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ARTICLE

Hypertension exhibits 5-HT_4 receptor as a modulator of sympathetic neurotransmission in the rat mesenteric vasculature

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Abstract

Sympathetic overdrive is a key player in hypertension, where the mesenteric vasculature plays a relevant role in modulating blood pressure. Although 5-HT inhibits noradrenergic mesenteric neurotransmission in normotensive rats, its effect on the mesenteric sympathetic drive in hypertensive rats has not been studied. We investigated the influence of in vivo 5-HT by characterizing the implicated serotonergic receptors on the mesenteric sympathetic outflow in rats with N-nitro-L-arginine methyl ester (L-NAME)-induced hypertension. Hypertension was induced in male Wistar rats by L-NAME administration (30 mg/kg per day; 21 days) in drinking water. The rats were anesthetized (sodium pentobarbital; 60 mg/kg, i.p.), prepared for the in situ autoperfused rat mesentery, and subjected for monitoring their systemic blood pressure (SBP), heart rate (HR), and mesenteric perfusion pressure (MPP). Electrical stimulation of mesenteric sympathetic nerves resulted in frequency-dependent increases in MPP without altering SBP or HR. The 5-HT and cisapride (5-HT_4 agonist) i.a. bolus (1–25 µg/kg) inhibited vasopressor responses by electrical stimulation of the mesenteric nerves, unlike an i.a. bolus (25 µg/kg each) of the agonist 5-carboxamidotryptamine (5-$HT_{1/7}$ agonist), α-methyl-5-HT (5-HT_2), or 1-PBG (5-HT_3). However, i.a. cisapride (25 µg/kg) did not affect the noradrenaline-induced vasoconstriction in the mesenteric vasculature. Administration of the selective 5-HT_4 receptor antagonist GR 125487 (1 mg/kg, i.v.) completely abolished cisapride- and 5-HT-evoked mesenteric sympatholytic effects. Additionally, ELISA analysis demonstrated higher 5-HT_4 receptor expression in mesenteric arteries from L-NAME-hypertensive compared with normotensive rats. Our findings suggest that L-NAME-induced hypertension modifies the 5-HT modulation of the rat mesenteric sympathetic drive: prejunctional 5-HT_4 receptors are involved in the serotonergic sympathoinhibitory effect.

Keywords 5-HT_4 receptor · hypertensive rat · L-NAME · mesenteric vasculature · sympathetic nervous system

Introduction

Hypertension is a main risk factor for cardiovascular diseases, which are still the most common cause of death worldwide. Although the pathogenesis of hypertension is multifactorial and highly complex, the influence of the sympathetic nervous system (SNS) on the heart and peripheral blood vessels has been shown to play a relevant role in hypertension, contributing not only to the origin but also to the maintenance of abnormally high blood pressure levels. Thus, a sympathetic overdrive has been evidenced in both animal models of hypertension and hypertensive patients, leading to chemical, molecular, and structural alterations affecting several vascular beds, and therefore to systemic vascular resistance [1–3].

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One of the vascular territories distinguished as being a large blood volume reservoir is the splanchnic bed, the vascular tone of which can be modified during periods of physiological adaptation or in pathological situations. Within its irrigation, the superior mesenteric artery (SMA) is the largest of the splanchnic arterial vessels, distributing >10% of the cardiac output and playing an important role in both physiological and pathophysiological states. The SMA controls the mesenteric vascular tone through, among others, sympathetic innervation regulating the tissue blood flow and, consequently, the systemic blood pressure [4, 5]. The homeostasis of the neurogenic control arising from the periarterial mesenteric innervation is disrupted in hypertension, increasing the vasopressor control (noradrenergic nerves) compared with the vasorelaxant responses (release of nitric oxide (NO) or calcitonin gene-related peptide (CGRP)). In this sense, reducing the massive release of noradrenaline (NA) could constitute a potential pharmacological strategy; unfortunately, the role of sympatholytic agents in the in vivo sympathetic neurotransmission has not yet been established in the mesenteric vasculature of hypertensive animals.

The biogenic amine 5-hydroxytryptamine (5-HT) intervenes in many cardiovascular disorders not only to achieve a direct effect on the heart and blood vessels but also to modulate diverse neurotransmission systems [6, 7]. This monoamine has been shown to modulate noradrenergic outflow in different vascular territories through the activation of different serotonergic receptors, depending on the studied vasculature [8–11]. In addition, our group has recently shown that 5-HT influences in vivo mesenteric periarterial sympathetic neurotransmission by exerting sympathoinhibitory effects through the activation of 5-HT$_{1D}$ receptors in normotensive rats [12].

Given that i) the mesenteric vasculature is a key player in cardiovascular homeostasis, in which both structural and functional alterations in the mesentery have been evidenced to contribute to the hypertensive process [13, 14], ii) SNS supplies an important contribution to the regulation of mesenteric vascular tone, iii) sympathetic hyperactivity predominates in the genesis as well as the cardiovascular-derived complications of hypertension, and iv) the serotonergic system downregulates noradrenergic neurotransmission in the rat mesenteric vascular bed, we hypothesized that hypertension could alter the 5-HT-induced modulation of mesenteric sympathetic neurotransmission by modifying the implicated serotonergic receptor. Thus, the present work was performed to investigate whether 5-HT could modulate, in addition to the involved serotonergic receptors, sympathetic neurotransmission in the mesenteric vascular bed in an experimental rat model of hypertension.

