*Originals***Possible modulation by endothelin-1, nitric oxide, prostaglandin I₂ and thromboxane A₂ of vasoconstriction induced by an α -agonist in mesenteric arterial bed from diabetic rats****A. Makino, K. Kamata**

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Summary We hypothesized that in diabetes arterial reactivity to constrictors is attenuated by certain endothelium-derived substances. We examined the vasoconstriction induced by methoxamine (α_1 -agonist) in isolated mesenteric arterial beds from streptozotocin (STZ)-induced diabetic rats and age-matched control rats. The dose-response curve for methoxamine was shifted to the right and the maximum contractile response was impaired in mesenteric arterial beds from diabetic rats. The methoxamine vasoconstriction was reduced in endothelium-denuded preparations from control rats, but increased in those from diabetic rats. Treatment with the nitric oxide synthase inhibitor N^G-nitro-L-arginine enhanced the vasoconstrictions induced by methoxamine in both control and diabetic rats. Indomethacin had no effect on the methoxamine vasoconstriction in control rats, but it shifted the dose-response curve to the left in diabetic rats. Whether given with or without indomethacin, BQ-123, (an ET_A-receptor antagonist) plus BQ-788 (an ET_B-receptor antagonist)

shifted the dose-response curve for methoxamine to the right in control rats (while reducing the maximum response) but to the left in diabetic rats. The methoxamine-stimulated release of 6-keto-prostaglandin F_{1 α} from the mesenteric arterial bed in diabetic rats was approximately four times that seen in the control rats, while the methoxamine-induced release of thromboxane B₂ (TXB₂), a metabolite of thromboxane A₂ (TXA₂), was less in diabetic rats than in the control animals. These results suggest that an increased production of prostaglandin I₂ (PGI₂) and decreased formation of TXA₂ could be responsible for the attenuation of the methoxamine-induced mesenteric vasoconstriction seen in diabetic rats, and these changes in the diabetic state could be partly responsible for the lower blood pressure seen in our diabetic rats. [Diabetologia (1998) 41: 1410–1418]

Keywords α_1 -Agonist, diabetic rat, mesenteric arterial bed, endothelium-derived contracting factor.

Diabetes mellitus is associated with vascular complications, including an impairment of vascular function and alterations in the reactivity of blood vessels to

neurotransmitters in the macro- and microvasculature. The effect of diabetes on vascular morphology and function has been studied using a wide variety of

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Abbreviations: Ach, acetylcholine; BQ-123, cyclo (D- α -aspartyl-L-propyl-D-valyl-L-leucyl-D-tryptophyl); BQ-788, N-[N-[2,6-dimethyl-1-piperidinyl]carbonyl]-4-methyl-L-leucyl]-1-(methoxy-carbonyl)-D-tryptophyl]-D-norleucine monosodium; COX-2, cyclooxygenase; EDRF, endothelium-derived re-

laxing factor; EDTA, ethylenediaminetetraacetic acid; ET_A, endothelin A receptor; ET_B, endothelin B receptor; ET-1, endothelin-1; KHS, Krebs-Henseleit solution; L-NOARG, N^G-nitro-L-arginine; NO, nitric oxide; NOS, nitric oxide synthase; PGH₂, prostaglandin H₂; PGI₂, prostaglandin I₂; SQ 29548, [1S-[1 α , 2 α (Z), 3 α , 4 α]]-7-[[2-[(phenylamino)carbonyl]hydrazino]methyl]-7-oxabicyclo(2.2.1)hept-2-yl]-5-heptenoic acid; STZ, streptozotocin; TXA₂, thromboxane A₂; TXB₂, thromboxane B₂; ANOVA, analysis of variance; TFA, trifluoroacetic acid.

animal and human models in a number of different vascular beds [1]. Conduit vessels, such as the aorta, from diabetic rats have been studied previously to show either a reduction in contractility or an enhancement of responses to vasoconstrictors [2–4]. Studies using mesenteric conduit arteries from diabetic rats have also found either an increased or decreased response to vasoconstrictors [5–11]. These experiments were almost all carried out using ring preparations of the mesenteric artery, whereas the latter experiments involved mostly perfused mesenteric arterial beds. It is important to indicate which type of preparation was being used when examining the vascular dysfunction induced by the diabetic state.

