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ORIGINAL PAPER

Endothelin-induced changes in blood flow in STZ-diabetic and non-diabetic rats: relation to nitric oxide synthase and cyclooxygenase inhibition

Sven-Olof Granstam · Elisabet Granstam

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Abstract In this study, using the microsphere method, the hemodynamic response to endothelin-1 (ET-1) in healthy and streptozotocin (STZ)-diabetic rats was evaluated as well as the influences of inhibition of nitric oxide (NO)-synthase using L-NAME (Nω-nitro-L-arginine methyl ester) and the cyclooxygenase inhibitor indomethacin. Blood flow (Q) was measured in tissues of interest for vascular complications in diabetes such as kidney, eye, brain, heart and skeletal muscle with the main focus on ophthalmic circulation. Under resting conditions, evidence for renal vasoconstriction was found in diabetic animals. In both groups, administration of L-NAME reduced Q in all investigated tissues indicating a basal NO influence. In the normal rats, ET-1 induced a significant increase in blood pressure and intense vasoconstriction in all tissues except in the choroid of the eye and in the brain, where it induced an increased Q. In the STZ-diabetic rats, effects of ET-1 were less pronounced. Pretreatment with L-NAME, but not the cyclooxygenase inhibitor, abolished the ET-1-induced vasodilation in the choroid of both groups. Administration of ET A receptor antagonist BQ-123 reduced the ET-1-induced vasodilation in the choroid only in diabetic animals. In conclusion, evidence for altered vascular endothelial response to ET-1 in STZ-diabetic animals was found particularly in the ophthalmic circulation. The findings suggest differential involvement of receptors in the response to ET-1 in normal and STZ-diabetic animals.

Keywords Endothelin · Nitric oxide · Diabetes mellitus · Rat · Blood flow

S.-O. Granstam
Department of Medical Sciences, Clinical Physiology, Uppsala University, Uppsala, Sweden

S.-O. Granstam (✉)
Department of Clinical Physiology/Cardiology, Uppsala University Hospital, 751 85 Uppsala, Sweden
e-mail: Sven-Olof.Granstam@medsci.uu.se

E. Granstam
Department of Neuroscience/Ophthalmology, Uppsala University, Uppsala, Sweden

Introduction

Impairment of endothelial cell function has been implicated in the development of vascular complications to diabetes mellitus such as renal failure and diabetic retinopathy [1]. The peptide hormone endothelin-1 (ET-1), produced by the vascular endothelial cells, is a potent vasoconstrictor [2]. In addition, ET-1 has been shown to interact with vasodilating nitric oxide (NO) [1, 3]. ET-1 has also been found to have pro-inflammatory properties and to promote vascular smooth muscle cell proliferation [4]. Furthermore, ET-1 has been shown to increase endothelial cell permeability and extracellular matrix protein expression [5], and can contribute to remodeling of resistance arteries [6] with changes in the microvasculature.

Increased expression of ET-1 has been demonstrated in diabetes [5], and circulating levels of ET-1 have been reported to be elevated in patients with type 2 diabetes [7]. In patients with proliferative diabetic retinopathy, increased levels of ET-1 have been found in both plasma and vitreous humor [8], suggesting involvement of ET-1 in the development of proliferative diabetic retinopathy. In the kidney, ET-1 is secreted by glomerular endothelial cells, mesangial cells and epithelial cells. In humans, a correlation between plasma or urinary levels of ET-1 and signs of diabetic nephropathy such as increased glomerular

filtration rate, mesangial expansion, macro- and/or microalbuminuria and uremia have been found [7, 9, 10].

ET-1 exerts its effects through activation of ET A and B-receptors [11]. In the vasculature, the ET A-receptor is mainly located on vascular smooth muscle cells and mediates vasoconstriction. The ET B-receptor is primarily located on endothelial cells, but may also be present on vascular smooth muscle cells. Stimulation of the endothelial ET B-receptor induces vasodilation through release of NO and prostacyclin whereas stimulation of the vascular smooth muscle cell ET B-receptors results in vasoconstriction [12].

In the rat model of streptozotocin (STZ)-induced diabetes, changes in ET-1 production and function in line with findings in humans have been found, such as elevated plasma ET-1 levels [13], increase in immunofluorescence to anti-ET-1 in retinal capillaries [14] and reduced progression of diabetic nephropathy by ET A-receptor inhibition [15]. In the present study, the microsphere method was applied to investigate the hemodynamic response to ET-1 before and after administration of ET A-receptor antagonist BQ-123 in normal rats, and in rats with STZ-induced diabetes, particularly in tissues of interest for the development of diabetic microvascular complications such as the kidney, eye, brain, heart and skeletal muscle. The possible involvement of NO and prostacyclin pathways was examined.

