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The nitric oxide synthesis/pathway mediates the inhibitory serotoninergic responses of the pressor effect elicited by sympathetic stimulation in diabetic pithed rats

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Abstract

We investigated the involvement of the nitric oxide pathway in the inhibitory mechanisms of 5-hydroxytryptamine (5-HT) in the pressor responses induced by stimulation of sympathetic vasopressor outflow in diabetic pithed rats.

Diabetes was induced in male Wistar rats by a single s.c. injection of alloxan. Four weeks later, the animals were anaesthetized, pretreated with atropine, and pithed. Electrical stimulation of the sympathetic outflow from the spinal cord (0.1, 0.5, 1 and 5 Hz) resulted in frequency-dependent increases in blood pressure.

The inhibition of electrically induced pressor responses by 5-HT (10 μg/kg/min) in diabetic pithed rats could not be elicited after i.v. treatment with 1*H*-[1,2,4]Oxadiazolo[4,3-a]quinoxalin-1-one (ODQ) (10 μg/kg), a guanylyl cyclase inhibitor, or *N*-ω-L-Arginine methyl ester hydrochloride (L-NAME) (10 mg/kg), a nitric oxide synthase (NOS) inhibitor.

The inhibitory effect produced by infusion of the selective 5-HT_{1A} receptor agonist 8-hydroxydipropylaminotretalin hydrobromide (8-OH-DPAT) (20 μg/kg/min) was abolished in the presence of ODQ (10 μg/kg), or L-NAME (10 mg/kg) in diabetic pithed rats.

The administration of L-Arginine (100 mg/kg) 30 min after L-NAME reproduced the inhibitory effect caused by 5-HT (10 μg/kg/min) and 8-OH-DPAT (20 μg/kg/min) on the electrically induced pressor responses, whereas in the presence of D-Arginine (100 mg/kg)+L-NAME the 5-HT or 8-OH-DPAT inhibitory effect on the pressor responses was abolished.

In conclusion, in diabetic pithed rats, the inhibition produced by prejunctional 5-HT_{1A} activation on electrically induced sympathetic pressor responses is mediated by the NO synthesis/pathway.

Keywords: 5-hydroxytryptamine; Nitric oxide; Experimental diabetes; 5-HT_{1A} receptors; Prejunctional inhibition

1. Introduction

Serotonin exerts different types of cardiovascular responses, including bradycardia and tachycardia, hypotension and hypertension, vasodilatation and vasoconstriction. These actions depend on several factors such as the animal species used, the basal vascular tone, the experimental conditions, the dose employed and, above all, the nature of the receptors involved.

We have previously reported that 5-HT can modulate cardiovascular responses to sympathetic stimulation in normoglycaemic (Morán et al., 1994, 1998; Fernández et al., 2000) and diabetic animals (García et al., 2005). Other authors (Villalón et al., 1998) working with normoglycaemic pithed male Wistar rats have reported evidence of the involvement of 5-HT_{1A} receptors, rodent 5-HT_{1B} and 5-HT_{1D} receptors in the prejunctional inhibition of electrically induced sympathetic

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outflow. However, in diabetic pithed rats only the 5-HT_{1A} receptor subtype is involved in the serotonergic prejunctional inhibition of electrically induced sympathetic outflow (García et al., 2005).

Diabetes is a disease currently considered to be an important public health problem owing to its increasing prevalence, and it causes and exacerbates macro- and microvascular complications that alter the responsiveness of different vascular beds to several vasoconstrictors and vasodilators (Miranda et al., 2000, 2002; El Kasef, 1996). It has been hypothesised that endothelial dysfunction could partially explain many of these altered responses (Cohen, 1993; De Vriese et al., 2000). Although the mechanisms by which the impaired endothelial regulatory functions contribute to the abnormal vascular reactivity have not been completely elucidated, one of the major vasoactive mediators involved is nitric oxide.

Thus, using Western blot techniques, Choi et al. (1997) demonstrated that the three enzyme isoformes involved in NO synthesis (neuronal NOS, endothelial NOS and inducible NOS) are clearly impaired one week after the onset of experimental diabetes. Additionally, nitric oxide synthase-dependent reactivity is impaired in large and small cerebral blood vessels during diabetes (Mayhan and Patel, 1998). Some findings have shown that endothelium-dependent relaxation is enhanced after streptozotocin-induced diabetes in the rat renal artery (Bhardwaj and Moore, 1988), in rat mesenteric arteries (Heygate et al., 1996), and in rat aorta (Tolins et al., 1993).

5-hydroxytryptamine has also been implicated in the pathophysiology of diabetic complications. Chronic diabetes is associated with modifications in 5-HT concentrations, the 5-HT receptor population, and the vascular responses induced by this amine. In this sense, 5-HT plasma levels have been reported to be enhanced during diabetes mellitus (Barradas et al., 1988; Martin et al., 1995). Regarding vascular responses, Miranda et al. (2000, 2002) suggested the existence of a diabetes-induced hyper-reactivity to 5-HT of rabbit renal and carotid arteries, whereas James and Hodgson (1995) reported attenuated responses to 5-HT in diabetic rat autoperfused hindquarters.

