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Research report

Cholinergic/opioid interaction in anterior cingulate cortex reduces the nociceptive response of vocalization in guinea pigs

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ABSTRACT

The anterior cingulate cortex (ACC) is crucial in the modulation of the sensory, affective and cognitive aspects of nociceptive processing. Also, it participates in the planning and execution of behavioral responses evoked by nociceptive stimuli via descending projections to the brainstem. In laboratory animals nociceptive experimental tests evaluate behavioral responses that preferentially express the sensory-discriminative or affective-motivational component of pain. The objective of this study was to investigate the participation of opioid and cholinergic neurotransmission in the ACC on different nociceptive responses in guinea pigs. We used nociceptive tests of formalin and vocalization evoked by peripheral noxious stimuli (electric shock) to evaluate the behavioral expression of the sensory-discriminative and affective motivational components, respectively. We verified that the microinjection of morphine (4.4 nmol) in the ACC of guinea pigs promotes antinociception in the two experimental tests investigated. This effect is blocked by prior microinjection of naloxone (2.7 nmol). On the other hand, the microinjection of carbachol (2.7 nmol) in the ACC induces antinociception only in the vocalization test. This effect was prevented by prior microinjection of atropine (0.7 nmol) and naloxone (2.7 nmol). In fact, the blockade of μ-opioids receptors with naloxone in ACC prevented the antinociceptive effect of carbachol in the vocalization test. Accordingly, we suggest that the antinociception promoted by carbachol was mediated by the activation of muscarinic receptors on local ACC opioid interneurons. The release of endogenous opioids seems to inhibited the expression of the behavioral response of vocalization. Therefore, we verified that the antinociceptive effect of morphine microinjection in ACC is broader and more robust than that promoted by carbachol.

1. Introduction

The role of the anterior cingulate cortex (ACC) on the affective-motivational and sensory-discriminative components of nociception has been experimentally explored with different behavioral tests (Fuchs et al., 1996; Johansen and Fields, 2004; Zugaib et al., 2014; Cao et al., 2016). Accordingly, the ACC seems to be crucial for the development of nociceptive stimulus-induced aversive memory (Johansen et al., 2001; Fuchs et al., 2014). Furthermore, the acquisition of aversive memory seems to depend on glutamatergic (via NMDA receptors) and cholinergic neurotransmission on ACC neurons (Johansen and Fields, 2004; Malin et al., 2007). On the other hand, GABAergic, opioidergic and dopaminergic stimulation of ACC neurons inhibits the acquisition of aversive memory secondary to nociceptive stimulation (López-Avila et al., 2004; LaGraize et al., 2006; LaGraize and Fuchs, 2007; Pezze et al., 2016).

Avoidance conditioning paradigms with nociceptive stimuli have been used to investigate the effects of the modulation of the ACC on the affective-motivational component of nociception (LaGraize et al., 2004; Pellicer et al., 2007; Yan et al., 2012; Cao et al., 2014; Fuchs et al., 2014; Lu et al., 2015). However, other studies have demonstrated that nociceptive motor behaviors and/or reflexes are modulated by electrical and pharmacological manipulation of the ACC (Fuchs et al., 1996; Lee et al., 1999; Calejesan et al., 2000; Zhang et al., 2005; Yi et al., 2011). In fact, these responses are often related to the sensory-discriminative component of nociception (Tjølsen et al., 1992; Le Bars et al., 2001). Interestingly, the ACC seems to preferentially modulate nociceptive behaviors that express an affective-motivational component (Fuchs et al., 2014).

In the ACC, the excitation/inhibition balance in response to noxious stimuli is mediated by several neurotransmission systems that

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are activated by afferents from sensory and emotional pathways that converge in the ACC (Vogt, 2005; Xie et al., 2009; Shackman et al., 2011). The vocalization response evoked by peripheral noxious stimuli in unanesthetized guinea pigs has been used to investigate nociception brain substrates (Menescal-de-Oliveira and Lico, 1977). This response has been accepted as an expression of the motivational-affective component of nociception (da Silva et al., 2010; Ferreira and Menescal-de-Oliveira, 2014). Specifically, in our previous study, we demonstrated that the nociceptive response of vocalization is modulated by the glutamate/GABA balance in the ACC (Zugaib et al., 2014). During nociceptive stimulation occurs glutamatergic excitation in descending projection neurons from the ACC, which trigger the nociceptive responses (Calejesan et al., 2000; Zhang et al., 2005; Yang et al., 2006; Zheng, 2010). Moreover, the blockade of the GABAergic activity that counteracts this excitation amplifies the nociceptive response (Wang et al., 2005; Zugaib et al., 2014).