Materials and methods

Experiments were performed in accordance with the Declaration of Helsinki and the guide for the care and use of laboratory animals (DHEW publication, NIH 80-23) and approved by the Animals Ethic Committee of the University of Uppsala. The animals were housed at the Hospital Research Center with free access to water and food. A total of 36 Sprague-Dawley (SpD) rats, obtained from Möllegaard (Denmark), were included in the study. 16 animals were made diabetic with an intravenous injection of streptozotocin (Zanosar®; Pharmacia Upjohn, USA) 40 mg/kg. Animals were fasted overnight before STZ administration and buprenorphin (Temgesic®; Meda, Sweden) was used as analgesia. One week after the STZ-injection, blood glucose levels were determined by tail vein samples (Hemocue, Sweden) and all rats with blood glucose levels greater than 20 mmol/l were considered diabetic and were included in the study. Blood flow (Q) determinations were performed 31 ± 2 days after STZ administration, i.e. after 3 weeks of established hyperglycemia. The age was between 13 and 17 weeks and the weight between 0.43 ± 0.01 kg for normal and 0.29 ± 0.02 kg for the diabetic rats ($P < 0.05$). Blood glucose measured immediately before the microsphere procedure was 35.2 ± 1.3 mmol/l.

On the day of Q determination, animals were anaesthetized with thiobutabarbital 120 mg/kg (Inactin®; RBI, USA). Body temperature was kept constant by a skin thermistor and heating pad (Atew, Sweden). Animals were tracheostomised and breathed spontaneously. Both femoral arteries were cannulated with polyethylene tubing for continuous blood pressure and heart rate recordings and for collection of reference blood samples. Both femoral veins were cannulated for infusion of drugs. A tube was also placed in the left heart ventricle through the right common carotid artery. At the end of operative procedure, heparin (500 U/kg; Lövens, Denmark) was given to prevent clotting. During the surgical preparation, a slow infusion of saline (5 ml/kg/h) was given to avoid dehydration.

Q was determined with 15 μm radioactive microspheres (Perkin Elmer Life Sciences, USA) [16]; the technique has previously been described in detail [17, 18]. Spheres labeled with three radionuclides, 141Ce, 103Ru and 95Nb, were used in all series, allowing three Q determinations. Approximately 150,000 spheres diluted to 0.3 ml saline were administered at each injection over 15 s, and sampling of reference blood in one femoral artery using a peristaltic pump continued for 1 min at a rate of 0.6 ml/min. The amount of radioactivity given was calculated by taking small aliquots of the initial sphere volume.

At the end of the experiments, the animals were given an overdose of anaesthesia and KCl. The choroid, anterior uvea, retina, heart, left and right kidneys, left masseter muscle and left brain hemisphere were dissected for measurements of counts per minute (CPM) in a gamma spectrometer (Modified model; Nuclear Chicago, USA). In order to obtain correct activity, background activity and cross-over between energy channels were considered for each sphere measurement; and in all experiments the same three nuclides were administered to give similar cross-over influences in all series. Regional blood flow (Q) was calculated by multiplying tissue CPM with the reference flow and dividing by the CPM of the reference blood sample and expressing it as mg/min for ophthalmic tissues and as g/min/g(tw) for other tissues. Vascular resistance (VR) was calculated by dividing the average mean arterial pressure (MAP) during sphere injection with Q and expressing it in arbitrary units [u = mmHg/g/min/g(tw)]. Cardiac index (CI) in g/min/kg(bw) and total peripheral resistance (TPRI) in arbitrary units [U = mmHg/g/min/kg(bw)] were calculated by knowing the total amount of administered radioactivity and the reference femoral flow and CPM and MAP [17].

In the first series of experiments (normal rats, $n = 7$; STZ rats, $n = 5$), the first Q determination was performed after a 15-min stabilization period. Five minutes later, an iv

infusion of ET-1, 60 pmol/min/kg(bw), was started and maintained throughout the experiment using a infusion pump (P-2000; IVAC Medical Systems, UK) at a rate of 0.1 ml/min. After 15 min of ET-1 infusion, the second Q determination was performed. After an additional 5 min, BQ 123, 1 mg/kg(bw), was administered as a bolus injection. Five minutes later, the third Q determination was performed.

In the second series of experiments (normal rats, $n = 8$; STZ, $n = 6$), animals were pretreated with the NO-synthase inhibitor L-NAME using a sequence of bolus injection [10 mg/kg(bw)] followed by an iv infusion [10 mg/h/kg(bw)] as previously described [18]. After 10 min of L-NAME infusion, the first microsphere injection was performed. Five minutes later, iv infusion of ET-1 [60 pmol/min/kg(bw)] was started and maintained throughout the experiment. Additional microspheres were given 15 and 25 min later. However, the third was only given for cross-over adjustments to resemble the other series.

