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Involvement of 5-HT_2 serotonergic receptors of the nucleus raphe magnus and nucleus reticularis gigantocellularis/paragigantocellularis complex neural networks in the antinociceptive phenomenon that follows the post-ictal immobility syndrome

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Abstract

The post-ictal immobility syndrome is followed by a significant increase in the nociceptive thresholds in animals and men. In this interesting post-ictal behavioral response, endogenous opioid peptides-mediated mechanisms, as well as cholinergic-mediated antinociceptive processes, have been suggested. However, considering that many serotonergic descending pathways have been implicated in antinociceptive reactions, the aim of the present work is to investigate the involvement of 5-HT_2-serotonergic receptor subfamily in the post-ictal antinociception. The analgesia was measured by the tail-flick test in seven or eight Wistar rats per group. Convulsions were followed by statistically significant increase in the tail-flick latencies (TFL), at least for 120 min of the post-ictal period. Male Wistar rats were submitted to stereotaxic surgery for introduction of a guide-cannula in the rhombencephalon, aiming either the nucleus raphe magnus (NRM) or the gigantocellularis complex. In independent groups of animals, these nuclei were neurochemically lesioned with a unilateral microinjection of ibotenic acid (1.0 μg/0.2 μL). The neuronal damage of either the NRM or nucleus reticularis gigantocellularis/paragigantocellularis complex decreased the post-ictal analgesia. Also, in other independent groups, central administration of ritanserin (5.0 μg/0.2 μL) or physiological saline into each of the reticular formation nuclei studied caused a statistically significant decrease in the TFL of seizing animals, as compared to controls, in all post-ictal periods studied. These results indicate that serotonin input-connected neurons of the pontine and medullary reticular nuclei may be involved in the post-ictal analgesia.

Keywords: Post-ictal analgesia; Pentylenetetrazole; GABA-A receptor; Serotonin; 5-HT_2 serotonergic receptor; Epilepsy; Pain

Introduction

The study of GABAergic, cholinergic, serotonergic and noradrenergic systems may contribute with elucidative data concerning the neurochemical bases of the post-ictal analgesia, and the investigation of the neurotransmitters involved in antinociceptive processes has been the aim of study of neuroscientists interested in the pain modulation (Dubner, 1978; Mason et al., 1988; Mason, 1997; Rosa et al., 1998; Dubner and Gold, 1999; Wei et al., 1999; Li et al., 2000; De Freitas et al., 2004; Freitas et al., 2005; Coimbra et al., 2006). The periaqueductal gray matter (PAG) and some nuclei of the reticular formation have been identified as the main structures of the supraspinal pain modulation (Reynolds, 1969; Nashold et al., 1969; Fields and Basbaum, 1989; Rosenfeld, 1994; Wei et al., 1999; Tracey et al., 2002; Dunckley et al., 2005; Coimbra et al., 2006). The electrical stimulation or microinjections of morphine in the nucleus raphe magnus (Dickenson et al., 1979), as well as in the

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dorsal raphe nucleus (Oliveras et al., 1979) or in the periaqueductal gray matter (Nashold et al., 1969; Mayer and Liebeskind, 1974; Hosobuch et al., 1979; Richardson and Akil, 1997; Morgan et al., 1989; Cui et al., 1999) are followed by intense antinociception. Descending pathways from these structures modulate the synaptic contact between the first and the second neurons of the ascending nociceptive pathways, in the dorsal horn of the spinal cord, using the dorsolateral funiculus. Interestingly, the discharge of pontomedullary serotonergic neurons is slow and steady, suggesting that these neurons may have a role in the tonic, rather than phasic, modulation of spinal processes (Mason, 1997).

In these descending outputs, the nucleus reticularis gigantocellularis/paragigantocellularis complex contributes with an important contingent of neuronal projections (Mohrland et al., 1982; Azami et al., 1982; Fields and Basbaum, 1989). Many of such nuclei receive connective outputs from PAG (Beitz, 1987; Mantyh, 1983). Raphe nuclei are situated into the median/paramedian axis from mesencephalon to medulla oblonga. Especially important for pain controlling are the raphe-spinal pathways, originated in the nucleus raphe magnus (NRM) and targeted to gelatinous substance of the spinal cord, inhibiting noxious inputs. Outputs from PAG to NRM, and serotonergic pathways from NRM, reaching the spinal tract of the spinal trigeminal nucleus or dorsolateral fasciculus toward the gelatinous layer of the caudal trigeminal nucleus or the Rexed's lamina I and II of the spinal cord, activate enkephalinergic inhibitory interneurons that hyperpolarize the second neuron of the spinal-thalamic nociceptive pathway (Fields et al., 1977; Willis and Westlund, 1997). In addition, the NRM and adjacent structures, such as the gigantocellularis/paragigantocellularis nuclei and nucleus raphe pallidus, exert influence on spinal cord neurons involved with the neurotransmission of noxious stimuli in the spinal thalamic pathway (Fields and Basbaum, 1978; Besson and Chaouch, 1987).

