Please cite this article in press as: Lyubashina OA, Sivachenko IB. The 5-HT$_4$ receptor-mediated inhibition of visceral nociceptive neurons in the rat caudal ventrolateral medulla. Neuroscience (2017), http://dx.doi.org/10.1016/j.neuroscience.2017.07.039

Neuroscience xxx (2017) xxx–xxx

THE 5-HT$_4$ RECEPTOR-MEDIATED INHIBITION OF VISCERAL NOCICEPTIVE NEURONS IN THE RAT CAUDAL VENTROLATERAL MEDULLA

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Abstract—Activation of the serotonin type 4 (5-HT$_4$) receptors has been reported to improve abdominal pain in patients with functional gastrointestinal disorders and reduce visceral nociception in animal models. Earlier studies have proposed that 5-HT$_4$ agonist can produce visceral analgesia by acting at the supraspinal level, but the underlying neuronal mechanisms remain unclear. The caudal ventrolateral medulla (CVLM) is the first site for processing of visceral nociceptive signals ascending via spinal pathways and an important component of the endogenous pain modulatory system. Therefore, the objective of the present study was to examine whether activation of 5-HT$_4$ receptors can affect the visceral pain-related neurons in the CVLM. In urethane-anesthetized adult male Wistar rats, we evaluated the effects of a 5-HT$_4$ receptor agonist, BIMU8 on ongoing firing of the CVLM neurons and their excitatory responses to noxious colorectal distension (CRD, 80 mmHg). The drug's effect was also tested on blood pressure reactions induced by CRD–a general physiological measure of visceral nociception. Intravenous administration of BIMU8 (0.5, 1 or 2 mg/kg) produced dose-dependent suppression of both the ongoing and CRD-evoked activities of the CVLM neurons and simultaneously attenuated the depressor hemodynamic reaction to CRD. The compound's inhibitory effect was almost completely eliminated by intracerebroventricular pretreatment with GR113808, a selective 5-HT$_4$ antagonist, indicating the preferential involvement of supraspinal 5-HT$_4$ receptors. Results indicate that visceral nociceptive transmission through the caudal medulla is negatively modulated by descending 5-HT$_4$-dependent mechanisms. These findings can contribute to a deeper understanding of supraspinal processing of pain signals from the abdomen.

Key words: abdominal pain, 5-HT$_4$ receptors, caudal ventrolateral medulla, neuronal activity, colorectal distension.

INTRODUCTION

Abdominal pain is a major symptom and a defining characteristic of functional gastrointestinal disorders (FGID) such as functional dyspepsia and irritable bowel syndrome (Azpiroz et al., 2007; Buéno et al., 2007; Farmer and Aziz, 2009; Wilder-Smith, 2011). In these disorders, pain and discomfort in the abdomen are associated with indigestion, nausea, bloating, and altered bowel habits (constipation and/or diarrhea), which may be related to disturbances in gut motility and secretion (Longstreth et al., 2006; Drossman, 2016). The underlying mechanisms are thought to involve a dysregulation of the brain–gut interactions at the peripheral, spinal, and cerebral levels, all of which contribute to the symptoms (Jones et al., 2006; Mayer et al., 2006; Elsenbruch, 2011; Drossman, 2016). Although the exact causes of the dysfunction are not fully understood, numerous studies have implicated altered peripheral and central serotonin signaling in FGID pathophysiology (reviewed in Gershon and Tack, 2007; Spiller, 2008; Camilleri, 2009; Manocha and Khan, 2012).

Serotonin (5-HT) is an important neurotransmitter in the brain-gut axis, where it directly and indirectly modulates gastrointestinal motor and secretory functions and affects perception of visceral stimuli at peripheral and central sites (Gershon and Tack, 2007; Camilleri, 2009; Cirillo et al., 2011). Accordingly, changes in 5-HT metabolism and signaling may lead to both abnormal gut physiology and visceral hypersensitivity commonly associated with FGID (Spiller, 2008; Farmer and Aziz, 2009; Cirillo et al., 2011; Manocha and Khan, 2012). 5-HT exerts its action in the brain and periphery by binding to multiple receptor subtypes (5-HT$_{1–7}$) and physiological consequence of such interaction is determined by which receptors are activated. Therefore, specific 5-HT receptors are of considerable interest as possible therapeutic targets for relieving various FGID symptoms, including abdominal pain (Buéno et al., 2007; Spiller, 2008; Camilleri, 2009; Mawe and Hoffman, 2013).

Over the past decade, the serotonin type 4 (5-HT$_4$) receptors have been extensively studied. It has been found that activation of these receptors enhances gastrointestinal motility by affecting all components of the peristaltic reflex and augments intestinal water and electrolyte secretion (Beattie and Smith, 2008; Budhoo

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Abbreviations: 5-HT, serotonin; 5-HT$_4$ receptors, serotonin type 4 receptors; CRD, colorectal distension; CVLM, caudal ventrolateral medulla; FGID, functional gastrointestinal disorders; RVM, rostroventral medulla.

