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Diabetes-induced changes in the 5-hydroxytryptamine inhibitory receptors involved in the pressor effect elicited by sympathetic stimulation in the pithed rat

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1 We investigated the effect of alloxan-induced diabetes on the inhibitory mechanisms of 5-hydroxytryptamine (5-HT) in the pressor responses induced by stimulation of sympathetic vasopressor outflow in pithed rats, and analysed the type and/or subtype of 5-HT receptors involved.

2 Diabetes was induced in male Wistar rats by a single s.c. injection of alloxan, then 4 weeks later, they 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.

3 Intravenous infusions of 5-HT (1–80 $\mu g\,kg^{-1}\,min^{-1}$) reduced the pressor effects obtained by electrical stimulation. The 5-HT_1 receptor agonist 5-carboxamidotryptamine, 5-CT (5 $\mu g\,kg^{-1}\,min^{-1}$), caused an inhibition of the pressor response, whereas the selective 5-HT_2 receptor agonist, α-methyl-5-HT (5 $\mu g\,kg^{-1}\,min^{-1}$) and the selective 5-HT_3 receptor agonist, 1-phenylbiguanide (40 $\mu g\,kg^{-1}\,min^{-1}$), did not modify the sympathetic pressor responses. 5-HT had no effect on exogenous noradrenaline (NA)-induced pressor responses.

4 The inhibition of electrically induced pressor responses by 5-HT (10 $\mu g\,kg^{-1}\,min^{-1}$) was unable to be elicited after i.v. treatment with methiothepin (100 $\mu g\,kg^{-1}$) because of the marked inhibition produced by methiothepin alone. The 5-HT-induced inhibition was blocked after i.v. administration of WAY-100,635 (100 $\mu g\,kg^{-1}$) and not affected by ritanserin (1 $mg\,kg^{-1}$), MDL 72222 (2 $mg\,kg^{-1}$).

5 The selective 5-HT_{1A} receptor agonist, 8-hydroxydipropylaminotretalin hydrobromide (8-OH-DPAT) (5–20 $\mu g\,kg^{-1}\,min^{-1}$) but neither the rodent 5-HT_{1B} receptor agonist, CGS-12066B (5 $\mu g\,kg^{-1}\,min^{-1}$), nor the selective nonrodent 5-HT_{1B} and 5-HT_{1D} receptor agonist, L-694,247 (5 and 40 $\mu g\,kg^{-1}\,min^{-1}$), inhibited the electrically induced pressor response. The selective 5-HT_{1A} receptor antagonist, WAY-100,635 (100 $\mu g\,kg^{-1}$), blocked the inhibition induced by 8-OH-DPAT (10 $\mu g\,kg^{-1}\,min^{-1}$). 8-OH-DPAT had no effect on exogenous NA-induced pressor responses.

6 Experimental diabetes produces changes in the inhibitory effect induced by 5-HT on electrically induced sympathetic pressor responses, such that the inhibitory action induced by 5-HT in diabetic pithed rats is mediated by prejunctional 5-HT_{1A} receptors.
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Abbreviations: Bpm, beats $minute^{-1}$; 5-CT, 5-carboxamidotryptamine maleate; EC_{50}, median effective concentration; MBP, mean blood pressure; NA, noradrenaline; 8-OH-DPAT, 8-hydroxydipropylaminotretalin hydrobromide; S–R curve, stimulation–response curve

Introduction

Diabetes causes and exacerbates macro- and microangiopathies, which contribute to the vascular deterioration associated with this pathology. However, the pathological processes underlying vascular complications are still poorly understood.

Several authors 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 an increase in the population of several types of 5-HT receptors in the rat brain (Sandrini *et al*., 1997). Likewise, diabetes reduces the 5-HT concentration in the gut and in platelets of alloxan-treated rats and these effects are reversed by treatment of the animals with the serotonin precursor 5-hydroxytryptophan (Cicin-Sain & Jernej, 1996). Also, the enterochromaffin cell 5-HT content is altered in diabetes, some studies found an increased level of serotonin in small intestine of STZ-diabetic rats (Martin *et al*., 1995; Takahara *et al*., 2001), but other showed decreased (Cicin-Sain & Jernej, 1996) or normal levels of serotonin (Richter *et al*., 1986) in the small intestine of alloxan- and

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STZ-induced diabetic rats. Recently, a clinical study carried out in noninsulin-dependent diabetes mellitus patients has suggested the potential benefits of the 5-HT_2 receptor antagonist sarpogrelate in the treatment of diabetic nephropathy (Ogawa *et al*., 1999; Doggrell, 2004). In addition, Sandrini *et al*. (1997) have proposed changes in 5-HT_{1A} and 5-HT_2 receptor concentrations in the diabetic rat brain. Miranda *et al*. (2000; 2002) have also suggested the existence of a diabetes-induced hyper-reactivity of rabbit renal and carotid arteries to 5-HT.

Dysfunction of the autonomic nervous system is a common complication of diabetes mellitus and functional changes in the sympathetic nervous system have been reported in several animal models of diabetes as well as in human diabetic subjects. Alterations in sympathetic-mediated vascular tone as a cause of diabetic vascular disease had been suggested by different authors and thus, an increase of vascular reactivity to adrenoceptors agents (Abebe & McLeod, 1990; Taylor *et al*., 1994; Tesfamariam & Cohen, 1995) and sympathetic nerve stimulation had been reported (Agrawal *et al*., 1987), but also some reports indicate diminished sympathetic contractile responses (Takiguchi *et al*., 1988; Andersson *et al*., 1992) or no change (Ralevic *et al*., 1993), both in streptozotocin- or alloxan-induced diabetes. The discrepancies among the above-mentioned results could be attributable to differences in the duration of the diabetes, the strain of rats or the experimental conditions (Ralevic *et al*., 1995; Kamata & Kirisawa, 1998).

