Available online at www.sciencedirect.com

SCIENCE DIRECT

European Journal of Pharmacology 502 (2004) 239–242

www.elsevier.com/locate/ejphar

Short communication

5-HT_7, but not 5-HT_{2B}, receptors mediate hypotension in vagosympathectomized rats

David Centurión[a], Erika Glusa[b], Araceli Sánchez-López[a], Luis F. Valdivia[a], Pramod R. Saxena[c], Carlos M. Villalón[a,*]

[a]*Departamento de Farmacobiología, CINVESTAV-IPN, Czda. Tenorios 235, Col. Granjas-Coapa, México DF 14330, México*
[b]*Friedrich-Schiller-University Jena, Nordhäusser Strasse 78, Erfurt D-99089, Germany*
[c]*Department of Pharmacology, Cardiovascular Research Institute "COEUR," Erasmus Medical Centre Rotterdam, P.O. Box 1738, Rotterdam 3000 DR, The Netherlands*

Received 3 August 2004; accepted 27 August 2004
Available online 21 September 2004

Abstract

This study evaluated the possible involvement of 5-HT_{2B} receptors in long-lasting hypotension to 5-hydroxytryptamine (5-HT), which is predominantly mediated by 5-HT_7 receptors, in anaesthetised vagosympathectomized rats. Intravenous injections of 5-HT and 5-carboxamidotryptamine (5-CT) elicited a dose-dependent hypotension that was dose-dependently antagonised by (*R*)-1-{(3-hydroxyphenyl)sulfonyl}-2-{2-(4-methyl-1-piperidinyl) ethyl} pyrrolidine (SB-269970; a selective 5-HT_7 receptor antagonist), but not by saline. Interestingly, α-methyl-5-(2-thienylmethoxy)-1*H*-indole-3-ethanamine (BW723C86; a 5-HT_{2B} receptor agonist) produced vasopressor responses without affecting hypotension to 5-HT. These results suggest that hypotension to 5-HT and 5-CT is mainly mediated by 5-HT_7 receptors, whilst the role of 5-HT_{2B} receptors seems unlikely.

Keywords: Hypotension; 5-Hydroxytryptamine; 5-HT_7 receptor; 5-HT_{2B} receptor; BW723C86; SB-269970

1. Introduction

5-Hydroxytryptamine (5-HT) produces a triphasic blood pressure response in anaesthetised rats with intact vagus nerves (Saxena and Lawang, 1985). This triphasic response consists of an initial hypotension associated with a brief bradycardia via the von Bezold–Jarisch reflex (mediated by 5-HT_3 receptors located on afferent vagal fibres), followed by a vasopressor response (via vascular 5-HT_{2A} receptors) and, finally, a longer-lasting hypotension (Saxena and Villalón, 1990). This late hypotension to 5-HT, which remains unaffected after 5-HT_2 receptor blockade and bilateral vagotomy, seems to be predominantly mediated by 5-HT_7 receptors since: (1) the rank order of agonist potency to produce hypotension was: 5-carboxamidotryptamine (5-CT)>5-HT≥5-methoxytryptamine; and (2) lisuride, methiothepin, mesulergine, metergoline, and clozapine antagonised the hypotensive responses to both 5-HT and 5-CT (De Vries et al., 1997). However, this study also recognized that endothelium-dependent vasorelaxation mediated by other 5-HT receptors (i.e., 5-$HT_{1B/1D}$, 5-HT_{2B}, orphan, etc.) cannot be categorically excluded because stimulation of these endothelial receptors can be: (1) overshadowed by the above rank order of agonist potency, and (2) also blocked by the aforementioned antagonists.

In view of the development of more selective ligands at 5-HT_7 [(*R*)-1-{(3-hydroxyphenyl)sulfonyl}-2-{2-(4-methyl-1-piperidinyl) ethyl} pyrrolidine] (SB-269970;

* Corresponding author. Tel.: +52 55 5061 2854; fax: +52 55 5061 2863.
E-mail address: carlos_villalon@infosel.net.mx (C.M. Villalón).

0014-2999/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2004.08.050

Lovell et al., 2000) and 5-HT_{2B} [α-methyl-5-(2-thienylmethoxy)-1*H*-indole-3-ethanamine] (BW723C86; Baxter, 1996) receptors, this study has further characterized the pharmacological profile of the receptors mediating 5-HT- and 5-CT-induced vasodepressor responses in anaesthetised vagosympathectomized rats. Particular emphasis was made on verifying the possible coinvolvement of 5-HT_7 and 5-HT_{2B} receptors.