http://dx.doi.org/10.1016/j.neuroscience.2017.07.039

et al., 1996; Grider et al., 1998; Hoffman et al., 2012; Ning et al., 2004; Prins et al., 2000, 2001). On this basis, 5-HT_4 agonists are primarily used as prokinetic agents for treating FGID associated with hypomotility (Lee et al., 2014; Quigley, 2015; Spiller, 2002). However, recently obtained data suggest that these receptors can also be involved in the modulation of visceral sensitivity. For example, it has been shown that a partial 5-HT_4 receptor agonist tegaserod substantially decreases rectal sensitivity in healthy subjects and patients with irritable bowel syndrome (Coffin et al., 2003; Sabaté et al., 2008) as well as reduces visceral nociception in animal models, including those of intestinal hypersensitivity (Schikowski et al., 2002; Liang et al., 2005; Greenwood-Van Meerveld et al., 2006; Chi et al., 2012; Hoffman et al., 2012; Sengupta et al., 2014;). Another drug selectively activating 5-HT_4 receptors, mosapride, had an inhibitory effect on the visceromotor response to gastric and colorectal noxious distensions in rats (Seto et al., 2011; Lee et al., 2012) and improved abdominal pain in patients with functional dyspepsia (Curran and Robinson, 2008). Novel compounds naronapride and YKP10811 exhibited antinociceptive action in colonic hypersensitivity triggered by inflammation and, as shown for YKP10811, by acute stress (Hoffman et al., 2012; Gilet et al., 2014). In our previous study, the visceral analgesic effect of prucalopride, a highly selective 5-HT_4 agonist, has been demonstrated in conscious dogs (Lyubashina et al., 2015).

It was initially suggested that the antinociceptive effects of 5-HT_4 receptor agonists are peripherally mediated (Schikowski et al., 2002; Greenwood-Van Meerveld et al., 2006; Hoffman et al., 2012). Meanwhile, the recent study by Sengupta and co-authors (2014) provided convincing evidence that tegaserod produces visceral analgesia by activation of opioidergic neurons in the rostral ventromedial medulla and exciting the brainstem noradrenergic system. These data suggest that various supraspinal sites can be involved in antinociceptive action of 5-HT_4 agonists in visceral pain, but the underlying neuronal mechanisms remain unclear and special studies are warranted to elucidate them.

The caudal medulla oblongata is the first site for supraspinal processing of visceral nociceptive signals ascending via pathways within the anterolateral quadrant of the spinal cord (Al-Chaer and Willis, 2007; Almeida et al., 2004, 2006). A region of the caudal medullary reticular formation located lateral to the lateral reticular nucleus and medial to the ventral tip of the spinal trigeminal nucleus is known as the caudal ventrolateral medulla (CVLM). This area is responsible for integrating cardiovascular and motor reactions to noxious events and is considered as an important component of the endogenous pain modulatory system (Lima et al., 2002; Almeida et al., 2006; Tavares and Lima, 2007). Certain populations of CVLM neurons have been shown to encode visceral noxious stimuli in a specific, excitatory fashion and thereby can act as monitors of ascending pain transmission (Ness et al., 1998, 1999; Robbins et al., 2005; Pinto-Ribeiro et al., 2011). These cells were referred to as visceral nociceptive neurons, the activity of which appears to undergo descending inhibition from the paraventricular nucleus of the hypothalamus and be suppressed by systemic analgesics (Ness et al., 1995, 1999; Pinto-Ribeiro et al., 2011). Whether activation of 5-HT_4 receptors can affect the visceral pain-related CVLM neurons is not yet known.

Considering the above, in this work we evaluated the effects of intravenously administered BIMU8—a potent and efficacious 5-HT_4 receptor full agonist that enters the central nervous system—on ongoing firing of the CVLM neurons and their responses to visceral noxious stimulation (colorectal distension, CRD). In parallel, we studied the compound's action on blood pressure reactions to noxious CRD that can be used as a physiological measure of visceral nociception (Ness and Gebhart, 1988) and are known to be CVLM-mediated (Lima et al., 2002). To determine if the effects of BIMU8 involve supraspinal 5-HT_4-dependent mechanisms, a series of experiments was performed in the presence of intraventricularly administered GR113808—a selective 5-HT_4 receptor antagonist.

EXPERIMENTAL PROCEDURES

Experiments were performed on male Wistar rats (body weight 250–330 g) that were bred in house and maintained 2–5 per cage (1782 cm^2 floor area) under standard laboratory conditions (12:12 light–dark cycle with lights on from 08:00 to 20:00; access to food and water *ad libitum*) and 16-h food deprivation on the day before experiments. The study protocol was approved by the Institutional Animal Care and Use Committee of the Pavlov Institute of Physiology and followed the Ethical Guidelines of the International Association for the Study of Pain and European Community Council Directive (86/609/EEC). All possible efforts were made to minimize animal suffering and to use only the number of animals necessary to produce reliable data.

Anesthesia and surgical preparation

The main experimental procedures were performed in an electrically enclosed and specially equipped room as described previously (Panteleev et al., 2015; Lyubashina et al., 2016). Briefly, rats were anesthetized with urethane (1.5 g/kg, i.p.; ICN Biomedicals, Aurora, OH, USA). Surgical sites were shaved gently with an electric shaver and cleaned with 1% iodine. The femoral venous and arterial catheters were inserted to enable drug administration and continuous monitoring of blood pressure, respectively. To measure and display blood pressure in real time, the pressure transducer (MLT0670, ADInstruments Ltd., UK) connected to a computer A/D converter was used. The trachea was intubated and the animal was placed in a stereotaxic frame (Medicor, Hungary) with the head tilted downwards 45°. The occipital surface of the skull was exposed and occipital craniotomy (0.3 × 0.6 cm) was performed, uncovering the caudal medulla at the level of the obex. The dura mater was removed and warm mineral oil was dripped

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