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Mechanisms underlying diabetes enhancement of endothelin-1-induced contraction in rabbit basilar artery

José A. Alabadi^{a,*}, Francisco J. Miranda^a, Silvia Lloréns^a, José M. Centeno^a,
Vannina G. Marrachelli^a, Enrique Alborch^{a,b}

^aDepartamento de Fisiología, Facultad de Farmacia, Universitat de València, Avda. Vicent Andrés Estellés s/n, 46100 Burjassot, Valencia, Spain

^bCentro de Investigación, Hospital La Fe, Valencia, Spain

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Abstract

The influence of alloxan-induced diabetes on the reactivity of rabbit basilar artery to endothelin-1 was examined. Endothelin-1 induced concentration-dependent contraction of basilar arteries that was higher in diabetic than in control rabbits. Endothelium removal produced a higher enhancement of the endothelin-1-induced contraction in control than in diabetic rabbits. *N*^G-nitro-L-arginine (L-NOArg) enhanced the maximal contraction induced by endothelin-1 in control rabbits and potentiated this response in diabetic rabbits. Endothelin ET_A receptor antagonist, cyclo(D-Asp-Pro-D-Val-Leu-D-Trp) (BQ-123), inhibited endothelin-1-induced contraction in both rabbit groups. Endothelin ET_B receptor antagonist, 2,6-Dimethylpiperidinecarbonyl- γ -Methyl-Leu-N_{in}-(Methoxycarbonyl)-D-Trp-D-Nle (BQ-788), enhanced endothelin-1-induced contraction in control rabbits and decreased the potency of endothelin-1 in diabetic rabbits. Sodium nitroprusside-induced relaxation of basilar arteries was lower in diabetic than in control rabbits. These results suggest that mechanisms underlying rabbit basilar artery hyperreactivity to endothelin-1 include decreased endothelial modulation of endothelin-1-induced contraction, with impaired endothelial endothelin ET_B receptor activity; decreased sensitivity to nitric oxide (NO) in vascular smooth muscle; and enhanced participation of muscular endothelin ET_A and ET_B receptors.

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1. Introduction

Several studies have demonstrated the correlation between diabetes and cerebrovascular diseases. Diabetic patients have increased susceptibility to hypoxic and ischemic injury in cerebral vessels (Mankovsky et al., 1996). Diabetes increases the risk of stroke and stroke mortality, and decreases recovery after stroke (Lukovits et al., 1999). Because of the rising prevalence of diabetes mellitus, its role as a risk factor for stroke and other vascular diseases may take increasing importance in the coming years (Lukovits et al., 1999).

Endothelin-1 could play a role in the pathophysiology of diabetic complications. Diabetes increases plasma endothelin-1 levels (Schneider et al., 2002) and basal endothelin-1

constrictor tone in diabetic patients (Mather et al., 2002). Also, endothelin-1 has a role in the pathophysiology of cerebral ischemia. It has been shown that endothelin-1 concentration is increased in plasma of patients with acute ischemic stroke (Estrada et al., 1994). Moreover, the use of endothelin-1 receptor antagonists increases cerebral perfusion and reduces ischemic damage in focal cerebral ischemia (Barone et al., 1995). The link between endothelin-1, diabetes and ischemic stroke has not yet been sufficiently investigated. The hyperglycemia of diabetes induces a “pseudohypoxic” state in vascular tissue (Williamson et al., 1993). Hypoxia enhances the contractile actions of endothelin-1 (Douglas et al., 1991). Thus, it is possible that the diabetic state potentiates this effect, contributing to the greater susceptibility to ischemic damage in diabetic patients (Hopfner and Gopalakrishnan, 1999).

Endothelial dysfunction could explain, at least partially, the altered response to several vasoconstrictors and vasodilators observed in diabetes. Recently, it has been sug-

* Corresponding author. Tel.: +34-963544904; fax: +34-963543395.

E-mail address: Jose.A.Alabadi@uv.es (J.A. Alabadi).

gested that endothelin contributes to the endothelial dysfunction in type 2 diabetic subjects (Mather et al., 2002). Nevertheless, it still remains a task to elucidate the mechanisms by which the impaired endothelial regulation produces an abnormal vascular reactivity in diabetes (De Vriese et al., 2000).

We have previously described the existence of hyper-reactivity to endothelin-1 of cerebral arteries after cerebral ischemia (Salom et al., 2000) and subarachnoid haemorrhage (Alabadi et al., 1997). On the other hand, we have also reported alterations in the vascular response of diabetic animals to 5-hydroxytryptamine (Miranda et al., 2000a, 2002) and acetylcholine (Miranda et al., 2000b; Alabadi et al., 2001). The aim of the present study was to analyse the influence of alloxan-induced diabetes on the reactivity of the rabbit basilar artery to endothelin-1, including attention to endothelin-1 receptors and the endothelial modulatory mechanisms regulating this response.