Many 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* (Ireland & Jordan, 1987; Molderings *et al*., 1987; Villalón *et al*., 1995a, b, c) and cats (Jones *et al*., 1995). Working in *in vivo* conditions, we have previously shown that, in normoglycaemic 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 have shown that the prejunctional 5-HT_{1D} heteroceptors are involved in sympathetic neurotransmission but this inhibition is also modulated by 5-HT_{1A} receptors (Morán *et al*., 1998), and also other authors (Villalón *et al*., 1998), in normoglycaemic pithed male Wistar rats, provide evidence for the involvement of 5-HT_{1A}, rodent 5-HT_{1B} and 5-HT_{1D} in the prejunctional inhibition of electrically induced sympathetic outflow. The present study was carried out to determine the effects of 5-HT in the pressor responses induced by stimulation of sympathetic vasopressor outflow in pithed atropine-treated alloxan diabetic rats. To do so, we analysed the possible changes induced by experimental diabetes in vascular reactivity to 5-HT in comparison with the results obtained in this work in a normoglycaemic group of animals and also with the results previously obtained by us in normoglycaemic rats (Morán *et al*., 1994; 1998). We also studied the 5-HT type/subtype receptors involved in these changes.

Methods

General

A total of 145 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 purpose (86/609/EEC, Article 5, Appendix II) and enacted by Spanish legislation on March 14, 1988 (R.D.223/1988).

Diabetes was induced in 125 rats by a single injection of alloxan (150 mg kg^{-1} s.c.) dissolved in 0.9% NaCl. Then, the animals were maintained on tap water and regular food *ad libitum* for 4 weeks. A second group of 20 rats was maintained under the same conditions for the same time period to serve as age-matched controls. Weight and blood glucose levels were determined before and at 2, 7, 14, 21 and 28 days after alloxan administration. Only rats with elevated blood glucose levels (>11 mM) at all time points were considered diabetic.

The animals, both diabetic and normoglycaemic, were anaesthetized with sodium pentobarbital (60 mg kg^{-1}, i.p.), and after cannulation of the trachea the rats were pithed by inserting a stainless steel rod through the orbit and foramen magnum (Gillespie & Muir, 1967) and artificially respirated with room air by a Harvard respiratory pump (1 ml air 100 g^{-1}, 50 strokes min^{-1}). 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 (Cibertec, Spain), displaying the recordings on one channel of a Letica Polygraph 4000 (Cibertec, Spain) to record blood pressure. Heart rate was measured by analysis of the blood pressure data by a CAR 1000 tachograph (Cibertec, Spain) connected to the same PRS 205 amplifier.

The entire sympathetic outflow from the spinal cord was stimulated using 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^{-1}), and then received *d*-tubocurarine (2 mg kg^{-1}, i.v.) to prevent electrically induced muscular twitching, and atropine (1 mg kg^{-1}, i.v.) to prevent the cholinergic effects.

Experimental protocols

After a stable haemodynamic condition for at least 10 min, baseline values of mean blood pressure (MBP) were determined. Then, sympathetic vasopressor 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 (S–R curve E0) was completed in about 20 min. At this point, the animals ($n = 145$) were divided into five different groups, and each group into different subgroups, taking into account that each animal was used to evaluate only a respective dose of agonist or antagonist, and each dose was repeated five times up to a total of $n = 5$ experiments.

The first group of experiments was carried out to confirm previous results from our laboratory (Morán *et al*., 1994; 1998) in normoglycaemic control rats. In this group ($n = 20$), each animal received a continuous i.v. infusion of one of the following: saline solution (1 ml h^{-1}, $n = 5$, control group for all the agonist treatments); 5-HT (20 μg kg^{-1} min^{-1}, $n = 5$);

8-hydroxydipropylaminotretalin hydrobromide (8-OH-DPAT) (20 μg kg^{-1} min^{-1}, $n=5$), a selective 5-HT$_{1A}$ receptor agonist or L-694,247 (1 μg kg^{-1} min^{-1}, $n=10$), a selective nonrodent 5-HT$_{1B}$ receptor and 5-HT$_{1D}$ receptor agonist, with a Harvard model 122 pump (Cibertec, Spain). After 5 min of the corresponding infusion, three new S–R curves (E1, E2 and E3) were obtained as described above for the S–R curve E0. Each infusion was maintained for 1 h.

In the first alloxan-treated diabetic group ($n=65$), the rats each received a continuous i.v. infusion of one of the following: saline solution (1 ml h^{-1}, $n=5$, control group for all the agonist treatments); 5-HT (1, 10 or 80 μg kg^{-1} min^{-1}, $n=15$); 5-carboxamidotryptamine maleate (5-CT) (5 μg kg^{-1} min^{-1}, $n=5$), a selective 5-HT$_1$ receptor agonist; 8-OH-DPAT (5, 10 or 20 μg kg^{-1} min^{-1}, $n=15$), a selective 5-HT$_{1A}$ receptor agonist; CGS-12066B (5 μg kg^{-1} min^{-1}, $n=5$), a selective rodent 5-HT$_{1B}$ receptor agonist; L-694,247 (5 or 20 μg kg^{-1} min^{-1}, $n=10$), a selective nonrodent 5-HT$_{1B}$ receptor and 5-HT$_{1D}$ receptor agonist; α-methyl-5-HT (5 μg kg^{-1} min^{-1}, $n=5$), a selective 5-HT$_2$ receptor agonist or 1-phenylbiguanide (40 μg kg^{-1} min^{-1}, $n=5$), a selective 5-HT$_3$ receptor agonist, in the same conditions as the infusions for the normoglycaemic control group.

The second diabetic group ($n=20$) was run in parallel with the above group in order to investigate, during the continuous infusion of saline solution (1 ml kg^{-1}), the effect *per se* of methiothepin (100 μg kg^{-1}, $n=5$), a nonselective 5-HT$_1$ receptor antagonist; ritanserin (1 mg kg^{-1}, $n=5$), a selective 5-HT$_2$ receptor antagonist; MDL-72222 (2 mg kg^{-1}, $n=5$), a selective 5-HT$_3$ receptor antagonist or WAY-100,635 (100 μg kg^{-1}, $n=5$), a selective 5-HT$_{1A}$ receptor antagonist, on the electrically induced pressor responses. All the antagonists were administered 5 min prior the saline infusion.