Some studies conducted under different experimental conditions have demonstrated the existence of regulatory 5-HT receptors located on postganglionic and possibly preganglionic sympathetic nerve terminals in rats, both in vitro and in vivo (Molderings et al., 1987; Ireland and Jordan, 1987; Villalón et al., 1995a,b,c), and cats (Jones et al., 1995). Working in in vivo conditions, we have previously shown that in pithed rats 5-HT inhibits the sympathetic transmission of the systemic vascular system by the activation of 5-HT_1 receptors (Morán et al., 1994). More recent studies by us in normoglycaemic animals have revealed that prejunctional 5-HT_{1D} heteroceptors are involved in sympathetic neurotransmission, although this inhibitory effect is also modulated by 5-HT_{1A} receptors (Morán et al., 1998). In contrast, in diabetic animals the inhibitory effect on sympathetic pressor outflow exerted by 5-HT is mainly produced via activation of 5-HT_{1A} receptors (García et al., 2005).

The present study was carried out to determine whether there is any indirect mechanism involved in the inhibitory effect of 5-HT on the pressor responses induced by stimulation of sympathetic vasopressor outflow in pithed atropine-treated alloxan diabetic rats. To do so, we analysed the possible involvement of the NO pathway (synthesis) in the inhibitory serotoninergic responses of the pressor effect elicited by sympathetic stimulation in diabetic pithed rats.

2. Methods

2.1. General

A total of 140 male Wistar rats (250–350 g) were used in our experiments. The animals were kept and supplied by the Animalarium of the Faculty of Pharmacy of the University of Salamanca (P.A.E.-SA001). Housing conditions and experimental procedures were in accordance with European Union regulations on the use of animal for scientific purposes (86/609/ECC, Article 5, Appendix II) and enacted by Spanish legislation on March 14, 1988 (R.D. 223/1988) and October 10, 2005 (R.D. 1201/2005).

Diabetes was induced by a single injection of alloxan (150 mg/kg, s.c.) in NaCl 0.9%. Weight and blood glucose levels were determined before administration, and 2, 7, 14, 21 and 28 days after administration. Only rats with elevated blood glucose levels (>11 mM) at all time points were considered diabetic. Normal rats served as controls, and the two groups, control and alloxan-diabetic rats, were all aged-matched.

All the animals were anaesthetized with sodium pentobarbital (60 mg/kg, i.p.), and had their trachea cannulated. The rats were pithed by inserting a stainless steel rod through the orbit and foramen magnum (Gillespie and Muir, 1967) and artificially respirated with room air by a Harvard respiratory pump (1 ml air/100 g, 50 strokes/min).

The right and the left jugular veins were cannulated for the infusion of agonists and for the administration of antagonists respectively, and the left carotid artery was connected to a PRS 205 amplifier, displaying the recordings on one channel of a Letica Polygraph 4000 for recording blood pressure. Heart rate was measured by analysis of the blood pressure data with a Car 1000 tachograph connected to the same PRS 205 amplifier.

The entire sympathetic outflow from the spinal cord was stimulated with a Cibertec Stimulator CS-9. Two electrodes were employed: one was connected to the pithing rod (the stimulating electrode), while the other one (the indifferent electrode) was inserted subcutaneously into a leg.

Before electrical stimulation, the animals were treated with heparin (1000 UI/kg), and then received d-tubocurarine (2 mg/kg, i.v.) to avoid electrically induced muscular twitching and atropine (1 mg/kg) to prevent cholinergic effects.

2.2. Experimental protocols

When the animals had been in a stable haemodynamic condition for at least 10 min, the baseline values of mean blood pressure were determined. Then, sympathetic outflow was stimulated by applying trains of 25 s, consisting of monophasic pulses of 1 ms duration and supramaximal intensity

(27.5±2.5 V for diabetic rats and 15±3 V for normoglycaemic animals) at increasing frequencies (0.1, 0.5, 1 and 5 Hz).

Thus, the control stimulation–response curve (curve E0) was completed in about 20 min. At this point the animals were divided into 7 different groups, and each group was split into different subgroups, taking into account that each animal was used to evaluate only one respective dose of agonist or antagonist, and that each dose was repeated five times, up to a total of $n=5$ experiments.

The first group represented the group to confirm the results from our previous works (Morán et al., 1994, 1998; García et al., 2005). This group ($n=35$) was subdivided in two different subgroups: the normoglycaemic one and the diabetic one.

In the normoglycaemic subgroup ($n=20$) each animal received a continuous i.v. infusion of one of the following: saline solution (1 ml/h, $n=5$, control group for all the agonist treatments), 5-HT (20 μg/kg/min, $n=5$), 8-OH-DPAT (20 μg/kg/min, $n=5$), a selective 5-HT_{1A} receptor agonist, or 2-[5-[3-(4-methylsulfonylamino)benzyl-1,2,4-oxadiazol-5-yl]-1*H*-indol-3-yl]ethanamine, L-694,247 (1 μg/kg/min, $n=5$), a selective non-rodent 5-HT_{1B} receptor and 5-HT_{1D} receptor agonist, all through a Harvard model 122 pump (Cibertec, Spain).