Endothelial cells regulate vascular tone by releasing potent vasoconstrictors such as endothelin-1 (ET-1) and TXA_2 , and vasodilators such as prostacyclin, nitric oxide and endothelium-derived hyperpolarizing factor [12–16]. Alterations in the production and effect of these endothelial cell products are at least partly responsible for the abnormal modulation of vascular tone that occurs in certain disease states [2–4]. In particular, the molecular and physiological disturbances caused by diabetes could result in changes in endothelial cell function, leading to, for example, altered responses to α -adrenoceptor agonists.

It has been reported that, although the endothelium-dependent relaxation of the aorta is attenuated in STZ-induced diabetic rats, blood pressure is not different between age-matched control and diabetic rats [17]. While the aorta does offer some resistance to flow, it is obvious that the contribution made by vessels of smaller diameter to peripheral vascular resistance is much greater. The mesenteric circulation of the rat receives approximately one-fifth of the cardiac output [18] and, thus, regulation of this bed makes a significant contribution towards the regulation of both systemic blood pressure and circulating blood volume. The goal of this study was to determine whether, and in what way, STZ-induced diabetes alters the responsiveness of the mesenteric arterial bed (in this case, to the α_1 -adrenoceptor agonist methoxamine) and to examine the causal relation, if any, between those changes in vasoreactivity and changes in endothelial function. We focused on the biosynthesis of ET-1, prostacyclin, TXA_2 and nitric oxide and we used methoxamine as the α_1 -agonist because this drug does not undergo uptake into sympathetic nerve endings. In addition, we examined whether any changes in vasoreactivity in the mesenteric bed were reflected by a change in resting blood pressure.

Materials and methods

Drugs. Streptozotocin, methoxamine hydrochloride, N^G -nitro-L-arginine (L-NOARG), indomethacin, papaverine hydrochloride, bovine serum albumin (Fraction V) and Triton X-100 were purchased from Sigma Chemical Co. (St. Louis, Mo., USA). Acetylcholine chloride was purchased from Daiichi Pharmaceutical Co. Ltd. (Tokyo, Japan). Tetraethylammonium was purchased from Wako Pure Chemical Industries Ltd. (Tokyo, Japan). Cyclo (D- α -aspartyl-L-propyl-D-valyl-L-leucyl-D-tryptophyl) (BQ-123), N-[N-[N-[(2,6-dimethyl-1-piperidiny)carbonyl]-4-methyl-L-leucyl]-1-(methoxycarbonyl)-D-tryptophyl]-D-norleucine monosodium (BQ-788) and [1S-[1 α , 2 α (Z), 3 α , 4 α]-7-[3-[[2-[(phenylamino)carbonyl]hydrazino]-methyl]-7-oxabicyclo(2.2.1)hept-2-yl]-5-heptenoic acid (SQ 29548) were purchased from Research Biochemicals International (Natick, Mass., USA).

Animals. Male Wistar rats, 8 weeks old and weighing 220–250 g, received a single injection via the tail vein of STZ 60 mg/kg, 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 [19]. Blood pressure and heart rate were recorded by the tail-cuff method. 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 Hoshi University (which is accredited by the Ministry of Education, Science, Sports and Culture, Japan).

