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Short communication

5-HT$_7$ receptor-mediated dilatation in the middle meningeal artery of anesthetized rats

José A. Terrón *, Esther Martínez-García

Sección Externa de Farmacología, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Apdo. Postal 14-740, Zacatenco 07000, México D.F., Mexico

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Abstract

Topical administration of 5-carboxamidotryptamine (5-CT; 0.01–1000 μM) to the exposed dura mater encephali of anesthetized rats produced decreases in blood pressure and dilatation in the middle meningeal artery. Pretreatment with the 5-HT$_{1B/1D}$ receptor antagonist, *N*-[4-methoxy-3-(4-methyl-1-piperazinyl) phenyl]-2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl) [1,1-biphenyl]-4-carboxamide hydrochloride monohydrate (GR-127935; 1 mg/kg, i.v.), unmasked meningeal dilator responses to lower concentrations of 5-CT, and attenuated those to higher concentrations; GR-127935 also inhibited 5-CT-induced hypotension. The 5-HT$_7$ receptor antagonist, (*R*)-1-{(3-hydroxyphenyl)sulfonyl}-2-{2-(2-(4-methyl-1-piperidinyl) ethyl} pyrrolidine (SB-269970; 1 mg/kg, i.v.), strongly inhibited dilator and hypotensive responses to 5-CT; the combination of GR-127935 + SB-269970 (1 mg/kg, i.v., each) further inhibited meningeal and hypotensive responses. Thus, 5-CT may produce dilatation in the middle meningeal artery via 5-HT$_7$ receptors; complex effects appear to involve 5-HT$_{1B/1D}$ receptors.

Keywords: 5-HT$_7$ receptor; Dilatation; Middle meningeal artery; (Rat)

1. Introduction

Evidence has been provided that cranial vasodilatation may be an important mechanism in migraine (Saxena and Ferrari, 1989; Terrón and Falcón-Neri, 1999) and that attacks could be evoked by a massive release of 5-HT in the brain (Fozard and Kalkman, 1994; Hamel and Saxena, 2000; Humphrey, 1991). However, a key issue to be resolved is how such increased levels of 5-HT could promote dilatation of cranial vessels. In this regard, our group demonstrated for the first time that 5-HT is actually capable of producing vasodilatation in cranial vessels via the activation of 5-HT$_7$ receptors (Terrón and Falcón-Neri, 1999), a finding which could explain, at least in part, the beneficial effects of migraine prophylactic 5-HT receptor antagonists (Terrón, 2002).

Among the various cranial vessels implicated in migraine headache, the middle meningeal artery has been considered as one of the most important since it is the largest artery supplying the dura mater (Castelli and Huelke, 1965), its stimulation leads to activation of second order trigeminal nociceptive pathways in the brain stem of mammals, including cats and monkeys (Hoskin et al., 1999), and it is pain-producing in humans (Ray and Wolff, 1940). Thus, on the basis of the above information it seems reasonable to hypothesize that dilatation in the middle meningeal artery could be evoked by activation of 5-HT$_7$ receptors. Therefore, the purpose of the present study was to investigate the effects of the mixed 5-HT$_{1B/1D}$ and 5-HT$_7$ receptor agonist, 5-carboxamidotryptamine (5-CT), which also displays substantial affinity at 5-HT$_{5A/5B}$ receptors (Matthes et al., 1993), on blood flow and conductance in the middle meningeal artery of anesthetized rats by topically applying the agonist on the exposed dura mater encephali, and characterize the responses by the use of selective antagonists. A preliminary account of this investigation was recently presented in an abstract form (Martínez-García and Terrón, 2006).

* Corresponding author. Tel.: +52 55 5061 3800x5436; fax: +52 55 5061 3394.
E-mail address: jterron@cinvestav.mx (J.A. Terrón).

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2. Materials and methods

Male Wistar rats (250–300 g; $n=27$) were anesthetized with sodium pentobarbitone (50 mg/kg, i.p.) and additional doses (5–10 mg/kg, i.v.) were provided as required. Depth of anesthesia was routinely assessed and held at the level in which noxious stimulation failed to elicit nociceptive motor reflexes or changes of systemic arterial pressure. The trachea was cannulated for ventilation with a Harvard Apparatus rodent ventilator (60 cycles/min; volume 2 ml/100 g body weight) and PE-50 and PE-10 catheters were inserted into the femoral artery and vein for continuous blood pressure recording and drug administration, respectively. Arterial blood pressure was monitored with a TSD104A pressure transducer connected to a Universal Interfase Module (UIM100C; Biopac Systems, Inc., Santa Barbara, California).

