European Journal of Pharmacology 754 (2015) 140–147

Contents lists available at ScienceDirect

European Journal of Pharmacology

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

Neuropharmacology and analgesia

5-Hydroxytryptamine does not reduce sympathetic nerve activity or neuroeffector function in the splanchnic circulation

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ARTICLE INFO

Article history:
Received 26 November 2014
Received in revised form
9 January 2015
Accepted 17 February 2015
Available online 28 February 2015

Keywords:
5-HT
Sympathetic nerve activity
Blood pressure

ABSTRACT

Infusion of 5-hydroxytryptamine (5-HT) in conscious rats results in a sustained (up to 30 days) fall in blood pressure. This is accompanied by an increase in splanchnic blood flow. Because the splanchnic circulation is regulated by the sympathetic nervous system, we hypothesized that 5-HT would: 1) directly reduce sympathetic nerve activity in the splanchnic region; and/or 2) inhibit sympathetic neuroeffector function in splanchnic blood vessels. Moreover, removal of the sympathetic innervation of the splanchnic circulation (celiac ganglionectomy) would reduce 5-HT-induced hypotension. In anaesthetized Sprague-Dawley rats, mean blood pressure was reduced from 101 ± 4 to 63 ± 3 mm Hg during slow infusion of 5-HT (25 μg/kg/min, i.v.). Pre- and postganglionic splanchnic sympathetic nerve activity were unaffected during 5-HT infusion. In superior mesenteric arterial rings prepared for electrical field stimulation, neither 5-HT (3, 10, 30 nM), the 5-HT_{1B} receptor agonist CP 93129 nor 5-$HT_{1/7}$ receptor agonist 5-carboxamidotryptamine inhibited neurogenic contraction compared to vehicle. 5-HT did not inhibit neurogenic contraction in superior mesenteric venous rings. Finally, celiac ganglionectomy did not modify the magnitude of fall or time course of 5-HT-induced hypotension when compared to animals receiving sham ganglionectomy. We conclude it is unlikely 5-HT interacts with the sympathetic nervous system at the level of the splanchnic preganglionic or postganglionic nerve, as well as at the neuroeffector junction, to reduce blood pressure. These important studies allow us to rule out a direct interaction of 5-HT with the splanchnic sympathetic nervous system as a cause of the 5-HT-induced fall in blood pressure.

1. Introduction

While 5-HT is well known as a vasoconstrictor, we found that 5-HT infused over 7–30 days in conscious rats produces a lasting reduction in mean arterial blood pressure. This was observed in multiple strains and in both male and female rats (Diaz et al., 2008; Tan et al., 2011; Davis et al., 2012, 2013). Unexpectedly, 5-HT nearly normalized arterial blood pressure in the deoxycorticosterone acetate (DOCA) salt hypertensive rat (Diaz et al., 2008). These findings make it important to identify the mechanism(s) by which 5-HT reduces blood pressure. 5-HT causes a robust fall in total peripheral resistance that is not caused by direct relaxation of arteries, including mesenteric arteries (Davis et al., 2012). However, microsphere studies demonstrated the ability of 5-HT to increase blood flow to the splanchnic circulation (Seitz and Watts, 2014), providing the regional focus for the current study. Since the sympathetic nervous system contributes significantly to total peripheral resistance regulation, removal of sympathetic tone is a means by which blood pressure could be reduced by 5-HT, and this is the focus of the current study. Another possibility is that 5-HT increases venous capacitance to increase blood flow, but this was not investigated presently.

There are several levels at which 5-HT could act to inhibit the function of the sympathetic nervous system. First, 5-HT can inhibit norepinephrine (NE) release at the sympathetic neuroeffector junction in blood vessels through interactions with presynaptic inhibitory 5-HT receptors (Gothert et al., 1991; Molderings et al., 1990, 2006). Second, 5-HT modifies sympathetic outflow centrally

Abbreviations: 5-CT, 5-carboxamidotryptamine; 5-HT, 5-hydroxytryptamine; 8-OH-DPAT, (±)-8-hydroxy-2-dipropylaminotetralin; CP93129, 1,4-dihydro-3-(1,2,3,6-tetrahydro-4-pyridinyl)-5H-pyrrol[3,2-b]pyridin-5-one; EFS, electrical field stimulation; TTX, tetrodotoxin

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http://dx.doi.org/10.1016/j.ejphar.2015.02.032
0014-2999/

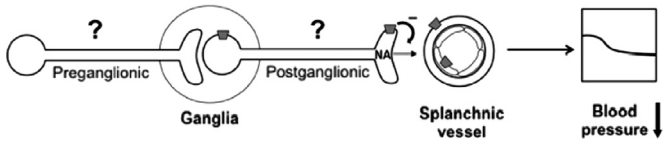

Fig. 1. Depiction of potential sites of action of 5-HT to reduce sympathetic outflow to the splanchnic bed. ?=questions examined in the present manuscript.

(Barnes and Sharp, 1999; Ramage and Villalon, 2008), reflected in changes in activity of preganglionic sympathetic nerves. Third, 5-HT both decreases and increases sympathetic ganglionic activity such that postganglionic sympathetic nerve activity is influenced (Hertzler, 1961; Jones et al., 1995; Meehan and Kreulen, 1991; Pickering et al., 1994; Ramage and Villalon, 2008; Sheng-Rong et al., 1999; Watkins and Newberry, 1996).