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 of heparin. Following this injection, the mesenteric arterial bed was rapidly dissected out and placed into modified Krebs-Henseleit solution (KHS, composition in mmol/l: 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; 0.25 % bovine serum albumin). The mesenteric artery and vein were tied off near the caecum and the remaining intestine was then separated from the arterial bed along the intestinal wall. The mesenteric arterial bed was then perfused using the method described previously by us [20–22]. Briefly, warm (37°C), oxygenated (95 % O_2 -5 % CO_2) KHS was pumped into the mesenteric arterial bed, using a peristaltic pump operating at a rate of 5 ml/min, through a cannula inserted into the superior mesenteric artery. Vascular responses were detected as changes in perfusion pressure; this was monitored continuously by way of 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 thence recirculating it through the mesenteric arterial bed. The total volume of the closed system was 50 ml, and agents were administered via the bath. In some preliminary experiments, the mesentery preparation was constricted by perfusion with a solution containing 5×10^{-6} to 4×10^{-5} mol/l methoxamine, which resulted in an increase in perfusion pressure of approximately 115–130 mmHg, and then maximally relaxed with a perfusion solution containing 10^{-6} mol/l acetylcholine (ACh), a response which confirmed the integrity of the endothelium in our preparation. Dose-response curves for methoxamine (10^{-7} to 3×10^{-4} mol/l) were obtained by cumulatively increasing the total concentration of the agonist in the bath. To investigate the influence of 10^{-6} mol/l BQ-788, 10^{-6} mol/l BQ-123, 10^{-4} mol/l L-NOARG and 10^{-5} mol/l 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 methoxamine. In some experiments, the mesentery preparation was perfused with Triton X-100 for 1 min to functionally remove the endothelial cells lining the resistance vessels. This treatment reduced by more than 90% the relaxation that had previously been induced by the maximally effective dose of ACh (10^{-6} mol/l) without reducing the contractile effects of methoxamine.

Sample preparation. We measured the content in the perfusate of 6-keto-prostaglandin $F_{1\alpha}$ (a metabolite of prostaglandin I_2 , PGI_2), thromboxane B_2 (TXB_2), a metabolite of thromboxane A_2 (TXA_2), and ET-1. For this purpose, the perfusate was collected on two occasions, each over a 30 s period, as follows: sample 1, before application of methoxamine; sample 2, after increasing concentrations of methoxamine had been applied and the methoxamine-induced contractile response had stabilized (5 min). The samples were mixed with 237.5 μ l of an ethylenediaminetetraacetic acid (EDTA) solution and 12.5 μ l of an 0.04 mol/l indomethacin solution only for the determination of 6-keto-prostaglandin $F_{1\alpha}$ and thromboxane B_2 . The samples were then stored at -80°C until the assay was done.

Measurements of 6-keto-prostaglandin $F_{1\alpha}$ and TXB_2 . The perfusates were extracted using Amprep C2 columns (Amersham International plc., Buckinghamshire, UK) following Amprep activation by 2 ml of 100% methanol and then 2 ml water. One ml of each sample was acidified to pH 3 and loaded onto the column. The column was washed with 5 ml of water, 5 ml ethanol and hexane. Immunoreactive prostanoids were eluted with 5 ml of methyl formate. Then, the eluate was dried down under nitrogen and the resulting pellet was reconstituted using assay buffer (0.05 mol/l phosphate buffer, pH 7.3, with 0.1% gelatin and 0.1% azide). The concentration of prostanoids in the eluate was determined by radioimmunoassay using commercially available kits (a 6-keto-prostaglandin $F_{1\alpha}$ [^{125}I] assay system and a TXB_2 [^{125}I] assay system; Amersham International plc.).

Measurement of ET-1. The perfusates were extracted using Amprep C2 columns (Amersham International plc.) following Amprep activation by 2 ml of 100% methanol and then 2 ml water. One ml of each sample was acidified with 0.25 ml 2 mol/l HCl, centrifuged at 10000 g for 5 min at room temperature, then 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 was reconstituted using assay buffer (0.02 mol/l borate buffer, pH 7.4, containing 0.1% sodium azide). The concentration of ET-1 in the effluent was determined by radioimmunoassay using a commercially available kit (endothelin 1–21 specific [^{125}I] assay system; Amersham International plc.).

Statistical analysis. Data are expressed as means $\pm$ SEM. In some experiments, statistical differences were assessed by Dunnett's test for multiple comparisons, after a one-way analysis of variance, a probability level of p less than 0.05 being regarded as significant. Statistical comparison between concentration-response curves was made by a two-way analysis of variance (ANOVA) with the Bonferroni correction performed post-hoc to correct for multiple comparisons: p less than 0.05 was considered significant.

