European Journal of Pharmacology 714 (2013) 65–73

Contents lists available at SciVerse ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Cardiovascular pharmacology

Peripheral 5-HT_{1D} and 5-HT_7 serotonergic receptors modulate sympathetic neurotransmission in chronic sarpogrelate treated rats

José Ángel García-Pedraza [a], Mónica García [a], María Luisa Martín [a], Jesús Gómez-Escudero [b], Alicia Rodríguez-Barbero [b], Luis San Román [a], Asunción Morán [a,*]

[a] Laboratorio de Farmacología, Departamento de Fisiología y Farmacología, Facultad de Farmacia, Universidad de Salamanca, 37007 Salamanca, Spain
[b] Departamento de Fisiología y Farmacología, Instituto "Reina Sofía" de Investigación Nefrológica, Universidad de Salamanca, 37007 Salamanca, Spain

ARTICLE INFO

Article history:
Received 4 March 2013
Received in revised form
21 May 2013
Accepted 29 May 2013
Available online 11 June 2013

Keywords:
5-HT
5-CT
Sarpogrelate
5-HT_2
5-HT_{1D}
5-HT_7 receptors
Prejunctional sympathoinhibition
Pithed rat

ABSTRACT

5-HT_2 receptor activation induces vasoconstriction, hypertension and platelet aggregation; therefore, its blocking may be useful in cardiovascular diseases, probably due to alterations in the modulation of serotonergic system. The aim of this study was to evaluate whether 5-HT_2 receptor blockade changes serotonergic modulation of sympathetic neurotransmission in pithed rats. Serotonergic modulation of sympathetic neurotransmission was investigated in Wistar rats treated with sarpogrelate, a 5-HT_2 receptor antagonist, during 14 days (30 mg/kg/day). After central nervous system destruction, we conducted electrical stimulation throughout the spinal cord flow to study the 5-HT-related products action on adrenergic system. 5-Hydroxytryptamine exerted inhibition of sympathetic outflow in sarpogrelate-treated pithed rats. This effect was mimicked and enhanced by 5-CT (5-$HT_{1/7}$ receptor agonist). L-694,247 and AS-19, 5-HT_{1D} and 5-HT_7 receptor agonists respectively, reproduced this action. Pretreatment with LY310762+SB258719 (5-HT_{1D} and 5-HT_7 receptor antagonists, respectively) completely abolished 5-CT inhibitory action. The nature of this action was prejunctional since these agonists did not modify the pressor responses induced by exogenous noradrenaline. Western Blot analysis confirmed a higher expression of 5-HT_{1D} receptors in sarpogrelate-treated rats. Experimental 5-HT_2 receptor blockade induces changes in the 5-HT receptors involved in the serotonergic inhibition of sympathetic-induced pressor responses. Prejunctional activation of 5-HT_{1D} and 5-HT_7 receptors induces a significantly higher serotonergic inhibition on adrenergic neurotransmission in sarpogrelate-treated pithed rats. The antagonism of 5-HT_2 receptors produces an enhancement of serotonergic sympathoinhibitory effect, which may explain the beneficial effects of this blockade in cardiovascular disorders where 5-hydroxytryptamine plays a crucial role.

1. Introduction

5-Hydroxytryptamine (5-HT) produces a wide array of activities on many organ systems including the central nervous, gastrointestinal and cardiovascular systems (Jonnakuty and Gragnoli, 2008). Biological effects of 5-HT at these levels are mediated by seven major families of 5-HT receptors (from 5-HT_1 to 5-HT_7), which, in turn, contains at least 14 different 5-HT receptor subtypes, each encoded by a separate gene and with different roles in cardiovascular system in humans (Kaumann and Levy, 2006). Among them, G_q-coupled 5-HT_2 receptors, located in platelets and vascular smooth muscle cells, are mainly associated with the regulation of cardiac and vascular events (Doggrell, 2003).

Studies conducted under different experimental conditions have demonstrated the existence of regulatory 5-HT receptors on postganglionic and possibly preganglionic sympathetic nerve terminals in rats *in vitro* and *in vivo* (Villalón et al., 1995a, 1995b) as well as in cats (Jones et al., 1995). Our research team has also demonstrated that 5-hydroxytryptamine exerts an inhibitory action on sympathetic neurotransmission in pithed rats by activation of 5-HT_1 receptors (Moran et al., 1994, 1998). The induction of diabetes and the duration of this pathology modify the receptor type/subtype involved (Garcia et al., 2005, 2006; Moran et al., 2010; Restrepo et al., 2012). On the other hand, 5-HT_2 receptor activation is involved in sympathetic stimulation that leads to vasoconstriction and increases blood pressure (BP) and heart rate (HR) (Ramage, 2001; Cote et al., 2004; Ramage and Villalón, 2008); hence, they are considered as sympathoexcitatory receptors.

