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European Journal of Pharmacology 401 (2000) 397–402

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Diabetes-induced changes in endothelial mechanisms implicated in rabbit carotid arterial response to 5-hydroxytryptamine

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Received 28 February 2000; received in revised form 29 June 2000; accepted 4 July 2000

Abstract

The influence of diabetes on endothelial mechanisms implicated in the response of isolated rabbit carotid arteries to 5-hydroxytryptamine (5-HT) was studied. 5-HT induced a concentration–dependent contraction that was potentiated in arteries from diabetic rabbits with respect to that in arteries from control rabbits. Endothelium removal potentiated 5-HT contractions in arteries from both control and diabetic rabbits but increased the maximum effect only in arteries from diabetic rabbits. Incubation of arterial segments with N^G-nitro-L-arginine (L-NA) enhanced the contractile response to 5-HT. This L-NA enhancement was greater in arteries from diabetic rabbits than in arteries from control rabbits. Aminoguanidine did not modify the 5-HT contraction in arteries from control and diabetic rabbits. Indomethacin inhibited the 5-HT-induced response, and this inhibition was higher in arteries from control rabbits than in arteries from diabetic rabbits. In summary, diabetes enhances the sensitivity of the rabbit carotid artery to 5-HT. In control animals, the endothelium modulated the arterial response to 5-HT by the release of both nitric oxide (NO) and a vasoconstrictor prostanoid. Diabetes enhances endothelial constitutive NO activity and impairs the production of the endothelial vasoconstrictor. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Endothelium; Diabetes; Nitric oxide (NO); 5-HT (5-Hydroxytryptamine, serotonin); Carotid artery

1. Introduction

Several studies have demonstrated a correlation between diabetes and brain ischemia. Diabetes causes and exacerbates macroangiopathies, increases the severity of ischemia and increases stroke mortality (Caplan, 1996). The epidemiological data obtained by the Epidemiology of Diabetes Interventions and Complications (EDIC) (1999) have demonstrated that type-1 diabetic patients display an increased intimal-medial thickness in the common and internal carotid arteries. In addition, carotid occlusive disease is directly linked to 20–30% of strokes that occur each year in the United States (Eugene et al., 1999).

The endothelium contributes to the regulation of vascular reactivity through the release of different constrictor (angiotensin II, endothelin-1, thromboxane A_2, prostaglandin H_2) or relaxant (nitric oxide (NO), prostacyclin, endothelium-derived hyperpolarising factor) substances, the equilibrium of which results in vascular tone. Diabetes alters the responsiveness of different vascular beds to several vasoconstrictors and vasodilators, and it has been hypothesised that endothelial dysfunction could partially explain many of these altered responses. Nevertheless, the mechanisms by which the impaired endothelial regulatory function contributes to the abnormal vascular reactivity have not been completely elucidated.

Several reports have suggested a possible role for 5-hydroxytryptamine (5-HT) in the pathophysiology of diabetic complications. Chronic diabetes is associated with a decrease in the cerebral concentration of 5-HT and with an increase in the population of 5-HT_{1A} and 5-HT_2 receptors in the rat brain (Sandrini et al., 1997). Diabetes reduces the 5-HT concentration in the gut and platelets of alloxan-treated rats, these effects being reversed by treatment of the animals with the serotonin precursor 5-hydroxytryptophan (Cicin-Sain and Jernej, 1996). In contrast, a relationship between increased 5-HT plasma levels and the devel-

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PII: S0014-2999(00)00469-6

opment of diabetic nephropathy has been suggested (Kasho et al., 1998).

We have previously described several aspects of the vasoconstrictor response of cerebral arteries to 5-HT, including the modulatory influence of the endothelium (Miranda et al., 1993), its dependence on the entry of extracellular Ca^{2+} (Torregrosa et al., 1994) and the receptors involved (Miranda et al., 1995). In addition, we have reported that cerebral arteries exhibit hyperreactivity to 5-HT after subarachnoid hemorrhage via a mechanism that involves the absence of the modulatory role of endothelial NO (Miranda et al., 1996). The aim of the present study was to analyse diabetes-induced changes in the reactivity of the carotid artery to 5-HT, including the study of the effects of the disease on the endothelial mechanisms that regulate this response. To do this, we studied the influence of alloxan-induced diabetes on the modulatory role of endothelial-derived factors in the constrictor response of rabbit carotid artery to 5-HT.