Table 1. Haemodynamic variables in STZ-induced diabetic rats

	control rats	diabetic rats
Body weight (g)	500.7 $\pm$ 9.7	249.1 $\pm$ 9.4 ^b
Plasma glucose (mmol/l)	0.84 $\pm$ 0.01	3.39 $\pm$ 0.07 ^b
Systolic blood pressure (mm Hg)	151.8 $\pm$ 3.7	140.1 $\pm$ 2.9 ^a
Heart rate (beats/min)	378.5 $\pm$ 13.1	291.2 $\pm$ 13.6 ^b

Values are means $\pm$ SEM; $n = 10$

Significantly different from control, ^a $p < 0.05$, ^b $p < 0.001$

Results

The blood glucose concentration in STZ-induced diabetic rats was approximately four times that seen in the control rats ($p < 0.001$). Both systolic blood pressure and heart rate were lower in diabetic rats than in the control rats (respectively, $p < 0.05$, $p < 0.001$) (Table 1).

Perfused mesenteric arterial bed. The basal perfusion pressures in mesenteric arterial beds from STZ-induced diabetic rats and age-matched control rats were 58.9 ± 1.3 mmHg, $n = 6$, and 61.5 ± 1.5 mmHg, $n = 6$, respectively ($p > 0.05$). Methoxamine (10^{-7} to 10^{-3} mol/l) caused a dose-dependent rise in perfusion pressure in both diabetic and control mesenteric arterial beds. The maximum response was significantly reduced in STZ-induced diabetic rats (Fig. 1), the pEC_{50} ($-\log EC_{50}$) values for methoxamine being 5.01 ± 0.05 mol/l ($n = 10$) and 4.74 ± 0.08 mol/l ($n = 9$, $p < 0.01$), in control and diabetic rats, respectively (Table 2). Unexpectedly, the methoxamine-induced vasoconstriction was considerably reduced in endothelium-denuded preparations from control rats, but not in those from diabetic rats (Fig. 2). In fact, in contrast to the situation in the control animals, the vasoconstriction induced by methoxamine was notably increased, rather than decreased in endothelium-denuded preparations from STZ-induced diabetic rats; i.e., the dose-response curve was shifted to the left and the maximal constriction was greater (Fig. 2, lower panel).

The subsequent experiments were carried out using endothelium-intact preparations. Treatment with L-NOARG (10^{-4} mol/l), a nitric oxide synthase (NOS) inhibitor (Fig. 3), greatly enhanced the vasoconstrictions induced by methoxamine in both control and diabetic rats. Indomethacin (10^{-5} mol/l) produced a slight inhibition of the vasoconstriction induced by methoxamine in control rats, but it shifted the dose-response curve to the left in diabetic rats (Fig. 4 and Table 2). SQ 29548 (3×10^{-6} mol/l), an antagonist of prostaglandin H_2 -thromboxane A_2 receptors, produced only a slight inhibition of the methoxamine response in control rats, and it had no effect on the methoxamine response in diabetic rats (Fig. 4 and Table 2). Treatment with indomethacin (10^{-5} mol/l) plus TXB_2 (125 pg/ml) considerably enhanced,

Fig. 1. Dose-response curves for methoxamine-induced vasoconstriction of mesenteric arterial beds taken from age-matched control ($n = 10$) and STZ-induced diabetic rats ($n = 9$). Ordinate shows peak increase in perfusion pressure at each dose. Each data point represents means $\pm$ SEM. *** $p < 0.001$, -■- control animals, -●- diabetic animals

while indomethacin (10^{-5} mol/l) plus PGI_2 (147 pg/ml) enhanced and attenuated the vasoconstrictions induced by methoxamine in control rats (Fig. 5). On the other hand, these treatments had no effects on methoxamine-induced vasoconstriction in diabetic rats. Whether injected with or without indomethacin, a mixture of BQ-123 (10^{-6} mol/l) (an ET_A -receptor antagonist) and BQ-788 (10^{-6} mol/l) (an ET_B -receptor antagonist) shifted the dose-response curve for the methoxamine-induced vasoconstriction to the right in control rats and reduced the maximum response (Fig. 6 and Table 2). In diabetic rats, however, such treatments shifted the dose-response curve slightly to the left (Fig. 6 and Table 2). The data shown in Figure 7, for the concentrations of TXB_2 and PGI_2 in control and diabetic groups were calculated by subtracting indomethacin-treated basal control values from methoxamine-stimulated values.