In a third series of experiments (normal rats, $n = 5$; STZ, $n = 5$), animals were pretreated with the cyclooxygenase inhibitor indomethacin as a bolus 5 mg/kg(bw) followed by infusion 0.1 mg/h/kg(bw). In this series, the first sphere was given after a resting period and the second after 11 min indomethacin treatment. Five minutes later ET-1 [60 pmol/min/kg(bw)] was started and maintained for 15 min following a third sphere injection.

Drugs and statistical analysis

Endothelin-1 (human; Bachem UK, UK) was dissolved in saline containing 0.05% rat serum albumin (Sigma-Aldrich, Sweden). L-NAME (Nω-nitro-L-arginine methyl ester; Sigma-Aldrich), BQ-123 sodium salt (cyclo-D-Trp-D-Asp-Pro-D-Val-Leu; Neosystem, France) and indomethacin (Confortid) were diluted in saline. Statistical analysis was performed with non-parametric tests. Within each animal group, Wilcoxon signed rank test and Mann–Whitney U test was applied between the normal and STZ groups within each series. However, statistics was not applied between the three different groups of series since groups were considered too small for such cross-over analysis. Non-parametric tests were chosen because of limited group sizes. All values are given as mean ± SEM. $P < 0.05$ was regarded as statistically significant.

Results

Resting conditions

Both kidneys were investigated in all series of experiments and similar blood flow measurements in the kidneys were regarded as an indication of reliability of the microsphere procedure (Table 1). Renal Q under resting conditions was significantly higher in the normal rats compared to the STZ-diabetic animals. VR in the right and left kidneys were 23 ± 2 and 23 ± 1 u in normal rats and significantly higher in STZ-diabetic animals (31 ± 1 and 32 ± 2 u, respectively), indicating renal vasoconstriction in diabetic animals. In the heart and skeletal muscle (masseter muscle), Q and VR were comparable in normal and diabetic rats. In the ophthalmic circulation, Q tended to be slightly higher in normal rats as compared to STZ-rats (Table 1) but there was no difference in VR. Retina had a very low Q and was therefore not further evaluated. Cerebral Q was only studied in the first series of experiments and showed similar levels in the two groups of animals.

Following treatment with the NO-synthase inhibitor L-NAME, resting Q was reduced in all tissues investigated, indicating the presence of a generalized vasodilator NO-tone. Following L-NAME, resting renal Q was comparable in normal and diabetic rats (Table 1). In the kidneys of both normal and diabetic rats, VR following L-NAME was increased compared to findings in rats without pretreatment (71 ± 9 and 71 ± 9 u in right and left kidneys compared to 23 ± 2 and 23 ± 1 u, respectively, in normal rats and 49 ± 3 and 52 ± 2 u compared to 31 ± 1 and 32 ± 2 u in diabetic rats). Resting Q was not affected by cyclooxygenase inhibition with indomethacin.

Endothelin-1 without pretreatment

In the normal rats of the first series of experiments, MAP was 129 ± 6 mmHg during first sphere injection and increased significantly during ET-1-infusion to 146 ± 5 mmHg at the second sphere injection ($P < 0.05$). In the diabetic rats, MAP was 120 ± 6 and 115 ± 8 mmHg, respectively. Total peripheral resistance was similar in both groups at baseline 0.36 ± 0.03 U in normal rats and 0.35 ± 0.02 U in diabetic rats (Fig. 1a). ET-1 markedly increased the TPRI to 0.77 ± 0.14 U in normal rats ($P < 0.05$) whereas no significant increase was observed in diabetic rats 0.41 ± 0.05 U (Fig. 1a). This response was significantly different between the groups. CI was reduced from 360 ± 20 to 220 ± 30 g/min/kg(bw) in normal rats and from 350 ± 20 to 290 ± 20 g/min/kg(bw) in diabetic rats. However, the difference between groups was not statistically significant (Fig. 1b).