2. Materials and methods

Thirty-three male New Zealand white rabbits were used in the present study. Animals were randomly divided into two experimental groups: 17 in the control group and 16 destined for induction of experimental diabetes. Housing conditions and experimental procedures were in accordance with the European Union regulations on the use of animals for scientific purposes (86/609/EEC, Article 5, Appendix II) and promulgated by Spanish legislation on March 14, 1988 (R.D. 223/1988).

2.1. Induction of diabetes and control animals

For induction of experimental diabetes, rabbits weighing 2.0–3.2 kg were sedated with intramuscular 40 mg of ketamine (Ketolar®). Diabetes was induced by injecting alloxan (100 mg kg^{-1}) into the lateral ear vein. To prevent hypoglycaemia, 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. A second group of rabbits (2.1–3.0 kg) was maintained under the same conditions for the same time period to serve as age-matched controls (henceforth ''control rabbits''). Diabetic rabbits showed a marked increase in serum glucose and a failure to increase their body weight when compared with control rabbits. Table 1 shows the mean values of body weight and glycaemia before and 6 weeks after diabetes induction for the rabbits in the diabetic group and for the rabbits in the control group. Two rabbits that received alloxan injection failed to become diabetic and were excluded of the study.

Table 1
Body weight and glycaemia in control and diabetic rabbits

	Body weight (kg)	Glycaemia (mmol l^{-1})	*n*
Control rabbits			
Initial time	2.55 ± 0.09	5.9 ± 0.2	17
6 weeks after	3.55 ± 0.08	6.0 ± 0.2	17
Diabetic rabbits			
Initial time	2.49 ± 0.09	6.0 ± 0.2	16
6 weeks after	3.00 ± 0.07[a]	20.4 ± 1.3[a]	14

Results are means ± S.E.M.
[a] Significantly different from corresponding value in control rabbits, $P < 0.05$.

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 mEq, 0.5 ml kg^{-1}, i.v.). The common carotid arteries were dissected free and cut into cylindrical segments measuring 4 mm in length. Each segment was prepared for isometric tension recording in an organ bath. Two stainless steel L-shaped pins (diameter, 125 μm) were introduced through the arterial lumen. One pin was fixed to the organ bath wall and 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 2 g was applied to the arterial segments, and they were allowed to equilibrate for a period of 60–90 min before the experiments were started. 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 was checked by depolarisation with 50 mM KCl. There were not significant differences in the response to KCl between arteries from control and diabetic rabbits.

2.3. Concentration–response curves of 5-HT

The experiments were carried out with carotid arteries from both control and diabetic rabbits. Concentration–response curves for 5-HT (10^{-8} to 10^{-5} M) were obtained by its cumulative addition to the organ bath. To assess the influence of the endothelium on the effect of 5-HT, concentration–response curves were obtained with arteries from which the endothelium had been removed by rubbing the intimal surface with a scored stainless steel rod (rubbed arteries). The absence of endothelium was checked by silver staining (Caplan and Schwartz, 1973). To assess the participation of NO in the response of carotid arteries to

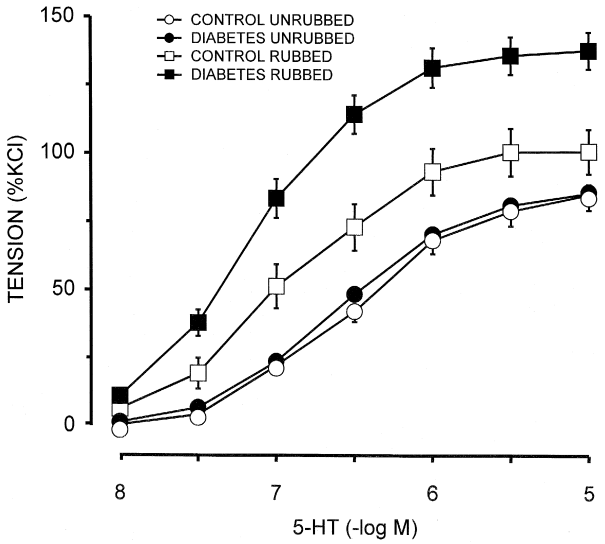

Fig. 1. Concentration–contraction response curves for 5-HT in carotid arteries isolated from control and diabetic rabbits with (unrubbed) and without (rubbed) endothelium. Values represent means ± S.E.M.