Methods

Ethical approval of the study protocol

A total of 95 male Wistar rats (350 ± 25 g) were housed under a 12-h light–dark cycle at a constant temperature (22 ± 2 °C) and humidity (50%) with food and water provided ad libitum. Both the housing conditions and experimental procedures were approved by the University of Salamanca Institutional Bioethics committee (project number 0000029, approved on July 28, 2015). Maintenance and manipulation protocols were performed following European guidelines (Directive 2010/63/EU) and Spanish legislation (R.D. 53/2013) for the use and care of animals in Biomedical Research and were in accordance with the ARRIVE guidelines for reporting experiments involving animals [15].

Drugs

The following drugs were used: heparin sodium was from Roche (Madrid, Spain); pentobarbital sodium, 5-HT, α-methyl-5-HT, N(ω)-L-arginine methyl ester hydrochloride (L-NAME), and 1-phenylbiguanide (1-PBG) were from Sigma-Aldrich (St Louis, MO, USA); atropine sulfate was from Scharlau, Barcelona (Spain); 5-carboxamidotryptamine maleate (5-CT), 5-fluoro-2-methoxy-d_3-[1-[2-[(methylsulfonyl)amino]ethyl]-4-piperidinyl]-1_H-indole-3-methylcarboxylate sulfamate (GR 125487) and cisapride were from Tocris Bioscience (Bristol, UK), and prazosin was from Pfizer (New York, NY, USA).

All drugs were dissolved in physiological saline at the time of experimentation, with the exception of cisapride (dissolved in 0.01 M HCl). These vehicles had no effect on the basal mesenteric perfusion pressure (MPP) or systemic blood pressure (SBP). The doses of all drugs (referring to their free base) were chosen on the basis of our previous experience [12, 16–19].

Animal preparation

Hypertension was induced by administration of L-NAME (30 mg/kg per day in the drinking water) for 21 days. On day 21 of L-NAME treatment, systolic blood pressure and heart rate (HR) were measured using the tail–cuff method with a photoelectric sensor (NIPREM 546, Cibertec S.A, Madrid, Spain). Several determinations were made in each session for each rat ($n = 5$ normotensive and $n = 90$ L-NAME hypertensive rats). Values were considered valid if five consecutive measurements were <10 mmHg.

After 21 days of L-NAME treatment, rats were anesthetized with sodium pentobarbital (60 mg/kg, i.p.) and prepared for in situ perfusion of the mesenteric territory as

we have previously described [12, 18]. The adequacy of anesthesia was monitored by the absence of ocular reflexes, a negative toe pinch test and muscle relaxation. A tracheotomy was performed, and catheters were placed in the right and left carotid arteries. The right carotid artery was cannulated for SBP and HR measurements using a pressure transducer connected to an e-corder 410 amplifier (Model ED410, Cibertec, Spain), with Chart™ and Scope™ software. The intact mesenteric vascular bed was perfused using an extracorporeal circuit and a constant flow Gilson peristaltic pump [12, 18]. Heparin (5 mg/kg) was then administered by a cannula placed in the left jugular vein. An i.v. (femoral vein) infusion of saline was initiated at a rate of 2 mL/h and continued throughout the experiment. The circuit was established with no interruption of blood flow to the mesenteric bed, pumping from the left carotid artery to the SMA. The distal portion of the external circuit was connected to a pressure transducer connected to an e-corder 410 amplifier (Model ED410, Cibertec, Spain) for measurement of the MPP. At the beginning of each experiment, the flow was adjusted to render the MPP equal to the SBP; it was kept constant throughout the experiment. Thus, the changes in the perfusion pressure reflected the changes in the vascular resistance. The flow rate through the mesenteric vascular bed ranged from 1.5 to 2 mL/min, depending on the SBP of the rat [12, 18]. In all experiments, atropine (1 mg/kg, i.v.) was administered prior to saline infusion with the aim of avoiding potential muscarinic actions. Drugs were administered intra-arterially via the distal cannula by a bolus injection of a maximum of 10 µL using a microsyringe (Exmire, Japan).

Hence, the 90 hypertensive animals were first distributed into two sets to examine the effects induced by diverse 5-HT agents on the vasoconstrictor responses produced by (i) electrical stimulation of sympathetic mesenteric nerves ($n = 75$; set 1) or (ii) i.a. bolus injections of exogenous NA ($n = 15$; set 2). In the vasoconstrictor stimulus-response (S-R) and dose-response (D-R) curves evoked by electrical stimulation and exogenous NA, respectively, each response was produced under unchanged values of basal SBP. The electrical stimuli (32.5 ± 2.5 V; 1 ms; 2, 4 and 8 Hz), as well as the NA doses (0.1, 0.3 and 1.0 µg/kg), were administered using a sequential schedule at 3–5 min intervals. At each frequency, electrical stimulation was continued until the response was maximal (20 s), returning to the basal MPP immediately after the end of the stimulation.