Both kidneys showed a similar response with a significant marked reduction of Q in both animal groups during ET-1 infusion (shown for the right kidney in Fig. 2) by −64 ± 11 and −47 ± 7%, respectively, compared to baseline ($P < 0.05$). VR increased significantly from 23 ± 2 to 220 ± 110 u in the normal rats ($P < 0.05$) and from 31 ± 1 to 60 ± 12 u in STZ-rats ($P < 0.05$), indicating

Table 1 Local blood flow in the examined tissues under resting condition before endothelin-1 administration in normal and STZ-diabetic rats

Tissue (unit)	Type of rat	Blood flow No pretreatment normal ($n = 7$), STZ ($n = 5$)	Blood flow L-NAME pretreated normal ($n = 8$), STZ ($n = 6$)	Blood flow indomethacin pretreated normal ($n = 5$), STZ ($n = 5$)
Right kidney [g/min/g(tw)]	Normal	5.8 ± 0.3*	2.3 ± 0.3	6.1 ± 0.3
	STZ	4.0 ± 0.4	2.7 ± 0.1	3.3 ± 0.4
Left kidney [g/min/g(tw)]	Normal	5.7 ± 0.3*	2.3 ± 0.2	5.7 ± 0.3
	STZ	3.9 ± 0.4	2.5 ± 0.1	3.1 ± 0.5
Heart [g/min/g(tw)]	Normal	4.2 ± 0.4	2.8 ± 0.2*	4.8 ± 0.5
	STZ	3.6 ± 0.4	2.2 ± 0.1	3.6 ± 0.4
Skeletal muscle [g/min/g(tw)]	Normal	0.16 ± 0.03	0.06 ± 0.01*	0.13 ± 0.04
	STZ	0.15 ± 0.03	0.12 ± 0.02	0.13 ± 0.05
Choroid (mg/min)	Normal	59 ± 14	6 ± 2	47 ± 15
	STZ	45 ± 6	10 ± 3	52 ± 12
Anterior uvea (mg/min)	Normal	49 ± 6	16 ± 2	48 ± 7
	STZ	38 ± 2	18 ± 2	41 ± 6
Retina (mg/min)	Normal	2 ± 1	2 ± 1	3 ± 1
	STZ	4 ± 1	2 ± 1	1 ± 1
Brain (left hemisphere) [g/min/g(tw)]	Normal	0.84 ± 0.07		
	STZ	0.80 ± 0.07		

Data are the mean ± SEM. Mann–Whitney *U* test between groups

STZ Streptozotocin, *L-NAME* a NO synthase inhibitor, *Indomethacin* a cyclooxygenase inhibitor

* $P < 0.05$

major renal vasoconstriction in both groups, although less pronounced in the diabetic rats.

In the heart, ET-1 induced a significant decrease in Q in normal rats compared to baseline (−38 ± 13%, $P < 0.05$), whereas no change was observed in diabetic rats (+1 ± 14%). VR increased in the normal rats significantly from 27 ± 3 to 39 ± 5 u ($P < 0.05$) but not in the STZ-rats (30 ± 3 to 32 ± 5 u), indicating coronary vasoconstriction only in the normal rats. A similar response was found in the skeletal muscle with significantly reduced Q only in normal rats (−52 ± 12%) and no change in diabetic rats (+2 ± 26%; Fig. 2). In the normal rats, VR was significantly increased from 1.0 ± 0.3 to 3.4 ± 0.9 u, but not significantly in diabetic rats (1.3 ± 0.2 to 1.7 ± 0.7 u). Thus, similar findings of vasoconstriction only in normal rats were found in the skeletal muscle and the heart.

Choroidal Q significantly increased compared to baseline by 126 ± 71% ($P < 0.05$) in the normal rats and by 72 ± 36% (ns) in the diabetic rats, respectively (Fig. 2). VR was significantly reduced in normal rats from 3.3 ± 0.9 to 1.8 ± 0.4 u ($P < 0.05$), but the reduction from 2.9 ± 0.5 to 2.0 ± 0.5 u was not statistically significant in diabetic rats. The difference in response between normal and diabetic rats was not statistically significant for either Q or VR.

In the anterior uvea of normal rats, ET-1 significantly decreased Q by −32 ± 5% compared to baseline ($P < 0.05$), whereas it was not significantly reduced (−18 ± 10%) in the STZ-rats (Fig. 2). VR was consequently increased from 2.8 ± 0.3 to 4.7 ± 0.3 u ($P < 0.05$) in normal rats, but not significantly in STZ-rats (3.2 ± 0.2 to 4.1 ± 0.7 u).

The findings from choroid differed markedly from findings in other investigated tissues including other vascular beds within the eye, such as the anterior uvea. Similar to the choroid, an increase in Q induced by ET-1 was observed in the brain of normal rats (+38 ± 20%, ns) and of diabetic rats (+32 ± 17%, $P < 0.05$).