2. Materials and methods

Forty-nine male New Zealand white rabbits were used in the present study. Animals were randomly divided into two experimental groups: 25 in the control group and 24 for induction of experimental diabetes. Experiments were performed according to the guidelines from the Council of the European Union (86/609/EEC, Article 5, Appendix II), promulgated by the Spanish legislation on March 14, 1988 (R.D. 223/1988).

2.1. Induction of diabetes and control animals

For induction of experimental diabetes, the rabbits ($n=24$, body weight 2.63 ± 0.05 kg, glycemia 5.8 ± 0.2 mM) were previously sedated with intramuscular 40 mg of ketamine (Ketolar®). Diabetes was induced by injecting alloxan (100 mg kg^{-1}) into the lateral ear vein. Alloxan is a diabetogenic agent which induces in the animal a syndrome resembling Type-I diabetes mellitus and is commonly used as a valid experimental model of diabetes in the rabbit (Chan et al., 2000). To prevent hypoglycemia, 10 ml of 5% glucose was injected (i.v.) after the alloxan and drinking water was supplemented with 10% glucose for the first 24 h after the alloxan injection. Thereafter, the animals were maintained on tap water and regular food ad libitum for 6 weeks. At this time, rabbits (diabetic rabbits) showed a significant increase in serum glucose (21.1 ± 0.6 mM) and their body weight (3.01 ± 0.07 kg) increased slightly. A second group of rabbits ($n=25$, body weight 2.57 ± 0.05 kg, glycemia 5.7 ± 0.1 mM) was maintained under the same conditions for the same time period to serve as age-matched controls (henceforth “control rabbits”). In these rabbits (control rabbits), after 6 weeks, the values for glycemia were similar (6.0 ± 0.2 mM) and the body weight had

increased to 3.54 ± 0.06 kg. This body weight increase was significantly higher than that observed in the diabetic rabbits.

2.2. Isometric tension recording

Six weeks after diabetes induction, the diabetic and the age-matched control rabbits were anaesthetised with sodium thiopental (sodium pentothal, 2% i.v.) and killed by injection of potassium chloride ($10 \text{ mEq, } 0.5 \text{ ml kg}^{-1}$, i.v.) into the lateral ear vein. The entire brain, including the brainstem, was removed, and the basilar artery was dissected free and cut into cylindrical segments measuring 3 mm in length. For isometric tension recording, the segments were mounted in an organ bath using tungsten wires ($89 \mu\text{m}$ in diameter). Two pins were introduced through the arterial lumen. One pin was fixed to a stationary support, while the other pin was connected to a strain gauge for isometric tension recording. The organ bath contained 5 ml of Ringer–Locke solution that was bubbled continuously with 95% O_2 and 5% CO_2 to provide a pH of 7.3–7.4. Temperature was kept at 37°C . A resting tension of 0.5 g was applied to the arterial segments, which were allowed to equilibrate for a period of 60–90 min before the start of the experiments. Tension was readjusted when necessary and the bath fluid was changed every 15 min. After this period of equilibration, the reactivity of the arterial segments from the control and diabetic groups was checked by depolarisation with 50 mM KCl. There were no significant differences (unpaired Student's *t* test) in the response to KCl between arteries from control (1216 ± 42 mg) and diabetic (1200 ± 53 mg) rabbits.

2.3. Concentration–response curves

The experiments were carried out with basilar arteries from both control and diabetic rabbits. Concentration–response curves for endothelin-1 (10^{-12} to 3×10^{-8} M) were obtained by its cumulative addition to the organ bath. To assess the influence of the endothelium on the effect of endothelin-1, concentration–response curves were obtained from arteries in which the endothelium had been removed by gentle rubbing of the intimal surface with a scored stainless-steel rod (rubbed arteries). The absence of endothelium was tested for by silver staining and by checking the absence of relaxant response to acetylcholine. To study the participation of nitric oxide (NO) in the response of basilar arteries to endothelin-1, the concentration–response curves to this peptide were obtained after incubation (20 min) of the arteries with the inhibitor of NO synthase, N^G -nitro-L-arginine (L-NOArg, 10^{-4} M). The possibility that some arachidonic acid derivative could modulate the arterial response to endothelin-1 was examined by obtaining concentration–response curves for endothelin-1 after incubation (20 min) of the arteries with indomethacin (10^{-5} M),