The third alloxan-treated group was used to analyse the 5-HT$_1$ receptor subtype involved in the inhibitory 5-HT effect. These animals ($n=25$) were subdivided into several treatment subgroups: methiothepin (100 μg kg^{-1}), ritanserin (1 mg kg^{-1}) or MDL-72222 (2 mg kg^{-1}) 5 min before the infusion of 5-HT (10 μg kg^{-1} min^{-1}, $n=5$ for each antagonist); or WAY-100, 635 (100 μg kg^{-1}) 5 min before the infusion of 5-HT (10 μg kg^{-1} min^{-1}, $n=5$) or 5 min before the infusion of 8-OH-DPAT (10 μg kg^{-1} min^{-1}, $n=5$).

Finally, in the last group ($n=15$) blood pressure dose–response curves by i.v. administration of exogenous nonadrenaline (NA) (0.01, 0.05, 0.1 and 0.5 μg kg^{-1}) were obtained before (E′0) and during (E′1, E′2 and E′3) the continuous infusion of either saline solution (1 ml h^{-1}, $n=5$), 5-HT (10 μg kg^{-1} min^{-1}, $n=5$) or 8-OH-DPAT (10 μg kg^{-1} min^{-1}, $n=5$), respectively. Accordingly, four dose–response curves for NA were obtained per animal. The infusions were started 5 min after the first curve-response (E′0) was elicited and were continued over 1 h.

Drugs employed

Apart from the anaesthetic (pentobarbital sodium, Sigma Chemical Company, St Louis, MO, U.S.A.), the drugs used in the present study (obtained from the indicated sources) were as follows: heparin sodium (Roche, Madrid, Spain); alloxan monohydrate, noradrenaline bitartrate, 5-HT-creatinine sulphate, *d*-tubocurarine hydrochloride (Sigma Chemical Company, St Louis, MO, U.S.A.); atropine sulphate (Scharlau, Barcelona, Spain); methiothepin mesylate, 5-CT, 8-OH-DPAT, CGS-12066B maleate, L-694,247, 1-phenylbiguanide, ritanserin, α-methyl-5HT, MDL-72222 (Research Biochemicals International, Natick, MA, U.S.A.); (*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl)cyclohexanecarboxamide trihydrochloride): WAY-100,635 (Pharmacia, Milan, Italy).

All drugs used were dissolved in distilled water at the time of the experiments, with the exception of ritanserin, which was dissolved in 0.04 mol l^{-1} of lactic acid.

Expression and analysis of results

Modifications in mean blood pressure were expressed as mmHg above the mean control blood pressure, measured both before electrical stimulation and as the stabilized maximum poststimulation.

All data were expressed as mean values ± s.e.m. of at least five experiments. Comparison of the results from the different experimental groups and their corresponding control group was carried out by ANOVA, followed by the Newman–Keuls multiple comparison test. The differences were considered significant when $P<0.05$. Since the data obtained for S–R curves E1, E2 and E3 were essentially the same, for simplicity, only the S–R curve corresponding to E2 stimulation or E′2 NA-administration are shown in the figures.

Results

Systemic haemodynamic variables

The alloxan-induced diabetes elicited a marked increase in serum glucose and failure of the animals to increase their body weight in comparison with control rats. Table 1 shows the mean values of body weight and glycaemia before and at 4 weeks after the induction of diabetes for the rats in the diabetic group and for the animals in the control group.

The mean resting blood pressure and heart rate in diabetic anaesthetized pithed rats in these studies were 41 ± 1 mmHg and 270 ± 4 beats minute^{-1} (bpm), respectively; and 39 ± 1 mmHg and 283 ± 6 b.p.m. for normoglycaemic anaesthetized pithed rats. These values were not significantly altered by the intravenous infusion of saline, the 5-hydroxytryptamine receptor agonists (5-HT, 5-CT, CGS-12066B, 8-OH-DPAT, L-694, 247, α-methyl-5-HT and 1-phenylbiguanide) or the 5-HT receptors antagonists (methiothepin, ritanserin, MDL-72222 and WAY-100,635).

Table 1 Values of body weight and glycaemia in control and diabetic rats

	Body weight (g)	*Glycaemia* (mM)	n
Control rats			
Initial time	313 ± 6	5.2 ± 0.2	20
4 weeks after	422 ± 13	5.2 ± 0.1	20
Diabetic rats			
Initial time	335 ± 4	4.9 ± 0.1	125
4 weeks after	368 ± 4*	27.0 ± 0.7*	125

Results are means ± s.e.m.: for '*n*' rats.
*Significantly different from the corresponding value in control rats, $P<0.05$.

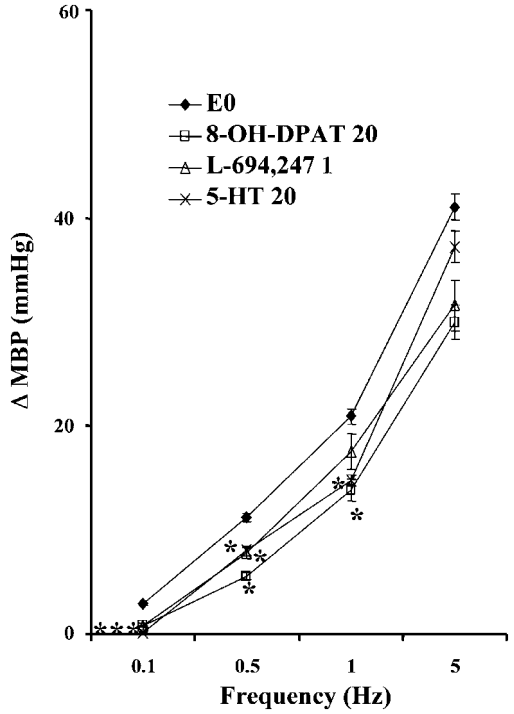

Figure 1 Effect of i.v. infusion of saline ($1\,ml\,h^{-1}$), 5-HT ($20\,\mu g\,kg^{-1}\,min^{-1}$), 8-OH-DPAT ($20\,\mu g\,kg^{-1}\,min^{-1}$) or L-694,247 ($1\,\mu g\,kg^{-1}\,min^{-1}$) on electrically induced pressor responses in normoglycaemic pithed rats. Data are shown as mean ± s.e.m. $^*P<0.05$ vs E0 control.