ET-1 effects during administration of BQ-123

Administration of BQ-123 during the ET-1-infusion did not induce any additional effect on MAP in the healthy animals (146 ± 5 mmHg before and 150 ± 5 mmHg after BQ-123) or in the diabetic rats (115 ± 8 mmHg before and 118 ± 10 mmHg after BQ-123), although MAP at this time was significantly different between animal groups. TPRI remained at a high level in healthy rats (0.82 ± 0.07 U) and increased significantly in the diabetic rats (0.52 ± 0.04 U, $P < 0.05$; Fig. 1a). CI remained

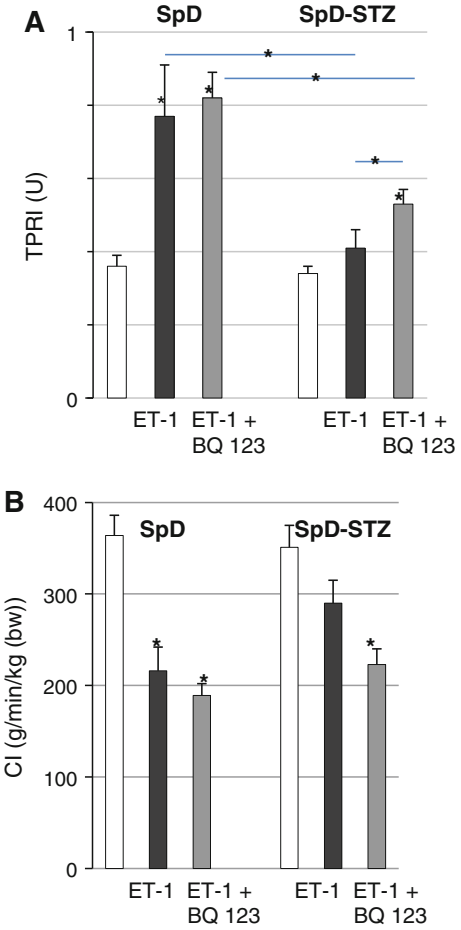

Fig. 1 Effects of endothelin-1 (ET-1) (*black columns*) and the combined infusion of BQ-123 and ET-1 (*gray columns*) on **a** total peripheral resistance index (*TPRI*) and **b** cardiac index (*CI*). Normal Sprague-Dawley rats (*SpD*) (*left*) and streptozotocin diabetic rats (SpD-STZ) (*right*). Data are the mean ± SEM. *Asterisk above column* shows significance at $P < 0.05$ compared to baseline within each animal group; *asterisk on line* shows significance between groups

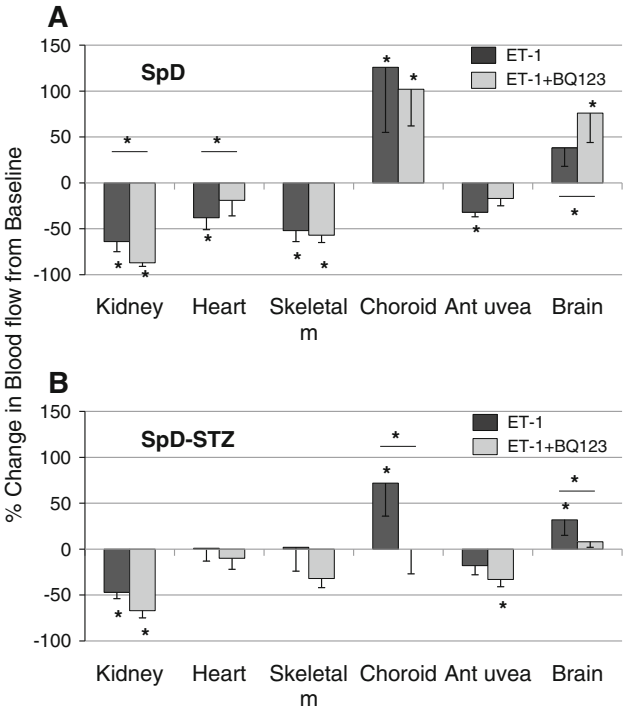

Fig. 2 Effects of endothelin-1 (ET-1) (*black columns*) and the combined infusion of BQ-123 and ET-1 (*gray columns*) on blood flow in different organs presented as % change from baseline. **a** Normal *SpD*-rats and **b** diabetic rats (*SpD-STZ*). Data are the mean ± SEM. *Asterisk above column* shows significance at $P < 0.05$ regarding change in blood flow compared to baseline; *asterisk on line* shows significance between ET-1 and after BQ-123 administration within each group of animals

reduced at 190 ± 10 and 220 ± 20 g/min/kg(bw) in normal and diabetic rats, respectively (Fig. 1b).

In the right kidney, administration of BQ-123 augmented the ET-1-induced Q reduction in normal rats, whereas in the STZ-rats, Q reduction was not significantly further affected by BQ-123. The level of Q reduction was significantly different between normal and diabetic animals with a more pronounced effect in the normal animals.