* Correspondence to: Laboratorio de Farmacología, Departamento de Fisiología y Farmacología, Facultad de Farmacia, Universidad de Salamanca, Campus Miguel de Unamuno, ES-37007 Salamanca, Spain. Tel.: +34 923 294530; fax: +34 923 294515.
E-mail address: amoran@usal.es (A. Morán).

0014-2999/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ejphar.2013.05.045

Since 5-HT_2 receptor blockade has been found to have protective effects in the treatment of peripheral vascular occlusive diseases (Igarashi et al., 2000), thromboangitis obliterans (Nakamura et al., 2001), effort angina (Tanaka et al., 1998), congestive heart failure (Brasil et al., 2002), atherosclerosis (Hayashi et al., 2003), restenosis after coronary stenting (Fujita et al., 2003), pulmonary hypertension (Miyata et al., 2000) and ischemia/reperfusion induced myocardial injury (Temsah et al., 2001), it seems likely that the antagonism of 5-HT_2 receptor may be a therapeutical approach to ameliorate cardiovascular abnormalities due to 5-HT_2 receptor activation. Thus, particular emphasis is currently put on the role of 5-HT in heart diseases and blood vessels disorders and on the development of 5-HT_2 receptor antagonists as potential drugs with clinical interest for the treatment of 5-HT-related cardiovascular complications.

In this line, our group investigates the possible effect of 5-HT_2 receptor antagonism in cardiovascular function. The blockade of 5-HT_2 receptors may exhibit its beneficial effect by modulating serotonergic action on sympathetic neurotransmission. Based on this fact, our study was conducted to determine the effects of 5-HT on pressor responses induced by stimulation of sympathetic vasopressor outflow in sarpogrelate-treated pithed rats. We analyzed the possible changes evoked by chronic 5-HT_2 receptor blockade in vascular reactivity to 5-HT in comparison with non-treated pithed rats (current data; Moran et al., 1994, 1998). We also determined the 5-HT receptor type/subtypes and the pre and/or postjunctional nature involved in these changes.

2. Material and methods

2.1. Ethical approval of the study protocol

Housing conditions and experimental procedures were in accordance with regulations provided by the European Union on the use of animals for scientific purposes (86/609/EEC, Article 5, Appendix II). This was enacted by Spanish legislation on 14 March 1988 (R.D.223/1988).

2.2. Drugs used

The drugs used in the present study were as follows: Sarpogrelate hydrochloride was from ABBLIS Chemical LLC (Houston TX, US); Heparin sodium was from Roche (Madrid, Spain); Pentobarbital sodium, 5-HT, d-tubocurarine hydrochloride, 7-Trifluoromethyl-4-(4-methyl-1-piperazinyl)pyrrolo[1,2-a]-quinoxaline dimaleate (CGS-12066B), 1-phenylbiguanide (1-PBG) and noradrenaline bitartrate were from Sigma-Aldrich (St Louis, MO, USA); Atropine sulfate from Scharlau (Barcelona, Spain); 5-carboxamidotryptamine maleate (5-CT), 8-hydroxy-2-dipropylaminotetralin hydrobromide (8-OH-DPAT), 2-[5-[3-(4-methylsulfonylamino)benzyl-1,2,4-oxadiazol-5-yl]-1H-indol-3-yl]ethanamine (L-694,247), (2S) (+)-5-(1,3,5-trimethylpyrazol-4-yl)-2-(dimethylamino)tetralin (AS-19), 3-methyl-N-[(1R)-1-methyl-3-(4-methyl-1-piperidinyl)propyl]-N-methylbenzenesulfonamide hydrochloride (SB258719) and 1-[2-[4-(4-Fluorobenzoyl)-1-piperidinyl]ethyl]-1,3-dihydro-3,3-dimethyl-2H-indol-2-one hydrochloride (LY310762) were from Tocris Bioscience (Bristol, UK).

All drugs were dissolved in distilled water at the time of experimentation, with the exception of AS-19 (dissolved in ethanol 12.5%).