In the diabetic subgroup ($n=15$) each animal received a continuous i.v. infusion of one of the following: saline solution (1 ml/h, $n=5$, control group for all the agonist treatments); 5-HT (10 μg/kg/min, $n=5$); 8-OH-DPAT (10 μg/kg/min, $n=5$), a selective 5-HT_{1A} receptor agonist all through a Harvard model 122 pump (Cibertec, Spain). After 5 min of the corresponding infusion, three new stimulation–response curves (E1, E2 and E3) were obtained, as described above for the stimulation–response curve E0. Each infusion was maintained for 1 h.

In the second group ($n=15$), in diabetic rats blood pressure dose–response curves obtained by i.v. administration of exogenous noradrenaline (0.01, 0.05, 0.1 and 0.5 μg/kg) were collected before (E′0) and during the continuous infusion of either saline solution (1 ml/h, $n=5$), 5-HT (10 μg/kg/min, $n=5$) or 8-OH-DPAT (10 μg/kg/min, $n=5$) respectively. Thus, four dose–response curves for noradrenaline were obtained per animal. The infusions were started 5 min after the first dose–response curve (E′0) had been elicited and were continued over 1 h.

The third group ($n=30$), which represented the control normoglycaemic group, received either L-NAME (10 mg/kg, i.v.) or ODQ (10 μg/kg, i.v.). 30 or 10 min respectively after the administration of these agents, the corresponding stimulation–response curve–$E0_{L\text{-NAME}}$ or $E0_{ODQ}$–was completed. The animals were then subdivided into three treatment groups for each agent: infusion of saline (1 ml/h, $n=5$ for each), L-694,247 (1 μg/kg/min, $n=5$ for each), or 8-OH-DPAT (20 μg/kg/min, $n=5$ for each). After 5 min, three new stimulation–response curves (E1, E2 and E3) were obtained as described above. Each infusion was maintained for 1 h.

The fourth group (diabetic rats) of experiments ($n=15$) was treated with L-NAME (10 mg/kg, i.v.). 30 min after the administration of this agent, the $E0_{L\text{-NAME}}$ stimulation–response curve was obtained. Then, these animals were subdivided into three treatment groups: infusion of saline (1 ml/h, $n=5$), 5-HT (10 μg/kg/min, $n=5$) or 8-OH-DPAT (10 μg/kg/min, $n=5$). After 5 min, three new stimulation–response curves (E1, E2 and E3) were obtained as described above. Each infusion was maintained for 1 h.

The fifth group ($n=15$, diabetic rats) received L-Arginine (100 mg/kg, i.v.) 30 min after the administration of L-NAME (10 mg/kg, i.v.). After its corresponding $E0_{L\text{-Arginine}}$ stimulation–response curve had been completed, the animals were subdivided into three groups that received an infusion of either saline (1 ml/h, $n=5$), 5-HT (10 μg/kg/min, $n=5$) or 8-OH-DPAT (10 μg/kg/min, $n=5$) respectively.

The sixth group ($n=15$, diabetic rats) received D-Arginine (100 mg/kg, i.v.) 30 min after the administration of L-NAME (10 mg/kg). After its $E0_{D\text{-Arginine}}$ stimulation–response curve had been obtained, the animals were subdivided into three groups: one of them received an infusion of saline (1 ml/h, $n=5$) and the others received an infusion of 5-HT (10 μg/kg/min, $n=5$) or 8-OH-DPAT (10 μg/kg/min, $n=5$) respectively.

The seventh group ($n=15$, diabetic rats) received an intravenous dose of ODQ (10 μg/kg) 10 min before its stimulation–response curve, $E0_{ODQ}$, had been obtained. The animals then were subdivided into three treatment groups, receiving an intravenous infusion of saline (1 ml/h, $n=5$), 5-HT (10 μg/kg/min, $n=5$), or 8-OH-DPAT (10 μg/kg/min, $n=5$) and after 5 min, three new stimulation–response curves (E1, E2 and E3) were obtained.

2.3. Drugs employed

Apart from the anaesthetic (pentobarbital sodium, Sigma Chemical Company, St Louis, MO, USA), the drugs used in the present study (obtained from the indicated sources) were as follows: heparin sodium (Roche, Madrid, Spain); alloxan monohydrate, 5-HT-creatinine sulphate, d-tubocurarine hydrochloride (Sigma Chemical Company, St Louis, MO, USA); atropine sulphate (Scharlau, Barcelona, Spain); 8-hydroxydipropylaminotretalin hydrobromide: 8-OH-DPAT, 2-[5-[3-(4-methylsulfonylamino)benzyl-1,2,4-oxadiazol-5-yl]-1*H*-indol-3-yl]ethanamine: L-694,247 (Research Biochemicals International, Natick, MA, USA); D-Arginine hydrochloride crystalline, L-Arginine HCl, *N*-ω-L-Arginine methyl ester hydrochloride: L-NAME; noradrenaline bitartrate (Sigma-Aldrich Chimie, Madrid, Spain); 1*H*-[1,2,4]Oxadiazolo[4,3-a]quinoxalin-1-one: ODQ (Biogen Cientifica, Madrid, Spain).