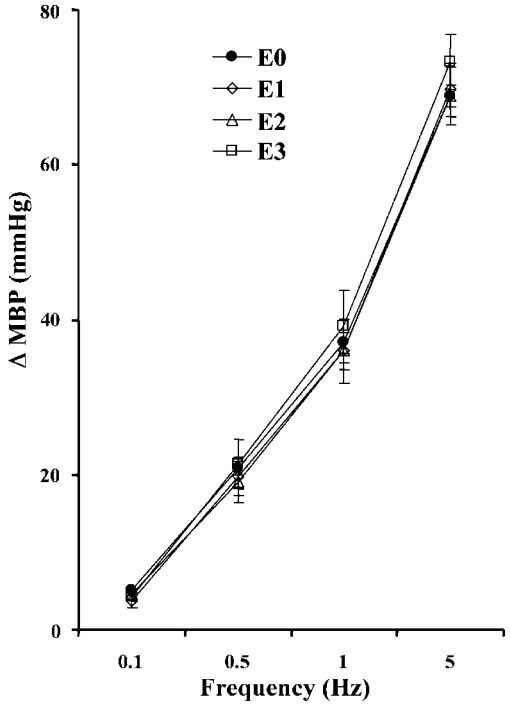

Figure 2 Effect of i.v. infusion of saline ($1\,ml\,h^{-1}$) on electrically induced pressor responses in diabetic pithed rats. E0 control, E1 first, E2 second and E3 third S–R curves. Data are shown as mean ± s.e.m. There were no statistically significant differences from the corresponding E0 control values ($P>0.05$). Vertical lines show s.e.m.

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

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 rats

Electrical stimulation of the preganglionic sympathetic outflow from the spinal cord in normoglycaemic pithed rats resulted in frequency-dependent increases in MBP. At the frequencies used, the increases in MBP in S–R curve E0 were 2.9 ± 0.2; 11.2 ± 0.6; 20.9 ± 0.8 and 41.1 ± 1.3 mmHg. These rises in MBP remained stable in S–R curves E1, E2 and E3 in control animals receiving an infusion ($1\,ml\,h^{-1}$, $n=5$) of saline solution (Figure 1).

Continuous infusion of 5-HT ($20\,\mu g\,kg^{-1}\,min^{-1}$, $n=5$) inhibited the sympathetic-induced pressor responses (Figure 1). Likewise, intravenous infusion of the selective 5-HT_{1A} receptor agonist, 8-OH-DPAT ($20\,\mu g\,kg^{-1}\,min^{-1}$, $n=5$), or L-694,247 ($1\,\mu g\,kg^{-1}\,min^{-1}$, $n=5$), a selective nonrodent 5-HT_{1B} receptor and 5-HT_{1D} receptor agonist, also inhibited the sympathetic-induced pressor responses (Figure 1).

Effects of physiological saline or 5-HT receptor agonists (5-HT, 5-CT, 8-OH-DPAT, CGS-12066B, L-694,247, α-methyl-5-HT and 1-phenylbiguanide) on the electrically induced increases in mean blood pressure

Electrical stimulation of the preganglionic sympathetic outflow from the spinal cord in diabetic pithed rats resulted in frequency-dependent increases in MBP. At the frequencies used, the increases in MBP in S–R curve E0 were 5.1 ± 0.2; 20.8 ± 0.9; 37.0 ± 1.4 and 68.9 ± 11.4 mmHg. These rises in MBP remained stable in S–R curves E1, E2 and E3 in control animals receiving an infusion ($1\,ml\,h^{-1}$, $n=5$) of saline solution (Figure 2).

Continuous infusion of 5-HT (5, 10 and $80\,\mu g\,kg^{-1}\,min^{-1}$, $n=15$) inhibited the sympathetic-induced pressor responses (Figure 3). The inhibition was more pronounced at lower stimulation frequencies in a dose-dependent way. Likewise, intravenous infusion of the selective 5-HT_1 receptor agonist, 5-CT ($5\,\mu g\,kg^{-1}\,min^{-1}$, $n=5$), or the selective 5-HT_{1A} receptor agonist, 8-OH-DPAT (5, 10 and $20\,\mu g\,kg^{-1}\,min^{-1}$, $n=15$) also inhibited the sympathetic–induced pressor responses (Figure 4a,5).

By contrast, CGS-12066B ($5\,\mu g\,kg^{-1}\,min^{-1}$, $n=5$), L-694,247 (5 and $20\,\mu g\,kg^{-1}\,min^{-1}$, $n=10$) (Figure 4b), α-methyl-5-HT ($10\,\mu g\,kg^{-1}\,min^{-1}$, $n=5$) or 1-phenylbiguanide ($40\,\mu g\,kg^{-1}\,min^{-1}$, $n=5$) (Figure 4a) failed to inhibit the pressor responses evoked by sympathetic stimulation.