2. Materials and methods

Experiments were carried out in a total of 25 male Wistar rats (250–300 g). After anaesthesia with thiobutabarbital sodium (100 mg/kg, i.p.) and cannulation of the trachea, a catheter was placed in the right external jugular vein. Subsequently, bilateral cervical vagosympathectomy was performed and the right carotid artery was cannulated for the recording of blood pressure, using a Statham pressure transducer (P23 ID). The animals were artificially ventilated (56 strokes/min; stroke volume: 20 ml/kg) with room air using a rodent ventilator (Ugo Basile Biological Research Apparatus, Comerio, VA, Italy), and heart rate was measured with a tachograph (7P4F; Grass Instrument, Quincy, MA, USA) triggered from the blood pressure signal. Both blood pressure and heart rate were recorded simultaneously by a model 7D Grass polygraph (Grass Instrument). Additional doses of the anaesthetic were given when required.

After a stabilization period of 30 min, baseline values of mean blood pressure and heart rate were determined. The animals (n=25) were then divided into two main groups. The first group (n=16) received ketanserin (56 μg/kg, i.v.) followed by consecutive intravenous bolus injections of 5-HT (1, 3, 10, and 30 μg/kg). At this point, this group was subdivided into three subgroups that received subsequent intravenous administrations of, respectively, saline (1 ml/kg, three times; n=5), SB-269970 (30, 100, and 300 μg/kg; n=5), or BW723C86 (3, 10, 30, 100, 300, and 1000 μg/kg; n=6). Then, the responses produced by 5-HT were elicited again after: (i) each dose of saline; (ii) each dose of SB-269970; or (iii) the complete dose–response curve to BW723C86 had been concluded (total dose=1443 μg/kg). Moreover, the second group (n=9) received consecutive intravenous bolus injections of 5-CT (0.01, 0.03, 0.1, and 0.3 μg/kg) and it was subsequently subdivided into two subgroups that received saline (n=5) or SB-269970 (n=4) at the doses previously described. Then, the responses to 5-CT were elicited again after each dose of saline or SB-269970.

The dose intervals between the different doses of 5-HT, 5-CT, and BW723C86 ranged between 1 and 15 min; as in each case, we waited until the haemodynamic parameters had returned to baseline values. For saline, ketanserin, SB-269970, or the highest dose of BW723C86, a period of 10 min was allowed to elapse before the dose–response curves to 5-HT or 5-CT were elicited again. The dosing with all compounds used was sequential. The experimental protocol of this study was approved by the Ethical Committee of CINVESTAV-IPN.

The compounds used (obtained from the sources indicated) were: 5-HT creatinine sulphate, thiobutabarbital sodium, and BW723C86 (Sigma, St. Louis, MO, USA); 5-carboxamidotryptamine maleate and SB-269970 (GlaxoSmithKline, Stevenage, Hertfordshire, UK); and ketanserin (a gift from Janssen Pharmaceutica, Beerse, Belgium). All compounds were dissolved in saline, except for SB-269970, BW723C86, and ketanserin, which were dissolved in, respectively, dimethyl sulfoxide (20% vol/vol), propylene glycol (20% vol/vol), and ascorbic acid (2% wt/vol). These vehicles had no effect on baseline mean blood pressure or heart rate (not shown). The doses of the agonists and antagonists refer to their corresponding free base.

All data in the text and illustrations are presented as mean±S.E.M. The changes from baseline in mean blood pressure elicited by 5-HT, 5-CT, or BW723C86 in the different groups of animals were compared by using the Student–Newman–Keuls' test, once a two-way repeated measures analysis of variance (randomized block design) had revealed that the samples represented different populations (Steel and Torrie, 1980). Statistical significance was accepted at P<0.05 (two-tailed).