For the recording of meningeal blood flow, the head of the animals was fixed in a stereotaxic frame, the skull exposed, and the left parietal bone trepanized with a saline-cooled micromotor drill. A cranial window of 4×6 mm was drilled to expose the dura mater and a needle type probe (tip diameter 0.8 mm) of a laser Doppler flow module coupled to the UIM100C was positioned over a secondary branch of the middle meningeal artery without touching it. A small light-weight polyethylene ring of 1 cm in diameter was placed on the dura around the tip of the flow probe in order to maintain fluids around the probe tip and the dura; placement of this device did not modify baseline blood flow. Diastolic blood pressure, heart rate, and meningeal blood flow were simultaneously recorded in a computer using the Acqknowledge 3.8.0 software (Biopac Systems, Inc., Santa Barbara, California). The body temperature of the animals was maintained at 37–37.5 °C with a thermostatically regulated homeothermic blanket.

After a stabilization period of at least 45 min, baseline values of diastolic blood pressure, heart rate, and meningeal blood flow were determined. After collection of these data, the animals were divided into 4 groups ($n=6$–8 each) which were treated respectively with either vehicle (1 ml/kg, i.v.), the selective 5-$HT_{1B/1D}$ receptor antagonist, *N*-[4-methoxy-3-(4-methyl-1-piperazinyl) phenyl]-2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl) [1,1-biphenyl]-4-carboxamide hydrochloride monohydrate (GR-127935; 1 mg/kg, i.v.), the selective 5-HT_7 receptor antagonist, (*R*)-1-{(3-hydroxyphenyl)sulfonyl}-2-{2-(2-(4-methyl-1-piperidinyl) ethyl} pyrrolidine (SB-269970; 1 mg/kg, i.v.), or the combination of GR-127935+SB-269970 (both at 1 mg/kg, i.v.). The doses of GR-127935 and SB-269970 were high enough to block their respective receptors (Centurion et al., 2004; Sanchez-Lopez et al., 2003). After an additional period of 15–20 min, a concentration-response relationship for the effects of 5-CT (0.01, 0.1, 1, 10, 100 and 1000 μM) on blood pressure, heart rate, and meningeal blood flow was constructed. Each concentration of 5-CT was applied topically in a volume of 100 μl within the ring around the tip of the flow probe and left there for 5–10 min; after this period, 3–4 100 μl washes with physiological saline were made in order to completely remove each concentration of 5-CT. In all cases, the response produced by a concentration of 5-CT was obtained only after arterial pressure and blood flow (heart rate was not modified by 5-CT) had completely returned to the baseline values. The experimental protocols were approved by the Cinvestav-IPN ethics committee.

The drugs used in the present study (obtained from the sources indicated) were the following: 5-CT maleate and GR-127935 (Tocris Bioscience, Ellisville, MO, U.S.A.); and SB-269970 (Sigma-Aldrich, St. Louis, MO, U.S.A.). The compounds were dissolved in distilled water (GR-127935) or physiological saline (5-CT and SB-269970). These vehicles had no effect on baseline blood pressure, heart rate or meningeal blood flow. The doses of GR-127935 and SB-269970 refer to their salts.

All data in the text and figures are presented as the mean± S.E.M. Conductance was calculated from the ratio between meningeal blood flow and diastolic blood pressure. The peak changes of diastolic blood pressure, meningeal blood flow and conductance induced by 5-CT in vehicle- and antagonist-treated animals were compared by two-way analysis of variance for repeated measures followed by a Newman–Keuls test to determine differences. Baseline values of the above variables (and heart rate) between vehicle- and antagonist-treated animals were compared using an unpaired *t* test. Statistical significance was accepted at $P \leq 0.05$ (two-tailed).