Effect of 5-HT receptor antagonists on the 5-HT- and 8-OH-DPAT-induced sympathoinhibitory effect

Pretreatment of diabetic pithed rats with a nonselective 5-HT_1 receptor antagonist, methiothepin ($100\,\mu g\,kg^{-1}$, $n=5$), produced a marked inhibition *per se* of the pressor response in S–R curve E0 (Figure 6). This inhibitory effect is higher than the inhibition produced by 5-HT ($10\,\mu g\,kg^{-1}\,min^{-1}$), so the action produced on electrically induced pressor responses by

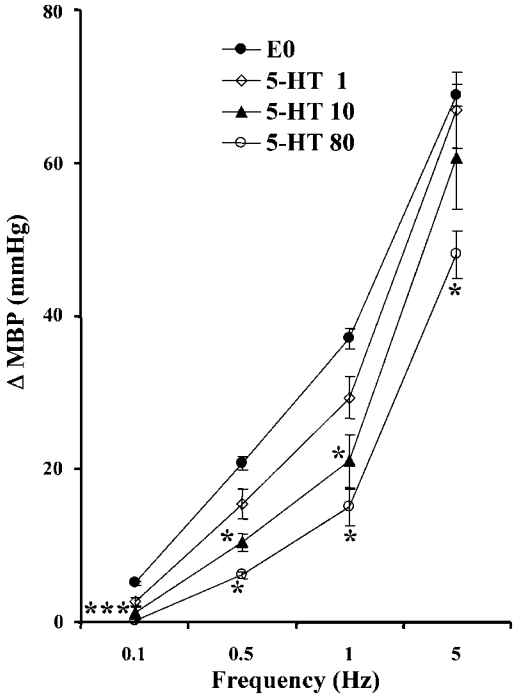

Figure 3 Effect of the i.v. infusions of 5-HT (1, 10 and 80 $\mu g\,kg^{-1}\,min^{-1}$) on electrically induced pressor responses in diabetic pithed rats (S–R E2). Data are shown as mean ± s.e.m. $^*P<0.05$ vs E0 control.

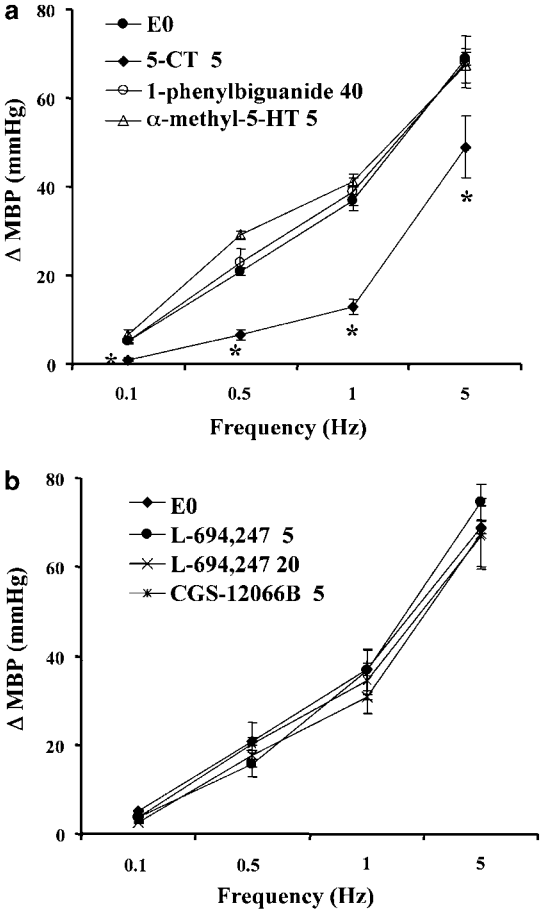

Figure 4 Effect of the i.v. infusions of (a) 5-CT ($5\,\mu g\,kg^{-1}\,min^{-1}$), α-methyl-5-HT ($5\,\mu g\,kg^{-1}\,min^{-1}$) or 1-phenylbiguanide ($40\,\mu g\,kg^{-1}\,min^{-1}$) and (b) L-694,247 (5 and $20\,\mu g\,kg^{-1}\,min^{-1}$) or CGS-12066B ($5\,\mu g\,kg^{-1}\,min^{-1}$) on electrically induced pressor responses in diabetic pithed rats (S–R E2). Data are shown as mean ± s.e.m. $^*P<0.05$ vs E0 control.

5-HT ($10\,\mu g\,kg^{-1}\,min^{-1}$) was unable to be elicited after i.v. treatment with methiothepin ($100\,\mu g\,kg^{-1}$, $n=5$) (Figure 6).

The inhibitory effects of 5-HT ($10\,\mu g\,kg^{-1}\,min^{-1}$, $n=5$) and 8-OH-DPAT ($10\,\mu g\,kg^{-1}\,min^{-1}$, $n=5$) were completely abolished by $100\,\mu g\,kg^{-1}$ of WAY-100,635 (Figure 7 and 8), a selective 5-HT_{1A} receptor antagonist, which, by itself, did not modify the pressor responses obtained by electrical stimulation.

The other antagonists tested, ritanserin ($1\,mg\,kg^{-1}$, $n=5$) a selective 5-HT_2 antagonist, or MDL-72222 ($2\,mg\,kg^{-1}$, $n=5$) (Figure 9a), a selective 5-HT_3 antagonist, did not modify *per se* the pressor responses in S–R curve E0, and were not able to antagonize the inhibitory effect of $10\,\mu g\,kg^{-1}\,min^{-1}$ of 5-HT ($n=5$ for each antagonist) (Figure 9b).

Effects of saline solution, 5-HT or 8-OH-DPAT on the NA-induced increases in mean blood pressure

The increases in mean arterial blood pressure (in S–R curve E′0) caused by exogenous NA ($0.01–0.5\,\mu g\,kg^{-1}$) in diabetic pithed rats were 15.0 ± 1.8, 20.0 ± 2.3, 32.0 ± 4.5 and 42.7 ± 5.0 mmHg. These rises in MBP remained stable in S–R curves E′1, E′2 and E′3 in control animals receiving an infusion of $1\,ml\,h^{-1}$ of saline ($n=5$). Continuous infusion of 5-HT ($10\,\mu g\,kg^{-1}\,min^{-1}$, $n=5$) or 8-OH-DPAT ($10\,\mu g\,kg^{-1}\,min^{-1}$, $n=5$) failed to inhibit the pressor responses to exogenous administration of NA (Figure 10).