an inhibitor of cyclooxygenase. To examine the participation of specific endothelin ET_A receptors, concentration–response curves for endothelin-1 were obtained in the presence (20 min of incubation) of the selective endothelin ET_A receptor antagonist, cyclo(D-Asp-Pro-D-Val-Leu-D-Trp) (BQ-123, 10^{-6} M). To study the participation of specific endothelin ET_B receptors in this response, concentration–response curves for endothelin-1 were obtained in the presence (20 min of incubation) of the selective endothelin ET_B receptor antagonist, 2,6-Dimethylpiperidinecarbonyl- γ -Methyl-Leu- N_{in} -(Methoxycarbonyl)-D-Trp-D-Nle (BQ-788, 10^{-6} M). Finally, to check the possibility that endothelin ET_B receptors located in the smooth muscle cells participate in the response of the basilar artery to endothelin-1, concentration–response curves for endothelin-1 were obtained in rubbed arteries incubated (20 min) with BQ-788 (10^{-6} M).

To detect possible changes in the sensitivity of the vascular smooth muscle cells to NO, concentration–response curves for sodium nitroprusside (10^{-9} – 10^{-4} M) were obtained cumulatively with basilar arteries from both control and diabetic rabbits, previously precontracted with UTP (10^{-4} M).

2.4. Drugs and solutions

Alloxan, BQ-123, BQ-788, endothelin-1, indomethacin and UTP were obtained from Sigma-Aldrich Química. L-NOArg was obtained from Peptide Institute and sodium nitroprusside from RBI. Alloxan was dissolved in saline solution. BQ-123, BQ-788, UTP and L-NOArg were dissolved in twice-distilled water and diluted with saline solution. The L-NOArg solution required sonication to dissolve completely. Endothelin-1 was dissolved in 0.1% aqueous acetic acid and diluted in a phosphate-buffered saline solution with 0.05% bovine serum albumin, fraction V (Sigma). Indomethacin was dissolved in ethanol and diluted in saline solution. Sodium nitroprusside was dissolved and diluted in saline solution. Care was taken to protect sodium nitroprusside solutions from light due to its light sensitivity. The composition of the Ringer–Locke solution was (mM): NaCl, 120; KCl, 5.4; $CaCl_2$, 2.2; $MgCl_2$, 1.0; $NaHCO_3$, 25 and glucose, 5.6. To prepare the KCl-depolarising solution, NaCl was replaced by an equimolar amount of KCl in the normal Ringer–Locke solution.

2.5. Statistical analysis

Comparisons of body weight and glycemia between control and diabetic rabbits were made by using an unpaired Student's *t* test. For the concentration–response curves, contraction values were expressed as percentages of the previous depolarisation induced by 50 mM KCl, and relaxation values as percentages of the active tone induced by UTP 10^{-4} M. For each concentration–response curve, the

maximum effect (E_{max}) and the concentration of drug which produced half of E_{max} (EC_{50}) were calculated. Maximum effects were expressed as means \pm S.E.M. and EC_{50} as the geometric mean with its confidence limits (95%) for repeated experiments. Statistical comparisons of E_{max} and $-\log EC_{50}$ (pD_2) values between arteries from control rabbits and arteries from diabetic rabbits subjected to the same experimental treatment were achieved by using an unpaired Student's *t* test. Comparisons between the values of E_{max} and pD_2 for the concentration–response curves for endothelin-1 obtained with the different treatments of arteries from control rabbits were made using an analysis of variance (ANOVA) followed by the Newman–Keuls test. The same tests were used to compare E_{max} and pD_2 values of the curves obtained with the different treatments of arteries from diabetic rabbits. A probability value of less than 5% was considered significant.

3. Results

3.1. Concentration–response curves for endothelin-1

Cumulative addition of endothelin-1 (10^{-12} to 3×10^{-8} M) produced a concentration-dependent contraction of the basilar artery from either control or diabetic rabbits (Fig. 1). In arteries from diabetic rabbits, the E_{max} of the concentration–response curve for endothelin-1 was significantly higher than that obtained with arteries from control rabbits, while there were no significant differences between the EC_{50} values (Fig. 1; Table 1).

Endothelium removal displaced to the left (significantly lower EC_{50}) the concentration–response curve for endothe-

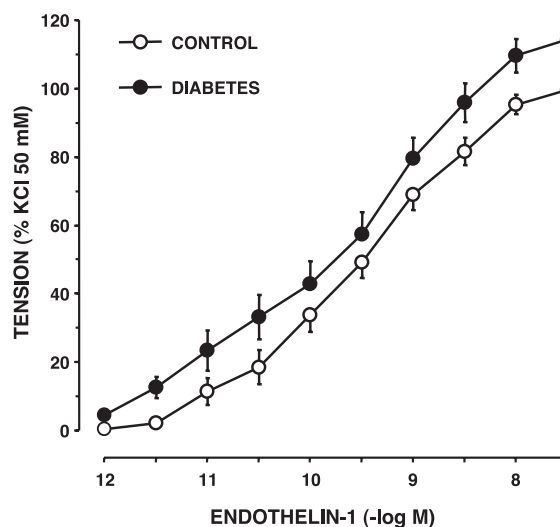


Fig. 1. Concentration–response contraction curves for endothelin-1 in basilar arteries isolated from control and diabetic rabbits. Contraction values are expressed as percentages of the previous depolarisation induced by 50 mM KCl and represent means \pm S.E.M.