In the heart of normal rats, ET-1-induced Q reduction was attenuated by administration of BQ-123, from −38 ± 13 to −19 ± 17% compared to baseline, but there was no significant change in VR. In diabetic rats, the coronary Q was not affected by either ET-1 alone or ET-1/BQ-123.

In the skeletal muscle of normal rats, Q remained reduced after BQ-123 administration. In STZ-rats, no significant change was observed in skeletal muscle Q (Fig. 2).

In the choroid of normal rats, BQ-123 did not significantly affect the ET-1-induced increase in Q. However, in the STZ-rats, administration of BQ-123 abolished the ET-1-induced increase in Q (Fig. 2). VR was significantly increased from 2.0 ± 0.5 to 3.3 ± 0.7 u ($P < 0.05$) in the diabetic rats, indicating vasoconstriction. The difference between normal and STZ-rats was significant regarding both Q and VR.

In the anterior uvea of normal rats, administration of BQ-123 during ET-1-infusion did not statistically affect Q, whereas in the diabetic rats, Q was significantly reduced to −33 ± 8% of baseline Q ($P < 0.05$; Fig. 2). However, there was no significant difference between the groups.

In the brain of normal rats, Q significantly increased during combined BQ-123/ET-1 administration compared to ET-1 alone to +76 ± 32% of baseline blood flow (Fig. 2a). In contrast, in the STZ-rats, BQ-123 abolished the ET-1-induced increase in cerebral Q (Fig. 2b). The difference in response to BQ-123 during ET-1 infusion in the brain was significant between the animal groups.

ET-1 effects during inhibition of NO synthase and cyclooxygenase

MAP in normal rats during NO-synthase inhibition with L-NAME was 149 ± 6 mmHg and did not increase further when ET-1 was administered (142 ± 10 mmHg). In diabetic rats, MAP was 129 ± 2 and 122 ± 3 mmHg, respectively. In the series of experiments with indomethacin, MAP was 121 ± 7 mmHg before and 129 ± 5 mmHg during ET-1-infusion in normal rats, and 121 ± 7 and 124 ± 6 mmHg, respectively, in the diabetic rats.

In the right kidney, where marked vasoconstriction was induced by ET-1 alone in both normal and STZ-rats, the ET-1-induced reduction in Q was maintained following both L-NAME and indomethacin pretreatment in normal and STZ-rats (Fig. 3).

In the heart, where ET-1-induced vasoconstriction was only observed in normal rats, the reduction of Q by ET-1 was less pronounced after L-NAME. Following indomethacin pretreatment, no effect on Q was observed (Fig. 3). In the heart of STZ-rats, a slight but significant reduction in Q was only observed following L-NAME.

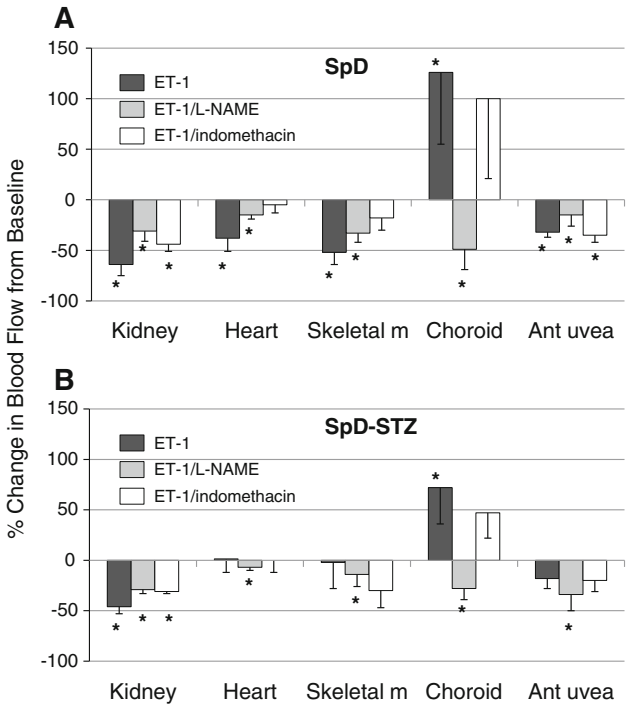

Fig. 3 Endothelin-1 (ET-1)-induced effects on blood flow in different organs, ET-1 alone (*black columns*), ET-1 with L-NAME (*gray columns*), and ET-1 with indomethacin (*white columns*). Data presented as % change from baseline. **a** Normal *SpD*-rats and **b** diabetic rats (*SpD-STZ*). Data are the mean ± SEM. *Asterisk above column* shows significance at $P < 0.05$ regarding change in blood flow compared to baseline within each group of animals

In skeletal muscle of normal rats, ET-1 alone induced vasoconstriction, which was less pronounced following pretreatment with L-NAME and abolished by pretreatment with indomethacin. In the skeletal muscle of STZ-rats, Q was significantly slightly reduced only after L-NAME (Fig. 3).