All drugs used were dissolved in distilled water at the time of the experiments.

2.4. Expression and analysis of results

Modifications in mean blood pressure were expressed as mm Hg above the mean control blood pressure, measured both before electrical stimulation and as the stabilized maximum post-stimulation.

All data are expressed as means±S.E.M. of at least five experiments. Comparison of the results from the different

Table 1
Body weight and glycaemia values in control and diabetic rats

	Body weight (g)	Glycaemia (mM)	*n*
Control rats			
Initial time	310±8	5.3±0.1	45
4 weeks after	426±10	5.2±0.2	45
Diabetic rats			
Initial time	331±6	5.0±0.3	75
4 weeks after	370±5[a]	27.3±0.9[a]	75

Results are means±S.E.M.: for "*n*" rats.
[a] Significantly different from the corresponding value in control rats, $P<0.05$.

experimental groups and their corresponding control group was carried out by a two-way analysis of variance (ANOVA), followed by the Newman–Keuls multiple comparison test. The differences were considered significant when $P<0.05$. Since the data obtained for the stimulation–response curves E1, E2 and E3 were essentially the same, for simplicity only the stimulation–response curve corresponding to E2 stimulation is shown in the figures.

3. Results

3.1. Systemic haemodynamic variables

Alloxan-induced diabetes elicited a marked increase in serum glucose levels and a failure of the animals to increase their body weight in comparison with control rats four weeks after the induction of diabetes (Table 1).

The mean resting blood pressure and heart rate in diabetic anaesthetized pithed rats in these studies were 40±2 mm Hg and 271±3 beats/min (bpm), respectively; and 38±3 mm Hg and 281±7 bpm for normoglycaemic anaesthetized pithed rats. In the presence of L-NAME or in the joint presence of L-NAME plus D-Arginine, the mean blood pressure values were increased to 68±3 mm Hg for normoglycaemic anaesthetized pithed rats and 58±4 mm Hg for diabetic anaesthetized pithed rats; whereas intravenous bolus administration of ODQ or L-NAME+L-Arginine did not significantly alter these values. Likewise, the intravenous infusion of saline, 5-HT, 8-OH-DPAT, or L-694,247 did not modify mean blood pressure or heart rate values.

Basal heart rate remained unchanged before and throughout all infusions of the agonists and antagonists.

3.2. Effects of physiological saline or 5-HT receptor agonists (5-HT, 8-OH-DPAT, L-694,247) on the electrically induced increases in mean blood pressure in control normoglycaemic pithed rats

Electrical stimulation of the preganglionic sympathetic outflow from the spinal cord in normoglycaemic pithed rats resulted in frequency-dependent increases in mean blood pressure. At the frequencies used, the increases in mean blood pressure in the E0 stimulation–response curve were 3.0±0.1; 11.4±0.3; 21.0±0.9 and 41.7±0.6 mm Hg. These rises in mean blood pressure remained stable in the E1, E2 and E3 stimulation–response curves in control animals receiving an infusion (1 ml/h, $n=5$) of saline solution. Continuous infusion of 5-HT (20 μg/kg/min, $n=5$) inhibited the sympathetic-induced pressor responses (Fig. 1A). Likewise, intravenous infusion of the selective 5-HT_{1A} receptor agonist 8-OH-DPAT (20 μg/kg/min, $n=5$) or L-694,247 (1 μg/kg/min, $n=5$), a selective non-rodent 5-HT_{1B} receptor and 5-HT_{1D} receptor agonist, also inhibited the sympathetic-induced pressor responses (Fig. 1A).

3.3. Effects of physiological saline or 5-HT receptor agonists (5-HT or 8-OH-DPAT) on electrically induced increases in mean blood pressure in diabetic pithed rats

Electrical stimulation of the preganglionic sympathetic outflow from the spinal cord in diabetic pithed rats resulted in frequency-dependent increases in mean blood pressure. At the frequencies used, the increases in mean blood pressure in the E0 stimulation–response curve were 5.2±0.1; 21.0± 0.7; 37.5± 0.9 and 69.0±10.0 mm Hg. These increases in mean blood pressure remained stable in the E1, E2 and E3 stimulation–response curves in control animals receiving an infusion (1 ml/h, $n=5$) of saline solution.

Continuous infusion of 5-HT (10 μg/kg/min, $n=5$) inhibited the sympathetic-induced pressor responses (Fig. 1B). The inhibition was more pronounced at lower stimulation frequencies in a dose-dependent way. Likewise, intravenous infusion of the selective 5-HT_{1A} receptor agonist, 8-OH-DPAT (10 μg/kg/min, $n=15$) also inhibited the sympathetic-induced pressor responses (Fig. 1B).