Table 1
The effect of increasing concentrations of 5-CT on diastolic blood pressure (DBP) and meningeal blood flow (MBF) in anesthetized rats pretreated either with vehicle (physiological saline; 1 ml/kg, i.v.), GR-127935 (GR; 1 mg/kg, i.v.), SB-269970 (SB; 1 mg/kg, i.v.), or GR-127935+SB-269970 (GR+SB; 1 mg/kg, i.v. each)

Treatment	Parameter	5-CT concentration (μM)					
		0.01	0.1	1	10	100	1000
Vehicle ($n=8$)	DBP	−0.2±1	2±1	−1±2	−2±2	−27±7[a]	−51±7[a]
	MBF	−2±1	1±2	−1±1	−0.1±4	−17±7[a]	−20±9[a]
GR ($n=7$)	DBP	−0.4±1	−0.1±1	1±1	−2±1	−15±4[a b]	−35±3[a b]
	MBF	8±3	6±2[a]	5±1[a]	7±3[a]	−8±5	3±12
SB ($n=6$)	DBP	−0.4±2	−2±1	1±1	−2±1	−3±1[b c]	−15±4[a b c]
	MBF	−1±2	−1±1	−0.4±1	−2±3	−0.3±2[b]	−1±6[b]
GR+SB ($n=6$)	DBP	−1±1	−1±0.3	−1±1	−1±1	3±2[b c]	−4±1[a b c d]
	MBF	1±1	−1±1	−3±2	3±3	2±2[b]	1±2[b]

5-CT was administered as a topical solution (100 μl) directly on the exposed dura mater encephali. Data are presented as the percent change from baseline values (mean±S.E.M.).
[a]$P<0.05$ vs baseline; [b]$P<0.05$ vs vehicle; [c]$P<0.05$ vs GR; [d]$P<0.05$ vs SB.

3. Results

The baseline values of diastolic blood pressure, heart rate, meningeal blood flow and conductance were, respectively, 108±3 mm Hg, 320±12 beats/min, 907±88 blood perfusion units (BPU) and 8.7±0.8 BPU/mm Hg (n=27). These variables were not significantly changed 15–20 min after intravenous administration of vehicle or antagonist drugs, which produced either a transient decrease (GR-127935) or a small increase (SB-269970) in blood pressure and blood flow (not shown). As depicted in Table 1, topical administration of 5-CT produced concentration-dependent decreases in diastolic blood pressure and blood flow in the middle meningeal artery. These changes were reflected as increases in conductance in the middle meningeal artery at the highest concentrations of the agonist (Fig. 1A). In contrast, 5-CT had no effect on heart rate (data not shown).

In the interaction experiments, pretreatment with GR-127935 not significantly attenuated the maximum dilator response to 5-CT, and unmasked modest increases in blood flow (Table 1) and conductance (Fig. 1B) to low and intermediate concentrations (0.01 to 10 μM) of the agonist; also, GR-127935 significantly inhibited the hypotensive responses produced by high concentrations (100 and 1000 μM) of 5-CT (Table 1). On the other hand, pretreatment with SB-269970 strongly blocked meningeal vasodilator (Fig. 1C) and hypotensive responses (Table 1) produced by 5-CT at 100 and 1000 μM; unlike the results in GR-127935-pretreated animals (Fig. 1B; Table 1), no increases in blood flow (Table 1) or conductance (Fig. 1C) to low and intermediate concentrations of 5-CT were observed in animals that received SB-269970. Finally, the administration of both antagonists, i.e. GR-127935+SB-269970, further inhibited the meningeal vasodilator (Fig. 1D) and systemic hypotensive responses (Table 1) produced by 5-CT as compared to the responses observed in animals that received a single treatment with GR-127935 (Fig. 1B) or SB-269970 (Fig. 1C) (see also Table 1).

