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Research Report

Dorsal raphe nucleus acetylcholine-mediated neurotransmission modulates post-ictal antinociception: The role of muscarinic and nicotinic cholinergic receptors

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ABSTRACT

The dorsal raphe nucleus (DRN) is a key structure of the endogenous pain inhibitory system. Although the DRN is rich in serotoninergic neurons, cholinergic neurons are also found in that nucleus. Both ictal and inter-ictal states are followed by post-ictal analgesia. The present study investigated the role of cholinergic mechanisms in postictal antinociceptive processes using microinjections of atropine and mecamylamine, muscarinic and nicotinic cholinergic receptor antagonists, respectively, in the DRN of rats. Intraperitoneal injection of pentylenetetrazole (PTZ) (at 64 mg/kg) caused tonic and tonic-clonic seizures. The convulsive motor reactions were followed by an increase in pain thresholds, a phenomenon known as post-ictal analgesia. Pre-treatment of the DRN with atropine or mecamylamine at 1 μg, 3 μg and 5 μg/0.2 μL decreased the post-ictal antinociceptive phenomenon. The present results showed that the post-ictal analgesia was mediated by muscarinic and nicotinic cholinergic receptors in the DRN, a structure crucially involved in the neural network that organises post-ictal hypoalgesia.

1. Introduction

Gamma-amino-butyric-acid (GABA) is an inhibitory neurotransmitter that is involved in distinct physiological and behavioural mechanisms, including locomotor activity, fear-related behaviour and pain modulation (Biagioni et al., 2013; de Freitas et al., 2014a, 2014b). Moreover, GABA is involved in presynaptic inhibition and modification of the regulation of encephalic excitability that occurs in epilepsy (Kanemoto et al., 2010; Ito et al., 1999, 2000).

Systemic injection of pentylenetetrazole (PTZ), a GABAergic antagonist, causes tonic-clonic seizures in rats (de Lima

Abbreviations: DRN, dorsal raphe nucleus; GABA, gamma-aminobutyric acid; i.p., intraperitoneal; LC, locus coeruleus; PTZ, pentylenetetrazole; PPTN, pedunculopontine tegmental nucleus; RN, raphe nuclei; TFL, tail-flick latency; TFT, tail-flick test

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and Rae, 1990; Schofield, 1987), which are widely used as an animal model of epileptic crises (Cevik et al., 2015; Coimbra et al., 2001a, 2001b; Tsuitsui et al., 1992). These tonic-clonic seizures are followed by an increase in the nociceptive threshold, a phenomenon known as post-ictal antinociception (de Oliveira et al., 2006; Felippotti et al., 2011a, 2011b, 2012; Freitas et al., 2005, 2008; Guieu et al., 1992; Myslobodsky et al., 1981; Szücs et al., 2015).

The cholinergic system has been suggested to be involved in the control of nociceptive perception (Chen et al., 2014; Decker and Meyer, 1999; Irusta et al., 2001; Joshi et al, 2008; Mui et al., 1997; Munro et al., 2010). Regarding post-ictal antinociception, systemic administration of atropine, a competitive antagonist of muscarinic cholinergic receptors, decreases tail-flick latencies in seizing animals, suggesting the involvement of acetylcholine in this inter-ictal antinociceptive response (Coimbra et al., 2001a). Additionally, both muscarinic and nicotinic receptors seem to be recruited in cholinergic neurotransmission during post-ictal antinociception. This hypothesis is supported by the fact that peripheral administrations not only of atropine but also of mecamylamine, a nicotinic cholinergic receptor antagonist, attenuate tonic-clonic seizure-induced antinociception (de Freitas et al., 2004). Moreover, treatment of the dorsal hippocampus (dH) with muscarinic and nicotinic cholinergic receptor antagonists attenuates antinociception following tonic-clonic seizures (de Freitas et al., 2013).

Outputs to the dH have been identified from the linear raphe nucleus, the nucleus raphe magnus (NRM), the dorsal raphe nucleus (DRN), and the locus coeruleus. All of these structures comprise the endogenous pain modulatory system and may be involved in the elaboration of post-ictal antinociception. Indeed, administration of cholinergic and nicotinic receptor antagonists into the NRM impairs the post-ictal antinociception phenomenon (de Oliveira et al., 2011).

