GR-127935-sensitive Mechanism Mediating Hypotension in Anesthetized Rats: Are 5-HT_{5B} Receptors Involved?

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Abstract: The 5-$HT_{1B/1D}$ receptor antagonist, GR-127935, inhibits its hypotensive responses produced by the 5-HT_{1A}, 5-$HT_{1B/1D}$ and 5-HT_7 receptor agonist, and 5-$HT_{5A/5B}$ receptor ligand, 5-carboxamidotryptamine (5-CT), in rats. This work further characterized the above mechanism using more selective 5-HT_{1B} and 5-HT_{1D} receptor antagonists. Also, expression of 5-HT_{5A} and 5-HT_{5B} receptor mRNAs in blood vessels was searched by reverse transcription polymerase chain reaction. Decreases in diastolic blood pressure induced by 5-CT (0.001–10 μg/kg, intravenously) were analyzed in anesthetized rats that had received intravenous vehicle (1 mL/kg), SB-224289 (5-HT_{1B} antagonist; 0.3 and 1.0 mg/kg), BRL15572 (5-HT_{1D} antagonist; 0.3 and 1.0 mg/kg), SB-224289 + BRL15572 (0.3 mg/kg, each), or SB-224289 + BRL15572 (0.3 mg/kg, each) + GR-127935 (1 mg/kg). Because only the latter treatment inhibited 5-CT–induced hypotension, suggestive of a mechanism unrelated to 5-$HT_{1B/1D}$ receptors, the effects of antagonists/ligands at 5-HT_{5A} (SB-699551, 1 mg/kg), 5-HT_6 (SB-399885, 1 mg/kg), and 5-$HT_{1B/1D/5A/5B/7}$ receptors (ergotamine, 0.1 mg/kg) on 5-CT-induced hypotension were tested. Interestingly, only ergotamine blocked 5-CT–induced responses; this effect closely paralleled that of SB-224289 + BRL-15572 + GR-127935. Neither did ergotamine nor GR-127935 inhibit hypotensive responses induced by the 5-HT_7 receptor agonist, LP-44. Faint but clear bands corresponding to 5-HT_{5A} and 5-HT_{5B} receptor mRNAs in aorta and mesenteric arteries were detected. Results suggest that the GR-127935-sensitive mechanism mediating hypotension in rats is unrelated to 5-HT_{1B}, 5-HT_{1D}, 5-HT_{5A}, 5-HT_6, and 5-HT_7 receptors. This mechanism, however, resembles putative 5-HT_{5B} receptors.

Key Words: 5-$HT_{1B/1D}$ receptors, 5-$HT_{5A/5B}$ receptors, hypotension, systemic blood vessels, anesthetized rats

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INTRODUCTION

It has been described that the long-lasting hypotensive response produced by serotonin (5-hydroxytryptamine; 5-HT) in rats is mediated by 5-HT_7 receptors.[1,2] More recently, it was reported that the 5-$HT_{1B/1D}$ receptor antagonist, GR-127935,[3] inhibits the hypotensive response produced by the 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, and 5-HT_7 receptor agonist, 5-carboxamidotryptamine (5-CT), in anesthetized rats through a mechanism unrelated to 5-HT_7 receptors, thus suggesting that 5-$HT_{1B/1D}$ receptors also mediate hypotension.[4,5] It is worth noting, however, that activation of 5-$HT_{1B/1D}$ receptors with sumatriptan did not evoke decreases of blood pressure in rats,[1,2] thus raising the possibility that inhibition of 5-CT-induced hypotensive responses by GR-127935[4] could be accounted for by a mechanism unrelated to 5-$HT_{1B/1D}$ (and 5-HT_7) receptors. This would imply that the peripheral vasodilator mechanisms involving 5-HT receptors could be more complex than previously anticipated.[1,2] Of potential clinical relevance may be the fact that the long-lasting nature of the 5-HT receptor-mediated hypotensive responses relies on the activation of the GR-127935–sensitive hypotensive mechanism, which is predominantly sensitive to the highest doses of 5-CT.[4] In fact, this is the reason why a previous study evaluating the hypotensive effects of 5-CT in rats failed to detect such novel hypotensive mechanism.[1] Then, the ability of 5-CT provided as a chronic intravenous treatment to significantly decrease blood pressure in spontaneously, and deoxycorticosterone acetate–salt hypertensive rats[6,7] might suggest that the GR-127935–sensitive 5-HT receptor mechanism mediating hypotension could be the target of novel antihypertensive therapies. Thus, on the bases of the above observations, the purpose of this study was to reanalyze, using more selective antagonists, the involvement of 5-HT_{1B} and 5-HT_{1D} receptors in mediating 5-CT–induced hypotension in anesthetized rats. Because 5-CT reportedly displays relatively high affinity at 5-$HT_{5A/5B}$ receptors,[8–10] efforts were made to elucidate the potential role of these receptors in 5-CT–induced hypotensive responses by means of the selective 5-HT_{5A} receptor antagonist, SB-699551,[11] and the nonselective 5-$HT_{1B/1D/5A/5B/7}$ receptor ligand, ergotamine.[8–10] Attempts were also made to detect, by reverse transcription polymerase chain reaction (RT-PCR), expression of the mRNA encoding for 5-HT_{5A} and 5-HT_{5B} receptors in rat blood vessels.

