10^{-5} M indomethacin-treated groups ($n=5$). (b) Diabetic groups: untreated group ($n=4$), 10^{-4} M L-NOARG treated group ($n=5$), isotonic high K^+ group ($n=6$), 10^{-5} M indomethacin treated group ($n=5$). The ordinate scales show the increase in perfusion pressure measured at the peak of the response. Each data point represents the mean and vertical lines show s.e.mean.

significantly augmented the ET-1-induced vasodilatation in the age-matched controls but not in STZ-induced diabetic rats (Figure 7).

Treatment of the mesentery with 10^{-4} M L-NOARG significantly decreased the ET-1-induced vasodilatation, the percentage inhibition being significantly greater in the controls than in the STZ-induced diabetic rats ('untreated' response reduced in controls by $59.6 \pm 2.9\%$ and in diabetic rats by $27.9 \pm 6.7\%$, $P < 0.01$). To exclude the involvement of EDHF in the ET-1-induced vasodilatation, the mesentery was depolarized with isotonic high K^+ solution (60 mM) in the presence of nicardipine (10^{-7} M); the mesenteric arterial bed was then contracted with methoxamine (4×10^{-6} to 3×10^{-5} M). Under these conditions, the ET-1-induced vasodilatation was significantly reduced, the percentage inhibition being significantly greater in the STZ-induced diabetic rats than in the controls ('untreated' response reduced in controls by $46.1 \pm 15.3\%$ and in diabetic rats by $91.3 \pm 3.4\%$, $P < 0.05$). Incubating the mesentery with 10^{-5} M indomethacin significantly decreased the ET-1-induced vasodilatation, extent of the inhibition being almost the same in the controls as in the STZ-induced diabetic rats ('untreated' response reduced in controls by $54.0 \pm 8.4\%$ and in diabetic rats by $52.6 \pm 6.4\%$) (Figure 8).

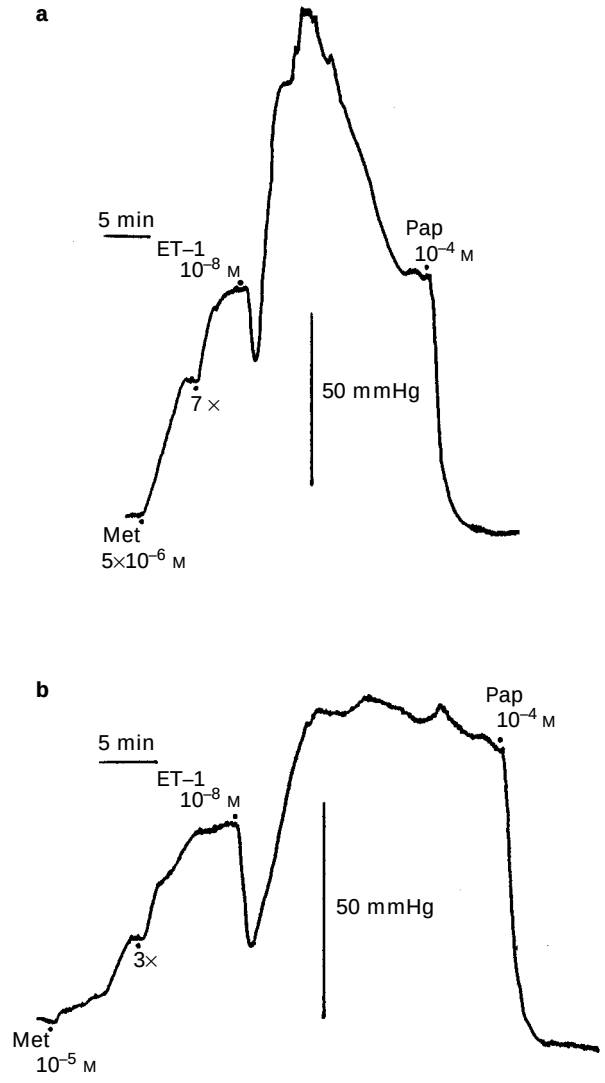

Figure 6 Typical records showing the response of methoxamine (5×10^{-6} to 3×10^{-5} M)-preconstricted mesenteric arterial beds taken from (a) age-matched controls and (b) STZ-induced diabetic rats to a single application of 10^{-8} M ET-1. When the methoxamine-induced contraction had reached a plateau, vasodilator and vasoconstrictor responses to ET-1 were evoked by a single concentration of ET-1. At the end of the experiment, the ability of papaverine (10^{-4} M) to induce maximal vasodilatation was confirmed.