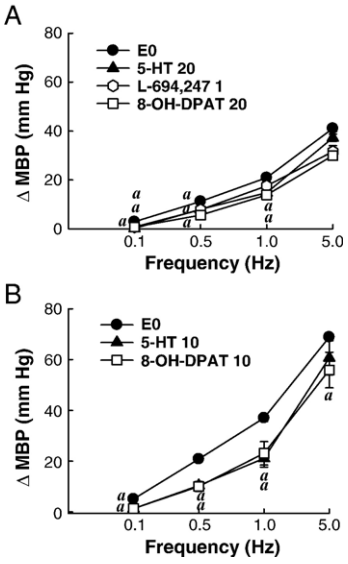

Fig. 1. Effect of i.v. infusion of (A) saline (1 ml/h), 5-HT (20 μg/kg/min), 8-OH-DPAT (20 μg/kg/min) or L-694,247 (1 μg/kg/min) on electrically induced pressor responses in normoglycaemic pithed rats or (B) saline (1 ml/h), 5-HT (10 μg/kg/min) or 8-OH-DPAT (10 μg/kg/min) on electrically induced pressor responses in diabetic pithed rats. Data are shown as means±S.E.M. [a]$P<0.05$ vs. E0 control (saline solution).

3.4. Effects of saline solution, 5-HT or 8-OH-DPAT on noradrenaline-induced increases in mean blood pressure in diabetic pithed rats

The increases in mean arterial blood pressure (in the E′0 stimulation response curve) caused by exogenous noradrenaline (0.01 to 0.5 μg/kg) remained stable in the E′1, E′2 and E′3 stimulation response curves in control animals receiving an infusion of 1 ml/h of saline ($n=5$). Continuous infusion of 5-HT (10 μg/kg/min, $n=5$) or 8-OH-DPAT (10 μg/kg/min, $n=5$) failed to inhibit the pressor responses to exogenous administration of noradrenaline (data not shown).

3.5. Effects of physiological saline or 5-HT receptor agonists (8-OH-DPAT or L-694,247) in the presence of L-NAME or ODQ on the electrically induced increases in mean blood pressure in control normoglycaemic pithed rats

Electrical stimulation of the preganglionic sympathetic outflow from the spinal cord in normoglycaemic pithed rats resulted in frequency-dependent increases in mean blood pressure (Fig. 2). The intravenous administration of ODQ (10 μg/kg) did not modify the E0 stimulation–response curve ($E0_{ODQ}$) (Fig. 2), whereas intravenous bolus administration of L-NAME (10 mg/kg) led to an increase in the frequency-dependent increases in mean blood pressure ($E0_{L\text{-}NAME}$) after electrical stimulation (Fig. 2). These rises in mean blood pressure remained stable in the stimulation–response curves E1, E2 and E3 in control animals receiving an infusion (1 ml/h) of saline solution.

In the presence of ODQ or L-NAME, continuous infusion of both L-694,247, a selective non-rodent 5-HT_{1B} receptor and 5-HT_{1D} receptor agonist (1 μg/kg/min), and 8-OH-DPAT, a selective 5-HT_{1A} agonist (20 μg/kg/min) continued to exert the inhibitory effect of these drugs (Fig. 3A and B).

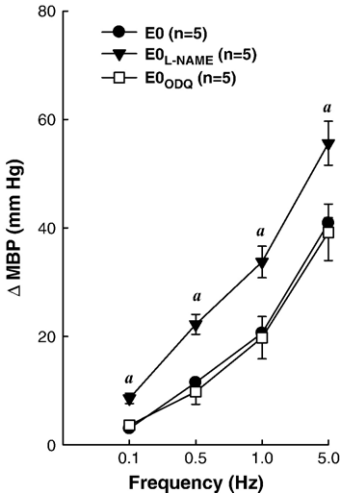

Fig. 2. Effect of i.v. infusion of saline (1 ml/h) on electrically induced pressor responses before and after intravenous bolus injection of L-NAME (10 mg/kg) or ODQ (10 μg/kg) in normoglycaemic pithed rats. Data are shown as means±S.E.M. $^{a}P<0.05$ vs. E0 control (saline solution).

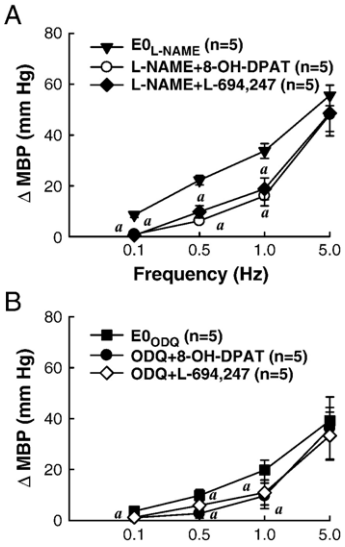

Fig. 3. Effect of i.v. infusion of 8-OH-DPAT (20 μg/kg/min) or L-694,247 (1 μg/kg/min) on electrically induced pressor responses in the presence of (A) L-NAME (10 mg/kg) or (B) ODQ (10 μg/kg) in normoglycaemic pithed rats (stimulation response E2). Data are shown as means±S.E.M. $^{a}P<0.05$ vs. $E0_{L\text{-}NAME}$ (saline solution+L-NAME) or $E0_{ODQ}$ (saline solution+ODQ) respectively.