5-HT, the concentration–response curves for this amine were obtained after incubation (20 min) of the unrubbed arteries with the inhibitor of NO synthase (NOS) N^G-nitro-L-arginine (L-NA, 10^{-4} M). Moreover, we examined the possibility that NO synthesised by the inducible NOS (iNOS) participated in the 5-HT-induced response by obtaining concentration–response curves for 5-HT in unrubbed arteries after incubation (20 min) with aminoguanidine (10^{-5} M), a selective inhibitor of the iNOS. To examine the possibility that some arachidonic acid derivative could modulate the arterial response to 5-HT, we obtained concentration–response curves for 5-HT after incubation (20 min) of the unrubbed arteries with indomethacin (10^{-5} M), an inhibitor of cyclooxygenase. Finally, we also obtained concentration–response curves for 5-HT in the presence of both L-NA (10^{-4} M) and indomethacin (10^{-5} M).

2.4. Drugs and solutions

Alloxan, 5-HT, aminoguanidine and indomethacin were obtained from Sigma and L-NA from Peptide Institute. Alloxan was dissolved in saline solution. 5-HT, aminoguanidine and L-NA were dissolved in twice-distilled water and diluted in saline solution; the L-NA solution required sonication to dissolve completely. Indomethacin was dissolved in ethanol and diluted in saline solution. 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 glycaemia between control and diabetic rabbits were made by using unpaired Student's *t*-test. 5-HT-induced contractions are expressed as a percentage of the previous depolarisation induced by 50 mM KCl. For each concentration–response curve the maximum effect (E_{max}) and the concentration of 5-HT which produced half of E_{max} (EC_{50}) were calculated. Maximum effects are expressed as means ± standard error of the mean (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 and diabetic rabbits receiving the same experimental treatment were achieved by using unpaired Student's *t*-test. Comparisons between the values of E_{max} and pD_2 of the concentration–response curves for 5-HT obtained with the different treatments in the arteries from the control rabbits were made using analysis of variance (ANOVA) followed by the Newman–Keuls test. The same tests were used to compare the E_{max} and pD_2 values of the curves obtained with the different treatments in the arteries from diabetic rabbits. A probability value of less than 5% was considered significant.

Table 2
EC_{50} and maximum effect (E_{max}) values for concentration–response curves for 5-HT in rabbit carotid artery

	EC_{50} ($\times 10^{-7}$ M)	E_{max} (%)	*n*
Control rabbits			
Control	3.1(2.9–3.5)	86 ± 6	51
Rubbed	1.2(1.0–1.5)[a]	101 ± 8	15
L-NA 10^{-4} M	0.8(0.7–0.9)[a]	120 ± 5[a]	15
Indomethacin 10^{-5} M	5.4(5.0–5.7)[a,b,c]	37 ± 3[a,b,c]	19
L-NA 10^{-4} M + Indomethacin 10^{-5} M	0.9(0.8–0.9)[a,d]	106 ± 4[d]	14
Aminoguanidine 10^{-5} M	2.6(2.2–2.9)	74 ± 6	18
Diabetic rabbits			
Control	2.3(2.1–2.6)[e]	85 ± 3	47
Rubbed	0.6(0.5–0.8)[a,e]	138 ± 7[a,e]	17
L-NA 10^{-4} M	0.5(0.4–0.6)[a]	138 ± 5[a,e]	10
Indomethacin 10^{-5} M	5.0(4.4–5.7)[a,b,c]	57 ± 3[a,b,c,e]	11
L-NA 10^{-4} M + Indomethacin 10^{-5} M	2.0(1.8–2.3)[b,c,d,e]	111 ± 5[a,b,c,d]	6
Aminoguanidine 10^{-5} M	2.8(2.6–3.0)	77 ± 4	20

E_{max} values are expressed as a percentage of a previous depolarisation with KCl 50 mM.
EC_{50} values are means and confidence limits; E_{max} values are means ± S.E.M.
[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 L-NA value, $P < 0.05$.
[d] Significantly different from corresponding indomethacin value, $P < 0.05$.
[e] Significantly different from corresponding value in control rabbits, $P < 0.05$.

