European Journal of Pharmacology 749 (2015) 49–55

Contents lists available at ScienceDirect

European Journal of Pharmacology

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

Neuropharmacology and analgesia

The inhibitory effect of granisetron on ventrolateral medulla neuron responses to colorectal distension in rats

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ARTICLE INFO

Article history:
Received 17 September 2014
Received in revised form
23 December 2014
Accepted 6 January 2015
Available online 13 January 2015

Keywords:
Irritable bowel syndrome
Abdominal pain
Granisetron
Colorectal distension
Ventrolateral medulla
Neurons

Chemical compounds studied in this article:
Granisetron(PubChem CID: 6918003)

ABSTRACT

Irritable bowel syndrome (IBS) is one of the most widespread functional gastrointestinal disorders characterized by abdominal pain. A key pathophysiological mechanism of abdominal pain is associated with disturbances of serotonergic transmission in feedback control loops of endogenous pain modulation in which the ventrolateral medulla (VLM) plays an important role. The receptors to serotonin (5-HT), and particularly the serotonin 3 (5-HT_3) receptors have been extensively used as a potential target for abdominal pain treatment of IBS patients due to antinociceptive features of the 5-HT_3 receptor antagonists. The precise mechanisms underlying the antinociceptive action of these antagonists remain unclear. The main objective of our study was to evaluate the involvement of the 5-HT_3 receptors in abdominal pain transmission within the VLM. Experiments were carried out on urethane-anaesthetized rats using the animal model of abdominal pain. Noxious colorectal distension (CRD) with a pressure of 80 mmHg induced a significant increase in VLM neuron-evoked activity and depressor reactions ($171.1 \pm 12.7\%$ and $64 \pm 1.8\%$ to baseline, accordingly). Selective blockade of the 5-HT_3 receptors with granisetron at doses of 1.0 or 2.0 mg/kg (i.v) resulted in long-lasting (90 min) dose-dependent inhibition of VLM neuron-evoked activity and depressor reactions. When brainstem dorsal surface applications of granisetron (10 or 20 μM) were used, the changes were more pronounced. These results suggest involvement of the 5-HT_3 receptors in abdominal pain transmission within the VLM, which will be discussed in relation to the central antinociceptive effect of granisetron.

1. Introduction

Irritable bowel syndrome (IBS) is one of the most widespread functional gastrointestinal disorders characterized by abdominal pain. It has been supposed that several pathophysiological mechanisms play a role in the genesis of this pain. Among them an important role belongs to central abnormalities of the serotoninergic system (Kim and Camilleri, 2000; Spiller, 2007; Bueno et al., 2007; Azpiroz et al., 2007; Sikander et al., 2009). Serotonin (5-HT) is involved in a wide range of physiological processes via at least seven distinct classes of receptors (5-HT_1 to 5-HT_7). Among these, the 5-HT_3 receptors contribute to the abdominal pain that attends IBS and have been extensively used as a potential target for abdominal pain treatment (Camilleri, 2002; Kuiken et al., 2005; Bueno et al., 2007). In the spinal cord, 5-HT_3 receptors are densely localized in the superficial dorsal horn on terminals of primary afferent fibers (Kia et al., 1995).

Within the brainstem, the highest density of 5-HT_3 receptors is found in the nucleus tractus solitaries, the dorsal motor nucleus of the vagus nerve, and the area postrema (Laporte et al., 1992). The ventrolateral medulla (VLM) is a functional heterogeneous brain area, which is involved in integrating visceral responses to nociceptive peripheral stimulation (Siddall and Dampney, 1989; Tavares and Lima, 2002). A significant increase in c-Fos-like immunoreactivity (c-FLI) after noxious colorectal distension (CRD) has been shown within the VLM (Monnikes et al., 2003; Wang et al., 2009). Stimulation of the VLM inhibits behavioral and dorsal horn nociceptive responses (Tavares and Lima, 2002, 2007). Thus, the VLM receives nociceptive inputs from the gut and, being one of the main components of the endogenous pain modulatory system, exerts spinal pain transmission control via descending projections to the spinal dorsal horns (Gebhart and Ossipov, 1986; Janss and Gebhart, 1988; Tavares and Lima, 2002, 2007; Heinricher et al., 2009). Numerous studies of 5-HT_3 receptors located on spinal neurons, which are responsive to noxious visceral stimuli, have confirmed that these receptors are involved in abdominal pain transmission. However, 5-HT_3 receptor involvement in supraspinal processing of abdominal pain, in particular within the VLM, has been poorly studied. Previously it was demonstrated that medullary neurons are responsive to noxious CRD and these responses can be

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http://dx.doi.org/10.1016/j.ejphar.2015.01.002
0014-2999/

used as biological markers of abdominal pain in studies of the antinociceptive properties of pharmacology drugs (Ness et al., 1998, 1999). Several 5-HT_3 receptor antagonists (alosetron, ondansetron, and granisetron) are characterized by antinocicepive effects. They are used for treatment of abdominal pain in IBS patients. The mechanisms underlying the antinociceptive action of these antagonists remain unclear. The objective of our study was to evaluate the involvement of the 5-HT_3 receptors in abdominal pain transmission within the VLM using the effects of selective blockade 5-HT_3 receptors with granisetron on VLM neuron responses and blood pressure reactions induced by noxious CRD.