Discussion

In the present work, we studied the influence of experimental diabetes on the inhibitory 5-hydroxytryptamine receptors involved in the pressor effect elicited by sympathetic stimulation in the pithed rat. To accomplish this, we have used an experimental model of chemical diabetes in the rat. The diabetogenic agent used was alloxan. This drug induces a syndrome resembling type 1 diabetes mellitus, characterized by hyperglycaemia, hypercholesterolaemia, glycosuria and a raised level of glycosylated haemoglobin in erythrocytes (Agrawal *et al*., 1987), and it is commonly used as a valid experimental model of diabetes in animal (Hodgson *et al*., 1990; Jamnicky *et al*., 1993; Öztürk *et al*., 1996; Chan *et al*., 2000; Miranda *et al*., 2002).

In our experiments in diabetic rats, it has been necessary to use a supramaximal voltage higher than that used in normoglycaemic rats. In the same way, we have obtained increments in mean blood pressure in diabetic rats higher than in normoglycaemic rats. These differences between both groups of animals (diabetic and normoglycaemic animals) can be due to a dysfunction of the autonomic nervous system reported in diabetes by many authors, although the results are controversial because several author proposed an increased vascular reactivity to adrenoceptors agents (Abebe & McLeod, 1990; Taylor *et al*., 1994), whereas others proposed an attenuation of sympathetic contractile responses (Takiguchi

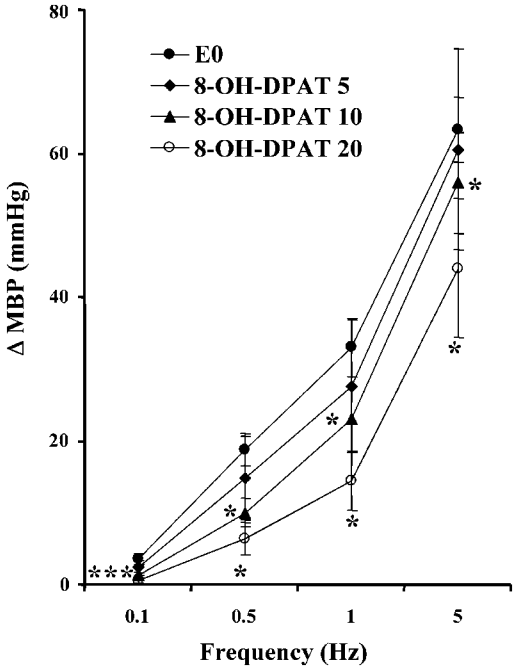

Figure 5 Effect of the i.v. infusion of 8-OH-DPAT (5, 10 and 20 $\mu g\,kg^{-1}\,min^{-1}$) on electrically induced pressor responses in diabetic pithed rats (S–R E2). Data are shown as mean ± s.e.m. $^*P<0.05$ vs E0 control.

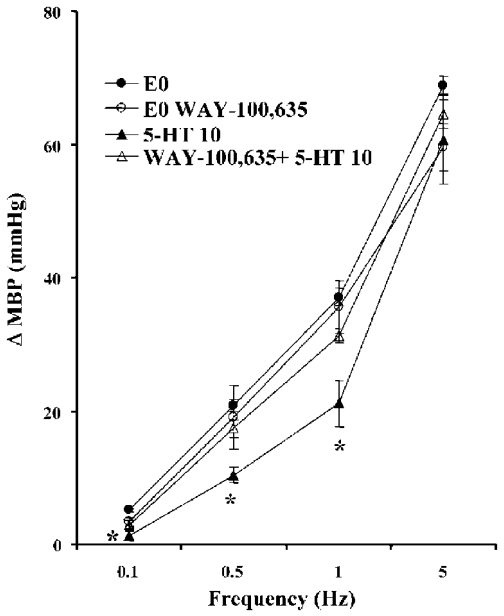

Figure 7 Effect of the i.v. administration of WAY-100,635 (100 $\mu g\,kg^{-1}$) on the inhibitory effect of 5-HT (10 $\mu g\,kg^{-1}\,min^{-1}$) on electrically induced pressor responses in diabetic pithed rats (S–R E2). Data are shown as mean ± s.e.m. $^*P<0.05$ vs E0 control.

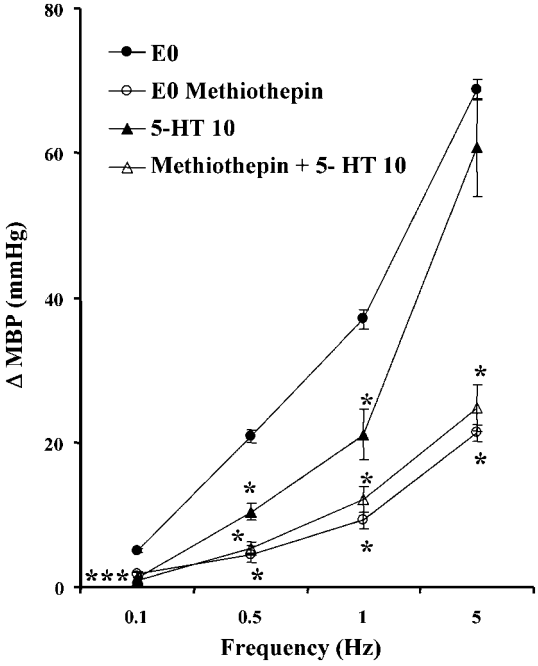

Figure 6 Effect of the i.v. administration of methiothepin (100 $\mu g\,kg^{-1}$) on the inhibitory effect of 5-HT (10 $\mu g\,kg^{-1}\,min^{-1}$) on electrically induced pressor responses in diabetic pithed rats (S–R E2). Data are shown as mean ± s.e.m. $^*P<0.05$ vs E0 control.