METHODS

All procedures and protocols complied with Federal Regulations for Animal Experimentation and Care (NOM-062-ZOO-1999, Ministry of Agriculture, Mexico) and were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals

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The authors report no conflicts of interest.

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(NIH Publications, number 80-23, revised 1978) and approved by the CINVESTAV-IPN ethics committee.

Animal Preparation

Male Wistar rats (250–300 g; n = 82) from the CINVESTAV-IPN inbred facilities were used. Animals were maintained at constant temperature and a 12-hour light:dark cycle (lights on 6:00–18:00 hours) with food and water provided ad libitum. Efforts were made to minimize unnecessary suffering of the animals and their number.

Measurement of Blood Pressure

Animals were anesthetized with sodium pentobarbital (50 mg/kg, intraperitoneally), and a continuous supporting dose (20 $mg \cdot kg^{-1} \cdot h^{-1}$, intravenously) was maintained throughout the experiments. Depth of anesthesia was routinely assessed and held at the level in which noxious stimulation failed to elicit nociceptive motor reflexes or changes in arterial pressure. The trachea was cannulated for ventilation with a rodent ventilator (60 cycles per minutes; volume 2 mL/100 g body weight), and PE-50 and PE-10 catheters were inserted into the left common carotid artery and both femoral veins for continuous blood pressure and heart rate recording and administration of drugs and anesthetic, respectively. Both vagus nerves were sectioned. Arterial blood pressure was monitored with a TSD104A pressure transducer connected to a Universal Interface Module (UIM100C; Biopac Systems, Inc., Santa Barbara, CA). Diastolic blood pressure and heart rate were simultaneously recorded in a computer using the Acqknowledge 3.8.1 software (Biopac Systems, Inc., Santa Barbara, CA). Diastolic blood pressure instead of mean arterial pressure was measured as the latter is partly determined by the blood pressure peaks achieved during systolic cardiac contraction. The body temperature of the animals was maintained at 37°C.

Experimental Protocol

After the animals had been in a stable hemodynamic condition for at least 15 minutes, baseline values of diastolic blood pressure and heart rate were determined. After collection of these data, the animals were divided into 9 groups, which received, respectively, intravenous administration of 20% dimethyl sulfoxide (DMSO) in distilled water (vehicle 1; 1 mL/kg; n = 6), 20% ethanol in distilled water (vehicle 2; 1 mL/kg; n = 6), physiological saline (vehicle 3; 1 mL/kg; n = 5), the 5-HT_{1B} receptor antagonist, SB-224289 (0.3 and 1.0 mg/kg; n = 6 and n = 5, respectively), the 5-HT_{1D} receptor antagonist, BRL-15572 (0.3 and 1.0 mg/kg; n = 6 and n = 5, respectively), the combination of SB-224289 + BRL15572 (0.3 mg/kg, each; n = 5), and the combination of SB-224289 + BRL15572 (0.3 mg/kg, each) + GR-127935 (1 mg/kg; n = 6). After an additional period of 15 minutes, a dose–response curve for the hypotensive effects produced by 5-CT (0.001–10 μg/kg, intravenously) was constructed. In view that the drug combination of SB-224289 + BRL15572 + GR-127935 was the only treatment that significantly inhibited 5-CT–induced hypotensive responses, which suggests the involvement of a mechanism unrelated to 5-$HT_{1B/1D}$ receptors, in 3 additional groups of animals, the effects of intravenous treatments with the 5-HT_{5A} receptor antagonist, SB-699551 (1 mg/kg; n = 7), the 5-HT_6 receptor antagonist, SB-399885 (1 mg/kg; n = 5), and the 5-$HT_{1B/1D/5A/5B/7}$ receptor ligand, ergotamine (0.1 mg/kg; n = 6), on 5-CT–induced responses were analyzed, respectively. Because, among these latter drugs, ergotamine was the only one that caused significant blockade of 5-CT-induced responses, its ability (0.1 mg/kg, intravenously; n = 5) and that of GR-127935 (1 mg/kg, intravenously; n = 5), to block the hypotensive effects produced by the 5-HT_7 receptor agonist, LP-44,[12] was tested. This latter series of experiments was performed to exclude the possibility that the antagonistic action of ergotamine (and that of GR-127935) against 5-CT–induced responses could be accounted for by interaction with 5-HT_7 receptors mediating hypotension in rats.[1,2]

With the lower agonist doses eliciting less than a 30-mm Hg decrease in diastolic blood pressure, full recovery was permitted before the next dose was injected. The depressor effects of higher doses producing a decrease in diastolic blood pressure of more than 30 mm Hg were evaluated by the method of stepwise cumulative administration, increasing each dose by 0.5 log unit increments, with each successive injection given immediately after the preceding dose had attained its maximum effect.[2] Only 1 agonist dose–response curve was obtained per animal.