3.6. Effects of physiological saline or 5-HT receptor agonists (5-HT or 8-OH-DPAT) in the presence of L-NAME on the electrically induced increases in mean blood pressure in diabetic pithed rats

Electrical stimulation of the preganglionic sympathetic outflow from the spinal cord in diabetic pithed rats resulted in frequency-dependent increases in mean blood pressure. At the frequencies used, the increases in mean blood pressure in the E0 stimulation–response curve were 5.3±0.4; 20.5±1.0; 37.3±1.2 and 68.5±3.0 mm Hg. Intravenous bolus administration of the NOS inhibitor L-NAME produced a rise in the frequency-dependent increases in mean blood pressure obtained by electrical stimulation (Fig. 4). These rises in mean blood pressure remained stable in the E1, E2 and E3 stimulation–response curves in control animals receiving an infusion (1 ml/h) of saline solution.

Intravenous bolus administration of L-NAME was able to abolish the inhibitory effect of both the continuous infusion of 5-HT (10 μg/kg/min) (Fig. 5) and the continuous infusion of 8-OH-DPAT (10 μg/kg/min) on the sympathetic-induced pressor responses (Fig. 5).

3.7. Effect of intravenous administration of L-Arginine or D-Arginine in the presence of L-NAME on the 5-HT-or 8-OH-DPAT-induced sympathoinhibitory effect in diabetic pithed rats

Pre-treatment of diabetic pithed rats with L-Arginine (100 mg/kg), a substrate for NO synthase, 30 min after the intravenous administration of L-NAME (10 mg/kg) led to a decrease in mean blood pressure, a similar value to that of the animals that had not received L-NAME being observed (40.9±1.0 mm Hg).

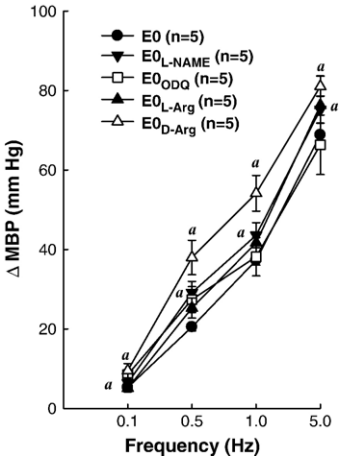

Fig. 4. Effect of i.v. infusion of saline (1 ml/h) on electrically induced pressor responses before and after intravenous bolus injection of L-NAME (10 mg/kg) or ODQ (10 μg/kg) or L-Arginine (100 mg/kg) or D-Arginine (100 mg/kg) in diabetic pithed rats. Data are shown as means±S.E.M. $^{a}P<0.05$ vs. E0 control (saline solution).

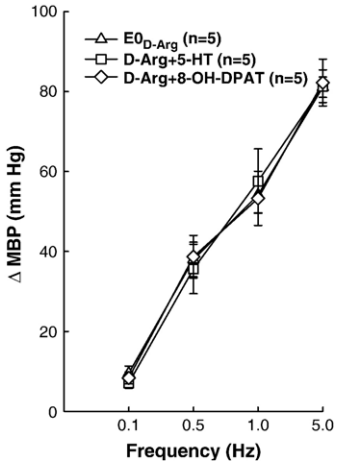

Fig. 6. Effect of i.v. infusion of 5-HT (10 μg/kg/min) or 8-OH-DPAT (10 μg/kg/min) on electrically induced pressor responses in the presence of D-Arginine (100 mg/kg) pretreated with L-NAME (10 mg/kg) in diabetic pithed rats (E2 stimulation response). Data are shown as means±S.E.M. $^{a}P<0.05$ vs. $E0_{D\text{-}Arg}$ (saline solution+D-Arginine).

The $E0_{L\text{-}Arginine}$ curve showed similar increases in mean blood pressure to those of E0 (Fig. 4), and slightly lower than the values for $E0_{L\text{-}NAME}$. In these experimental conditions, intravenous infusion of 5-HT (10 μg/kg/min) (Fig. 5), or 8-OH-DPAT (10 μg/kg/min) continued to produce the inhibitory effect on the mean blood pressure increases obtained by electrical stimulation (Fig. 5).

Intravenous administration of D-Arginine (100 mg/kg) 30 min after the i.v. administration of L-NAME (10 mg/kg) did not modify the augmentation in mean blood pressure produced after the administration of L-NAME (57.9±3.8 mm Hg) (Fig. 4). In these experimental conditions, both the intravenous infusion of 5-HT (10 μg/kg/min) and the intravenous infusion of 8-OH-DPAT (10 μg/kg/min) failed to inhibit the pressor responses elicited by sympathetic stimulation (Fig. 6).

3.8. Effects of physiological saline or 5-HT receptor agonists (5-HT or 8-OH-DPAT) in the presence of ODQ on the electrically induced increases in mean blood pressure in diabetic pithed rats

Intravenous administration of ODQ (10 μg/kg), a guanylyl cyclase inhibitor, did not significantly modify mean blood pressure in diabetic pithed animals (41.5±1.59 mm Hg). Likewise, ODQ was not able to change the increases in mean blood pressure obtained by electrical stimulation ($E0_{ODQ}$) with respect to E0 (Fig. 4).