2. Materials and methods

2.1. Animals

Experiments were performed on Wistar rats (body weight 280–350 g). The animals were housed 2–5 per cage and maintained on a 12-h light/dark schedule with unrestricted access to water and 12-h food deprivation on the day before experiments. The experimental procedures were performed in compliance with the Ethical Guidelines of the International Association for the Study of Pain and European Community Council Directive (86/609/EEC) and approved by OLAW of the NIH (#A5952-01) and Institutional Animal Care and Use Committee of the Pavlov Institute of Physiology of the Russian Academy of Sciences. All possible efforts were made to reduce the number of animals and to minimize their suffering.

2.2. Anesthesia and surgical preparation

The rats were anesthetized with urethane (1.5 g/kg; i.p., ICN Biomedical Inc, USA). After being given a surgical level of anesthesia, the left femoral artery was cannulated for continuous monitoring of blood pressure with a pressure transducer (MLT0670, ADInstruments Ltd., UK). The femoral vein was cannulated for intravenous injections of granisetron or saline. The head of a rat was fixed in a stereotaxic frame (Medicor, Hungary). The medulla was approached from the dorsal surface, as described previously (Panteleev et al., 2011). A midline incision was made on the dorsal surface of the neck. After bilateral neck muscle retraction, the occipital bone above the dorsal medulla surface and a small portion of the dura mater were carefully cut away. The exposed surface of the medulla was covered with warm mineral oil. The adequacy of the anesthesia level was judged by the stability of the arterial blood pressure in a range of 70–100 mmHg before each stimulation. Body temperature was maintained in a range of 37–38 °C with a heating pad driven by water thermostat (U-10, Germany).

2.3. Colorectal distension (CRD)

Colorectal distention of a rat's descending colon and rectum was performed with an 8-cm flexible latex balloon inserted via the anus and kept in position by taping the connecting catheter to the tail. The balloon was quickly inflated by an air-pump (DS-09, Visma-Planar, Belarus) up to a pressure of 20 or 80 mmHg which was maintained for 60 s.

2.4. Extracellular recordings

Extracellular recordings were made with tungsten microelectrodes (World Precision Instruments Inc, USA) with a tip diameter of 1 μm and an impedance of 1–2 MΩ. The electrode was dipped into the VLM using an electronic microdrive with steps of 4 μm until unit activity was recorded. The signal was amplified and filtered by a DAM 80 alternating current amplifier (World Precision Instruments Inc, USA). For real time display and storage data the VLM neuron activity was fed into a computer through an on-board sound card with Audition 3 software (Adobe Corp, USA). A subsequent off-line quantitative analysis was carried out using Spike 2 data analysis software (CED, UK) providing differential discrimination of spike sequences by shape. Calculation of a spike rate time course curve was created for each isolated spike sequence using Origin 7.5 graphic software (Origin Lab Corp, USA). The discharge rate of the VLM neurons was averaged for 60 s before (baseline) and for 60 s during the stimulus period. The VLM neurons were considered to be responsive if their mean discharge rate during CRD increased at least 10% above the baseline and remained increased for 30–60 s after completion of CRD. The location of the recorded VLM neurons was marked by passing a direct current (0.05 mA for 30 s) through the site of the electrode tip. After an electrolytic lesion was made, the rat was given a lethal injection of urethane (> 3 mg/kg, i.v.), and the brainstem was quickly removed and fixed in 10% buffered formalin. The brainstem was cut into 40-μm coronal sections on a freezing microtome, mounted on slides, and then stained with thionine (Sigma-Aldrich, Corp, USA). The lesion sites were identified on standardized sections from Paxinos and Watson (1998). Data were excluded if the recorded neurons were not located in the VLM area.

2.5. Experimental protocols

In the first series of experiments the ongoing neuron activity and the neuron activity evoked by the CRD with pressure of 20 or 80 mmHg of the VLM and blood pressure reactions were measured. In the second series of experiments, the VLM neuron activity and blood pressure changes to noxious CRD (80 mmHg) were measured before and 5, 15, 30, 45, 60, 75, 90 min after i.v. injection of granisetron (Sigma-Aldrich, Corp, USA) at doses of 1.0 or 2.0 mg/kg dissolved in 0.3 ml of saline. In the third series of experiments, the VLM neuron activity and blood pressure changes to noxious CRD were measured before and 5, 10, 15, 20, 30, 40, 50 and 60 min after application of 10 or 20 μM of granisetron dissolved in 1.0 μL of warm saline on the dorsal surface of the medulla. In control experiments, the equivalent values of saline for intravenous injection and application were used.

2.6. Quantitation of evoked neuronal activity and blood pressure reactions

For each unit, the CRD-induced evoked neuronal activity was defined as the ratio of the discharge rate per second during an interval of 60 s starting with the onset of the CRD (evoked response) to the discharge rate during the same interval immediately preceding the onset of the CRD (baseline activity) expressed as percentages of baseline activity. As responses of different neurons to the same CRD naturally varied in maximal response, each unit's evoked activity was normalized to the activity which was induced by CRD before granisetron administration (initial value). Blood pressure reactions were estimated for each CRD as the mean pressure for a 60 s interval preceding the onset of the CRD (baseline) and the same interval after the onset of CRD (response). (Based on the results of the) The Shapiro–Wilk test of normality, nonparametric Mann–Whitney test, and Friedman's and Kruskal–Wallis test with Dunn's post-hoc analyses were used to determine the statistical significance of CRD-induced changes of evoked neuron activity and blood pressure reactions using GraphPad InStat 3.02 (GraphPad Software Inc, USA). Differences were considered to be statistically significant when P was 0.05 or less. Results are presented as a mean of $\pm$ S.E.M, n.