Experimental design

Experiments were carried out after a 15-min period to allow blood pressure and perfusion pressure to stabilize, and the baseline values of SBP, HR, and MPP were calculated. Five rats were used to evaluate each dose of agonist or antagonist, as well as each animal preparation to evaluate only one agonist or antagonist.

Electrical stimulation of the sympathetic mesenteric nerves in anesthetized L-NAME hypertensive rats

The first set was performed to investigate the impact of serotonergic agents on mesenteric sympathetic outflow. Increments in MPP were obtained by electrical stimulation of the perivascular nerves from the SMA. Thus, a small bipolar electrode was located in the SMA using square wave pulses from a Cibertec Stimulator CS-9 at increasing frequencies of stimulation (2, 4, and 8 Hz). Thus, the control S-R curve (E0) was finalized in 15 min.

This set of L-NAME hypertensive rats was distributed into several groups. The first group ($n = 40$) received the following: (i) nothing (sham); (ii) saline (10 µL); (iii) 0.01 M HCl (10 µL); (iv) 5-HT (1–25 µg/kg); selective agonists of (v) 5-$HT_{1/7}$ receptors (5-CT; 25 µg/kg); (vi) 5-HT_2 receptor (α-methyl-5-HT; 25 µg/kg); (vii) 5-HT_3 receptor (1-PBG; 25 µg/kg); and (viii) 5-HT_4 receptor (cisapride; 1–25 µg/kg) *via* the distal cannula by i.a. bolus administration. After 5 min of the corresponding i.a. administration, a new S-R curve (E1) was achieved as described above for the S-R curve E0.

The second group ($n = 30$) was conducted to confirm the serotonergic receptors implicated in the 5-HT influence on mesenteric noradrenergic outflow. This group received i.v. vehicle (saline, 1 mL/kg) or GR 125487 (5-HT_4 receptor antagonist; 1 mg/kg, i.v.). The corresponding curve ($E0_{saline}$, $E0_{GR\ 125487}$) was finished after 10 min. Then, the animals were subdivided into three treatment groups for each agent: i.a. injection of saline (control group; 10 µL), 5-HT (25 µg/kg) or cisapride (25 µg/kg). After 5 min of i.a. injections, a new S-R curve (E1) was generated.

The third group ($n = 5$) was destined to confirm the α_1-adrenergic nature of the MPP increases obtained by electrical stimulation. Accordingly, these rats received an α_1-adrenergic antagonist, prazosin (250 µg/kg; i.v.), 10 min before the electrical stimulation.

Intra-arterial administration of noradrenaline in anesthetized L-NAME hypertensive rats

In the second set ($n = 15$), D-R curves generated by i.a. injections of exogenous NA (0.1, 0.3, and 1 µg/kg) were executed before (E'0) and 5 min after (E'1) the following (i.a.): saline (10 µL), 0.01 M HCl (10 µL) or cisapride (25 µg/kg).

5-HT_4 receptor ELISA bioassay

Mesenteric arteries from normotensive rats (control) and L-NAME hypertensive rats ($n = 5$ each group) were

Table 1 Transitory increases in mesenteric perfusion pressure (ΔMPP; mmHg) after an i.a. bolus of 5-HT (1, 6.25, 12.5, and 25 μg/kg) or α-methyl-5-HT (25 μg/kg) in L-NAME hypertensive rats. All values are expressed as the mean ± SEM from the baseline value ($n = 5$ each)

Treatment	Dose (μg/kg; i.a.)	ΔMPP from baseline (mmHg)
5-HT	1	19.7 ± 3.4
	6.25	45.4 ± 4.9
	12.5	64.0 ± 3.0
	25	75.0 ± 8.3
α-methyl-5-HT	25	78.0 ± 3.5

carefully isolated and stored at −80 °C. Mesenteric tissue was pulverized, and the tissue was homogenized in PBS and stored overnight at −20 °C. After two freeze-thaw cycles to break the cell membranes, the homogenates were centrifuged for 5 min at 5000×*g*, 2–8 °C. The supernatant was assayed immediately. The 5-HT$_4$ receptor was determined by enzyme immunoassay following the manufacturer's instructions (MyBiosource).

Statistical evaluation

All data are shown as the mean ± SEM. The peak changes in MPP by electrical stimulation or exogenous NA are expressed as increases (mmHg) in MPP from the corresponding baseline value. Correlation of the results from the experimental groups and their corresponding control group was evaluated by one-way ANOVA followed by the Student-Newman-Keuls' post hoc test. In the ELISA assay, statistical significance was carried by one-way ANOVA followed by the Student's *t*-test. Statistical significance was accepted at $P < 0.05$. Because the electrically and NA-induced increments in MPP in the sham group were similar to those obtained in the presence of vehicle (saline and 0.01 M HCl), the statistical evaluation only performed *vs* vehicle.