Table 1

Half-maximal effective concentration (EC_{50}) and maximum effect (E_{max}) values for concentration–response curves for endothelin-1 in rabbit basilar artery

	EC_{50} (M)	E_{max} (%)	n
<i>Control rabbits</i>			
Control	$2.3 (1.5–3.5) \times 10^{-10}$	100 ± 3	17
Rubbed	$2.6 (1.7–4.2) \times 10^{-11a}$	125 ± 5^a	11
L-NOArg (10^{-4} M)	$1.1 (0.8–1.7) \times 10^{-10}$	119 ± 6^a	12
Indomethacin (10^{-5} M)	$6.8 (5.6–8.1) \times 10^{-10}$	110 ± 6	12
BQ-123 (10^{-6} M)	$4.0 (3.1–5.1) \times 10^{-9a}$	111 ± 5	11
BQ-788 (10^{-6} M)	$2.3 (1.4–3.8) \times 10^{-10}$	126 ± 6^a	11
Rubbed + BQ-788 (10^{-6} M)	$1.4 (1.1–1.7) \times 10^{-10b}$	125 ± 10^a	9
<i>Diabetic rabbits</i>			
Control	$1.4 (0.9–2.2) \times 10^{-10}$	114 ± 4^c	18
Rubbed	$8.1 (6.2–10.7) \times 10^{-12ac}$	116 ± 7	10
L-NOArg (10^{-4} M)	$1.5 (0.8–2.9) \times 10^{-11ac}$	124 ± 11	10
Indomethacin (10^{-5} M)	$2.0 (1.3–3.3) \times 10^{-10c}$	113 ± 5	13
BQ-123 (10^{-6} M)	$3.2 (2.4–4.1) \times 10^{-9a}$	120 ± 6	11
BQ-788 (10^{-6} M)	$1.1 (0.8–1.7) \times 10^{-9ac}$	123 ± 7	10
Rubbed + BQ-788 (10^{-6} M)	$4.1 (3.4–4.9) \times 10^{-10bc}$	125 ± 5	9

E_{max} values are expressed as percentages of a previous depolarisation with KCl 50 mM. EC_{50} values are means and confidence limits; E_{max} values are means \pm S.E.M.

L-NOArg: N^G -nitro-L-arginine.

^a Significantly different from corresponding control value, $P < 0.05$.

^b Significantly different from corresponding rubbed value, $P < 0.05$.

^c Significantly different from corresponding value for control rabbits, $P < 0.05$.

lin-1 in arteries from either control or diabetic rabbits and also increased significantly the E_{max} of the concentration–response curve for endothelin-1 obtained with arteries from control rabbits (Figs. 2 and 3; Table 1). Moreover, in endothelium-denuded arteries from diabetic rabbits, the EC_{50} value of the concentration–response curve for endothelin-1 was significantly lower than that obtained in arterial segments without endothelium from control rabbits (Table 1).

In basilar arteries from control rabbits, incubation with L-NOArg (10^{-4} M) significantly enhanced the maximal contraction of the concentration–response curve for endothelin-1, without significantly changing the EC_{50} value (Fig. 2; Table 1). However, incubation of basilar arteries from diabetic rabbits with the same inhibitor of NO synthase, L-NOArg (10^{-4} M), did not significantly modify the E_{max} , but significantly decreased the EC_{50} value of the concentration–response curves for endothelin-1 (Fig. 3; Table 1). Moreover, comparison showed that the endothelin-1-induced contraction in arterial segments incubated with L-NOArg had an EC_{50} value of the concentration–response curve significantly lower in arteries from diabetic rabbits than that obtained in arteries from control rabbits (Table 1).