Analysis by RT-PCR of 5-HT_{5A} and 5-HT_{5B} Receptor mRNAs in Blood Vessels

Aorta and second and third order mesenteric arteries were excised from 4 animals. The potential expression of mRNAs encoding for 5-HT_{5A} and 5-HT_{5B} receptors was measured by RT-PCR. Total RNA was extracted from arteries using TRIzol reagent (Invitrogen, Carlsbad, CA). Reverse transcription was conducted in a reaction volume of 50 μL using 5 μg of total RNA and Super Script III One-Step RT-PCR system (Invitrogen), following manufacturer's protocol in an end point thermal cycler (Gene Cycler; Bio-Rad). For amplification of 5-HT_{5A} receptor cDNA, the sense and antisense primers were 5′-GCA CTA GTC AGT CTT TTC TCT CAG CTT TC-3′ and 5′-ATG GGG GAG ACG CTG TTG GTC TTC CTG GA-3′, respectively, and for amplification of 5-HT_{5B} receptor cDNA, the sense and antisense primers were, 5′-GGC CGC GAG CCG CCC TTC TCT GCC TTC AC-3′ and 5′-CGC CGT CTG CGG CCG AAT CGA AAC TTG-3′, respectively, according to previously reported sequences.[8] The amplification profile involved a cDNA synthesis cycle at 60°C for 30 minutes, a denaturation cycle at 94°C for 2 minutes, and 35 cycles involving denaturation at 92°C for 1 minute, annealing at 60°C for 1 minute, and extension at 74°C for 2 minutes. After amplification, PCR products were electrophoresed on a 1.5% agarose gel for 1 hour at 100 V. Bands were visualized with ethidium bromide by UV light after agarose gel electrophoresis and digitalized; then, their intensities were measured by densitometry using Quantity One 1-D Image Analysis Software (Bio-Rad).

Data Presentation and Statistical Evaluation

All data in the text and figures are presented as the mean ± SEM. The hypotensive activity calculated as $-\log ED_{50}$ (the negative logarithm of the agonist dose producing

50% of the maximum hypotensive response; pD_2) and the E_{max} (the maximum response) were calculated by nonlinear regression analysis using GraphPad Prism version 5.0 (GraphPad Software, San Diego CA); the logarithms of 5-CT doses were entered to perform the analysis. The peak changes in diastolic blood pressure induced by 5-CT were compared by 2-way analysis of variance with agonist dose and pharmacological treatment (ie, vehicle and antagonist drugs) as between-subject independent factors. Comparisons between pD_2 and E_{max} values in vehicle and antagonist-treated animal groups were performed using one-way analysis of variance. Post Hoc Newman–Keuls tests were performed to determine differences. Baseline values of diastolic blood pressure and heart rate before and after treatments were compared using paired *t* tests as appropriate. Statistical significance was accepted at $P < 0.05$ (2-tailed).

Drugs

The drugs used in this study (obtained from the sources indicated) were the following: 5-CT maleate, SB-224289 hydrochloride, BRL15572 hydrochloride, GR-127935 hydrochloride, SB-699551 dihydrochloride, and SB-399885 hydrochloride (Tocris Bioscience, Ellisville, MO); ergotamine tartrate (Sigma-Aldrich, St. Louis, MO); and LP-44 hydrochloride (gift from Dr Marcello Leopoldo, Dipartimento Farmaco-Chimico, Universitá degli Studi di Bari, Italy). The compounds were dissolved in physiological saline (5-CT and GR-127935), 20% DMSO in distilled water (SB-224289, BRL-15572, SB-399885, ergotamine tartrate, and LP-44 hydrochloride), or 20% ethanol in distilled water (SB-699551). The above vehicles had no effect on baseline values of diastolic blood pressure or 5-CT–induced hypotensive responses. However, 20% DMSO and 20% ethanol induced modest although significant decreases in heart rate (see Systemic Hemodynamic Variables in RESULTS).

