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Vasoconstrictor 5-HT receptors in the smooth muscle of the rat middle cerebral artery

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ABSTRACT

Serotonin (5-HT) can constrict cerebral arteries via activation of 5-HT_{1B} and 5-HT_{2A} receptors. Our goal was to reveal the importance and relative contribution of the two 5-HT receptor subtypes to the serotonin-induced vasoconstriction in the rat middle cerebral artery. The vasoconstrictor effects of 5-carboxamidotryptamine, sumatriptan and 5-HT were measured without and with pre-treatment with SB 216641 (5-HT_{1B} antagonist), or ritanserin, (5-HT_{2A} antagonist), in endothelium-denuded arteries, *in vitro*. All agonists caused vasoconstrictions. The order of potency (EC_{50}) of the compounds was: 5-carboxamidotryptamine (14 ± 3 nM) > 5-HT (270 ± 30 nM) > sumatriptan (5.8 ± 1.9 μM). The concentration–response curve of 5-carboxamidotryptamine resembled the sum of two sigmoid curves (EC_{50} 14 ± 3 nM and 15 ± 7 μM), and SB 216641 and ritanserin antagonized its low and high concentration components, respectively. Vasoconstrictions evoked by 5-HT at low and high concentrations were also fully antagonized by SB 216641 and ritanserin, respectively. Sumatriptan constricted the middle cerebral artery exclusively via 5-HT_{1B} receptors. The efficacy of 5-carboxamidotryptamine and sumatriptan was low in comparison to the maximum contractile force elicited by 120 mmol/l KCl, reaching only 18–23% for 5-HT_{1B} and 14% for 5-HT_{2A} receptor activation. In conclusion, 5-HT produced small vasoconstrictions in the rat middle cerebral artery that were mediated by 5-HT_{1B} receptors with high potency and by 5-HT_{2A} receptors with low potency. Thus, 5-HT may have a minor physiological role in blood flow regulation via 5-HT_{1B} receptor activation while 5-HT_{2A} receptors seem to have a pathophysiological role in this vessel.

1. Introduction

The serotonin-induced vasoconstriction is mostly mediated via the activation of 5-HT_{1B} and/or 5-HT_{2A} receptors located on the smooth muscle cells of various brain and peripheral vessels (Kaumann and Levy, 2006; Monassier et al., 2010; Razzaque et al., 2002). In general, vasoconstriction mediated via 5-HT_{2A} receptor activation is mainly characteristic for vascular smooth muscle responses in the periphery, while 5-HT_{1B} receptors have more prominent role in cerebral vessels (Kaumann and Levy, 2006). The magnitude of vascular responses to serotonin (5-HT) can be altered in pathophysiological conditions. In peripheral vessels the 5-HT_{1B} receptor-induced vasoconstrictor response greatly increases in different disease states (Kaumann and Levy, 2006; MaassenVanDenBrink et al., 2008; MacLean et al., 1996). Upregulation of 5-HT_{1B} receptors may occur in cerebral vessels particularly upon subarachnoid hemorrhage (Hansen-Schwartz et al., 2003a; Larsen et al., 2011), ischemia (Vikman and Edvinsson, 2006; Maddahi and Edvinsson, 2008; Ahnstedt et al., 2011) and also in hypertension (Nishimura, 1996).

There are some previous findings to show that sumatriptan, a 5-$HT_{1B/1D}$ agonist can induce either weak or strong vasoconstrictions in cerebral vessels (Edvinsson et al., 2005; Mehrotra et al., 2006; Nagai et al., 2007; Razzaque et al., 2002; Roon et al., 1999; Silva and Ribeiro, 2012). It is not entirely clear if these contradictory findings indicate species differences or are due to other reasons. It is well known that receptor affinity of compounds can be hugely species dependent (Braden et al., 2006). Some compounds can produce effects with diverse affinities in various tissues of the same species known as tissue selectivity (Sarsero et al., 1998). Further, the pattern of intracellular messenger systems activated by a compound can also be tissue specific termed as functional selectivity (Urban et al., 2007). Therefore, in drug development it is best to characterize the potency and efficacy of a compound in the targeted tissue *in vitro* before its pharmacological effects or side effects are tested in animals, *in vivo*. For that reason a suitable model would be useful for testing the vasoconstrictor effects of serotonergic drugs and drug candidates in rat cerebral arteries. Therefore, the aim of our study was to reveal the functional role of the 5-HT_{1B} and 5-HT_{2A}

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receptor subtypes in the 5-HT-induced vasoconstriction in the endothelium-denuded middle cerebral artery of the rat, *in vitro*. Vasoconstrictions produced by 5-HT, sumatriptan and 5-carboxamidotryptamine were measured with and without pre-treatment with a 5-HT_{1B} or a 5-HT_{2A} receptor antagonist.

2. Materials and methods

2.1. Animals

Male Spraque-Dawley rats (Charles River Hungary, 260–300 g) were housed in standard conditions, and food and water were available *ad libitum* at all times. The local Animal Ethics Committee approved all experiments. The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996), and care and use of the experimental animals were in accordance with the 86/609/EEC directive.