Discussion

The main conclusions from the present study are that the plasma ET-1 level is significantly higher in STZ-induced diabetic rats than in age-matched controls and that the contractile response to ET-1, via ET_A receptors, and the vasodilator response, via ET_B receptors were both desensitized. Moreover, the relative contributions made by the various endothelium-derived relaxing factors (EDRFs) to the ET-1-induced vasodilatation were different in the diabetic state than in non-diabetic state.

The contractile response of the mesenteric arterial bed to ET-1 was significantly attenuated in the diabetic state. It has also been shown that responsiveness to ET-1 was attenuated in thoracic aortae isolated from diabetic rats 2 weeks after STZ treatment (Fulton *et al.*, 1991) or 8 weeks after (Tada *et al.*, 1994). However, no significant reduction was found in the response to noradrenaline of thoracic aortae isolated from 2-week STZ-diabetic rats (Fulton *et al.*, 1991). These results imply that the changes in responsiveness due to diabetes may vary depending on the diabetic period, the tissues studied and the type of response examined. The vasoconstrictor action of ET-1 has been shown to be exerted primarily via an increase in intracellular calcium (Hirata *et al.*, 1988b). Interestingly, Fulton *et al.* (1991) showed that in diabetes there may be, at least under some circumstances, an impairment of the mobilization of intracellular calcium stores. Moreover, an elevation of ET-1 levels may result in a homologous down-regulation of its receptors (Hirata *et al.*, 1988a; Nayler *et al.*, 1989; Miasiro & Paiva, 1990). It will need to be determined by further research whether the decrease

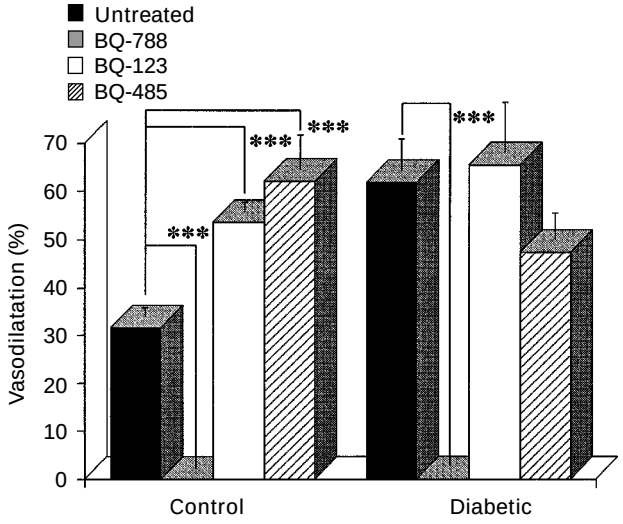

Figure 7 Effects of BQ-788, BQ-485 and BQ-123 on the ET-1-induced vasodilatation in methoxamine-preconstricted mesenteric arterial beds in age-matched controls and STZ-induced diabetic rats. Vasodilatation indicates percentage decrease and increase, respectively, in perfusion pressure in methoxamine-preconstricted beds. Untreated ($n = 6$), 10^{-6} M BQ-788-treated group ($n = 4$), 3×10^{-6} M BQ-123 treated groups ($n = 4$) 10^{-6} M BQ-485 ($n = 4$) ***$P < 0.001$.

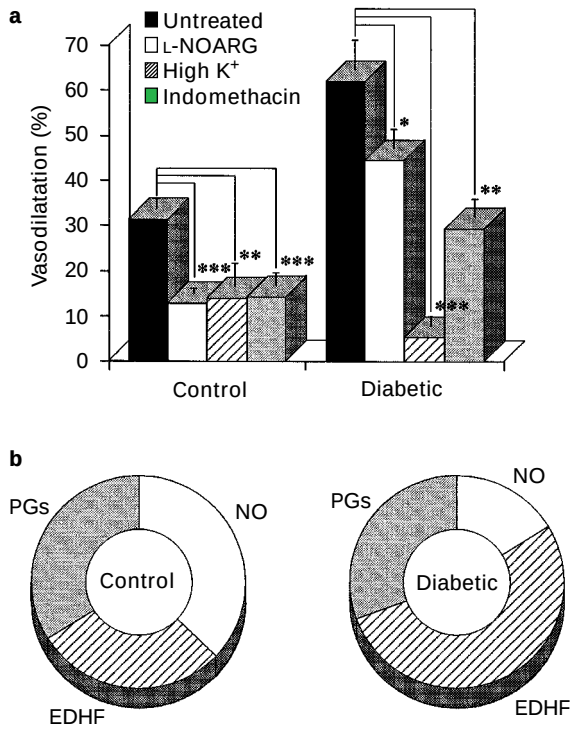

Figure 8 Effects of L-NOARG, isotonic high K^+ and indomethacin on the ET-1-induced vasodilatation in methoxamine-preconstricted mesenteric arterial beds taken from age-matched controls and STZ-induced diabetic rats. Vasodilatation indicates percentage decrease in perfusion pressure. (a) Untreated groups ($n=6$) and with 10^{-4} M L-NOARG ($n=6$), isotonic high K^+ (60 mM) ($n=6$), or 10^{-5} M indomethacin ($n=6$). Each data column represents the mean ± s.e. $^*P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$. (b) Relative involvement of various endothelium-derived relaxing factors in the ET-1-induced vasodilatation in controls and STZ-induced diabetic rats (based on data in (a)).

in the contractile response to ET-1 is mediated by the former effect or by the latter.