RESULTS

Systemic Hemodynamic Variables

The effects of pharmacological treatments on baseline values of diastolic blood pressure and heart rate are shown in Table 1. Thus, SB-224289 (0.3 mg/kg, intravenously), BRL-15572 (0.3 mg/kg, intravenously), the combination of SB-224289 + BRL-15572 (0.3 mg/kg, intravenously each), and SB-399885 produced modest but significant increases in diastolic blood pressure. In addition, significant decreases in heart rate were observed subsequent to treatment with 20% DMSO (vehicle 1), 20% ethanol (vehicle 2), BRL-15572, SB-699551, ergotamine, and SB-399885 (Table 1). It should be pointed out that the hemodynamic effects produced by the higher 1 mg/kg dose of SB-224289 and BRL15572 (data not shown) were rather similar to those induced by the lower 0.3 mg/kg dose.

TABLE 1. The effect of intravenous administration of 20% DMSO in distilled water (VEH 1; 1 mL/kg), SB-224289 (SB224; 0.3 mg/kg), BRL-15572 (BRL; 0.3 mg/kg), SB224 + BRL (0.3 mg/kg each), SB224 + BRL (0.3 mg/kg, each) + GR-127935 (GR; 1 mg/kg), SB-399885 (1 mg/kg), 20% ethanol in distilled water (VEH 2; 1 mL/kg), SB-699551 (1 mg/kg), ergotamine (0.1 mg/kg), physiological saline (VEH 3; 1 mL/kg) and GR (1 mg/kg) on baseline values of diastolic blood pressure and heart rate in anesthetized and vagosympathectomized rats

		DBP		HR	
Treatment	**n**	**Before**	**After**	**Before**	**After**
VEH 1	6	101 ± 6	106 ± 6	407 ± 16	387 ± 18*
SB224	6	105 ± 5	110 ± 5*	390 ± 22	377 ± 28
BRL	6	98 ± 3	106 ± 4*	393 ± 14	364 ± 15†
SB224 + BRL	5	103 ± 3	109 ± 3*	422 ± 9	391 ± 10
SB224 + BRL + GR	6	110 ± 5	109 ± 9	412 ± 12	392 ± 16
SB-399885	5	98 ± 4	116 ± 4†	397 ± 14	371 ± 14*
VEH 2	6	103 ± 4	98 ± 5	385 ± 18	361 ± 21†
SB-699551	7	98 ± 2	96 ± 3	379 ± 16	350 ± 17‡
Ergotamine§	11	110 ± 3	110 ± 5	430 ± 9	366 ± 13‡
VEH 3	5	93 ± 5	96 ± 6	369 ± 14	360 ± 12
GR¶	5	103 ± 7	108 ± 5	431 ± 20	421 ± 16

Data are presented as the mean ± SEM.
*$P < 0.05$ versus before.
†$P < 0.01$ versus before.
‡$P < 0.001$ versus before.
§5-CT and LP-44 experiments were pooled.
¶LP-44 experiments.

Effects of Selective 5-HT_{1B} and 5-HT_{1D} Receptor Antagonists on 5-CT–induced Hypotensive Responses

The dose–response curves for 5-CT in animals that received vehicle 1 and single or combined treatment with 5-HT_{1B} and 5-HT_{1D} receptor antagonists are shown in Figure 1. The corresponding parameters of potency (pD_2) and efficacy (E_{max}) are depicted in Table 2. As expected, intravenous administration of 5-CT evoked dose-dependent decreases in diastolic blood pressure. The single treatment with the selective 5-HT_{1B} and 5-HT_{1D} receptor antagonists, SB-224289 and BRL-55172, respectively, completely failed to inhibit 5-CT–induced hypotensive responses (Fig. 1; Table 2); the combined treatment with these drugs produced a small but not significant rightward displacement of the dose–response curve for 5-CT (Table 2). Interestingly, however, administration of GR-127935 to animals that had received SB-224289 and BRL55172 significantly blocked 5-CT–induced responses in a manner closely matching the effect of the single treatment with GR-127935,[4] thus suggesting the involvement of a mechanism unrelated to 5-HT_{1B} and 5-HT_{1D} receptors (Fig. 1). This effect was visualized as a significant decrease of the pD_2 and the corresponding E_{max} value for 5-CT as compared with control values (in vehicle 1–treated animals; Table 2).

