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Research report

5-Hydroxytryptamine$_{2A/2C}$ receptors of nucleus raphe magnus and gigantocellularis/paragigantocellularis pars α reticular nuclei modulate the unconditioned fear-induced antinociception evoked by electrical stimulation of deep layers of the superior colliculus and dorsal periaqueductal grey matter

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HIGHLIGHTS

- The electrical stimulation of dlSC and dlPAG neurons elicits fear-induced antinociception.
- Gi/PGiα 5-HT$_{2A/2C}$ receptors modulate fear-induced antinociception evoked by dlSC activation.
- Gi/PGiα 5-HT$_{2A/2C}$ receptors modulate fear-induced antinociception evoked by dlPAG activation.
- NRM 5-HT$_{2A/2C}$ receptors modulate fear-induced antinociception evoked by dlSC activation.
- NRM 5-HT$_{2A/2C}$ receptors modulate fear-induced antinociception evoked by dlPAG activation.

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ABSTRACT

The electrical stimulation of the dorsolateral columns of the periaquedutal grey matter (dlPAG) or deep layers of the superior colliculus (dlSC) evokes defensive behaviours followed by an antinociceptive response. Monoaminergic brainstem reticular nuclei are suggested to comprise the endogenous pain modulatory system. The aim of the present work was to investigate the role played by 5-HT$_2$ subfamily of serotonergic receptors of the nucleus raphe magnus (NRM) and the gigantocellularis/paragigantocellularis pars α reticular nuclei (Gi/PGiα) in the elaboration of instinctive fear-induced antinociception elicited by electrical stimulation of dlPAG or of dlSC. The nociceptive thresholds were measured by the tail-flick test in Wistar rats. The 5-HT$_{2A/2C}$-serotonergic receptors antagonist ritanserin was microinjected at different concentrations (0.05, 0.5 and 5.0 μg/0.2 μL) either in Gi/PGiα or in NRM. The blockade of 5-HT$_2$ receptors in both Gi/PGiα and NRM decreased the innate fear-induced antinociception elicited by electrical stimulation of the dlSC or the dlPAG. These findings indicate that serotonin is involved in the hypo-algesia induced by unconditioned fear-induced behavioural responses and the 5-HT$_{2A/2C}$-serotonergic receptor subfamily in neurons situated in the Gi/PGiα complex and NRM are critically recruited in pain modulation during the panic-like emotional behaviour.

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1. Introduction

Electrical and chemical stimulation of the mesencephalic tectum structures, such as the corpora quadrigemina and the dorsal periaqueductal grey matter elicit defensive behaviours which are expressed by defensive immobility and escape behaviour followed by antinociception [1–8].

Monoaminergic projections, particularly from the locus coeruleus and the ventromedial medulla oblongata, such as the nucleus raphe magnus, the gigantocelullaris and the paragigantocellularis pars alpha reticular nuclei (Gi/PGiα), are suggested to participate in antinociceptive responses [9–12].

These brainstem nuclei as well as the periaqueductal grey matter participate of endogenous pain inhibitory system that modulates the perception of nociceptive stimuli [13,14], recruiting interneurons that inhibit the first synaptic contact of the ascending sensory-discriminative pathways at the level of the dorsal horn of the spinal cord. Several neurotransmitters were demonstrated to be involved in neural inputs to this pain modulatory system, including endogenous opioid peptides, acetylcholine, serotonin, and noradrenaline [8,12,15–23].

Neurons of the periaqueductal grey matter are connected with the nucleus raphe magnus (NRM) and paragigantocellularis reticular nucleus (B_3 pars), and these connexions play an important role in the supraspinal antinociceptive processes [24,25].

Many reports based on pharmacological, immunohistochemical and radioautographic techniques have demonstrated the involvement of serotonin in antinociceptive mechanisms [8,22,23,26]. The NRM electrical stimulation also produces a potent antinociceptive effect [27,28].

The 5-hydroxytryptamine-2 (5-HT_2) subfamily of serotonergic receptors is widely represented by neurons from the basal prosencephalon and specific nuclei of the brainstem, such as the NRM, the paragigantocellular reticular nucleus, and the dorsal raphe nucleus [7,25,29,30], connected by the dorsolateral column of the periaqueductal grey matter (dlPAG) and deep layers of the superior colliculus (dlSC) neuronal cells [8]. This neural network plays a critical role in the central modulation of antinociception [12,22,31].

Therefore, the aim of this study was to investigate the involvement of serotonergic neurotransmission in the NRM and Gi/PGiα in the unconditioned fear-induced antinociception elicited by electrical stimulation of cranial division of the mesencephalic tectum, at the level of dlSC and dlPAG. The hypothesis of the present work is that the blockade of 5-HT_2 receptors of ventromedial medulla oblongata nuclei will cause impairment in unconditioned fear-induced hypo-algesia.