Results

Systemic hemodynamic variables

After 21 days, L-NAME treatment elicited a marked increase in systolic blood pressure (163.0 ± 3.0 mmHg, $n = 90$; $p < 0.05$) in comparison to non-treated rats (110 ± 4.0 mmHg, $n = 5$) and a decrease in HR (340.0 ± 9.3 bpm, $n = 90$; $p < 0.05$) in comparison to non-treated rats (367.0 ± 2.8 bpm, $n = 5$).

Under anesthesia, the baseline values of SBP, MPP and HR in L-NAME hypertensive rats were 139.0 ± 0.5 mmHg, 136.6 ± 6.0 mmHg and 300.0 ± 9.0 bpm, respectively. These parameters were not significantly changed after administration of agonists (i.a.), antagonists (i.v.) or their vehicles (both i.a. and i.v.) (not shown), except (i) i.v. prazosin injection significantly decreased SBP (119.5 ± 4.4 mmHg, $n = 5$; $p < 0.05$) and (ii) i.a. administration of increasing doses of 5-HT (1, 6.25, 12.5, and 25 μg/kg, $n = 5$) and α-methyl-5-HT (25 μg/kg, $n = 5$) significantly increased MPP (see Table 1); these increases, however, immediately returned to baseline levels.

Vascular responses produced by periarterial noradrenergic fiber stimulation or exogenous noradrenaline in the in situ autoperfused mesentery of L-NAME hypertensive rats

The responses to electrical stimulation (2, 4, and 8 Hz) of mesenteric sympathetic fibers were instantaneous and evoked frequency-dependent increases in MPP (16.0 ± 1.5, 41.4 ± 3.5 and 90.1 ± 3.6 mmHg (S-R curve E0). Likewise, i.a. injection of increasing doses of NA (0.1, 0.3, and 1.0 μg/kg) caused dose-dependent increments in MPP (20.7 ± 3.3, 50.2 ± 4.5 and 111.1 ± 5.9 mmHg (D-R curve E'0).

Since there were no changes in HR or SBP, these vasoconstrictor responses (induced by either sympathetic stimulation or by i.a. NA administration) were locally produced at the mesenteric vascular level. As expected, the electrically induced mesenteric vasoconstrictions were abolished with i.v. 250 μg/kg of prazosin (data not shown), validating the α_1-adrenergic nature of these responses.

Consequently, we examined the repercussion of serotonergic agonists/antagonists on the electrically or NA-evoked vasopressor effects on the mesenteric vasculature.

Mesenteric vascular effects of vehicle or the 5-HT receptor agonists 5-HT, 5-CT, α-methyl-5-HT, 1-PBG and cisapride in the in situ autoperfused mesentery of L-NAME hypertensive rats

In rats receiving i.a. vehicle (saline, 0.01 M HCl; 10 μL each), 5-CT, α-methyl-5-HT, or 1-PBG (25 μg/kg each agonist) (Fig. 1), the above S-R curve remained unchanged. In contrast, rats that received 5-HT showed a dose-dependent inhibition (1–25 μg/kg) (Figs. 1 and 2). Similarly, cisapride administration (25 μg/kg) mimicked the significant reduction of vasoconstriction at all assessed frequencies (Fig. 1).

Influence of selective 5-HT$_4$ receptor agonist on the electrically induced mesenteric vasoconstrictor responses in the in situ autoperfused mesentery of L-NAME hypertensive rats

As shown in Fig. 3, i.a. bolus injections of the selective 5-HT$_4$ agonist (cisapride) induced a dose-dependent

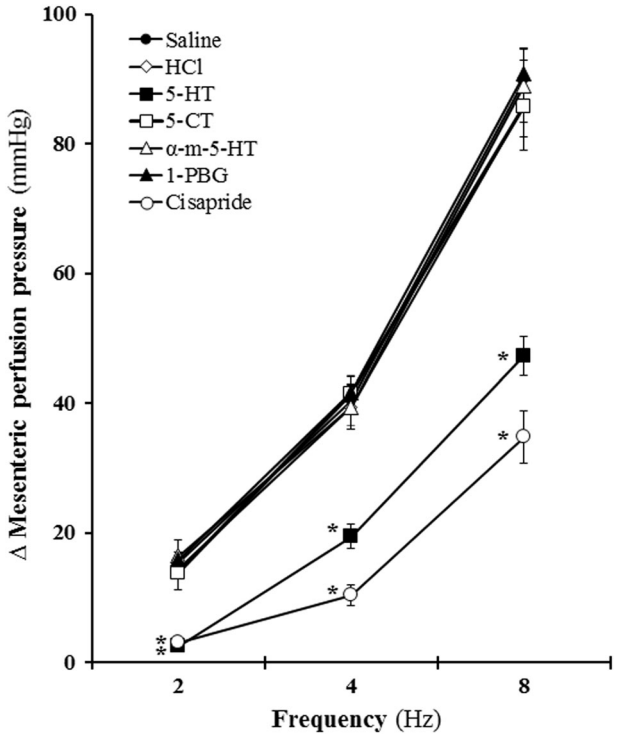

Fig. 1 Effect of vehicle (saline and HCl0.01 M HCl; 10 µL and $n = 5$ each), 5-HT, 5-CT, α-methyl-5-HT (α-m-5-HT), 1-PBG or cisapride (25 µg/kg and $n = 5$ each agonist) on the increase (Δ) in mesenteric perfusion pressure elicited by electrical stimulation of mesenteric sympathetic nerves. *$P < 0.05$ vs respective vehicle