Effects of Selective Antagonists at 5-HT_{5A} and 5-HT_6 Receptors and Ergotamine on 5-CT–induced Hypotension

The effects of selective 5-HT_{5A} and 5-HT_6 receptor antagonists and those of ergotamine on 5-CT–induced hypotensive responses are shown in Figure 2, whereas the corresponding pD_2 and E_{max} parameters for 5-CT in control (vehicle-treated) and antagonist-treated animals are depicted in

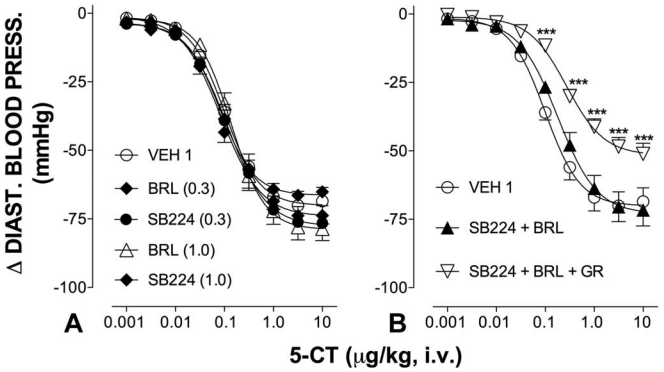

FIGURE 1. Changes in diastolic blood pressure (Δ DIAST. BLOOD PRESS) induced by 5-CT in anesthetized and vagosympathectomized rats pretreated with: A, 20% DMSO in distilled water (vehicle 1 [VEH 1]; 1 mL/kg, intravenously), SB-224289 (0.3 and 1.0 mg/kg, intravenously; SB224 (0.3), and SB224 (1.0), respectively), BRL-15572 (0.3 and 1.0 mg/kg, intravenously; BRL [0.3] and BRL [1.0], respectively); B, Vehicle 1 (VEH 1; 1 mL/kg, intravenously), the combination of SB224 + BRL (0.3 mg/kg, intravenously, each), and the combination of SB224 + BRL (0.3 mg/kg, intravenously, each) + GR-127935 (GR; 1 mg/kg, intravenously). The logarithms of 5-CT doses were entered to perform nonlinear regression analyses (see Methods and Table 2). Each point represents the mean ± SEM of 5–8 experiments. ***$P < 0.001$ versus VEH 1.

Table 2. Thus, pharmacological blockade of 5-HT_{5A} receptors with SB-699551 completely failed to inhibit the hypotensive effects produced by 5-CT (Fig. 2; Table 2). Similarly, pretreatment with the selective 5-HT_6 receptor antagonist, SB-399289, was without effect on 5-CT–induced responses (Fig. 2; Table 2). Interestingly, nevertheless, the 5-$HT_{1B/1D/5A/5B/7}$ receptor ligand, ergotamine, significantly blocked the hypotensive effects induced by 5-CT (Fig. 2; Table 2). Remarkably, the antagonistic action of ergotamine closely resembled that of the combined treatment with SB-224289 + BRL-15572 + GR-127935 (present results) and that of the single treatment with GR-127935,[4] as it inhibited the responses produced by intermediate and higher doses of the agonist only (ie, 0.1–10 μg/kg, intravenously; Fig. 2), thus suggesting a noncompetitive interaction. The effect of ergotamine was visualized as a significant decrease of both pD_2 and E_{max} values for 5-CT (Table 2). The blocking effect produced by the combined treatment with SB-224289 + BRL-15572 + GR-127935 was actually closely similar in magnitude to that of ergotamine (Figs. 1, 2); in fact, no significant differences between the pD_2 and E_{max} values for 5-CT in animals that received both treatments were detected (Table 2). Finally, both ergotamine and GR-127935 (at the same doses used in the experiments with 5-CT) were inactive as antagonists against the hypotensive effects produced by the selective 5-HT_7 receptor agonist, LP-44 (Fig. 2; Table 2).

Expression of 5-HT_{5A} and 5-HT_{5B} Receptor mRNAs in Blood Vessels

A typical experiment showing mRNA expression of 5-HT_{5A} (637 bp long) and 5-HT_{5B} receptors (620 bp long) is shown in Figure 3. To the best of our knowledge, this is the first time these receptors are described in blood vessels. We decided to test expression both in conductance (aorta) and distribution/resistance vessels (second and third order mesenteric arteries) to elucidate the presence of both transcripts in the cardiovascular system. From the low intensity of the bands, it seems that the expression levels are low; the bands, however, correspond to the expected molecular size.

TABLE 2. The effects of pharmacological treatments (intravenously) on the apparent potency ($pD_2 = -\log ED_{50}$) and the maximum hypotensive effect (E_{max}; mm Hg) of 5-CT and LP-44 in anesthetized and vagosympathectomized rats. Treatments, which were given individually or combined as indicated, included: 20% DMSO in distilled water (VEH 1; 1 mL/kg), SB-224289 (SB224; 0.3 mg/kg), BRL-15572 (BRL; 0.3 mg/kg), GR-127935 (GR; 1 mg/kg), SB-399885 (1 mg/kg), ergotamine (0.1 mg/kg), 20% ethanol in distilled water (VEH 2; 1 mL/kg), SB-699551 (1 mg/kg), and physiological saline (VEH 3; 1 mL/kg)