2. Material and methods

2.1. Subjects

Male Wistar albino rats (N = 128), weighing between 200 and 250 g, from the animal care facility of the University of São Paulo (USP) at Ribeirão Preto Campus were used. These animals were housed in groups of four in a Plexiglas-walled cage (41 × 34 × 16 cm), with free access to food and water throughout the experiment. The room temperature was controlled (22 ± 1 °C), and a light/dark cycle (lights on from 07:00 to 19:00 h) was maintained. The experiments were performed from 8:00 to 17:00 h. All protocols were used in compliance with the recommendations of the Brazilian Society for Neuroscience and Behaviour (SBNeC), as well as with the recommendations of the Committee for Ethics in Animal Experimentation (CEUA) of the Ribeirão Preto Medical School of the University of São Paulo (FMRP-USP) (CEUA-FMRP-USP process 017/2016), which are in accordance with the Animal Research Ethics guidelines adopted by the National Council for Animal Experimentation Control (CONCEA).

2.2. Stereotaxic surgery

The animals (6–9 per group) were anaesthetised by intraperitoneal (IP) administration of ketamine at 92 mg/kg (Ketamine Agener®, *União Química Farmacêutica Nacional*, Brazil) and xylasine at 9.2 mg/kg (Dopaser®, Hertape/Calier, Juatuba, Minas Gerais, Brazil) and were fixed in a stereotaxic frame (David Kopf, Tujunga, California, USA). A tungsten bipolar microelectrode (50 μm; impedance between 1 and 3 MΩ) was implanted in the midbrain, aimed at the dlPAG or dlSC. The electrode was insulated with Teflon, except at the cross-section of the tip. The electrode was vertically introduced using the following coordinates with the bregma serving as the reference for each plane: anteroposterior, −5.8 mm; mediolateral, 0.4 mm to dlPAG, and 1.2 mm to dlSC and dorsoventral, 5.0 mm to dlPAG and 4.6 mm to dlSC. A guide-cannula made of a stainless steel (o.d. 0.6 mm, i.d. 0.4 mm) was implanted in the brainstem, aimed at the NRM or at the Gi/PGiα. The upper incisor bar was set at 3.3 mm below the interaural line, such that the skull was horizontal between bregma and lambda. The guide-cannula was vertically introduced using the following coordinates, with the bregma serving as the reference for each plane: anteroposterior, −10.5 mm to NRM and −10.5 mm to Gi/PGi; mediolateral, 0.0 mm to NRM and 0.4 mm to Gi/PGiα; and dorsoventral, 9.2 mm to NRM and 9.0 mm to Gi/PGiα. The guide-cannula was fixed to the skull by means of acrylic resin and two stainless-steel screws. At the end of the surgery, each guide cannula was sealed with a stainless-steel wire to protect it from obstruction. After the surgery, each animal was treated with an intramuscular injection of 60.000 IU of penicillin G benzathine and a nonsteroidal anti-inflammatory drug (banamine meglumine; 2.5 mg/kg).

2.3. Nociceptive tests

Each animal was individually placed in a restraining apparatus, and its tail was inserted into a heating sensor (tail-flick Analgesia Instrument; Insight, RibeirãoPreto, Brazil). The heating sensor functions such that calorimetric progressive elevation is automatically interrupted at the moment when the animal removes its tail from the apparatus. The current raises the temperature of the coil (Ni/Cr alloy, 26.04 cm in length × 0.02 cm in diameter) from room temperature (approximately 20 °C) at the rate of 9 °C/s. A small current intensity adjustment could be performed at the beginning of the experiment to obtain three consecutive tail-flick latencies (TFLs) between 2.5 and 3.5 s. If the animal did not remove its tail from the heater within 6 s, the tail-flick device was turned off to prevent damage to the skin.

2.4. Procedure

One week after surgery, the animals were placed in a circular enclosure. This arena was situated in an experimental compartment illuminated with a 40 W fluorescent lamp (350 lx at the arena floor level). The rats were allowed a 10 min period of habituation in the enclosure in the beginning of each session. Afterwards, the midbrain was electrically stimulated by means of a sine wave stimulator. The stimulation current was monitored measuring the voltage drop across a 1 K resistor with an oscilloscope (MO- 1250S; Minipa do Brasil, São Paulo, Brazil). Midbrain stimuli (60 Hz) were presented at 1 min intervals, with the current intensity increasing by steps of 1.4 μA for measurements of the defensive threshold of escape behaviour. The current intensity-producing running from moderate to strong intensity (or jumping) in two successive trials was considered to be the escape behaviour threshold. Animals