4. Discussion

It has been demonstrated that activation of the 5-HT_7 receptor mediates dilatation in cranial vessels, including the dog basilar and middle cerebral artery (Terrón and Falcón-Neri, 1999), the porcine pial vein (Ishine et al., 2000) and the canine carotid circulation (Villalón et al., 1997). On the basis of these and other observations, it was postulated that the 5-HT_7 receptor

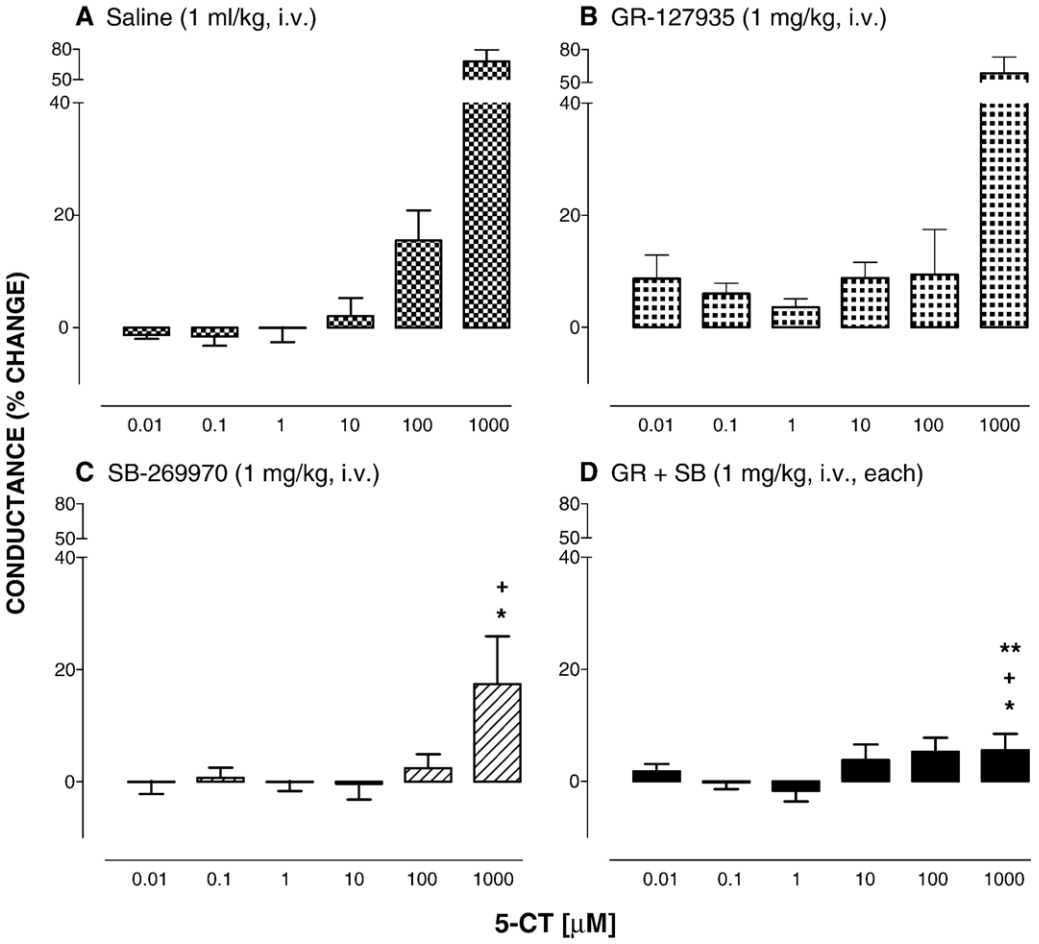

Fig. 1. Effect of the topical administration of 5-CT on conductance in the middle meningeal artery of anesthetized rats pretreated either with physiological saline (A), GR-127935 (B), SB-269970 (C), or GR-127935+SB-269970 (GR+SB; D). Each bar represents the mean±S.E.M. of 6-8 experiments. *P<0.05 vs vehicle; ^{+}P<0.05 vs GR-127935; **P<0.05 *vs* SB-269970.

could be a target of endogenous 5-HT to promote cranial vasodilatation during migraine attacks (Terrón and Falcón-Neri, 1999; Terrón, 2002). The present study in anesthetized rats provides evidence that such a dilator mechanism involving the 5-HT_7 receptor may operate in the middle meningeal artery and therefore be of relevance in the pathogenesis and treatment of migraine. These data also reveal a possible role of 5-$HT_{1B/1D}$ receptors mediating dilatation of systemic vessels and, perhaps also, complex effects involving constriction and dilatation in the middle meningeal artery.