	n	pD_2	E_{max}
5-CT			
VEH 1	6	1.010 ± 0.06	−70.1 ± 5.2
SB224	6	0.925 ± 0.07	−78.2 ± 6.0
BRL	6	1.122 ± 0.06	−66.2 ± 2.2
SB224 + BRL	5	0.780 ± 0.06	−71.6 ± 6.6
SB224 + BRL + GR	6	0.552 ± 0.06*	−52.0 ± 4.0†
SB-399885	5	1.147 ± 0.06	−68.1 ± 2.6
Ergotamine	6	0.549 ± 0.10*	−50.9 ± 4.9†
VEH 2	6	1.056 ± 0.05	−65.2 ± 4.5
SB-699551	7	0.977 ± 0.10	−58.1 ± 5.0
LP-44			
VEH 3	5	2.66 ± 0.03	−86.2 ± 2.2
Ergotamine	5	2.702 ± 0.107	−78.8 ± 4.4
GR-127935	5	2.579 ± 0.05	−85.4 ± 3.9

Results from the experiments with the 1 mg/kg dose of SB224 and BRL (not shown) were closely similar to those with the 0.3 mg/kg dose.
*$P < 0.0001$ versus the corresponding value in VEH 1.
†$P < 0.05$ versus the corresponding value in VEH 1.

DISCUSSION

The results of this work have unraveled a hypotensive mechanism unrelated to 5-HT_{1B} and 5-HT_{1D} (and 5-HT_7) receptors in rats. Indeed, in a previous study,[4] the hypotensive response induced by 5-CT in anesthetized rats was shown to be amenable to blockade not only by the 5-HT_7 receptor antagonist, SB-269970,[13] but also by the 5-$HT_{1B/1D}$ receptor antagonist, GR-127935,[3] thus suggesting the involvement of 5-HT_7 and 5-$HT_{1B/1D}$ receptors. The present experiments using selective antagonists at 5-HT_{1B} (SB-224289) and 5-HT_{1D} receptors (BRL-15572), however, do not support the above possibility, as both drugs were completely inactive as antagonists against 5-CT–induced hypotensive responses. These observations then raised the possibility that another receptor mechanism could be involved, so efforts were made to elucidate the nature of it. From the available affinity data for 5-CT at different 5-HT receptor subtypes, it has been reported that, in addition to displaying high affinity at 5-HT_{1A} (pKi = 8.6), 5-HT_{1B} (pKi = 7.9), 5-HT_{1D} (pKi = 8.1), and 5-HT_7 receptors (pKi = 9.1),[14,15] the compound also displays modest to high affinity at 5-HT_{5A} (pKi = 7.8–7.9)

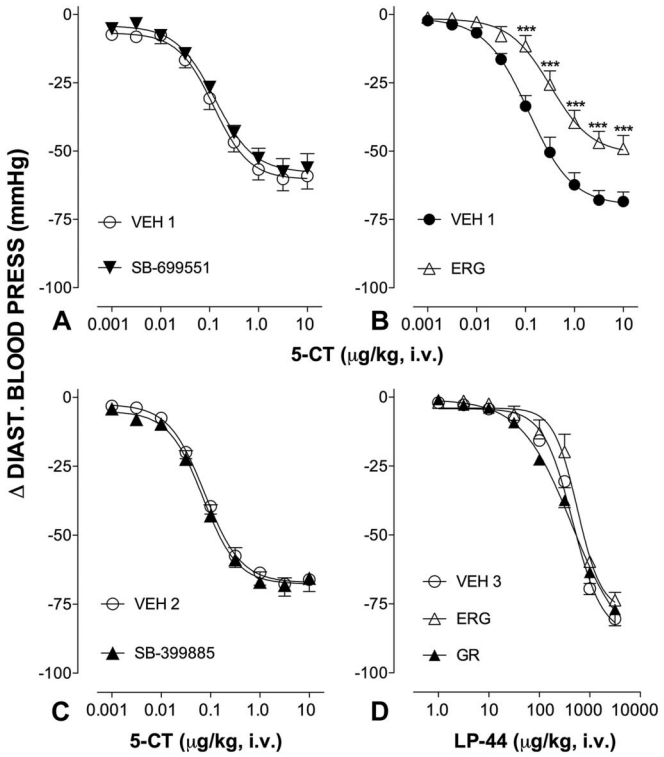

FIGURE 2. Changes in diastolic blood pressure (Δ DIAST. BLOOD PRESS) induced by 5-CT and LP-44 in anesthetized and vagosympathectomized rats pretreated with: A, 20% DMSO in distilled water (vehicle 1 [VEH 1]; 1 mL/kg, intravenously) and SB-699551 (1 mg/kg, intravenously); B, VEH 1 (1 mL/kg, intravenously) and ergotamine (ERG; 0.1 mg/kg, intravenously); C, 20% ethanol in distilled water (vehicle 2, VEH 2; 1 mL/kg, intravenously) and SB-399885 (1 mg/kg, intravenously) and (D) physiological saline (vehicle 3, VEH 3; 1 mL/kg, intravenously), ERG (0.1 mg/kg, intravenously) and GR-127935 (GR; 1 mg/kg, intravenously). The logarithms of 5-CT and LP-44 doses were entered to perform nonlinear regression analyses (see Methods and Table 2). Each point represents the mean ± SEM of 5–7 experiments. ***$P < 0.001$ versus VEH 1.