3. Results

Baseline values of mean blood pressure and heart rate were, respectively, 116±6 mm Hg and 367±9 beats/min (n=25). These values remained without significant changes after intravenous administration of each dose of saline or SB-269970 (not shown). As depicted in Fig. 1A, 5-HT (left panel) and 5-CT (right panel) produced dose-dependent hypotensive responses, without changing heart rate (not shown). At the doses used, 5-CT was about 2 log units more potent than 5-HT, and the duration of the responses to 5-CT (2.9±0.5, 4.7±0.3, 7.8±0.7, and 11.3±1 min) was longer than that of 5-HT (1.3±0.2, 2.7±0.2, 3.7±0.2, and 6±0.4 min). Fig. 1A also shows that the responses to 5-HT and 5-CT were reproducible, as they remained unchanged in control animals receiving saline. In contrast, Fig. 1B illustrates that SB-269970 dose-dependently blocked (and practically abolished at its highest dose) the responses elicited by 5-HT (left panel) and 5-CT (right panel).

Remarkably, the selective 5-HT_{2B} receptor agonist, BW723C86 (3, 10, 30, 100, 300, and 1000 μg/kg, i.v.) produced increases (rather than decreases) in mean blood pressure of, respectively, 12±1, 12±1, 12±1, 13±2, 42±7*, and 45±6* mm Hg (*P<0.05 vs. baseline) without affecting heart rate. Interestingly, the hypotensive responses to 5-HT remained without significant changes before (−12±2, −19±2, −26±3, and −32±6 mm Hg) and after (−5±2, −23±1, −33±8, and −42±7 mm Hg) the

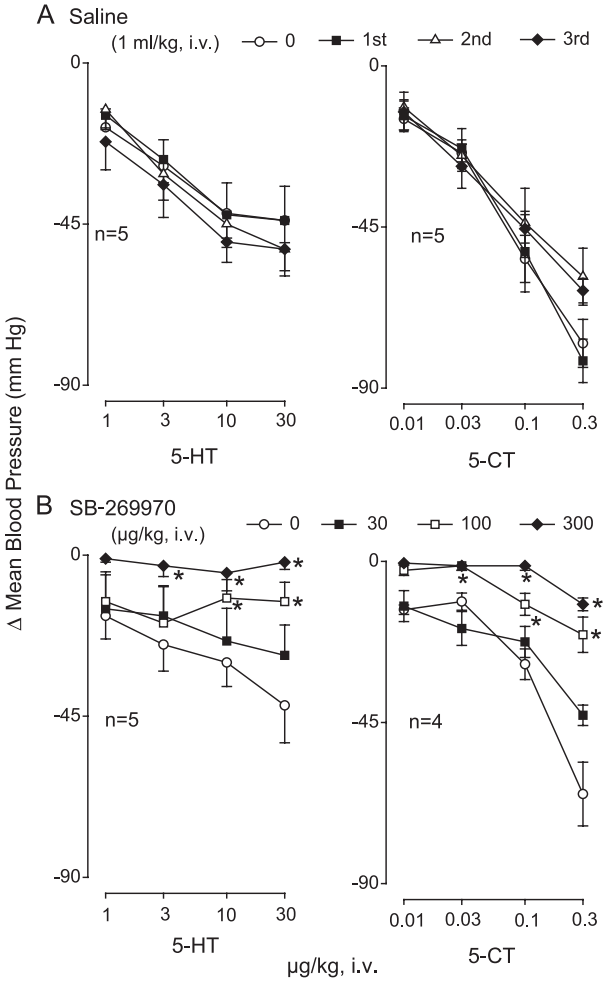

Fig. 1. Effect of (A) physiological saline or (B) SB-269970 on decreases in mean blood pressure induced by intravenous bolus injections of 5-HT (pretreatment with ketanserin; 56 μg/kg) or 5-CT (no pretreatment) in vagosympathectomized rats. *P<0.05 vs. control. Each point represents the mean±S.E.M.

complete dose–response curve to BW723C86 had been concluded (total dose: 1443 μg/kg).