2.3. Animal preparation

Male Wistar rats (240–300 g) were used in the present study ($n=200$). Rats were kept and supplied by the Animal House of the Faculty of Pharmacy of the University of Salamanca (P.A.E.-SA001; Salamanca, Spain).

Rats were maintained on tap-water and regular food *ad libitum* for 14 days. Sarpogrelate was administered dissolved in drinking water (30 mg/kg/day, p.o.) (Takishita et al., 2004; Kobayashi et al., 2008). A second group was maintained under the same conditions for the same time period to serve as age-matched controls. Body weight, systolic BP and HR were determined before, at 7 and 14 days of treatment. BP and HR were measured in awake rats periodically 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. Values were considered valid if five consecutive measurements did not differ by 10 mm Hg.

Animals were anaesthetized with sodium pentobarbital (60 mg/kg, i.p.), and had their trachea cannulated. Rats were pithed and artificially ventilated. Jugular veins were cannulated for the infusion/administration of agonists/antagonists and the left carotid artery was also cannulated to record BP and HR (using a pressure transducer connected to an e-corder 410 amplifier (Model ED410, Cibertec, Spain), using Chart™ and Scope™ software). The entire sympathetic outflow from the spinal cord was stimulated. Before electrical stimulation, the animals were intravenously treated with heparin (1000 UI/kg), d-tubocurarine (2 mg/kg) and atropine (1 mg/kg) (Gillespie and Muir, 1967; Garcia et al., 2005, 2006; Restrepo et al., 2012).

2.4. Experimental protocols

After reaching a stable haemodynamic condition for ≥ 10 min, baseline values of mean blood pressure (MBP) and HR were determined (68.0 ± 1.06 mm Hg and 327.0 ± 4.0 bpm, respectively). Then, sympathetic vasopressor outflow was stimulated by applying trains of 25 s, consisting of monophasic pulses of 1 ms duration and supra-maximal intensity (15 ± 3 V) at increasing frequencies (0.1, 0.5, 1 and 5 Hz) (Moran et al., 1998).

Thus, the control stimulation–response curve (S–R curve E0) was completed in ~20 min. At this point, rats were divided into five groups:

The first group (control/non-treated group; $n=30$) received a continuous intravenous infusion (using the Harvard model 122 pump, Cibertec) of one of the following: saline solution (1 ml/h, control group for all the agonist treatments; $n=5$), 5-HT (20 μg/kg/min; $n=5$), or the selective 5-$HT_{1/7}$ receptor agonist, 5-CT (5 μg/kg/min; $n=5$), or L-694,247, a selective 5-HT_{1D} receptor agonist (1 μg/kg/min; $n=5$), or 8-OH-DPAT, a selective 5-HT_{1A} receptor agonist (10 μg/kg/min; $n=5$) or AS-19, a selective 5-HT_7 receptor agonist (5 μg/kg/min; $n=5$). After 10 min of the corresponding infusion, two new S–R curves (E1 and E2) were obtained as described above for the S–R curve E0.

In the first sarpogrelate-treated group ($n=80$), each rat received a continuous intravenous infusion of one of the following: saline solution (1 ml/h, control group for all the agonist treatments; $n=5$), 5-HT (5, 20 or 80 μg/kg/min; $n=5$ for each dose), the selective 5-$HT_{1/7}$ receptors agonist, 5-CT (0.005, 0.1, or 5 μg/kg/min; $n=5$ for each dose), the selective 5-HT_{1A} receptor agonist, 8-OH-DPAT (5 μg/kg/min; $n=5$), the selective rodent 5-HT_{1B} receptor agonist, CGS-12066B (5 μg/kg/min; $n=5$), the selective 5-HT_{1D} receptor agonist, L-694,247 (0.1, 5 or 10 μg/kg/min; $n=5$ for each dose), the selective 5-HT_3 receptor agonist, 1-PBG (5 μg/kg/min; $n=5$) or the selective 5-HT_7 receptor agonist, AS-19 (0.1, 5, or 10 μg/kg/min; $n=5$ for each dose). The S–R curves were constructed in the same conditions as for the infusions in the non-treated group.

The second sarpogrelate-treated group ($n=10$) was run in parallel with the above group to investigate, during the continuous infusion of saline solution (1 ml/h), the effect *per se* of the selective 5-HT_{1D} receptor antagonist, LY310762 (1 mg/kg; $n=5$) or the selective 5-HT_7 receptor antagonist, SB258719 (1 mg/kg; $n=5$)