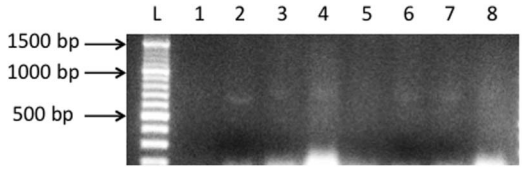

FIGURE 3. Amplification by RT-PCR of 5-HT_{5A} and 5-HT_{5B} receptor cDNAs in aorta and mesenteric arteries. 5-HT_{5A} and 5-HT_{5B} receptor RNAs were isolated and processed as described in Methods. L, 100 bp DNA ladder; 1, negative control; 2, 5-HT_{5A} receptor cDNA in aorta; 3 and 4, 5-HT_{5A} receptor cDNA in mesenteric arteries; 5, negative control; 6, 5-HT_{5B} receptor cDNA in aorta; 7 and 8, 5-HT_{5B} receptor cDNA in mesenteric arteries. The figure shows a typical experiment (n = 4).

and 5-HT_{5B} receptors (pKi = 6.6–7.4).[8–10] This pharmacological profile of 5-CT might therefore suggest that its hypotensive effects in rats could also be mediated, at least in part, by 5-HT_{1A}, 5-HT_{5A}, and/or 5-HT_{5B} receptors. Furthermore, it is interesting that SB-269970, which was reported as a highly selective antagonist at 5-HT_7 receptors, also displays considerable affinity at 5-HT_{5A} receptors (pKi = 7.2)[13] thus implying the possibility that its ability to block 5-CT–induced hypotension could be partly due to interaction with 5-HT_{5A} receptors. However, the role of 5-HT_{1A} receptors seems unlikely as the 5-HT_1 receptor antagonist, propranolol, had no effect on 5-CT–induced hypotensive responses in methoxamine-infused pithed rats,[2] and pretreatment with the selective 5-HT_{1A} receptor antagonist, WAY-100135 (0.5 mg/kg, intravenously), had no effect on 5-CT–induced hypotensive responses in anesthetized rats (our own unpublished observations).

The present experiments showing that the selective 5-HT_{5A} receptor antagonist, SB-699551, failed to modify 5-CT–induced hypotensive responses do not support a role for these receptors. Similarly, the involvement of 5-HT_6 receptors was excluded, as a selective antagonist for them (ie, SB-399885) had no effect on 5-CT–induced hypotension. Interestingly, however, it is worth noting that 5-CT–induced hypotensive responses were inhibited by the 5-$HT_{1B/1D/5A/5B/7}$ receptor ligand, ergotamine,[8–10] in a manner that closely resembled that of the combined treatment with SB-224289 + BRL-15572 + GR-127935 (present results) and that of the single treatment with GR-127935,[4] suggesting a noncompetitive interaction. In this regard, it seems therefore that the hypotensive response induced by 5-CT in rats is mediated by at least 2 mechanisms, namely, 5-HT_7 receptors, which are predominantly activated by lower doses of the agonist,[1] and GR-127935-sensitive receptors[4] that may resemble putative 5-HT_{5B} receptors and which are also sensitive to the blocking action of ergotamine (present study). The activation of both mechanisms by 5-CT may actually underlie the seemingly noncompetitive inhibitory effects of both GR-127935 and ergotamine on 5-CT–induced responses. A more accurate analysis of the antagonist-receptor interaction with the GR-127935–sensitive mechanism would however require the development of a selective agonist, which could theoretically represent a novel antihypertensive approach. The antihypertensive effects induced by chronic administration of 5-CT in spontaneously and deoxycorticosterone acetate–salt hypertensive rats[6,7] do indeed support this possibility.

It could be argued that the cardiovascular changes elicited by the antagonist drugs used in this study (Table 1) may have influenced their potential ability to modify 5-CT–induced responses. However, the decrease in heart rate produced by ergotamine (Table 1) is unlikely to have accounted for its ability to block 5-CT–induced hypotensive responses as diastolic blood pressure is unaffected by heart rate. However, an increase in blood pressure may be expected to increase the scope for agonist-induced hypotensive effects probably promoting their physiological facilitation. Although SB-224289, BRL-15572, and the combined treatment with both drugs produced significant increases in diastolic blood pressure (Table 1), the magnitude of these changes was rather small and therefore unlikely to have markedly prevented their potential ability to block 5-CT–induced hypotensive responses.