In the choroid of both normal and STZ-rats, the increase in Q induced by ET-1 was abolished by L-NAME pretreatment (Fig. 3), and instead a significant reduction in Q occurred in both groups. In contrast, following cyclooxygenase inhibition with indomethacin, choroidal Q was increased by ET-1 in both healthy and diabetic rats.

In the anterior uvea of normal rats, significant reductions of Q were induced by ET-1 irrespective of pretreatment. In diabetic rats, a similar response was observed; however, the reduction in Q was statistically significant only following L-NAME pretreatment (Fig. 3).

Discussion

In the present study, the microsphere method was applied to investigate hemodynamics in normal rats and in rats with STZ-induced diabetes mellitus. Evidence for renal vasoconstriction was found in diabetic animals under baseline conditions compared to non diabetic rats. ET-1 induced intense vasoconstriction in all tissues in the normal rats except in the choroid of the eye and in the brain. A similar response was observed in the STZ-diabetic rats, although the effect of ET-1 was less pronounced. Pretreatment with L-NAME abolished the ET-1-induced vasodilation in the choroid of both healthy and diabetic animals indicating involvement of NO, whereas administration of BQ-123 reduced the ET-1-induced vasodilation only in diabetic animals, suggesting differential involvement of receptors in the response to ET-1 in normal and STZ-rats.

Streptozotocin is an antibiotic, structurally a glucosamine derivative of nitrosurea. It causes hyperglycemia mainly by its direct cytotoxic action on the pancreatic beta cells [13, 19]. It is a widely used rat animal model for diabetes demonstrating many similarities with human diabetes regarding endothelial cell function. In the rat model of STZ-induced diabetes, elevated plasma ET-1 levels [13], increase in immunofluorescence to anti-ET-1 within the capillary bed and veins of the retina [14] and reduced progression of diabetic nephropathy by ET A-receptor inhibition [15] have been demonstrated. This model has previously been successfully used in conjunction with the microsphere technique for measurement of central and regional hemodynamics [16, 18, 20]. In the present study, although the duration of hyperglycemia was short, significant alterations in resting Q and response to ET-1 were identified in diabetic animals compared to the healthy rats.

However, the hyperglycemia per se might partly be responsible for differences in hemodynamic response, and the length of established diabetes should also be considered of possible importance.

Under resting conditions, evidence for renal vasoconstriction was found in the diabetic animals. In other investigated tissues, VR was comparable between normal and diabetic rats. Following treatment with the NO-synthase inhibitor L-NAME, resting Q was reduced in all investigated tissues, indicating the influence of vasodilating NO. This finding is well in line with previous studies in the STZ-rat [20]. Generalized vasoconstriction following inhibition of NO synthesis, although to a different extent in various tissues, has also been observed in other rat models of diabetes, such as the Zucker diabetic fatty rat [21] as well as in the ocular circulation of diabetic humans [22].

The ET-1-induced intense vasoconstriction in most non-ocular tissues could explain a compensatory reduction in CI, which was more pronounced in the normal than in the diabetic animals. The vasoconstrictive effect of ET-1 in the kidneys was less pronounced in this model of diabetes. Reduced ET-1-induced vasoconstriction in diabetes has previously been demonstrated in the rat isolated mesenteric arteries [13, 23] and coronary circulation of both experimental animals [24] and humans [25].

In the heart and the skeletal muscle, ET-1 induced vasoconstriction in normal animals, whereas in the diabetic animals, a small ET-1-induced reduction in regional blood flow was observed only following pretreatment with L-NAME. This finding suggests that, in the diabetic animals, continuous formation of NO by the vascular endothelial cells reduces the vasoconstrictive effect of ET-1. Also, in the mesenteric arteries of the diabetic Goto-Kakizaki rat, vasoconstriction induced by ET-1 was reduced compared to non-diabetic Wistar rats, and the effect of ET-1 was enhanced by inhibition of NO synthesis [23]. In the hearts of early STZ-diabetic rats, increased expression of NO-synthase was inhibited by treatment with an ET-A-receptor antagonist in support of an important interrelationship between ET-1 and NO [26].

In contrast to the ET-1-induced intense vasoconstriction in most tissues of both normal and diabetic rats, ET-1 was found to induce an increase in blood flow in the choroid of the eye and to some extent in the brain. Pretreatment with L-NAME abolished the ET-1-induced vasodilation in the choroid of both healthy and diabetic animals, indicating the involvement of NO in both animal groups.