Incubation of basilar arteries from either control and diabetic rabbits with indomethacin (10^{-5} M) did not sig-

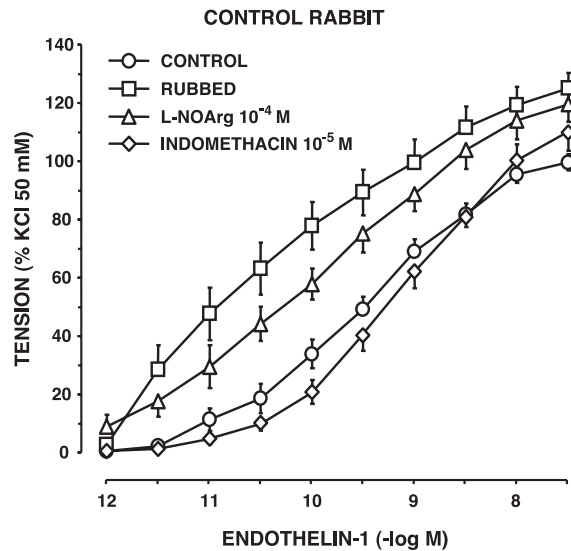


Fig. 2. Concentration–response contraction curves for endothelin-1 in basilar arteries isolated from control rabbits under the following experimental conditions: control, without endothelium (rubbed), incubation with L-NOArg (10^{-4} M) and incubation with indomethacin (10^{-5} M). Contraction values are expressed as percentages of the previous depolarisation induced by 50 mM KCl and represent means \pm S.E.M.

nificantly modify the contractile response to endothelin-1 (Figs. 2 and 3; Table 1).

The incubation of basilar arteries from either control or diabetic rabbits with the antagonist of the endothelin ET_A receptors, BQ-123 (10^{-6} M), significantly increased the EC_{50} value (displaced to the right) of the concentration–

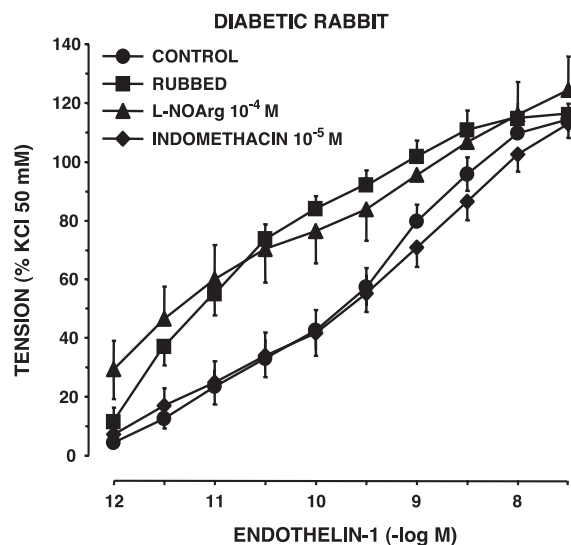


Fig. 3. Concentration–response contraction curves for endothelin-1 in basilar arteries isolated from diabetic rabbits under the following experimental conditions: control, without endothelium (rubbed), incubation with L-NOArg (10^{-4} M) and incubation with indomethacin (10^{-5} M). Contraction values are expressed as percentages of the previous depolarisation induced by 50 mM KCl and represent means \pm S.E.M.

response curve for endothelin-1, without modifying the E_{\max} value (Figs. 4 and 5; Table 1).

Incubation of basilar arteries from control rabbits with the antagonist of the endothelin ET_B receptors, BQ-788 (10^{-6} M), significantly increased the E_{\max} value of the concentration–response curve for endothelin-1, without modifying the EC_{50} value (Fig. 4; Table 1). However, for basilar arteries from diabetic rabbits, preincubation with BQ-788 did not significantly modify the E_{\max} value, but significantly increased the EC_{50} value (displaced to the right) of the concentration–response curve for endothelin-1 (Fig. 5; Table 1). In addition, comparing the endothelin-1-induced contraction in arterial segments incubated with BQ-788, the EC_{50} value for the concentration–response curve was significantly higher with arteries from diabetic rabbits than that obtained with arteries from control rabbits (Table 1).

The incubation of rubbed basilar arteries with BQ-788 (10^{-6} M) significantly increased the EC_{50} (displaced to the right) of the concentration–response curve for endothelin-1 as compared to the corresponding curve obtained with rubbed arteries in the absence of BQ-788 from either control (Fig. 4; Table 1) or diabetic (Fig. 5; Table 1) rabbits. Moreover, the EC_{50} value for the concentration–response curve for endothelin-1 obtained in rubbed arteries incubated with BQ-788 (10^{-6} M) from diabetic rabbits was significantly higher than that obtained with the corresponding arteries from control rabbits (Table 1).

Table 1 summarises the E_{\max} and EC_{50} values of concentration–response curves for endothelin-1 under the different experimental conditions.