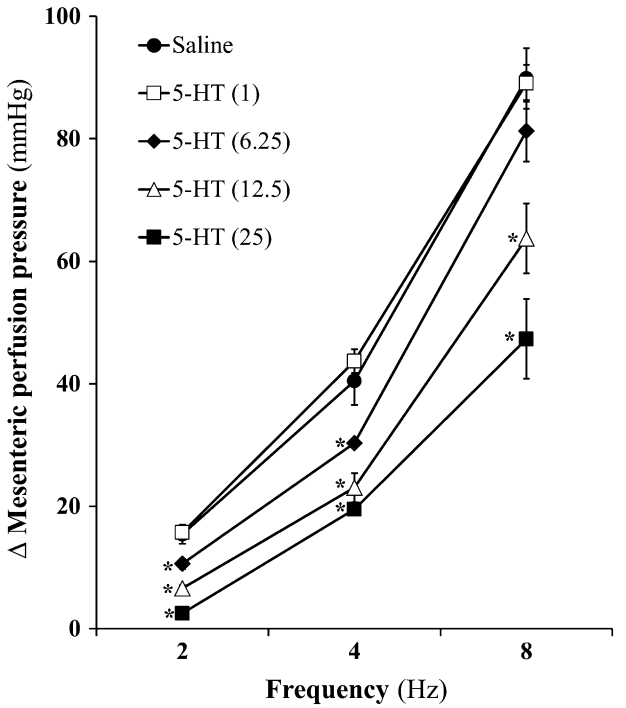

Fig. 2 Effect of an i.a. bolus of saline (10 µL; $n = 5$) and increasing doses of 5-HT (1–25 µg/kg; $n = 5$ each) on the increases (Δ) in mesenteric perfusion pressure elicited by electrical stimulation of mesenteric sympathetic nerves. *$P < 0.05$ vs saline

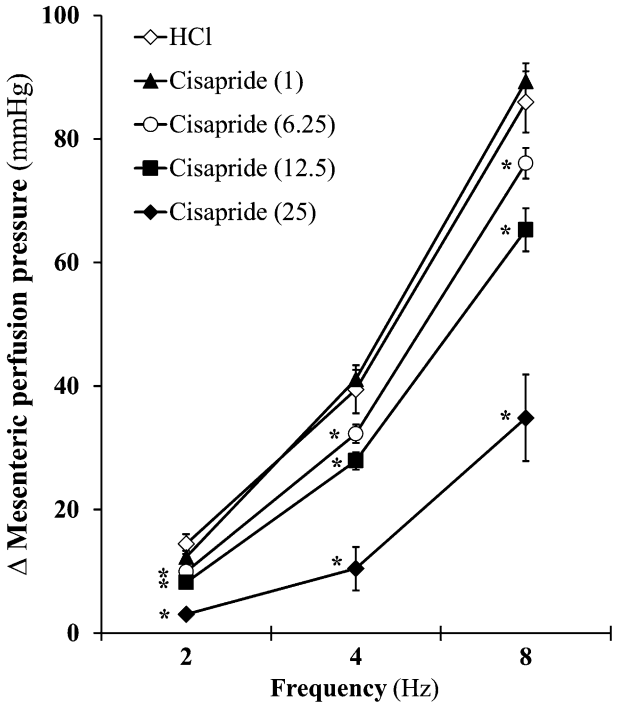

Fig. 3 Effect of an i.a. bolus of 0.01 M HCl (10 µL; $n = 5$) and increasing doses of cisapride (1–25 µg/kg; $n = 5$ each) on the increases (Δ) in mesenteric perfusion pressure elicited by electrical stimulation of mesenteric sympathetic nerves. *$P < 0.05$ vs 0.01 M HCl

sympathoinhibition (1–25 µg/kg) of the mesenteric vasopressor responses evoked by electrical stimulation.

Effect of an i.v. bolus of saline or GR 125487 on the effect of cisapride or 5-HT on the electrically induced mesenteric vasoconstrictor responses in L-NAME hypertensive rats

The administration of GR 125487 (1 mg/kg, i.v.), a selective 5-HT_4 receptor antagonist, did not modify per se the vasopressor responses in the sham group (not shown). However, either cisapride- or 5-HT-produced sympathoinhibition was abolished by i.v. injection of GR 125487, whereas i.v. saline injection did not alter the inhibitory effects of cisapride or 5-HT (Fig. 4).