4. Discussion

As described earlier in vagosympathectomized rats (De Vries et al., 1997), the present results reconfirm that the hypotensive responses to 5-HT and 5-CT are predominantly mediated by 5-HT_7 receptors since SB-269970, a selective 5-HT_7 receptor antagonist with low affinity for 5-HT_{2B} receptors (Lovell et al., 2000), potently blocked these responses. Consistent with these findings, 9-(tetrahydro-2-furanyl)-9*H*-purin-6-amine (SQ22536; 30 μg/kg, i.v.), an adenylyl cyclase inhibitor (Turcato and Clapp, 1999), significantly blocked the hypotensive responses to both 5-HT and isoprenaline without affecting those to acetylcholine (data not shown). Accordingly, the vasodepressor responses to 5-HT are most likely related to activation of adenylyl cyclase, a transduction mechanism associated with relaxation mediated by 5-HT_7 receptors (Hoyer et al., 1994). It must be emphasised that pretreatment with ketanserin, which has low affinity for 5-HT_{2B} receptors (pK_i: 5.5; Centurión et al., 2002) (to block the hypertension mediated by 5-HT_{2A} receptors), and bilateral vagosympathectomy (to avoid the von Bezold–Jarisch reflex) were required to induce a "pure" late hypotensive response to 5-HT. Indeed, the dose of 56 μg/kg ketanserin is high enough to block the cardiovascular responses mediated by 5-HT_{2A} receptors in rats (pK_i: 9.0; Centurión et al., 2002). This is the reason why 5-HT failed to elicit vasopressor and tachycardic responses in the present study.

Although, admittedly, ketanserin has an appreciable affinity at 5-HT_7 receptors (pK_i: 7.7; Hoyer et al., 1994), this drug did not affect the hypotensive responses to 5-HT (data not shown). Moreover, we decided not to pretreat with ketanserin the animals receiving 5-CT, as the latter compound does not produce 5-HT_2 receptor-mediated vasopressor (nor tachycardic) responses in rats (Saxena and Villalón, 1990; De Vries et al., 1997).

Interestingly, some findings show that 5-HT_{2B} receptors mediate endothelium-dependent vasorelaxation in the pig pulmonary artery (Glusa and Pertz, 2000) and rat jugular vein (Ellis et al., 1995); therefore, this mechanism may also be involved in the hypotension to 5-HT. Nevertheless, this is unlikely since the selective 5-HT_{2B} receptor agonist, BW723C86, produced hypertensive (rather than hypotensive) responses and failed to antagonize the hypotensive responses to 5-HT. Consistent with this view, the nitric oxide synthetase inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME), failed to attenuate 5-HT- or 5-CT-induced vasodepressor responses in pithed rats (Van Gelderen and Saxena, 1992). Considering the above, the complete blockade produced by SB-269970 on both 5-HT- and 5-CT-induced vasodepressor responses reconfirms the involvement of 5-HT_7 receptors without a discernible participation of other mechanisms. Admittedly, this conclusion is based on the assumption that species differences between the affinities of SB-269970 and BW723C86 do not play a major role.

Although we have no clear-cut explanation for the increases in blood pressure produced by BW723C86, one cannot ignore that 5-HT_{2B} receptors mediate vasoconstriction in rat mesenteric arteries (Watts and Fink, 1999). Nevertheless, a central mechanism is unlikely since intracerebroventricular administration of BW723C86 failed to increase blood pressure and heart rate in rats (Knowles and Ramage, 2000).

Finally, endothelial (Schoeffter and Hoyer, 1990) or prejunctional sympatho-inhibitory (Villalón et al., 1998) 5-$HT_{1B/1D}$ receptors as well as other 5-HT_1 receptor subtypes (i.e., 5-HT_{1A}, 5-HT_{1E}, and 5-HT_{1F}) do not seem to play a major role in the present study since, as previously shown, the hypotension to 5-HT and 5-CT in anaesthetised rats (De Vries et al., 1997) was: (i) not mimicked by sumatriptan, whereas 5-CT and sumatriptan display, respectively, low and

high affinities at the 5-HT_{1F} receptor; (ii) resistant to blockade by 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 (GR127935); and (iii) antagonised by clozapine or mesulergine, which does not bind potently to the 5-HT_1 receptor subtypes (Hoyer et al., 1994). Moreover, the 5-HT_1 family is, by definition, negatively coupled to adenylyl cyclase (Hoyer et al., 1994), a transduction mechanism usually associated with vasoconstriction and hypertension rather than hypotension.

In conclusion, the present results obtained in anaesthetised vagosympathectomized rats suggest that the late long-lasting hypotension to 5-HT is primarily mediated by 5-HT_7 receptors; the role of 5-HT_{2B} receptors, if any, seems to be unlikely.

Acknowledgements

The skillful technical assistance of Mr. Arturo Contreras Bustos is kindly acknowledged. The authors also thank CONACyT (México) for their support.

References

Baxter, G.S., 1996. Novel discriminatory ligands for 5-HT_{2B} receptors. Behav. Brain Res. 73, 149–152.