The choroidal vascular bed is characterized by relatively wide but flat capillaries with fenestrated capillary walls and a high blood flow. The high flow rate results in a high oxygen tension in the tissue [27, 28]. The capillary wall is permeable to plasma proteins such as vitamin A. Choroidal blood flow contributes significantly to the nourishment of the retina [27, 28] and may also contribute to temperature regulation. Retinal blood vessels, on the other hand, are the continuous type with tight junctions constituting the blood–retinal barrier. Retinal blood flow is low and is autoregulated in contrast to the choroidal blood flow [27, 28].

In a study of spontaneous oscillations in choroidal arterioles in rabbits, ET-1 was found to increase vasomotion [29]. In this model of in situ-perfused isolated rabbit eyes, ET-1 induced an initial short and weak vasodilating effect followed by a strong and long-lasting vasoconstrictor tone. Similar findings of transient choroidal vasodilation followed by vasoconstriction was observed using the laser Doppler technique in rabbits [30]. During the present experimental conditions, a vasodilating effect of ET-1 was observed in the choroid. The results are consistent with findings in a study in rabbits using the hydrogen clearance method where intravitreal administration of ET-1 induced a long-lasting increase in choroidal blood flow whereas retinal blood flow was reduced [31]. A difference in reactivity between vascular beds of the choroid and anterior uvea has previously been demonstrated in the STZ-diabetic model [18, 20].

ET-1 exerts its effect through ET A- and B-receptors. ET A-receptors are distributed on the vascular endothelial cells and activation generally induces vasoconstriction. ET B-receptors, on the other hand, are located on vascular endothelial cells causing vasodilation through release of NO and prostacyclin, but may also be present on vascular smooth muscle cells causing vasoconstriction [12]. The net effect of ET-1 depends mainly on overall ET receptor profile. In the rat retina, ET B-receptors have been shown to be the dominating receptor subtype [32]. The findings in the present study of ET-1-induced vasodilation in the choroid suggest a predominance of vasodilatory ET B-receptors in the choroid. It might also be that, with the dose of ET-1 used in the present study, the vasodilatory ET B-receptor response is dominant. ET B-receptors have been found in the outer choroid [33]. Efficient vasodilatory mechanisms in the choroid could be of importance to maintain retinal integrity and reduce the susceptibility of the retina to vasoconstrictive agents.

In diabetic patients, increased levels of ET-1 in plasma and vitreous humor have been found [8, 34]. In streptozotocin-diabetic rats, an increase in resistivitiy index for retinal blood flow indicating vasoconstriction was prevented by treatment with the ETA/B-receptor antagonist bosentan, suggesting endothelin involvement in this functional change [35]. Furthermore, in the same study, ET-1 and ET-3 immunoreactivity and endothelin receptor concentrations were increased in the retina [35]. In diabetic BB/W rats following 6 months of diabetes, significant increases in ET-1, ET-3, ET(A) and ET(B) mRNA expressions compared to age-matched control rats were

observed [36]. In a more recent study, glucose-induced ET-1 expression was found to be regulated by ERK5 in the endothelial cells and retina of diabetic rats [37]. Consistent findings of upregulation of ET-1 and increased expression of ET A- and ET B-receptors in the retinal vasculature have been found in diabetes [37]. Although there are no such observations in choroidal blood vessels, it seems likely that diabetes may also induce changes in expression of ET-1 and receptor profile of ET A- and ET B-receptors in this vascular bed.

In the present study, administration of the selective ET A-receptor antagonist BQ-123 reduced the ET-1-induced choroidal vasodilation only in diabetic animals, suggesting differential involvement of ET receptors in the response to ET-1 in normal and STZ-rats. A similar finding, but less pronounced, was observed in the brain of diabetic rats. It may be hypothesized that BQ-123 was less selective in the diabetic animals during the present experimental conditions with high glucose. In addition to ET A-receptor inhibition, BQ-123 has been shown to inhibit vascular smooth muscle cell release of vasoactive peptides and reduce oxidative stress under hyperglycemia [38].

Pretreatment with the cyclooxygenase inhibitor indomethacin did not significantly affect blood flow or response to ET-1 in either normal or diabetic animals. Although prostacyclins are produced by the vascular endothelium [39], the results of the present study suggest that, in this animal model, their influences are of less importance.

In summary, in the present study of early STZ-diabetic animals, evidence for altered vascular endothelial response to ET-1 with and without pretreatment with the NO-synthase inhibitor L-NAME was found. ET A-receptor inhibition using BQ-123 revealed differences in this diabetic model and non-diabetic rats, particularly in ocular circulation. Further studies are of importance to confirm the relationship to diabetic microvascular complications.

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