3. Results

5-HT (10^{-8} to 10^{-5} M) induced a concentration-dependent contraction of the carotid artery from either control or diabetic rabbits (Fig. 1). The EC_{50} of the concentration–response curve was significantly lower in arteries from diabetic rabbits with respect to that obtained in arteries from control rabbits, without significant differences between E_{max} values (Table 2). The maximal contraction induced by 5-HT was 2518 ± 121 mg (control rabbits) and 2445 ± 172 mg (diabetic rabbits). In arteries from control rabbits, mechanical removal of the endothelium displaced to the left the concentration–response curve for 5-HT without significantly changing the maximal contraction (Fig. 1, Table 2). In arteries from diabetic rabbits, removal of the endothelium enhanced the 5-HT induced contraction both in terms of E_{max} and EC_{50} values (Fig. 1, Table 2). The concentration–response curve for 5-HT obtained in rubbed arteries from diabetic rabbits exhibited a lower EC_{50} and a higher E_{max} value with respect to values obtained in rubbed arteries from control rabbits (Fig. 1, Table 2).

Incubation with L-NA (10^{-4} M) enhanced the contractions induced by 5-HT in arterial segments from both control (Fig. 2) and diabetic (Fig. 3) rabbits. The maximal contraction in response to 5-HT in L-NA-treated arteries from diabetic animals was significantly higher than that obtained in arterial segments from control rabbits (Table 2).

Incubation with aminoguanidine (10^{-5} M) did not significantly modify the contractile action of 5-HT in arterial segments isolated from either control (Fig. 2) or diabetic (Fig. 3) rabbits.

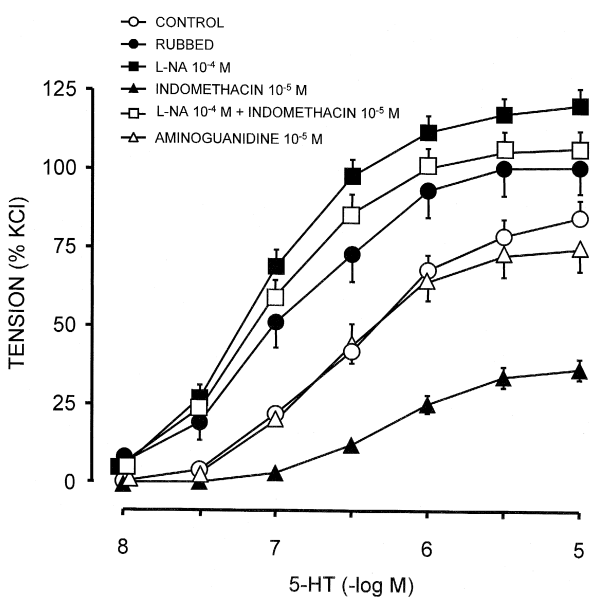

Fig. 2. Concentration–contraction response curves for 5-HT in carotid arteries isolated from control rabbits in different experimental conditions: control, without endothelium (rubbed), incubated with L-NA (10^{-4} M), incubated with indomethacin (10^{-5} M), incubated with L-NA (10^{-4} M) + indomethacin (10^{-5} M) and incubated with aminoguanidine (10^{-5} M). Values represent means ± S.E.M.