ET-1 stimulates the release of nitric oxide (NO) (Warner *et al*., 1989b). Indeed, in the present study L-NOARG, an inhibitor of NO synthase, slightly but significantly increased the vasoconstrictor responses to ET-1 in controls. This suggests that NO-synthase products play an important role in modifying the constrictor effects of ET-1 in the rat mesenteric arterial bed.

The ET_B receptor, which occurs predominantly on endothelial cells, mediates vasodilatation through the generation of endothelium derived relaxing factors (EDRFs) and prostacyclin (Thiemermann *et al*., 1988; De Nucci *et al*., 1988; Wright & Fozard, 1988; Warner *et al*., 1989a; Luscher *et al*., 1993). In the present study, we found that the vasodilatation induced by IRL-1620, an ET_B-receptor agonist, was significantly attenuated in STZ-induced diabetic rats. However, there was no significant difference between controls and diabetic rats in terms of EC_{50} values. In STZ-induced diabetic rats, the density of ET-1 receptors is reduced in cardiac tissue (Nayler *et al*., 1989). As both the contractile response via ET_A stimulation and the vasodilator response via ET_B stimulation were reduced in our diabetic rats, it is likely that the attenuation of responsiveness to ET-1 is the result of a general decrease in the number of ET-receptors.

ET-1 caused a transient vasodilatation in the mesenteric arterial bed when it had been preconstricted with methoxamine. This response was abolished by removal of the endothelium and by pretreatment with BQ-788, an antagonist of the ET_B receptor. Thus, in the mesenteric bed, ET-1-induced vasodilatation seems to be mediated by ET_B receptors localized on the endothelium.

The relaxation responses of aortic strips to endothelium-dependent agents are decreased in STZ-induced diabetic rats (Oyama *et al*., 1986; Pieper & Gress, 1988; Kamata *et al*., 1989a, b; Tomlinson *et al*., 1992; Abiru *et al*., 1993b; Poston & Taylor, 1995). Surprisingly, we found that the vasodilator response of the mesenteric arterial bed to ET-1 was significantly increased in diabetes. Furthermore, preincubating the mesentery with BQ-485, an antagonist of the ET_A receptor, or BQ-123, another antagonist of the ET_A receptor, significantly augmented the ET-1-induced vasodilatation in the controls, but not in STZ-induced diabetic rats. It is likely, therefore, that an attenuation of the contractile response to ET-1, which is mediated by the ET_A receptor, underlies the enhancement of the ET-1-induced vasodilatation that we saw in diabetic rats.

Treatment of the mesentery with L-NOARG significantly decreased the ET-1-induced vasodilatation, the percentage reduction being significantly greater in the controls than in STZ-induced diabetic rats. By contrast, in a mesenteric arterial bed already depolarized with K^+, the ET-1-induced vasodilatation was significantly decreased, the percentage reduction being significantly greater in STZ-induced diabetic rats than in the controls. Treatment of the mesentery with indomethacin significantly decreased the ET-1-induced vasodilatation to a similar degree in controls and STZ-induced diabetic rats. These results suggest that the enhancement of the ET-1-induced vasodilatation of the mesentery that was seen in the diabetic state may be due to an attenuation of ET_A-receptor-mediated vasoconstriction and to an increased release of EDHF.

It is surprising that the ET-1-induced vasoconstriction was significantly reduced in the endothelial-denuded preparation in control rats and was significantly increased in diabetic rats. While the ET-1-induced vasoconstriction was markedly inhibited by the detergent, Triton X-100, this may not be due to the destruction of ET-1 receptors. If Triton X-100 destroys the ET-1 receptors, the ET-1-induced vasoconstriction should be decreased in the diabetic rats. This was not the case. The precise mechanisms of ET-1-induced vasoconstriction will be necessary to understand these data more fully.

In conclusion, we found marked increases not only in plasma glucose but also in the plasma ET-1 level in STZ-induced diabetic rats. The decreased contractile response and the increased vasodilator response of the mesenteric arterial bed to ET-1 seen in diabetic rats may be due primarily to a desensitization of ET_A receptors, though ET_B receptors are also desensitized. This desensitization may be a consequence of the elevation in the plasma endothelin-1 level seen in STZ-induced diabetic rats.

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