In view that the blood flow responses produced by 5-CT in the middle meningeal artery were accompanied by important decreases in diastolic blood pressure, which have previously been shown to be mediated by 5-HT_7 receptors (De Vries et al., 1997; Terrón, 1997), vascular conductance (i.e. the reciprocal of resistance) was calculated in order to look at the direct effects of 5-CT in the middle meningeal artery without interference of the perfusion pressure changes derived from the decreases in blood pressure. Thus, concentration-dependent increases in conductance in the middle meningeal artery were observed after local administration of 5-CT (Fig. 1A). Since autoregulation has been reported to operate in the middle meningeal artery of cats (Michalicek et al., 1996), the possibility exists that the changes in conductance in the middle meningeal artery in our experiments may have been due in part to autoregulation. It is to be remarked in this regard that in some experiments (i.e. GR-127935-pretreated rats) 5-CT produced significant increases in blood flow without inducing significant changes in blood pressure (see Table 1) thereby implying that the agonist did produce direct vasodilator effects in the middle meningeal artery. Furthermore, preliminary experiments in our laboratory have shown that decreases in blood pressure (induced by blood loss from the femoral artery), similar to those induced by 5-CT in the present experiments, produced in most cases decreases in blood flow and no changes in conductance (data not shown); additional experiments however are in progress to further analyze this issue.

Regarding the role of 5-HT receptor mechanisms regulating blood flow in the middle meningeal artery, a previous study in anesthetized rats demonstrated that topical application of a very high concentration of sumatriptan (40 mM), as well as the i.v. administration of a very high dose of 3-(1,2,5,6-tetrahydropyrid-4-yl)pyrrolo[3,2-b]pyrid-5-one (CP-93129; 23 μmol/kg), both of which are agonists at 5-$HT_{1B/1D}$ receptors, produced modest decreases of blood flow in the middle meningeal artery, whereas 5-HT (at 1 mM) had no effect (Messlinger et al., 1997). Despite the fact that these effects of sumatriptan and CP-93129 were not characterized with selective antagonists, these data might imply activation of 5-$HT_{1B/1D}$ receptors mediating vasoconstriction in the rat middle meningeal artery. The present observations showing that pretreatment with GR-127935 unmasked increases in blood flow (Table 1) and conductance (Fig. 1B), which were apparently antagonized by co-treatment with SB-269970 (see Table 1 and Fig. 1D), suggest that at least two mechanisms are involved in the effects of 5-CT in the middle meningeal artery: 1) vasoconstriction mediated by 5-$HT_{1B/1D}$ receptors; and 2) vasodilatation mediated by 5-HT_7 receptors. Since pretreatment with GR-127935 attenuated the dilator responses produced by 5-CT (Fig. 1B), and no vasoconstrictor effects were unmasked by SB-269970 (as one could have expected from the result in GR-127935-pretreated animals), it could be hypothesized that 5-$HT_{1B/1D}$ receptors also mediate dilatation in the middle meningeal artery. In support of this hypothesis, a role for 5-$HT_{1B/1D}$ receptors in mediating vasodilatation has been reported in the rat middle cerebral artery (Hansen-Schwartz et al., 2003) and human cerebral intracortical arteries (Elhusseiny and Hamel, 2001), an effect that primarily involves nitric oxide generation. In spite of the above, further experiments are required to convincingly establish whether or not 5-$HT_{1B/1D}$ receptors mediating vasoconstriction and/or vasodilatation exist in the middle meningeal artery of rats.

A final remark is on the effect of the 5-HT receptor antagonists on 5-CT-induced hypotensive responses (Table 1). Thus, as expected, blockade of 5-HT_7 receptors with SB-269970 strongly inhibited 5-CT-induced decreases in blood pressure (Table 1). Interestingly, it was additionally found that blockade of 5-$HT_{1B/1D}$ receptors with GR127935 significantly decreased the hypotensive effects of 5-CT as well (Table 1). These observations may therefore suggest that, in addition to 5-HT_7 receptors (De Vries et al., 1997; Terrón, 1997), 5-$HT_{1B/1D}$ receptors also mediate dilatation of systemic resistance vessels in rats.

In conclusion, the present study suggests that 5-CT may produce modest dilator responses in the middle meningeal artery of anesthetized rats via activation of 5-HT_7 receptors. These data might provide support to the contention that 5-HT promotes cranial dilatation and migraine via activation of 5-HT_7 receptors (Terrón and Falcón-Neri, 1999; Terrón, 2002).

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