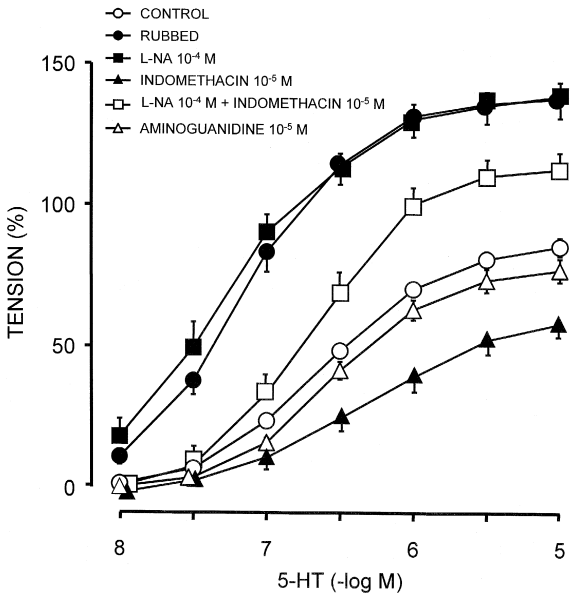

Fig. 3. Concentration–contraction response curves for 5-HT in carotid arteries isolated from diabetic rabbits in different experimental conditions: control, without endothelium (rubbed), incubated with L-NA (10^{-4} M), incubated with indomethacin (10^{-5} M), incubated with L-NA (10^{-4} M) + indomethacin (10^{-5} M) and incubated with aminoguanidine (10^{-5} M). Values represent means ± S.E.M..

The incubation of carotid arteries from both control (Fig. 2) and diabetic (Fig. 3) rabbits with indomethacin (10^{-5} M) significantly inhibited the concentration–response curve for 5-HT, both in terms of EC_{50} and E_{max} values, with respect to that obtained either in control, rubbed or L-NA preincubated arterial segments (Table 2). The maximal contraction in response to 5-HT in indomethacin-treated arteries from control animals was significantly lower than that obtained in arterial segments from diabetic rabbits (Table 2).

In the presence of both L-NA (10^{-4} M) and indomethacin (10^{-5} M), the concentration–response curve for 5-HT obtained in carotid arteries from control rabbits exhibited an E_{max} significantly higher than that obtained in arteries preincubated with indomethacin alone and an EC_{50} significantly lower than that obtained both in control and indomethacin-treated arteries (Fig. 2, Table 2).

In arteries isolated from diabetic rabbits (Fig. 3), the E_{max} of the concentration–response curve for 5-HT obtained in the presence of both L-NA and indomethacin was significantly higher than that obtained either in the absence of both inhibitors or in the presence of indomethacin alone and was significantly lower with respect to that obtained in either rubbed or L-NA-treated arteries (Table 2). The EC_{50} obtained in arterial segments preincubated with both inhibitors was significantly higher than that obtained in rubbed and L-NA-treated arteries and significantly lower than that obtained in indomethacin-treated arteries (Table 2). When compared with responses induced in L-NA +

indomethacin-treated arteries from control animals, the EC_{50} was significantly higher in arterial segments from diabetic rabbits without there being significant differences in the E_{max} values (Table 2).

Table 2 summarises the E_{max} and EC_{50} values of concentration–response curves for 5-HT under the different experimental conditions.

4. Discussion

The influence of alloxan-induced diabetes on the modulatory role of the endothelium in the constrictor response of rabbit carotid artery to 5-HT was examined. Alloxan is a diabetogenic agent that induces in the animal a syndrome resembling type-I diabetes mellitus, characterised by hyperglycaemia, hypercholesterolaemia, glycosuria, and raised levels of glycosylated haemoglobin in erythrocytes (Agrawal et al., 1987), and it is commonly used as a valid experimental model of diabetes in the rabbit (Öztürk et al., 1996).

The present results show that 5-HT strongly contracted carotid arteries isolated from control rabbits. Alloxan-induced diabetes potentiated the contractile action of 5-HT on the carotid artery, as can be deduced from the statistically significant displacement to the left of the concentration–response curve. This would be in agreement with the diabetes enhancement of the pressor response to 5-HT in the pulmonary circulation (El Kashef, 1996) and the enhanced sensitivity of the basilar artery to 5-HT observed in short-term diabetic rats (Van Buren et al., 1998). In arteries from control rabbits, the elimination of the endothelial cell layer by rubbing of the internal surface potentiated the contractile activity of 5-HT, thus indicating that the endothelium partially counteracts this response. The endothelial modulation of the 5-HT-induced contraction has been reported in previous works (Miranda et al., 1993; Valentin et al., 1996). In rubbed arteries from diabetic animals, a greater potentiation of the 5-HT-induced contraction with respect to control rabbits was observed. This result would indicate that diabetes enhances the inhibitory influence of the endothelium on 5-HT-induced contractions in rabbit carotid artery. This enhancement in the modulatory activity of the endothelium is in agreement with some reports that show an enhanced endothelium-dependent relaxation in response to histamine (White and Carrier, 1986) and to acetylcholine (Bhardwaj and Moore, 1988; Pieper, 1999) in diabetes. However, this result also suggests the existence of a diabetes-induced hyperreactivity of the carotid arterial smooth muscle to 5-HT that would be partially deadened by the enhanced modulatory activity of the endothelium.