Fig. 2. Effect of removal of endothelium on the dose-response curves for methoxamine-induced vasoconstriction of mesenteric arterial beds. Upper panel: age-matched control rats with ($n = 10$) or without endothelium ($n = 9$). Lower panel: STZ-induced diabetic rats with ($n = 9$) or without endothelium ($n = 6$). Ordinate shows peak increase in perfusion pressure at each dose. Each data point represents means $\pm$ SEM. * $p < 0.05$, *** $p < 0.001$, -■- + endothelium, -●- -endothelium

Table 2. Maximal increases and EC_{50} values for methoxamine-induced changes in perfusion pressure in mesenteric arterial beds from control and diabetic rats

	control rats			diabetic rats		
	n	Max. response (mm Hg)	$\log \text{EC}_{50}$	n	Max. response (mm Hg)	$\log \text{EC}_{50}$
Untreated	10	169.3 ± 3.7	-5.01 ± 0.05	9	102.3 ± 9.0	-4.74 ± 0.08
-EC	9	76.9 ± 11.7^c	-5.78 ± 0.06^c	6	130.3 ± 4.2^a	-5.29 ± 0.05^c
L-NOARG	6	189.4 ± 3.0^b	-5.98 ± 0.10^c	6	138.5 ± 8.1	-5.14 ± 0.09^b
L-NOARG + TEA	8	170.9 ± 11.4	-5.53 ± 0.09^c	7	160.4 ± 8.9^c	-5.06 ± 0.11^a
Ind	8	145.7 ± 11.1^a	-5.19 ± 0.10	8	110.1 ± 10.5	-5.19 ± 0.05^c
SQ29548	8	147.4 ± 5.7^b	-5.07 ± 0.06	6	92.3 ± 11.5	-4.68 ± 0.10
BQ123 + BQ788	5	119.8 ± 9.2^c	-5.14 ± 0.09	5	99.1 ± 14.3	-5.06 ± 0.07^a
BQ123 + BQ788 + Ind	5	112.8 ± 5.3^c	-4.71 ± 0.17^a	4	113.0 ± 5.8	-5.09 ± 0.13^a

Values are means $\pm$ SEM

n : number of experiments

-EC, without endothelium; L-NOARG, N^G -L-nitro-arginine (10^{-4} mol/l); TEA, tetraethylammonium (10 mmol/l); Ind, indomethacin (10^{-5} mmol/l)

Significantly different from untreated group, ^a $p < 0.05$, ^b $p < 0.01$, ^c $p < 0.001$

Fig. 3. Effects of L-NOARG on the dose-response curves for methoxamine-induced vasoconstriction of mesenteric arterial beds. Upper panel: age-matched control rats either untreated ($n = 10$) or 10^{-4} mol/l L-NOARG-treated ($n = 6$). Lower panel: STZ-induced diabetic either untreated ($n = 9$) or 10^{-4} mol/l L-NOARG-treated ($n = 6$). Ordinate shows peak increase in perfusion pressure at each dose. Each data point represents means $\pm$ SEM. $**p < 0.01$, $***p < 0.001$, $\blacksquare$ - untreated, $\bullet$ - L-NOARG

Changes in release of ET-1, 6-keto-prostaglandin $F_{1\alpha}$ and TXB_2 . The basal concentration of ET-1 in the perfusate was notably higher in beds from control rats than in those from diabetic rats, but there was no difference between control and diabetic rats in terms of the methoxamine (10^{-3} mol/l)-stimulated release of ET-1 (Fig. 7). The basal concentration of 6-keto-prostaglandin $F_{1\alpha}$, a metabolite of PGI_2 , in the effluent collected from the mesenteric arterial bed was markedly increased in diabetic rats, and the methoxamine (10^{-3} mol/l)-stimulated release of 6-keto-prostaglandin $F_{1\alpha}$ in diabetic rats was approximately four times that seen in the control rats. Treatment with indomethacin (10^{-5} mol/l) attenuated both the basal and methoxamine (10^{-3} mol/l)-stimulated release of 6-keto-prostaglandin $F_{1\alpha}$ in diabetic rats (Fig. 7). On the other hand, the methoxamine (10^{-3} mol/l)-induced release of TXB_2 , a metabolite of