In the presence of ODQ, the inhibitory effect produced by the intravenous infusion of 5-HT (10 μg/kg/min) or 8-OH-

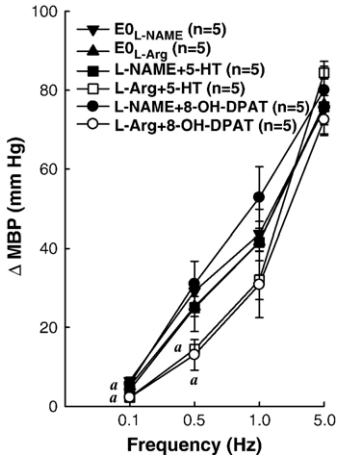

Fig. 5. Effect of i.v. infusion of 5-HT (10 μg/kg/min) or 8-OH-DPAT (10 μg/kg/min) on electrically induced pressor responses in the presence of L-NAME (10 mg/kg) or in the presence of L-Arginine (100 mg/kg) pretreated with L-NAME (10 mg/kg) in diabetic pithed rats (E2 stimulation response). Data are shown as means±S.E.M. $^{a}P<0.05$ for agonists in the presence of L-Arg vs. control $E0_{L\text{-}Arg}$ (saline solution+L-Arginine).

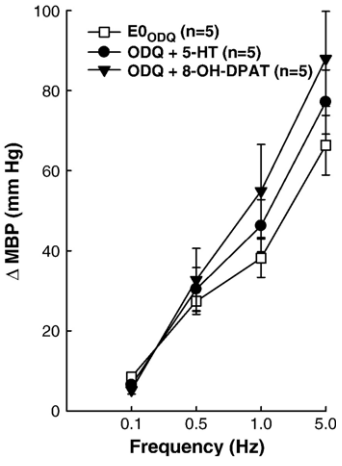

Fig. 7. Effect of i.v. infusion of 5-HT (10 μg/kg/min) or 8-OH-DPAT (10 μg/kg/min) on electrically induced pressor responses in the presence of ODQ (10 μg/kg) in diabetic pithed rats (stimulation response E2). Data are shown as means±S.E.M. $^{a}P<0.05$ vs. $E0_{ODQ}$ (saline solution+ODQ).

DPAT (10 μg/kg/min) in diabetic pithed rats was completely abolished (Fig. 7).

4. Discussion

In the present work, we studied the possible role of the NO pathway in the inhibitory 5-hydroxytryptaminergic action in the pressor effect elicited by sympathetic stimulation in diabetic pithed rats. To accomplish this, we used an experimental model of chemical diabetes in the rat, alloxan being the diabetogenic agent employed. This drug induces a syndrome resembling type 1 diabetes mellitus (Agrawal et al., 1987), and it is commonly used as a valid experimental model of diabetes in animals (Hodgson et al., 1990; Chan et al., 2000; Miranda et al., 2002; García et al., 2005).

In our experiments in diabetic rats, it was necessary to use a supramaximal voltage higher than that used in normoglycaemic rats. Similarly, we obtained increases in mean blood pressure in the diabetic rats that were higher than in the normoglycaemic rats. These differences between both groups of animals (diabetic and normoglycaemic) could be due to a dysfunction of the autonomic nervous system reported in diabetes by many authors, although the results are controversial because several authors have proposed increased vascular reactivity to adrenoceptor agents (Abebe and Mcleod, 1990; Taylor et al., 1994), whereas others have posited an attenuation of sympathetic contractile responses (Takiguchi et al., 1988; Andersson et al., 1992) or even no changes at all (Ralevic et al., 1993).

Our previous results revealed that in diabetic rats (García et al., 2005), as occurs in normoglycaemic Wistar rats in this and other works (Morán et al., 1994, 1998; Villalón et al., 1995c), 5-HT interferes with adrenergic neurotransmission and reduces the increases in blood pressure obtained by sympathetic stimulation.

Both in normoglycaemic and diabetic pithed rats, the results observed previously by us (Morán et al., 1994, 1998; García et al., 2005) demonstrated that the 5-HT receptors mediating the inhibition of pressor effects obtained by stimulation of sympathetic outflow are mainly prejunctional 5-HT_1 in nature, since 5-HT does not modify the increases in blood pressure elicited by exogenous administration of noradrenaline. In experimental alloxan-induced hyperglycaemia in rats, we have previously shown that there is a major involvement of 5-HT_{1A} receptors in the inhibitory serotoninergic responses of the pressor effect elicited by sympathetic stimulation, whereas 5-HT_{1D} receptor activation is devoid of this inhibitory effect in diabetes (García et al., 2005).

These results, obtained in diabetic rats, are in contrast with those obtained in normoglycaemic rats by Villalón et al., 1998, who reported that 5-HT_{1A}, rodent 5-hydroxytrytamine 5-HT_{1B} and 5-HT_{1D} receptors were involved in the 5-HT inhibitory effect. They are also in contrast with those obtained by our group in normoglycaemic rats, in which 5-hydroxytryptamine 5-HT_{1D} heteroceptors are mainly involved, although 5-HT_{1A} receptors may also be involved (Morán et al., 1998).

Several studies (Molderings et al., 1987; Schoeffter and Hoyer, 1990; Ralevic et al., 1995) carried out under different experimental conditions have shown the participation of EDRF in several serotonergic responses. Other authors have suggested the possible role of 5-HT in the physiopathology of hyperglycaemic complications (Sandrini et al., 1997). Miranda et al. (2000, 2002) demonstrated changes in 5-HT responses related to endothelial mechanisms in experimental diabetes.