Centurión, D., Ortiz, M.I., Saxena, P.R., Villalón, C.M., 2002. The atypical 5-HT_2 receptor mediating tachycardia in pithed rats: pharmacological correlation with the 5-HT_{2A} receptor subtype. Br. J. Pharmacol. 135, 1531–1539.

De Vries, P., Villalón, C.M., Heiligers, J.P., Saxena, P.R., 1997. Nature of 5-HT_1-like receptors mediating depressor responses in vagosympathectomized rats; close resemblance to the cloned 5-HT_7 receptor. Naunyn-Schmiedeberg's Arch. Pharmacol. 356, 90–99.

Ellis, E.S., Byrne, C., Murphy, O.E., Tilford, N.S., Baxter, G.S., 1995. Mediation by 5-hydroxytryptamine$_{2B}$ receptors of endothelium-dependent relaxation in rat jugular vein. Br. J. Pharmacol. 114, 400–404.

Glusa, E., Pertz, H.H., 2000. Further evidence that 5-HT-induced relaxation of pig pulmonary artery is mediated by endothelial 5-HT_{2B} receptors. Br. J. Pharmacol. 130, 692–698.

Hoyer, D., Clarke, D.E., Fozard, J.R., Hartig, P.R., Martin, G.R., Mylecharane, E.J., Saxena, P.R., Humphrey, P.P., 1994. International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (Serotonin). Pharmacol. Rev. 46, 157–203.

Knowles, I.D, Ramage, A.G., 2000. Evidence that activation of central 5-HT_{2B} receptors causes renal sympathoexcitation in anaesthetised rats. Br. J. Pharmacol. 129, 177–183.

Lovell, P.J., Bromidge, S.M., Dabbs, S., Duckworth, D.M., Forbes, I.T., Jennings, A.J., King, F.D., Middlemiss, D.N., Rahman, S.K., Saunders, D.V., Collin, L.L., Hagan, J.J., Riley, G.J., Thomas, D.R., 2000. A novel, potent, and selective 5-HT_7 antagonist: (*R*)-3-(2-(2-(4-methylpiperidin-1-yl)-ethyl)pyrrolidine-1-sulfonyl)phenol (SB-269970). J. Med. Chem. 43, 342–345.

Saxena, P.R., Lawang, A., 1985. A comparison of cardiovascular and smooth muscle effects of 5-hydroxytryptamine and 5-carboxamidotryptamine, a selective agonist of 5-HT_1 receptors. Arch. Int. Pharmacodyn. Ther. 277, 235–252.

Saxena, P.R., Villalón, C.M., 1990. Cardiovascular effects of serotonin agonists and antagonists. J. Cardiovasc. Pharmacol. 15 (Suppl. 7), S17–S34.

Schoeffter, P., Hoyer, D., 1990. 5-Hydroxytryptamine (5-HT)-induced endothelium-dependent relaxation of pig coronary arteries is mediated by 5-HT receptors similar to the 5-HT_{1D} receptor subtype. J. Pharmacol. Exp. Ther. 252, 387–395.

Steel, R.G.D., Torrie, J.H., 1980. Principles and Procedures of Statistics: A Biomedical Approach. 2nd ed. McGraw-Hill, Kogakusha, Tokyo.

Turcato, S., Clapp, L.H., 1999. Effects of the adenylyl cyclase inhibitor SQ22536 on iloprost-induced vasorelaxation and cyclic AMP elevation in isolated guinea-pig aorta. Br. J. Pharmacol. 126, 845–847.

Van Gelderen, E.M., Saxena, P.R., 1992. Effect of *N*(G)-nitro-L-arginine methyl ester on the hypotensive and hypertensive responses to 5-hydroxytryptamine in pithed rats. Eur. J. Pharmacol. 222, 185–191.

Villalón, C.M., Centurión, D., Rabelo, G., De Vries, P., Saxena, P.R., Sánchez-López, A., 1998. The 5-HT_1-like receptors mediating inhibition of sympathetic vasopressor outflow in the pithed rat: operational correlation with the 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D} subtypes. Br. J. Pharmacol. 124, 1001–1011.

Watts, S.W., Fink, G.D., 1999. 5-HT_{2B}-receptor antagonist LY-272015 is antihypertensive in DOCA-salt-hypertensive rats. Am. J. Physiol. 276, H944–H952.