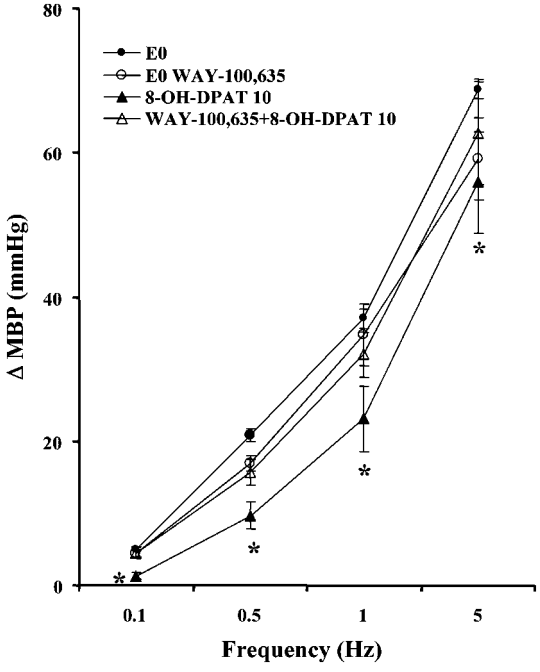

Figure 8 Effect of the i.v. administration of WAY-100,635 (100 $\mu g\,kg^{-1}$) on the inhibition produced by 8-OH-DPAT (10 $\mu g\,kg^{-1}\,min^{-1}$) on electrically induced pressor responses in diabetic pithed rats (S–R E2). Data are shown as mean ± s.e.m. $^*P<0.05$ vs E0 control.

et al., 1988; Andersson *et al*., 1992) or even no changes (Ralevic *et al*., 1993).

Our results revealed that in the diabetic rat, as occurs in normoglycaemic Wistar rats in this work and others (Morán *et al*., 1994; Villalón *et al*., 1995c; Morán *et al*., 1998), 5-HT interferes with adrenergic neurotransmission and reduces the increases in blood pressure obtained by sympathetic stimulation. However, it does not affect the increases in blood pressure elicited by exogenous administration of NA.

In the same way as in normoglycaemic rats, the higher degree of inhibition obtained with 5-CT in this work (in

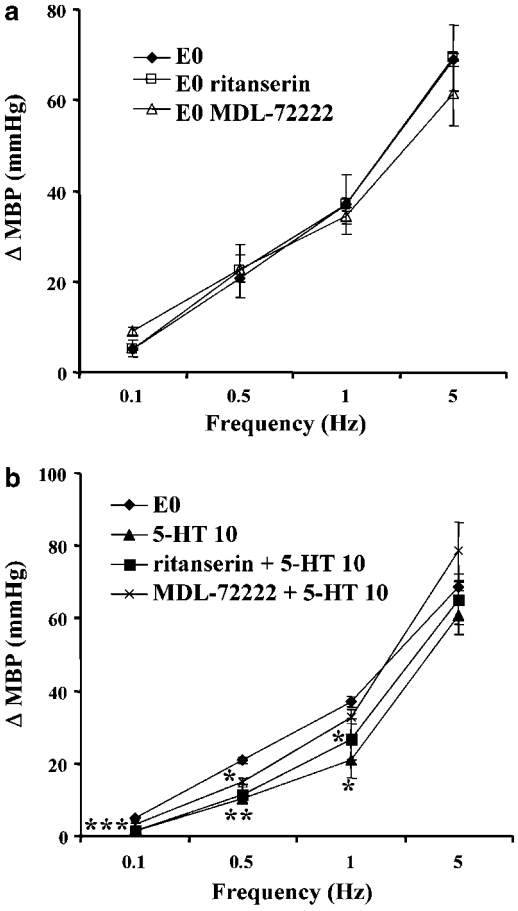

Figure 9 Effect of i.v. administration of (a) ritanserin (1 mg kg^{-1}) or MDL-72222 (2 mg kg^{-1}) on electrically induced pressor responses (S–R E0) and (b) ritanserin (1 mg kg^{-1}) or MDL-72222 (2 mg kg^{-1}) on the inhibition produced by 5-HT (10 $\mu g\,kg^{-1}\,min^{-1}$) on electrically induced pressor responses in diabetic pithed rats (S–R E2). Data are shown as mean ± s.e.m. $^*P<0.05$ vs E0 control.

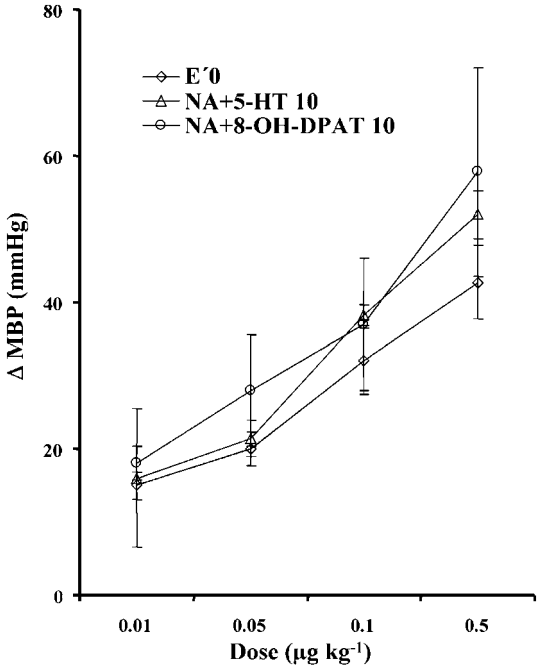

Figure 10 Effect of continuous infusion of 5-HT (10 $\mu g\,kg^{-1}\,min^{-1}$) and 8-OH-DPAT (10 $\mu g\,kg^{-1}\,min^{-1}$) on increases in mean blood pressure induced by exogenous i.v. administration of NA in diabetic pithed rats (S–R E′2) from E′0 dose–reponse curve. Data are shown as mean ± s.e.m. There were no statistically significant differences from the corresponding E′0 control values ($P>0.05$).

diabetic rats) with respect to that observed with 5-HT at a dose of 20 $\mu g\,kg^{-1}\,min^{-1}$ in this work and previously by us (Morán *et al*., 1994; 1998), together with the absence of activity with α-methyl-5-HT, a selective 5-$HT_{2A/2B/2C}$ receptor agonist (Baxter *et al*., 1995) and 1-phenylbiguanide, a selective 5-HT_3 receptor agonist (Ireland & Tyers, 1987; Chen *et al*., 1991), confirm that the main serotonergic receptor subtypes involved in this inhibitory activity in diabetic rats are 5-HT_1.