Fig. 4. Effects of indomethacin and SQ 29548 on the dose-response curves for methoxamine-induced vasoconstriction of mesenteric arterial beds. Upper panel: age-matched control rats, untreated ($n = 10$), 10^{-5} mol/l indomethacin-treated ($n = 8$) or 3×10^{-6} mol/l SQ 29548-treated ($n = 8$). Lower panel: STZ-induced diabetic rats, untreated ($n = 9$), 10^{-5} mol/l indomethacin-treated ($n = 8$) or 3×10^{-6} mol/l SQ 29548-treated ($n = 6$). Ordinate shows peak increase in perfusion pressure at each dose. Each data point represents means $\pm$ SEM. $*p < 0.05$ for indomethacin vs untreated and SQ29548 vs untreated, $\blacksquare$ - untreated, $\bullet$ - Indomethacin, $\blacktriangle$ - SQ29548

TXA_2 , was perceptibly smaller in mesenteric arterial beds from diabetic rats than in those from control rats. The methoxamine (10^{-3} mol/l) induced release of TXB_2 was considerably decreased by indomethacin in control and in diabetic rats.

Discussion

The major conclusions to be drawn from this study are that the vasoconstriction induced in the mesenteric arterial bed by methoxamine, an α_1 -adrenoceptor agonist, is markedly influenced by endothelium-derived factors and that it was measurably weaker in STZ-induced diabetic rats. The main mechanism underlying the decreased response seen in diabetes seems to involve an increased production of PGI_2 and a decreased formation of TXA_2 .

Fig. 5. Effects of indomethacin plus TXB₂ or indomethacin plus PGI₂ on the dose-response curves for methoxamine-induced vasoconstriction of mesenteric arterial beds. Upper panel: age-matched control rats, untreated ($n = 6$) or treated with either, 10^{-5} mol/l indomethacin plus 125 pg/ml TXB₂ ($n = 5$) or 10^{-5} mol/l indomethacin plus 147.2 pg/ml PGI₂ ($n = 6$). Lower panel: STZ-induced diabetic rats, untreated ($n = 6$), or treated with either 10^{-5} mol/l indomethacin plus 48.9 pg/ml TXB₂ ($n = 6$) or 10^{-5} mol/l indomethacin plus 675.3 pg/ml PGI₂ ($n = 8$). Ordinate shows peak increase in perfusion pressure at each dose. Each data point represents means $\pm$ SEM. * $p < 0.05$, $\blacksquare$ - untreated, $\bullet$ - indomethacin + TXB₂, $\blacktriangle$ - indomethacin + PGI₂.

Systolic blood pressure, as recorded using the tale-cuff technique, was lower in diabetic rats than in the control rats. This result is in agreement with a previous report [23], but there are also reports claiming an enhancement [24] or no change [25–26] in blood pressure. The lower blood pressure in diabetic rats may be due to a cardiac dysfunction or to a decreased contractility of resistance vessels, such as those of the mesenteric arterial bed, or both. Indeed, we recently reported that the β -adrenoceptor-mediated response is desensitized in the papillary muscle of STZ-induced diabetic rats [27].

Studies from several laboratories have shown that vascular responsiveness is changed in experimental diabetes; however, the conclusions drawn from these studies have not always been consistent [2–4]. The reasons for this controversy are not apparent, but

Fig. 6. Effects of BQ-788 plus BQ-123 (with or without indomethacin) on the dose-response curves for methoxamine-induced vasoconstriction of mesenteric arterial beds. Upper panel: age-matched control rats, untreated ($n = 10$) or treated with either 10^{-6} mol/l BQ-123 plus 10^{-6} mol/l BQ-788 ($n = 5$) or 10^{-6} mol/l BQ-123 plus 10^{-6} mol/l BQ-788 plus 10^{-5} mol/l indomethacin-treated ($n = 5$). Lower panel: STZ-induced diabetic rats, untreated ($n = 9$) or treated with either 10^{-6} mol/l BQ-123 plus 10^{-6} mol/l BQ-788 ($n = 5$) or 10^{-6} mol/l BQ-123 plus 10^{-6} mol/l BQ-788 plus 10^{-5} mol/l indomethacin-treated ($n = 4$). Ordinate shows peak increase in perfusion pressure at each dose. Each data point represents means $\pm$ SEM. ** $p < 0.01$, *** $p < 0.001$, $\blacksquare$ - untreated, $\bullet$ - BQ123 + BQ788, $\blacktriangle$ - BQ123 + BQ788 + indomethacin.