It should be emphasized that the doses of the antagonist drugs used in this study are within the dose ranges previously

demonstrated to be effective to block their respective receptor mechanisms in vivo. Thus, SB-224289 (0.3 mg/kg, intravenously) was shown to reverse the inhibitory effects produced by the 5-HT_{1B} receptor agonist, CP-93,129, on the vasodepressor responses induced by electrical stimulation of the spinal cord in rats.[16] In addition, BRL-15572 and SB-224289 (both at 0.1 mg/kg, intravenously) were reported to block significantly naratriptan- and alniditan-induced inhibition of electrically induced activity of trigeminocervical complex neurons in anesthetized cats[17]; also, at the 0.3 mg/kg dose (intravenously), these antagonists were shown to block 5-CT–induced inhibition of sympathetic nerve stimulation-induced tachycardia in rats.[18] That 5-HT_{1B} and 5-HT_{1D} receptors are not involved is further suggested by the fact that a higher dose (1 mg/kg, intravenously) of both SB-224289 and BRL-15572 was completely inactive to inhibit 5-CT–induced hypotensive responses (Fig. 1). Furthermore, the 5-HT_{5A} receptor antagonist, SB-699551, was found to significantly inhibit acoustic startle responses (with and without prepulse conditions) in goldfish (at 0.9 mg/kg, intraperitoneally),[19] increase (in the presence of a 5-HT_{1A} receptor antagonist) the extracellular levels of 5-HT in the guinea pig frontal cortex (at 0.3, 1, and 3 mg/kg, subcutaneously),[11] and decrease conditioned responses during short-term memory trails in the autoshaping associative learning task in rats.[20] These latter observations imply that although species differences have been reported regarding the affinity of SB-699551 at 5-HT_{5A} receptors, with the antagonist exhibiting nearly 100-fold lower affinity at rat, as compared with human and Guinea pig 5-HT_{5A} receptors,[11] the compound is functionally active at doses as low as 0.3 mg/kg in rats.[20] Finally, the selective 5-HT_6 receptor antagonist, SB-399885 (at a dose of 1 mg/kg, intraperitoneally), was shown to induce anxiolytic-like activity in the conflict drinking test in rats.[21]

Taken together, these data suggest that GR-127935 and ergotamine might share a common mechanism of action as to their ability to block noncompetitively 5-CT–induced hypotensive effects in rats. From the above interaction experiments and using exclusion criteria, it could be hypothesized that such a mechanism might correspond to 5-HT_{5B} receptors, but this will require confrontation using a selective antagonist, which has not become available thus far. The potential involvement of putative 5-HT_{5B} receptors in mediating 5-CT–induced hypotension in rats is actually consistent with the affinity profile of 5-CT at 5-HT receptor (sub)types, as the mechanism is activated by the higher doses of 5-CT only, which implies that the agonist may have relatively low affinity at it.

The potential involvement of 5-HT_{5B} receptors in mediating 5-CT–induced hypotensive responses in rats is further strengthened by our RT-PCR studies showing expression of 5-HT_{5A} and 5-HT_{5B} transcripts in rat blood vessels, including aorta and second and third order mesenteric arteries. Although the intensity of the bands obtained was faint, which suggests low expression levels, it is expected that detection may be improved by using other techniques with higher sensitivity.

CONCLUSIONS

This study using selective 5-HT_{1B} and 5-HT_{1D} receptor antagonists have ruled out the involvement of these receptor subtypes in mediating 5-CT–induced hypotensive responses in rats. Similarly, the involvement of 5-HT_{5A} and 5-HT_6 receptors was excluded as pharmacological blockade of these mechanisms with selective antagonist drugs was without effect on 5-CT–induced hypotensive responses. Interestingly, the ability of GR-127935, given both as a single treatment[4] and in combination with SB-224289 and BRL-15572 (present study), to block the above responses was closely mirrored by ergotamine because both drugs produced noncompetitive inhibition of 5-CT-induced hypotension within the same agonist dose range. It should be emphasized however that the suggested involvement of putative 5-HT_{5B} receptors in 5-CT–induced hypotension is based exclusively on exclusion criteria, and that confirmation of this hypothesis will be possible only with the advent of a selective antagonist. Nevertheless, our positive findings on the expression of 5-HT_{5A} and 5-HT_{5B} receptors in rat systemic blood vessels provide support to the above possibility. The antagonistic effects of GR-127935 in other cardiovascular responses and preparations could partly involve the mechanism described here.

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