CONTROL RABBIT

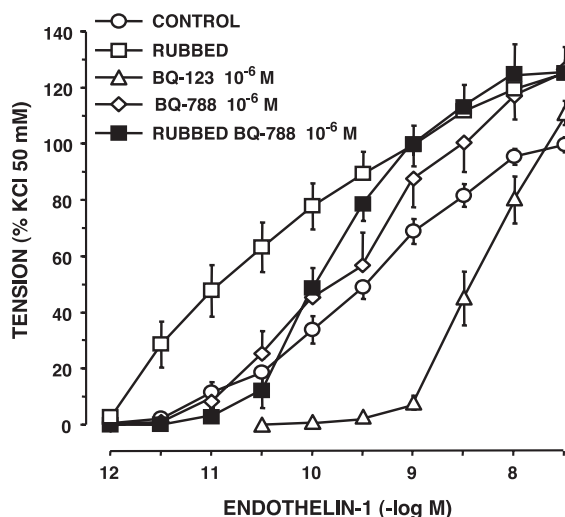


Fig. 4. Concentration–response contraction curves for endothelin-1 in basilar arteries isolated from control rabbits under the following experimental conditions: control, without endothelium (rubbed), incubation with BQ-123 (10^{-6} M), incubation with BQ-788 (10^{-6} M) and incubation of rubbed arteries with BQ-788 (10^{-6} M). Contraction values are expressed as percentages of the previous depolarisation induced by 50 mM KCl and represent means \pm S.E.M.

DIABETIC RABBIT

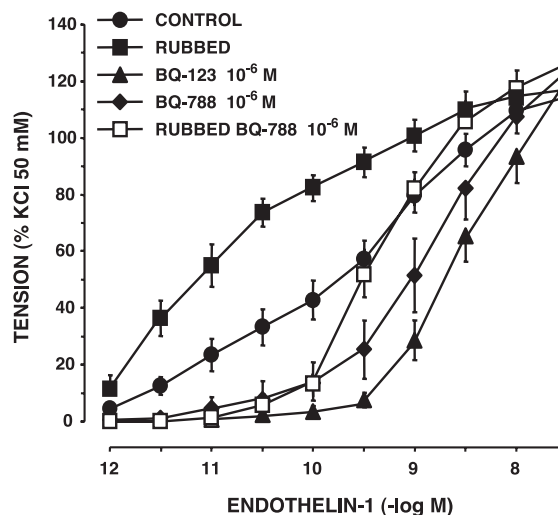


Fig. 5. Concentration–response contraction curves for endothelin-1 in basilar arteries isolated from diabetic rabbits under the following experimental conditions: control, without endothelium (rubbed), incubation with BQ-123 (10^{-6} M), incubation with BQ-788 (10^{-6} M) and incubation of rubbed arteries with BQ-788 (10^{-6} M). Contraction values are expressed as percentages of the previous depolarisation induced by 50 mM KCl and represent means \pm S.E.M.

3.2. Concentration–response curves for sodium nitroprusside

Cumulative addition of sodium nitroprusside (10^{-9} – 10^{-4} M) produced concentration-dependent relaxation of isolated basilar arteries previously contracted with UTP (10^{-4} M) from both control and diabetic rabbits (Fig. 6). In basilar arteries from control rabbits, the EC_{50} value was

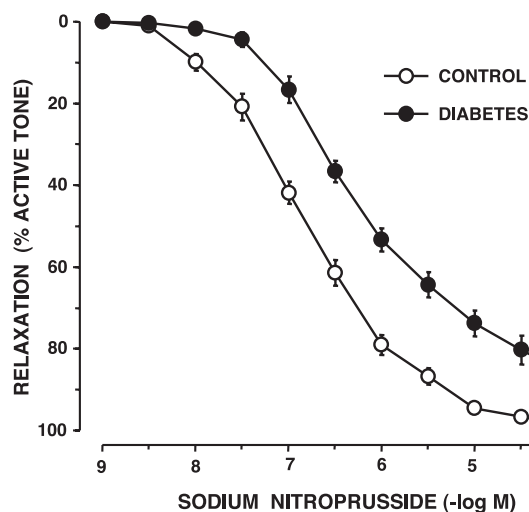


Fig. 6. Concentration–response relaxant curves for sodium nitroprusside in basilar arteries isolated from control and diabetic rabbits. Relaxation values are expressed as percentages of the active tone induced by UTP 10^{-4} M and represent means \pm S.E.M.

$1.6 (1.4–1.9) \times 10^{-7}$ M and the E_{\max} value was $99 \pm 1\%$ of the active tone ($n=11$). With arteries from diabetic rabbits, the EC_{50} value of the curves for sodium nitroprusside was $5.0 (4.2–6.0) \times 10^{-7}$ M and the E_{\max} value was $86 \pm 3\%$ of the active tone ($n=8$). Comparison of relaxations induced by sodium nitroprusside in basilar arteries showed that, for diabetic rabbits, the EC_{50} value was significantly higher and the E_{\max} value was significantly lower than was found for arteries from control rabbits.