the variability is generally attributed to differences in the diabetogen used, the sex of the animals and the techniques used for measuring and expressing contractile force. Most studies have been conducted using larger conduit arteries, primarily the aorta, in rats with spontaneous or chemically induced diabetes. In this study, we examined the contractile response of the mesenteric arterial bed to methoxamine, an α_1 -agonist, and found a markedly weaker dose-dependent vasoconstriction in STZ-induced diabetic rats.

The vasoconstriction induced by methoxamine in control rats was markedly inhibited by BQ123 + BQ788 (ET_A and ET_B receptor antagonists [28–29], respectively), and methoxamine caused a slight release of ET-1. In the mesenteric arterial bed from control rats, SQ29548, an antagonist of the

Fig. 7A–C. ET-1, 6-keto-prostaglandin $F_{1\alpha}$, and TXB_2 levels in effluent from perfused mesenteric arterial bed in age-matched control (open columns) and STZ-induced diabetic rats (filled columns), and effects of indomethacin. Basal, before application of methoxamine; after Met-stimulation, after increasing concentrations of methoxamine (10^{-3} mol/l) had been applied and the methoxamine-induced contractile response had stabilized. **A:** ET-1 release. **B:** 6-keto-prostaglandin $F_{1\alpha}$ release. **C:** TXB_2 release. Where indicated, the mesentery was incubated with 10^{-5} mol/l indomethacin (+ indomethacin). Values are means $\pm$ SEM ($n = 4 \sim 6$ animals). * $p < 0.05$, ** $p < 0.01$

PGH₂-TXA₂ receptor [30], inhibited slightly the methoxamine-induced vasoconstriction, and methoxamine caused a pronounced TXA₂ release. The methoxamine-induced vasoconstriction was markedly enhanced by treatment with L-NOARG, indicating that methoxamine releases NO from the endothelium, and confirming our report that methoxamine releases NO from the endothelium of the mesenteric arterial bed [21]. These results suggest that the endothelium plays an important role in determining the size of the methoxamine-induced vasoconstrictor response in control mesenteric arterial beds (i.e., NO, PGI₂, ET-1 and TXA₂ can all modulate this vasoconstrictor response).

To our surprise, the methoxamine-induced vasoconstriction was much weaker in the endothelium-denuded mesentery from control rats, whereas there was an enhanced response in the endothelium-denuded mesentery from diabetic rats. A slight leftward shift of the dose-response curve and marked decrease in the maximum response may be ascribed to the disappearance of the inhibitory effect of NO (Fig. 3) and of constrictor factors (ET, TXA₂, Fig. 4 and 5), respectively, in the mesenteric artery of control animals. The magnitude of the decrease in the maximum response following endothelium denudation, however, was greater than that expected from the elimination of the influence of ET and TXA₂. Therefore, a possible decrease in the contribution made by other unknown constrictor(s) or smooth muscle damage caused by the detergent (Triton X-100) cannot be excluded. In contrast, in the mesenteric arterial bed from STZ-induced diabetic rats, the vasoconstriction induced by methoxamine was considerably increased, rather than decreased in endothelium-denuded preparations from STZ-induced diabetic rats. This could reflect the removal of methoxamine-induced NO release. Conceivably, alternative explanations could include an increased release of other unknown constrictor(s) or less extensive damage to the smooth muscle by the detergent or both. Further investigations are required on this point.