Some structures and neural brainstem networks, such as the corpora quadrigemina, the dorsal periaqueductal gray matter, the dorsal raphe nucleus, the nucleus reticularis gigantocellularis, pars alpha and the locus coeruleus have endogenous opioid-, monoamine- and acetylcholine-mediated mechanisms involved in the control of pain (Basbaum and Fields, 1984; Mason et al., 1988; Rosa et al., 1998; Li et al., 2000; Azami et al., 2001), defense (Monassi et al., 1997; Coimbra et al., 2000; Eichenberger et al., 2002; Osaki et al., 2003; Ribeiro et al., 2005) and epilepsy (Garcia-Cairasco et al., 1993; Cardoso et al., 1994; Peterson et al., 2000; Coimbra et al., 2001b; De Freitas et al., 2004; Freitas et al., 2005). Many of these nuclei are interconnected (Fardin et al., 1984; Zeng et al., 1991; Freitas et al., 2005; Coimbra et al., 2006) and send projections to the dorsal horn of the spinal cord, controlling the synapses between the first and the second neurons of the spinal-thalamic nociceptive pathways (Basbaum and Fields, 1984; Fields and Basbaum, 1989; Azami et al., 2001). Noradrenergic and serotonergic systems of the locus coeruleus and raphe nuclei are particularly involved in the control of pain, seizures and epilepsy generated in prosencephalic structures (Wang and Nakai, 1994; Zhang et al., 1997; Shouse et al., 2001).

Considering that the serotonergic system, the nucleus raphe magnus and both the nucleus reticularis gigantocellularis and the nucleus reticularis paragigantocellularis exert an important role in the descending modulation of spino-thalamic nocicetive pathways (Mohrland et al., 1982; Fardin et al., 1984; Zhuo and Gebhart, 1990; Wei et al., 1999; Coimbra et al., 2006), the aim of the present study is to investigate the involvement of 5-HT_2-mediated mechanism of the nucleus raphe magnus and gigantocellularis complex neural networks in the antinociception that follows the post-ictal immobility syndrome.

Materials and methods

Animals

Male Wistar albino rats (n = 7–8 per group), weighing between 200 and 250 g, from the animal care facility of the Campus of Ribeirão Preto at the University of São Paulo (USP), were used. These animals were housed in groups of four in a plexiglass-walled cage, and given free access to food and water throughout the experiment. The room temperature was controlled (22 ± 1°C), and a light–dark cycle (07:00–19:00 h lights on) was maintained. All protocols were used in compliance with the recommendation of the Brazilian Society for Neuroscience and Behavior (SBNeC), which are based on the US National Institutes of Health Guide for Care and Use of Laboratory Animals.

Neurophysiological procedures

Neurochemical lesion of neurons situated into the nucleus raphe magnus and the gigantocellularis/paragigantocellularis complex

The animals were anaesthetized with sodium pentobarbital (45 mg/kg, IP) and fixed in a stereotaxic frame (David Kopf, USA). 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 nucleus raphe magnus (NRM) or at the nucleus reticularis gigantocelllaris/nucleus reticularis paragigantocellularis complex (Gi/PGi). 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/PGi; and dorsoventral, 9.2 mm to NRM and 9.0 mm to Gi/PGi. 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. Five days after the surgery, the animals received microinjections of 0.2 μL of the neurotoxin ibotenic acid (at 1.0 μg/0.2 μL). After the rats were anesthetized, the injection needle was linked to a 5.0 μL hand-drive syringe (Hamilton) by means of polyethylene tubing. The injection was made using a thin dental needle (Mizzy, o.d. 0.3 mm) introduced through the guide cannula until its lower end was 1 mm below the guide cannula. The needle was left in the place for an additional