Influence of vehicle (HCl) or cisapride on the mesenteric vasoconstrictor responses produced by exogenous noradrenaline in the in situ autoperfused mesentery of L-NAME hypertensive rats

The increments in MPP (D-R curve E′0) produced by i.a. NA (0.1, 0.3, and 1.0 µg/kg) persisted (D-R curves E′1) after receiving i.a. saline and 0.01 M HCl administration (Fig. 5). Cisapride (25 µg/kg, i.a.) was not able to

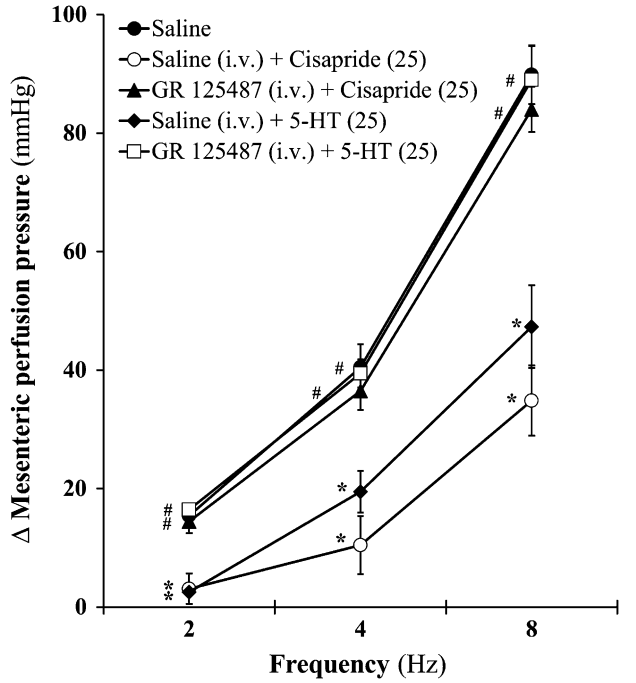

Fig. 4 Effect of an i.a. bolus of cisapride or 5-HT (25 µg/kg each) in the presence of i.v. pretreatment with vehicle (saline) or GR 125487 (1 mg/kg) on the increases (Δ) in mesenteric perfusion pressure elicited by electrical stimulation of mesenteric sympathetic nerves ($n = 5$ each group). *$P < 0.05$ vs saline i.a. #$P < 0.05$ *vs* cisapride or 5-HT in the presence of i.v. saline, respectively

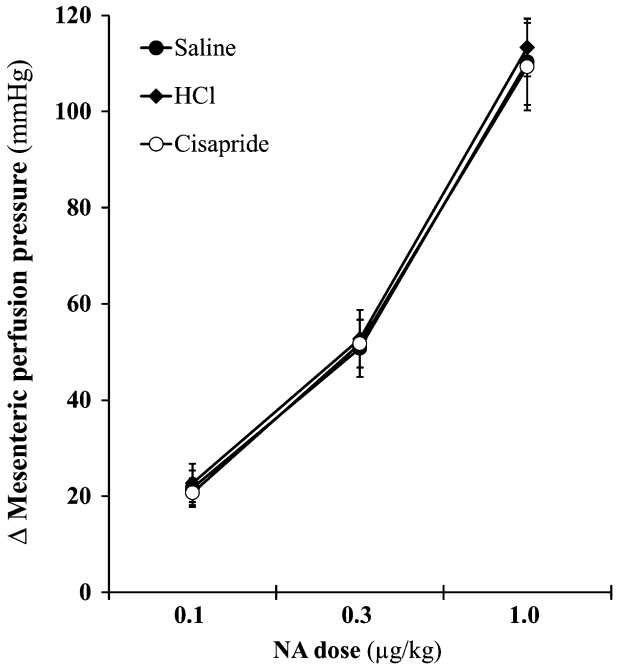

Fig. 5 Effect of an i.a. bolus of saline ($n = 5$), the vehicle 0.01 M HCl (10 µL; $n = 5$) or cisapride (25 µg/kg; $n = 5$) on the increases (Δ) in mesenteric perfusion pressure elicited by increasing i.a. doses of noradrenaline (NA) (0.1, 0.3, and 1.0 µg/kg). *$P < 0.05$ vs saline and HCl

reduce the vasoconstriction induced by i.a. NA injection (Fig. 5).

Study of 5-HT$_4$ receptor expression in the superior mesenteric artery in normotensive and L-NAME hypertensive rats

We examined the expression of the 5-HT$_4$ receptor in mesenteric arteries from normotensive (control) and L-NAME hypertensive rats by ELISA ($n = 5$ for each group). The 5-HT$_4$ receptor was expressed in mesenteric arteries of control rats. Interestingly, 5-HT$_4$ receptor expression was significantly increased in mesenteric arteries from L-NAME hypertensive rats (Fig. 6).