There were no significant differences in the UTP (10^{-4} M)-induced active tone between basilar arteries from control (751 ± 84 mg, $n=11$) and diabetic (634 ± 60 mg, $n=8$) rabbits.

4. Discussion

The present study showed enhancement of the contractile response of isolated rabbit basilar artery to endothelin-1 in diabetes. An increased vascular response to endothelin-1 has been reported in different pathophysiological conditions such as cerebral ischemia (Salom et al., 2000), subarachnoid hemorrhage (Alabadi et al., 1997) and hypertension (Cardillo et al., 1999). In diabetes, endothelin-1-induced contraction was enhanced in aorta (Hattori et al., 1999), and in renal and mesenteric (Kiff et al., 1991) rat arteries. More recently, an enhancement of the sensitivity to endothelin-1 in isolated subcutaneous resistance arteries of diabetic patients (McIntyre et al., 2001) was observed. Moreover, studies performed in vivo show an enhancement of the reactivity to endogenous endothelin-1 in resistance vessels of diabetic patients (Cardillo et al., 2002), and enhanced response to the peptide in resistance arterioles of diabetic hamsters (Mayhan et al., 1999). This cerebrovascular hyperreactivity to endothelin-1 could play a role in the pathogenesis of cerebral ischemia of diabetic patients.

In contrast to our results, the response to endothelin-1 in rat basilar artery in vivo was similar in control rats and in diabetic rats 3–4 months after injection of streptozotocin (Mayhan, 1998). Differences in the diabetes duration, animal species, in vivo vs. in vitro methodology and diabetogenic agent could explain this discrepancy.

The hyperreactivity to endothelin-1 is not the result of a non-specific increase in the reactivity of the rabbit basilar artery induced by diabetes, since we have observed that the contractile response of basilar arteries to other vasoconstrictors such as UTP and 5-hydroxytryptamine (data not shown) was similar in diabetic and in control rabbits.

In arteries from control rabbits, mechanical removal of the endothelium increased the maximal contraction and displaced to the left the concentration–response curve for endothelin-1, indicating an inhibitory endothelial modulation of the contractile action of this peptide. These results are in accordance with results of previous work performed with cerebral arteries of non-diabetic animals (Salom et al., 1991; Petersson et al., 1997). In arteries from diabetic

rabbits, endothelium denudation also displaced to the left the concentration–response curve for endothelin-1, without significant changes in the maximal contraction, suggesting a lower modulatory role of endothelium in the diabetic state. This fact could contribute to the hyperreactivity to endothelin-1 observed in diabetic rabbits. Consistent with our results, the endothelial modulation of the vascular responses to several stimuli is impaired in aorta and carotid arteries of gestational diabetic women (Hu et al., 1998) and in aorta (Pagano et al., 1998) and renal artery (Costa e Forti and Fonteles, 1998) of alloxan-induced diabetic rabbits.

The modulatory action of the endothelium could be effected, at least partially, through the release of NO. In control rabbits, incubation of the arterial segments with L-NOArg, an inhibitor of the constitutive NO synthase, significantly enhanced the maximal contraction induced by endothelin-1, indicating an inhibitory role for NO in the modulation of the contractile response of the basilar artery to endothelin-1. This inhibitory role of NO in the endothelin-1-induced vasoconstriction has been described for the cerebrovascular bed (Alabadi et al., 1997). On the other hand, incubation of basilar arteries from diabetic rabbits with L-NOArg did not significantly modify the maximal contraction but displaced to the left the concentration–response curve for endothelin-1, suggesting that, in diabetes, the inhibitory role of NO in the response of basilar artery to endothelin-1 differs from that seen in control rabbits. The alteration in the NO inhibitory role could be related to changes in NO production and/or sensitivity of vascular smooth muscle cells to NO induced by diabetes. There is a report of a decreased NO production in diabetes (Lagaud et al., 2001). In addition, our results for the relaxation induced by the NO donor, sodium nitroprusside (lower relaxation in arteries from diabetic rabbits), indicate a decreased sensitivity to NO in the smooth muscle cells of the basilar diabetic arteries. Therefore, these findings suggest that diabetes alters the endothelial NO modulation of the endothelin-1-induced contraction in basilar arteries by a mechanism that includes, at least, a decreased sensitivity to NO in the vascular smooth muscle cells. This fact could contribute to the hyperreactivity to endothelin-1 observed in the present work. Interestingly, this decreased sensitivity to NO of diabetic basilar arteries cannot be generalised to other vascular beds. We have previously reported that, in renal arteries from diabetic rabbits, the sensitivity of smooth muscle cells to NO is not altered (Alabadi et al., 2001).