These results are consistent with those from previous studies carried out by us and other authors in normoglycaemic rats (Morán *et al*., 1994; 1998; Villalón *et al*., 1995a) and in other animal species (see McGrath, 1977) and confirm that the prejunctional 5-HT receptor in the diabetic rat mediating the inhibition of pressor effects obtained by stimulation of sympathetic outflow in pithed rats are mainly 5-HT_1 in nature.

Accordingly, in order to establish the 5-HT_1 subtype receptor responsible for this action, different antagonists and selective 5-HT_1 agonists were used in our experiments.

A nonselective 5-HT_1 antagonist, methiothepin (Hoyer *et al*., 1994) blocked the electrically induced pressor responses *per se*, and this inhibitory effect may be accounted for the affinity of this antagonist for α_1-adrenoceptors (Leysen *et al*., 1985; Fernández *et al*., 2000). In this sense, the inhibition of electrically induced pressor responses by 5-HT (10 $\mu g\,kg^{-1}\,min^{-1}$) was unable to be elicited after i.v. treatment with methiothepin (100 $\mu g\,kg^{-1}$) because of the marked inhibition produced by methiothepin itself. In contrast the 5-HT_2 receptor antagonist ritanserin (Awouters *et al*., 1988) and the 5-HT_3 receptor antagonist MDL-72222 (see Fozard, 1984) were not able to block the inhibitory effect of 5-HT, hence these receptors are virtually devoid of any action in the inhibitory effect of 5-HT on the sympathetic pressor responses induced by electrical stimulation.

According to our results the inhibitory effect of 5-HT and 5-CT is mimicked by 8-OH-DPAT (Middlemiss & Fozard, 1983). This latter compound, a 5-HT_{1A} selective agonist that displays a similar agonist potency to that of 5-CT for this receptor subtype (pEC50 values of 8.2 and 8.6, respectively) (Hoyer *et al*., 1994), inhibited the pressor effect obtained by electrical stimulation, apparently with more intensity than that of 5-CT. A possible activation of α_2-presynaptic receptors by this agonist (Castillo *et al*., 1994) would explain this inhibition. However, the total reversibility of the 8-OH-DPAT-induced inhibitory effect following the administration of WAY-100,635, a selective 5-HT_{1A} antagonist (Fletcher *et al*., 1994) with no reported α-adrenoceptor antagonist activity of its own and which, at dose used by us, did not inhibit the pressor response, together with the blockade obtained with this antagonist on the inhibitory effect of 5-HT, confirm the participation in this inhibitory activity of the 5-HT_{1A} receptor subtype, although it is not possible to rule out the possible participation of the other receptor subtypes in this action. Therefore, these initial results suggest that 5-HT_{1A} receptor subtypes are mainly responsible for the inhibitory activity of 5-HT in diabetic rats.

The absence of activity observed with the selective rodent 5-HT_{1B} receptor agonist CGS-12066B (Neale *et al*., 1987) and a selective nonrodent 5-HT_{1B} receptor and the 5-HT_{1D} receptor agonist, L-694,247 (Beer *et al*., 1993), confirms that these subtypes of 5-HT_1 receptors do not participate in 5-HT-induced inhibitory sympathetic activity.

The results obtained in this work, in diabetic rats, are in contrast with those obtained in normoglycaemic rats reported by Villalón *et al*. (1998), who demonstrated that 5-HT_{1A}, rodent 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-HT_{1D} heteroceptors are mainly involved, although also modulated by 5-HT_{1A} receptors (Morán *et al*., 1998).

Thus, according to our results and in contrast to the situation seen in normoglycaemic rats, experimental alloxan-induced hyperglycaemia in rats produces a major involvement of 5-HT_{1A} receptors, whereas the 5-HT_{1D} receptor activation is devoid of this inhibitory effect in diabetes.

These differences could be related to vascular reactivity, alterations in peripheral nerve sensitivity or to endothelial dysfunction due to induced diabetes (Ralevic *et al*., 1995; De Vriese *et al*., 2000; Miranda *et al*., 2000; 2002), and they are in agreement with some data pointing to modifications in the 5-HT receptor population, based on an increase in the 5-HT_{1A} and 5-HT_2 receptor population in alloxan-treated diabetic rats (Sandrini *et al*., 1997).

All the agonists used in our experiments are more potent at lower stimulation frequencies. These results are consistent with those previously reported by Villalón *et al*. (1995a, b), following the pattern of other prejunctional modulators of NA release from sympathetic nerves. As previously reported by us in normoglycaemic rats (Morán *et al*., 1994; 1998), the agonists used by us here failed to inhibit the pressor response evoked by i.v. NA administration. We thus confirm the mainly prejunctional nature of this inhibition. Nevertheless, our results do not exclude, as has been proposed in anaesthetized cats (Jones *et al*., 1995) and rats (Ireland & Jordan, 1987), the possible ganglionic location of these receptors.

In conclusion, the present results suggest that diabetes induces changes in the inhibitory effect induced by 5-HT in the sympathetic pressor responses, such that the inhibitory action induced by 5-HT in diabetic pithed rats is mainly mediated by prejunctional 5-HT_{1A} receptors.

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