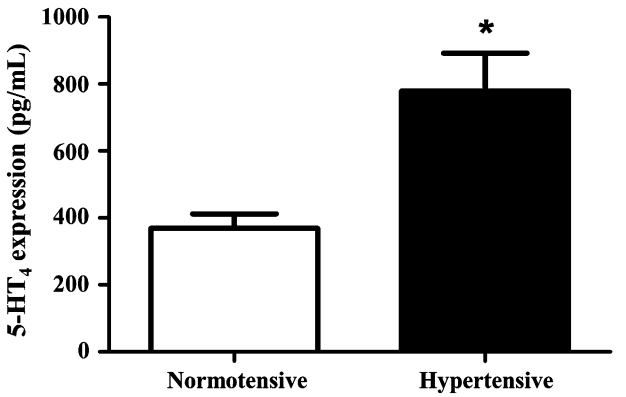

Fig. 6 The 5-HT$_4$ receptor expression (pg/mL tissue) in ex vivo mesenteric arteries of normotensive and L-NAME-hypertensive rats ($n = 5$ each group) measured by enzyme immunoassay. *$P < 0.05$ vs normotensive

Discussion

We have recently studied the in vivo influence of the serotonergic system on sympathetic neurotransmission in the rat mesenteric vascular bed, showing that 5-HT plays a neuroinhibitory role in mesenteric sympathetic neurotransmission due to the activation of pre and/or postjunctional 5-HT$_{1D}$ receptors [12]. Other researchers have also confirmed that 5-HT (through 5-HT$_{1D}$ receptors) is able to inhibit the release of other neurotransmitters, such as CGRP, in the rat mesentery [20]. Nevertheless, the role of the serotonergic system on noradrenergic control in the mesenteric vasculature in hypertensive animals has not yet been established. Thus, the present work was performed to investigate the effects of 5-HT on mesenteric sympathetic neurotransmission in an experimental model of arterial hypertension, such as L-NAME-hypertensive rats.

The model of hypertension induced by L-NAME was developed in 1992 by Baylis et al. [21], since then serving

widely as a model of experimental hypertension in research characterized by increased blood pressure and contractility in several vascular beds and decreased vascular relaxation. The NO deficiency leads to systemic vasoconstriction, endothelial dysfunction and increased blood pressure, mimicking hypertension in humans [19, 21–28]. In our study, oral administration of L-NAME resulted in systolic blood pressure values of ~160 mmHg after 21 days of treatment, without changing the gain in body weight in L-NAME-treated compared with non-treated rats. However, L-NAME treatment showed a slight, but significant, drop in HR. This bradycardic response might have been due to reflex mechanisms in response to the increase in blood pressure in the L-NAME hypertensive model, as previously described [29].

In situ autoperfusion of rat mesentery is an experimental technique that allows the evaluation of the in vivo influence of drugs on noradrenergic neurotransmission, specifically in the mesenteric vascular tree and without affecting the systemic hemodynamic parameters. Given that sympathetic periarterial nerves surround the SMA, the electrical stimulation of this artery provokes increases in MPP (frequency-dependent) through the local release of NA, which activates α_1 receptors to cause local vasoconstriction [4]. This phenomenon was confirmed in our experiments using the selective α_1-adrenoceptors antagonist prazosin [11, 12]. Interestingly, mesenteric vasoconstrictor responses are increased compared with those obtained in normotensive rats (recent outcomes demonstrated by our lab) under the same experimental conditions [12]. These results indicate that the L-NAME hypertensive model in rats is associated with greater activity of the sympathetic outflow in the mesenteric vasculature, as previously demonstrated in other experimental models of hypertension [30, 31]. Under our stimulation conditions and atropine pretreatment, we focused this study on sympathetic neurotransmission, since any response from the other possible innervations of the mesenteric artery have not been observed (such as nitrergic or sensitive neurotransmission) [4].

The i.a. administration of 5-HT (1–25 µg/kg) or α-methyl-5-HT (25 µg/kg) per se significantly increased MPP, which instantly returned to basal levels and, therefore, did not modify the ulterior responses in MPP, in agreement with our previous data [12, 18]. The 5-HT showed a significant dose- and frequency-dependent inhibition of electrically induced MPP increases, whereas α-methyl-5-HT did not have an effect. The mesenteric vasoconstriction by 5-HT_2 receptor activation has been previously demonstrated by our group in the in situ autoperfused mesentery of normotensive rats [12, 18] and by others in in situ autoperfused areas such as the kidney [11, 32–34] or hindquarters [10]. Although 5-HT_2 receptors are related to sympathoexcitatory effects [7, 35], we have established that vasoconstrictor effects specifically by 5-$HT_{2B/2C}$ receptors in the mesenteric territory are not mediated through the adrenergic system [18]. Thus, we have excluded a contribution of 5-HT_2 receptors in the serotonergic modulation of mesenteric sympathetic neurotransmission in L-NAME hypertensive rats.