2.2. Preparation of segments

Anaesthetized rats were infused with physiological saline via the left ventricle (8–10 ml) to remove the blood from the cerebral vessels, and then they were decapitated. The brain was quickly removed and put into an organ chamber containing carbogenated Krebs solution at 3–4 °C, and then the proximal parts of the right and left middle cerebral arteries were removed. The middle cerebral artery was cut into exactly 2 mm-long ring segments. The endothelium was removed mechanically by a thin thread. The right and left middle cerebral artery were studied randomly. The rings were threaded on two 40-μm-diameter tungsten wires under a preload of 1.2–1.5 mN according to the method described by Mulvany and Halpern (1977). This tension mimics the physiological blood pressure in rats (100 mmHg). The organ chamber (6 ml, Myo-01, Experimetria) was perfused (Minipuls 3, Gilson) with Krebs solution (1 ml/min) at 37 °C, and bubbled with carbogen (95% O_2–5% CO_2). After a 60–70-min incubation period changes in isometric tension were measured by a force displacement transducer (FSG-01, Experimetria) and analyzed by Isosys 1.0 software program.

2.3. Experimental protocol

The vessels were primed with 0.1 μmol/l serotonin 2–3 times to develop their pharmacological sensitivity. The removal of endothelium was verified by lack of response to 10^{-5} mol/l acetylcholine in rings pre-constricted with 3 μmol/l prostaglandin $F_{2\alpha}$. The concentration–response curves for various 5-HT agonists (at concentrations between 1 nmol/l and 10 μmol/l) were obtained by cumulative application. Constrictions evoked by 5-carboxamidotryptamine were studied separately at low (1 nM–1 μM) and high concentrations (1 μM–100 μM) in order to avoid desensitization. The effects of various 5-HT agonists in the absence (control) or in the presence of antagonists were studied in separate groups of tissues. All antagonists were applied 30 min before treatments with the agonists. At the end of experiment the maximum contractile response of the artery was obtained by applying a modified Krebs solution containing 120 mmol/l K^+.

2.4. Drugs and solutions

Krebs solution of the following composition was used (in mmol/l): NaCl 118, KCl 4.7, $NaHCO_3$ 27.2, glucose 11.1, $MgSO_4$ 1.2, KH_2PO_4 1.0, $CaCl_2$ 2.6 (pH=7.4). Krebs solution containing 120 mmol/l KCl had the same composition except that NaCl was exchanged for KCl. Serotonin creatinine sulfate, 8-hydroxy-2-(di-n-propylamino)tetralin and ritanserin were purchased from Research Biochemicals International (Natick, USA), 5-carboxamidotryptamine and SB 216641 (N-[3-(2-dimethylamino)ethoxy-4-methoxyphenyl]-2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)-(1,1′-biphenyl)-4-carboxamide were purchased from TOCRIS (Avonmouth, Bristol, U.K.), sumatriptan (Imigran 6 mg/0.5 ml injections) was made by Glaxo Wellcome Operations, U.K., and acetylcholine and prostaglandin $F_{2\alpha}$ were obtained from Sigma-Aldrich Co. (St. Louis, USA).

2.5. Statistical analysis

Data are given as contractile force in mN. EC_{50} and midpoint slope values (Hill slope) were determined by nonlinear curve fitting (GraphPad Prizm software, La Jolla, California, USA). Potencies of antagonists are given as dose ratio (DR) values (EC_{50} of the agonist in the presence of antagonist/EC_{50} of the agonist in the absence of antagonist) expressing the rightward shift of the concentration–contraction curves of the agonists in the presence of various concentrations of an antagonist.

3. Results

3.1. Agonist potencies of various serotonergic agonists

All agonists constricted the middle cerebral artery with strikingly different potencies and maximum contractile forces. According to the preliminary results with 5-carboxamidotryptamine the effect of this agonist could be described as the sum of two separate sigmoid curves in low (1 nM–1 μM) and high (1 μM–100 μM) concentration ranges. Therefore, in order to avoid desensitisation the vasoconstrictor effects of 5-carboxamidotryptamine were studied separately at low and high concentrations. Based on their EC_{50} values the order of potency of the test compounds was 5-carboxamidotryptamine (1st phase) > 5-HT > sumatriptan > 5-carboxamidotryptamine (2nd phase) (Table 1). 8-Hydroxy-2-(di-n-propylamino)tetralin, a non-selective 5-HT_{1A} receptor agonist was ineffective up to the concentration of 3 μM (data not shown).

3.2. Pharmacological characterization of the two phases of the 5-carboxamidotryptamine-evoked vasoconstrictions

The 1st phase (1 nM–1 μM) of the 5-carboxamidotryptamine-induced contractions could be characterized by high potency (EC_{50}: 14 ± 3 nM, n=13) but low maximum constriction (0.30 mN), and it was concentration-dependently (0.1, 0.3 and 1 μM; n=8/group) antagonized by SB 216641, a selective 5-HT_{1B} antagonist (Schlicker et al., 1997). SB 216641 at 1 μM almost completely depressed the 1st phase of the 5-carboxamidotryptamine-produced vasoconstrictions. In the 2nd phase (1 μM–100 μM) 5-carboxamidotryptamine elicited a small additional increase in the maximum contractile force (0.53 mN) with a much lower EC_{50} value

Table 1
Agonist potency of 5-HT and some serotonergic agonists in the middle cerebral artery of the rat, *in vitro*.

Compound	EC_{50}
5-HT	270 ± 30 nM (n=15)
5-Carboxamidotryptamine (phase 1)	14 ± 3 nM (n=13)
5-Carboxamidotryptamine (phase 2)	15 ± 7 μM (n=13)
Sumatriptan	5.8 ± 1.9 μM (n=12)