Treatment with indomethacin or SQ 29548 produced only a slight inhibition of the methoxamine-induced vasoconstriction in age-matched control animals, whereas indomethacin (but not SQ29548) augmented the vasoconstriction induced by methoxamine in diabetic rats. For this reason, we measured 6-keto-prostaglandin $F_{1\alpha}$ (a metabolite of PGI₂) and TXB_2 (a metabolite of TXA₂) in the effluent collected from the mesenteric arterial beds of control and diabetic rats. The basal concentration of 6-keto-prostaglandin $F_{1\alpha}$ was notably increased in diabetic rats and the methoxamine-stimulated release of 6-keto-prostaglandin $F_{1\alpha}$ in diabetic rats was approximately four times that seen in the control rats, suggesting that the enzyme cyclooxygenase is induced or activated in the diabetic state. Our findings are in agreement with

those of others [31–32] who showed that the release of 6-keto-prostaglandin $F_{1\alpha}$, TXB_2 and prostaglandin E_2 from mesenteric arterial bed were significantly greater in diabetic than in control rats. Of particular interest in this connection are the observations that cytokines such as interleukin- 1β , tumor necrosis factor- α and interferon- γ induce the expression of cyclooxygenase-2 (COX-2), and that the expression of COX-2 results in an overproduction of prostaglandins [33–34]. It is unclear at present, however, whether the increased production of PGI_2 in the diabetic state is due to an increased expression of COX-2. Although the concentration of 6-keto-prostaglandin $F_{1\alpha}$ in the effluent was markedly increased in our diabetic rats, the concentration of TXB_2 was decreased. These results could clearly explain our finding that the vasoconstriction induced by methoxamine was notably increased by treatment with indomethacin in diabetic rats.

ET-1, a vasoconstrictor peptide secreted from endothelial cells, is thought to have a role in a number of vascular diseases [35]. ET-1 constricts smooth muscle by activating ET_A receptors [36] and also leads to the release of endothelium-derived relaxing factor by way of the ET_B receptors located on the endothelium [37]. A number of studies have shown that ET-1 can activate phospholipase A_2 by way of various intracellular signalling pathways, leading to a release of arachidonic acid [38–40]. Moreover, it has been reported that in the aorta of normotensive rats, ETs cause an increase in those vasoconstrictors whose production is catalysed by cyclooxygenase in vascular smooth muscle cells [41]. In our study, the methoxamine-induced contractile response was markedly diminished by simultaneous pretreatment with ET_A and ET_B receptor antagonists in the beds from control rats but not in those from diabetic rats. Furthermore, there was only a slight decrease in release of ET-1 from the mesentery preparation in diabetic rats. These results suggest that the attenuated vasoconstriction by methoxamine seen in diabetic rats might not be due to a decrease in the release of ET-1 from the mesenteric arterial bed but in part to a desensitization of the ET-1 receptor. We recently reported that plasma ET-1 concentrations are actually increased in the diabetic state and that the contractile response of the mesenteric arterial bed to ET-1 was attenuated in STZ-induced diabetic rats by desensitization of its receptor [42]. Treatment with indomethacin and the two ET-receptor antagonists caused a more powerful attenuation in the control mesenteric arterial bed than treatment with the two ET-receptor antagonists alone. This may simply be evidence of an additive effect, although a better knowledge of the precise mechanisms underlying the methoxamine-induced vasoconstriction will be necessary before we can fully understand these data.

In conclusion, the vasoconstriction induced by the α_1 -agonist methoxamine is greatly influenced by the

endothelium in the mesenteric arterial bed of control rats. One of the important modulators in control rats seems to be ET-1, although TXA_2 could also be involved. In the diabetic state, both the basal concentration of PGI_2 in the mesentery and the formation of PGI_2 that occurs in response to the α_1 -agonist methoxamine are notably increased and this increased production of PGI_2 is largely responsible for the attenuation of the methoxamine-induced vasoconstriction in the mesenteric arterial bed of diabetic rats. Furthermore, the formation of TXA_2 in response to methoxamine is decreased in the diabetic state, and this decreased formation of TXA_2 will also attenuate the methoxamine-induced vasoconstriction. Such changes in PGI_2 and TXA_2 formation could be responsible for the lower blood pressure seen in diabetic rats. Based on our results, we propose that changes in prostanoid turnover in the diabetic state might be responsible for the lower blood pressure seen in our diabetic rats.

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