The i.a. administration of 5-$HT_{1/7}$ or 5-HT_3 receptor agonists (5-CT or 1-PBG, respectively, at 25 µg/kg each) was unable to inhibit the mesenteric vasopressor responses induced by electrical stimulation of the perivascular nerves. However, i.a. administration of cisapride (selective 5-HT_4 agonist) [36] inhibited (in a frequency- and dose-dependent manner) the MPP increases evoked by sympathetic stimulation. Therefore, we corroborate that the pharmacological profile of the sympatholytic receptors involved is 5-HT_4, since i) cisapride is a potent agonist at 5-HT_4 receptors [36], ii) this sympathoinhibition was completely blocked by the selective 5-HT_4 antagonist GR 125487 [37], iii) this antagonist also abolished the 5-HT-induced inhibitory effect, and iv) additionally, by ELISA assay, we detected an increased expression of the 5-HT_4 receptor in the SMA from L-NAME hypertensive compared with normotensive rats.

Our current findings demonstrate that, as in normotensive animals [12], 5-HT inhibited mesenteric sympathetic outflow in L-NAME hypertensive animals. However, the induction of this pathology resulted in a remarkable change in serotonergic modulation: 5-HT_4 receptors are those involved in the sympathoinhibitory effect. The nature of these 5-HT_4 receptors was prejunctional, since 25 µg/kg of cisapride reduced electrical-evoked mesenteric vasoconstriction but not those induced by exogenous NA. Furthermore, the higher 5-HT_4 receptor expression in the SMA from L-NAME hypertensive compared with normotensive rats indicated that L-NAME-induced hypertension increased the expression of 5-HT_4 receptors in the rat mesenteric artery.

Our research group has previously shown that the induction of hypertension by L-NAME causes significant changes in the serotonergic modulation of renal vascular tone [19], probably because of the alterations in the endothelium compared with the hypertensive animal. In fact, Vanhoutte et al. [38] have shown that endothelial damage can involve changes in the serotonergic influence, since 5-HT responses are modulated by the endothelium. Given that 5-HT_{1D} receptors are expressed in the endothelium [6], the endothelial disorder characteristics of cardiovascular diseases could affect the role of these serotonergic receptors by modifying the 5-HT modulation of vascular beds [17, 39, 40].

Although 5-HT_4 receptors are coupled to G_s proteins, the underlying transduction mechanism of which is related to an increased release of neurotransmitters [41], in our experimental conditions the activation of these receptors

reduced the release of NA. This circumstance could be justified by the involvement of indirect mechanisms reducing NA release by activation of the 5-HT$_4$ receptor. Similarly 5-HT$_4$ receptor activation has been shown to provoke sympathoinhibition in the rabbit pulmonary artery, mediated by acetylcholine release [42]; however, we discarded that indirect pathway under our experimental conditions due to the pretreatment with atropine. In contrast, McHale et al. have demonstrated that 5-HT inhibits the contraction of isolated sheep mesenteric lymphatic rings through 5-HT$_4$ activation, suggesting the involvement of a direct activation of these receptors in the vascular effect [43].

It is important to underline that the presence of 5-HT and of serotonergic receptors in the human gut is consistent with that found in laboratory animals, including the rat [44]. Additionally, a recent study by Seitz et al. has demonstrated that the continuous infusion of 5-HT in rats evokes an in vivo splanchnic venodilation and a systemic hypotensive effect, highlighting the relevance of the splanchnic vasculature in blood pressure regulation [45]. Nevertheless, other studies have affirmed that 5-HT does not reduce the activity of the sympathetic nerve in the in vitro splanchnic circulation [46]. However, as proposed by Jackson and Campbell regarding the mesenteric vasculature, the in vivo model is much more sensitive than its in vitro counterpart, since the mesenteric arterioles remain intact and the physiological blood supply is maintained [47]. Hence, our current results in both L-NAME hypertensive and normotensive animals [12] allowed us to conclude that the serotonergic system modulates sympathetic neurotransmission at the splanchnic level in an in vivo experimental model. Similarly, the importance of presynaptic regulation in mesenteric NA release in hypertension has been previously demonstrated, where an impairment of presynaptic adenosine receptors is involved in the potentiated mesenteric vasoconstriction in spontaneously hypertensive rats [48].

High blood pressure is a growing public health problem and a dominant cardiovascular risk factor. Despite the availability of many antihypertensive therapies, there is a high percentage of uncontrolled hypertensive subjects who are refractory to conventional treatments. Consequently, research focused on pathophysiological knowledge of this disease is crucial and necessary to identify novel therapeutic approaches aimed at controlling those impaired parameters, such as sympathetic overdrive. In this sense, it has been shown that sympathetic ablation of the splanchnic nerves can effectively reduce the established hypertension in Dahl salt-sensitive rats, although complete denervation leads to some gastrointestinal side effects [49]. Therefore, modifying the splanchnic sympathetic control without eliminating all of its innervation could represent a novel and powerful target for hypertension.

In conclusion, our study shows that the serotonergic system interferes with the control of mesenteric vascular homeostasis, modulating sympathetic neurotransmission. The 5-HT inhibits noradrenergic neurotransmission in the in situ autoperfused mesentery of L-NAME hypertensive rats through the activation of prejunctional 5-HT$_4$ receptor. Thus, modulation of the serotonergic system affecting noradrenergic overactivity in the mesenteric vasculature might provide a possible therapeutic target and a new research pathway for the development of new antihypertensive drugs.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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