We studied the possibility that the modulatory action of the endothelium could be achieved, at least partially, through the release of NO. NO is synthesised from the amino acid L-arginine by the Ca^{2+}-dependent enzyme NOS. There are three isoenzymes of NOS (Moncada et al., 1997): two constitutive isoforms, endothelial (eNOS) and neuronal (nNOS) and one iNOS. Incubation of carotid arteries with the inhibitor of the constitutive NOS L-NA significantly enhanced the contractile response of arterial segments to 5-HT. Because the response of the L-NA-treated carotid arteries to 5-HT was significantly higher in arterial segments from diabetic rabbits than in those from control rabbits, it can be deduced that NO was more effective at inhibiting diabetic than control arteries, indicating a greater modulatory role of NO in the diabetic state. This would be in agreement with studies of diabetic rats showing that diabetes-induced endothelial dysfunction results, in part, from an increase in NO production during the course of the disease (Pieper et al., 1998). An excessive NO production has also been suggested to contribute to the renal hyperfiltration and hyperperfusion that characterise early diabetic nephropathy (Sugimoto et al., 1998; Choi et al., 1999).

The expression of iNOS has been described in several pathological conditions. iNOS has been suggested to participate in the pathophysiology of certain vascular disorders (Sayama et al., 1998; Nogawa et al., 1998; Forster et al., 1999). It has also been suggested that iNOS could play a role in diabetic vasculopathy (Stevens et al., 1997). In the present work, we studied the possible induction by diabetes of NO synthesis in the carotid artery by obtaining concentration–response curves for 5-HT in the presence of aminoguanidine, an inhibitor of iNOS. The results obtained showed that aminoguanidine did not significantly modify the vascular response to 5-HT, thus excluding the participation of an inducible source of NO in the carotid arterial bed of diabetic rabbits. This result is in agreement with the absence of an inhibitory effect of aminoguanidine on the enhanced relaxant response to ACh of rat aortic rings in the early stages of the streptozotocin-induced diabetes (Pieper, 1999).

In the present work, we also examined the participation of a derivative from arachidonic acid via cyclooxygenase in the modulation of the response of the carotid artery to 5-HT by obtaining concentration–response curves for this amine in the presence of the inhibitor of the enzyme cyclooxygenase indomethacin (Moncada and Vane, 1979). The results obtained show that indomethacin significantly inhibited the 5-HT-induced contraction in arteries from both control and diabetic rabbits, thus suggesting that the contractile carotid response to 5-HT is modulated, in addition to NO, by the release of a vasoconstrictor prostanoid (i.e., thromboxane A_2). Because the response of the indomethacin-treated carotid arteries to 5-HT was significantly lower in arterial segments from control rabbits than in those from diabetic rabbits, it can be deduced that this vasoconstrictor prostanoid has lower modulatory activity in the diabetic state. This would be in agreement with studies performed in diabetic rats suggesting a decreased formation of thromboxane A_2 in the mesenteric arterial bed of diabetic rats (Makino and Kamata, 1998).

In the presence of both inhibitors, L-NA and indomethacin, the 5-HT-induced contraction of arteries from diabetic rabbits was significantly higher than that obtained both in the absence of the inhibitors and with indomethacin alone and was significantly lower than contractions obtained in the presence of L-NA alone. In spite of the absence of statistical differences in some comparisons, a similar tendency was obtained in arteries from control rabbits.

In summary, diabetes enhances the sensitivity of the rabbit carotid artery to 5-HT. In control animals the endothelium modulates the arterial response to 5-HT by the release of both NO and a vasoconstrictor prostanoid. Diabetes enhances endothelial constitutive NO activity and impairs the production of the endothelial vasoconstrictor.

Acknowledgements

The authors are grateful to Salvador Banacloche for his technical assistance.

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