The DRN is an important mesencephalic structure involved in pain modulation processes (Dai et al., 1992; Porro et al., 1991; Wang et al., 1988). Direct stimulation of the DRN neurons produce antinociception in rats (Mayer and Liebeskind, 1974; Mayer, 1984), whereas, the DRN neurons lesion partially attenuates the antinociceptive process induced by systemic administration of morphine (Garau et al., 1975). Although the DRN is rich in serotoninergic neurons, the presence of endogenous opioid peptide (Reyes-Vazquez et al., 1989), GABA (Nagai et al., 1983) and acetylcholine (Klamt and Prado, 1991) have also been shown to be present in its different subnuclei. While 5-hydroxytryptamine (5-HT) and opioid neurons play an inhibitory role on pain (Ruan et al., 1990), GABA neurons are anti-analgesic in the DRN (Moreau and Fields, 1986). Regarding the cholinergic system, acetylcholine seems to play an inhibitory role on pain, because microinjection of the muscarinic and nicotinic cholinergic receptors agonist carbachol into the DRN induces strong antinociception (Klamt and Prado, 1991). Despite no cholinergic neurons have been found in that mesencephalic tegmental nucleus (Ruan et al., 1990), the cholinergic pain inhibitory effect in the DRN may be due to the activity of cholinergic axon terminals from the laterodorsal tegmental area (Wang et al., 2000). In addition, that mesencephalic structure is the target and source of several monoaminergic projections to structures that are involved in pain modulation (Akil and Mayer, 1972; Hayes et al., 1977).

For these reasons, the aim of the present study was to investigate the involvement of the cholinergic system of the DRN in the elaboration of the antinociceptive process induced by seizures. To verify the role played by DRN muscarinic and nicotinic receptors on post-ictal antinociception, atropine or mecamylamine, muscarinic and nicotinic cholinergic receptor antagonists, respectively, was microinjected into the DRN, which was followed by intraperitoneal treatment with PTZ.

2. Results

Peripheral treatment with pentylenetetrazol at 64 mg/kg caused tonic and tonic-clonic seizures in all animals, and these convulsive motor reactions were followed by an increase in pain thresholds, a phenomenon known as post-ictal analgesia. The sham procedure consisted in the introduction of the needle without microinjection of any drug. We have noted that there was a sudden drop in the postictal antinociception after a 40-min plateau displayed by physiological saline plus PTZ-treated rodents. The sham group was performed considering that some structures situated above the DRN have a critical involvement in pain modulation as previously demonstrated (Reynolds, 1969; Coimbra et al., 1992, Coimbra and Brandão, 1997; Coimbra et al., 2006). However, there were no significant differences between these two experimental groups.

Microinjections of atropine (1 μg/0.2 μl, 3 μg/0.2 μl and 5 μg/0.2 μl) into the DRN decreased the post-ictal antinociception. Repeated measures two-way ANOVA showed a significant effect of the treatments (atropine 1 μg/0.2 μl, 3 μg/0.2 μl and 5 μg/0.2 μl, followed by PTZ i.p. x saline+saline x saline+PTZ i.p. × *sham*) [$F(5,39)=16$; $p<0.001$] and time (from 0 to 120 min) [$F(14,26)=63.99$; $p<0.001$] as well as a significant interaction between treatment and time [$F(70,122)=4.05$; $p<0.001$]. Duncan's post-hoc test showed a significant effect of the treatments on the nociceptive thresholds. Tonic and tonic-clonic seizures increased the nociceptive thresholds during 120 min of the post-ictal period [$F(5,40)$ varying from 1.22 to 20.92; $p<0.05$]. The post-hoc analyses showed that microinjection of atropine into the DRN caused a decrease in the postictal analgesia from 10 to 80 min after the convulsive seizures [$F(5,40)$ varying from 6.16 to 17.01; $p<0.05$] (Fig. 1).

Microinjection of different concentrations of mecamylamine into the DRN also decreased the post-ictal antinociception. Repeated measures two-way ANOVA showed a significant effect of the treatments (mecamylamine at 1 μg/0.2 μl, 3 μg/0.2 μl and 5 μg/0.2 μl, followed by PTZ i.p. x saline+saline x saline+PTZ i.p. x *sham*) [$F(5,39)=22.99$; $p<0.001$] and time (from 0 to 120 min) [$F(14,26)=17.94$; $p<0.001$] as well as a significant treatment versus time interaction [$F(70,122)=3.44$; $p<0.001$]. One-way ANOVAs showed a significant effect of the pre-treatment with the nicotinic cholinergic antagonist mecamylamine on the post-ictal analgesia immediately after seizures and from 10 to 120 min [$F(5,40)$ varying from 1.60 to 19.56; $p<0.05$]. The post-hoc analyses showed that the microinjection of mecamylamine into the DRN caused a decrease in the post-ictal