The participation of the arachidonic acid derivatives in the modulation of the response of the basilar artery to endothelin-1 was analysed by obtaining concentration–response curves for this peptide in the presence of indomethacin, an inhibitor of the enzyme cyclooxygenase. Our results show that incubation of basilar arteries from both control and diabetic rabbits with indomethacin did not significantly modify the endothelin-1-induced contraction, suggesting that in rabbit basilar arteries, the endothelin-1-

induced contraction is not modulated by arachidonic acid derivatives.

Vascular actions of endothelin-1 are primarily mediated by two distinct G-protein-coupled receptor subtypes named endothelin ET_A and ET_B receptors (Hopfner and Gopalakrishnan, 1999). Both endothelin ET_A and ET_B receptors are located in the smooth muscle cells and mediate vasoconstriction. Endothelin ET_B receptors are also located in the endothelium and mediate vasodilation through the activation of NO release. In the present work, incubation with the antagonist of the endothelin ET_A receptors, BQ-123, inhibited the contractile response of the basilar artery to endothelin-1, in both control and diabetic rabbits. These results confirm that the contraction of the rabbit basilar artery in response to endothelin-1 is mediated by endothelin ET_A receptors. The fact that differences in the arterial response to endothelin-1 between control and diabetic rabbits do not persist after incubation with BQ-123 suggests a greater participation of endothelin ET_A receptors in diabetes, which could contribute to the hyperreactivity to endothelin-1 observed in diabetic animals. A recent study performed in vivo shows that the activity of endogenous endothelin-1 on endothelin ET_A receptors is enhanced at the time that sensitivity to exogenous endothelin-1 is blunted in the resistance vessels of patients with type II diabetes (Cardillo et al., 2002). Moreover, diabetes induces upregulation of endothelin ET_A receptors in rat heart (Chen et al., 2000).

In control rabbits, incubation of unrubbed arteries with the antagonist of the endothelin ET_B receptors, BQ-788, enhanced the contractile response to endothelin-1. This result suggests the participation of endothelial endothelin ET_B receptors mediating vasodilatation in the response of basilar artery to endothelin-1. The presence of endothelin ET_B receptors that mediate endothelium-dependent relaxation in the rabbit basilar artery has been demonstrated previously (Zuccarello et al., 1998). Therefore, we can conclude that in rabbit basilar artery, endothelin-1 acts on the endothelial endothelin ET_B receptors, stimulating NO release which in turn counteracts the vasoconstrictor action of the peptide. In contrast, in diabetic rabbits, incubation of unrubbed arteries with BQ-788 did not modify the maximal contraction induced by endothelin-1. This result indicates that diabetes impairs the vasodilator action of endothelin-1 mediated by NO release through endothelial endothelin ET_B receptor activation, and could explain the lower modulatory role of the endothelium in the diabetic state described in the present work. In renal perfusates of diabetic rats, the impaired NO release in response to stimulation of endothelin ET_B receptors is attributed, at least in part, to a decrease in the number or the activity of endothelial endothelin ET_B receptors (Kakoki et al., 1999). The impaired activity of endothelin ET_B receptors could contribute to the hyperreactivity to endothelin-1 observed in diabetes.

In addition, our results show that in both control and diabetic rabbits, incubation of rubbed arteries with BQ-788 displaced to the right the concentration–response curve to

endothelin-1 with respect to the curves obtained with untreated rubbed arteries. This result suggests that endothelin-1 also acts on endothelin ET_B receptors located on the vascular smooth muscle that mediate vasoconstriction. The fact that differences in the response to endothelin-1 in rubbed arteries between control and diabetic rabbits (higher potency in diabetes) do not persist after incubation of rubbed arteries with BQ-788 (higher potency in control), suggests a greater participation of muscular endothelin ET_B receptors in diabetes, which could also contribute to the hyperreactivity of the basilar artery to endothelin-1 in diabetes. This could be consistent with upregulation of endothelin ET_B receptors reported for the heart of diabetic rats (Chen et al., 2000).

In summary, diabetes induces hyperreactivity of rabbit basilar artery to endothelin-1. At least three causes could contribute to this hyperreactivity: (1) the lower endothelial inhibitory modulation of this response, including impaired activity of the endothelial endothelin ET_B receptors; (2) lower sensitivity to NO in the vascular smooth muscle cells; and (3) the greater participation of muscular endothelin ET_A and ET_B receptors that mediate vasoconstriction. The cerebrovascular bed hyperreactivity to endothelin-1 could contribute to the greater susceptibility to cerebrovascular diseases of diabetic patients.

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