We investigated each of these potential levels in the splanchnic circulation of the rat, depicted in Fig. 1. We hypothesized that 5-HT would: 1) reduce sympathetic nerve activity directly at either the preganglionic (potential central effect) and/or postganglionic level (ganglionic inhibition); and/or 2) directly inhibit sympathetic neuroeffector function. We used agonists of 5-HT receptors that have either been shown to acutely lower blood pressure and/or inhibit sympathetic neurotransmission. These experiments were done with an acute infusion of 5-HT. To connect these findings with the role of the sympathetic nervous system in chronic infusion of 5-HT, we examined whether removal of the primary source of sympathetic innervation to the splanchnic bed would affect the hypotension caused by 5-HT during a one week administration.

2. Materials and methods

2.1. Animal

The Michigan State University Institutional Animal Care and Use Committee (IACUC) approved all protocols. Male Sprague Dawley rats (300–400 g, Charles River Laboratories) were used in this study.

2.2. Methods for studies of sympathetic nerve activity

2.2.1. Anesthesia

Sprague Dawley rats used for recordings of sympathetic nerve activity were anesthetized with urethane (1–1.1 g/kg i.p.) and paralyzed with gallamine triethiodide (initial dose of 20 mg/kg i.v.; supplemental doses administered as needed to maintain paralysis) following induction with isoflurane. The trachea was cannulated for maintenance of artificial respiration using positive pressure ventilation (Harvard Apparatus Inspira ASV ventilator). Rectal temperature was maintained near 37 °C with the aid of a heat lamp.

2.2.2. Catheterization

Arterial and venous catheters were introduced into the femoral artery and femoral vein. The arterial catheter was connected to a pressure transducer to monitor changes in blood pressure. Venous catheter(s) were used to deliver drugs to the anesthetized rat.

2.2.3. Nerve recording

Potentials were recorded monophasically from the cut central ends of the preganglionic or postganglionic sympathetic splanchnic nerve placed on platinum bipolar electrodes. The capacity-coupled preamplifier bandpass was set at 1–1000 Hz. Preganglionic or postganglionic sympathetic nerve activity (SNA) was then rectified and integrated (1-V reset). This signal was then quantified in 1- or 10-min data blocks at baseline and during 5-HT infusion. Hexamethonium (10 mg/kg, i.v.) was administered at the end of each experiment to verify that recordings were from preganglionic or postganglionic branches of the splanchnic nerve.

2.3. Methods for studies involving infusion of 5-HT in conscious animals

2.3.1. Anesthesia

All rats were anesthetized with isoflurane (2% in 100% O_2). Rats were treated with amoxicillin (150 mg/kg/i.m.) following surgery and 3 days thereafter. All rats were treated with rimadyl (5 mg/kg, s.c. for 2 days) for general analgesia.

2.3.2. Blood pressure probe

Radiotelemeters (DSI PhysioTel PA series transmitter model PA-C40) were implanted through subcutaneous incisions in the lower abdomen and catheters introduced into the left femoral artery. Incisions were closed with silk suture. Pressure sensing tips were advanced into the thoracic aorta. All rats were given 7 days to recover prior to any measure. Mean arterial pressure (MAP) and heart rate (HR) were recorded at 10-min intervals (10 s recording) for the duration of the study.

2.3.3. Alzet osmotic pump

A small incision was made at the base of the neck. Blunt dissection was used to create a small subcutaneous pocket between the scapulae. The pump (Alzet Osmotic Pump, Model 2ML1, Duret Corporation, Cupertino, CA, 10 µl/h 7 days) was inserted and the skin sutured closed. To each pump, a 5-HT creatinine complex [(25 µg/kg/min) in 1% ascorbic acid as antioxidant] or vehicle (1% ascorbic acid) was loaded. The solution was dissolved in 1 M HCl, and a pH-balance (~7) was achieved with 4 M NaOH.

2.4. Methods used for ganglionectomy and surgical validation

2.4.1. Celiac ganglionectomy

While rats were under general anesthesia (2% isoflurane in 100% O_2), a ventral midline abdominal incision was performed and the small intestines were gently retracted and placed on warm saline soaked gauze. The celiac plexus located between the aorta, celiac artery and mesenteric artery was dissected free and removed (CGx). The small intestines were placed back into the abdominal cavity and lavaged with warm saline. The midline abdominal incision was sutured closed in layers. The sham group (SGx) underwent a sham operation that was performed by accessing and exposing the celiac plexus only. All rats were given an intra-muscular injection of piperacillin. Animals were used after a recovery period of five days before implantation of a 5-HT or vehicle containing pump and radiotelemeters for blood pressure measurements (described above). Rats were sacrificed by pneumothorax following deep anesthesia induced by an intraperitoneal injection of sodium pentobarbital (60–80 mg/kg, i.p.). The liver, spleen, and small intestine were dissected and stored at −80 °C prior to isolation of amines for measurements in HPLC.

2.4.2. High pressure liquid chromatography

Catecholamine measures were made by homogenizing the tissue in four times their weight of 0.1 M percholoric acid, centrifugation and taking samples through a 30 kDa filtration tube; the filtrate was analyzed by HPLC. The HPLC system (ESA Biosciences, Chelmsford MA) consisted of a Coulochem III electrochemical detector set at −350 mV with separation of the analytes on an HR-80 reverse-phase column (Thermo Scientific, Waltham MA). Cat-A-Phase II (Thermo) was the mobile phase with a flow rate of 1.1 ml/min and the separation column was maintained at 35 °C. Quantification of the analytes was accomplished by performing a standard curve periodically and the limit of detection was 0.1 ng/ml for catecholamines.