Here we studied whether there is any indirect mechanism involved in the inhibitory effect of 5-HT, via 5-HT1A receptors, on the pressor responses induced by stimulation of sympathetic vasopressor outflow in pithed atropine-treated alloxan-induced diabetic rats. Some evidence supports increased NO production, including findings that endothelium-dependent relaxation is enhanced early on after the onset of diabetes in the rat renal artery (Bhardwaj and Moore, 1998), in rat mesenteric arteries (Heygate et al., 1995), in rat aorta (Pieper et al., 1998a) and in endothelial cell cultures (McDuffie et al., 1999; Ishida et al., 1998). In this sense, we analysed the possible involvement of NO pathway/synthesis on the inhibitory serotoninergic responses of the pressor effect elicited by sympathetic stimulation in diabetic and normoglycaemic pithed rats (García et al., 2005).

In both diabetic and normoglycaemic rats, intravenous administration of L-NAME, a NO synthase inhibitor, significantly increased mean blood pressure. In this sense, the increases in mean blood pressure obtained by electrical stimulation were also augmented after i.v. administration of L-NAME. This can be explained in terms of the inhibitory effect of basal NO release produced by L-NAME (Rees et al., 1990). However, our results did not reveal any differences between the pressor responses induced by electrical stimulation produced by the selective 5-HT_{1A} agonist, 8-OH-DPAT (Middlemiss and Fozard, 1983; Hoyer et al., 1994) or by the selective non-rodent 5-HT_{1B} receptor and the 5-HT_{1D} receptor agonist, L-694,247 (Beer et al., 1993) when we previously administered L-NAME to the normoglycaemic pithed rats.

In the hyperglycaemic rats, however, in the presence of L-NAME the inhibitory effect produced by 5-HT or 8-OH-DPAT was completely reversed. Accordingly, we suggest that the inhibitory serotoninergic responses of the pressor effect elicited by sympathetic stimulation in diabetic pithed rats would be mediated, at least in part, by the NO pathway. These results are in agreement with those reported by Miranda et al. (2000, 2002) for rabbits, which describe endothelial alterations in serotoninergic responses during experimental diabetes, and also with others (Pieper et al., 1998b; Fitzgerald and Brands, 2000; Komers and Anderson, 2003) who demonstrated the existence of endothelial dysfunction in diabetic rats related to enhanced NO production.

In confirmation of the data obtained in diabetic rats, the administration of L-Arginine, a NO synthase substrate (Palmer et al., 1988a,b; Tousoulis et al., 1999), after the administration of L-NAME reduced the augmentation in mean blood pressure to control values. Likewise, the inhibitory effect on the pressor responses elicited by electrical stimulation produced by 5-HT and 8-OH-DPAT was reversed again in the presence of both L-NAME and L-Arginine. In contrast, the administration of D-Arginine (Tousoulis et al., 1999), the non-active isomere of L-

Arginine, after the administration of L-NAME did not modify the responses produced by intravenous perfusion of 5-HT and 8-OH-DPAT in the presence of L-NAME. All these data suggest the direct involvement of the NO pathway in the inhibitory serotoninergic effect of the pressor effect elicited by sympathetic stimulation in diabetic pithed rats.

This demonstrates, as other authors have described for different experimental conditions (Ralevic et al., 1995; Miranda et al., 2000), that 5-HT is directly related to the alterations produced during the development of diabetes and reveals the participation of EDRF in the vascular serotoninergic responses in hyperglycaemic rats, in contrast to the results obtained in normoglycaemic rats, where EDRF is not involved.

The involvement of NO in the inhibitory serotoninergic actions of the pressor responses obtained by electrical stimulation are clearly confirmed with the experiments conducted in the presence of ODQ, a guanylyl cyclase inhibitor (Gimeno et al., 1998; Bryan-Lluka et al., 2004; Chen et al., 2005). In the experiments carried out in its presence, in diabetic rats the inhibitory action produced by 5-HT or by the selective 5-HT_{1A} agonist 8-OH-DPAT was completely reversed, as occurred in the presence of L-NAME. However, in normoglycaemic rats, ODQ did not abolish the inhibitory effect produced by L-694,247 or 8-OH-DPAT.

All these data and others previously reported by us (García et al., 2005) suggest that experimental diabetes induces changes in the intracellular signalling pathways activated by 5-HT_{1A} and 5-HT_{1D} receptors, responsible for the serotoninergic inhibitory action of the pressor effect elicited by sympathetic stimulation in normoglycaemic rats (Morán et al., 1994, 1998).

Further studies should be carried out to determine more precisely which step of the NO pathway is responsible for the serotoninergic inhibitory effect of the pressor effect elicited by sympathetic stimulation in diabetic rats.

In conclusion, our results suggest that during the development of experimental diabetes the inhibitory serotoninergic effect, via the 5-HT_{1A} receptors, of the pressor responses obtained by electrical stimulation of sympathetic outflow would be mediated by the NO pathway and guanylyl cyclase activation.

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