Other neurotransmitter systems have been described as mediators of nociceptive behavioral effects in the ACC (Xie et al., 2009; Fuchs et al., 2014; Pezze et al., 2016; Koga et al., 2017). In fact, opioidergic modulation in the ACC produces antinociception in experimental animals and humans (Zubieta et al., 2003; Zhuo, 2005; Vogt and Vogt, 2009; Eippert et al., 2009). Furthermore, cholinergic stimulation in the ACC has also been related to learning and nociceptive memory in models of peripheral neuropathy (Malin et al., 2007; Ortega-Legaspi et al., 2003, 2010). However, we did not find any study investigating cholinergic and opioidergic systems interaction in the ACC in nociceptive responses. Therefore, we examined the function and the possible interaction of these systems in different behavioral nociceptive responses in guinea pigs.

2. Results

2.1. The effect of cholinergic modulation in the ACC on the nociceptive response of vocalization in guinea pigs

We microinjected the nonspecific agonist of cholinergic receptors, carbachol (2.7 nmol), into the ACC of guinea pigs and verified an antinociceptive effect. In fact, the mean amplitude of vocalizations evoked by peripheral noxious stimuli was reduced in the animals treated with carbachol in the ACC (Control: n = 9; Carbachol: n = 8; Two-way ANOVA, p = 0.01; ($F_{(18, 174)} = 4.45$)). This effect was produced 15 min after the microinjection and remained for another 15 min (Figs. 1A and 2B). To test whether this effect was mediated by muscarinic receptors on ACC neurons, we microinjected atropine (0.7 nmol) prior to carbachol. We observed that the blockade of the muscarinic receptors with atropine prevented the antinociception promoted by carbachol (Figs. 1A and 2D, Control: n = 9, Atropine + Carbachol: n = 9; Two-way ANOVA, p > 0.05). We conclude that the activation of muscarinic receptors in the ACC promotes antinociception evidenced by the reduction in the VI. In addition, microinjection of atropine (0.7 nmol) alone did not change the expression of the nociceptive response of vocalization (Figs. 1A and 2C, Control: n = 9; Atropine: n = 7; Two-way ANOVA, p > 0.05).

2.2. Antinociception induced by opioid and cholinergic stimulation in the ACC is prevented by naloxone

We observed that microinjection of morphine (4.4 nmol) in the ACC of guinea pigs also reduced the mean amplitude of vocalizations induced by peripheral noxious stimuli throughout the recording period (Figs. 1B and 2F, Control: n = 9, Morphine: n = 9; Two-way ANOVA, p = 0.002; ($F_{(2, 138)} = 52.2$)). We also verify that the antinociception promoted by the microinjection of morphine was more pronounced than that promoted by the microinjection of carbachol (Fig. 1A and B, Morphine: n = 9; Carbachol: n = 8; Two-way ANOVA, p = 0.002; ($F_{(1, 105)} = 15.11$)). Accordingly, prior microinjection of the naloxone, an antagonist with higher affinity for the μ-opioid receptor, prevented morphine antinociception (Figs. 1B and 2G, Control: n = 9; Naloxone + Morphine: n = 8; Two-way ANOVA, p > 0.05).

Next, we showed that the prior microinjection of naloxone in the ACC prevents the antinociceptive effect of carbachol in the vocalization test (Figs. 1C and 2E, Control: n = 9, Naloxone + Carbachol: n = 8; Two-way ANOVA, p > 0.05). We suggest that opioid interneurons in the ACC are activated by muscarinic cholinergic stimulation and mediate the reduction in the nociceptive response of vocalization in guinea pigs.

2.3. Microinjection of morphine but not carbachol promotes antinociception evaluated by the formalin test

Finally, we also tested the functional role of cholinergic and opioidergic neurotransmission systems in the nociceptive formalin test. Invariably, we observed that in the first five minutes after the formalin injection, the guinea pigs responded with paw shakes and motor agitation. In the second phase, unlike in rats, the guinea pigs did not express a second peak in behaviors and followed with a drastic reduction in recovery behaviors and motor agitation five minutes after the injection. Still, in the second phase, we noticed that some animals exhibited stress responses, such as increased urination, less movement on the platform and coprophagia. The microinjection of carbachol in the ACC did not modify the licking and flinching behaviors of the paw (Fig. 1D). However, microinjection of morphine into the ACC of guinea pigs caused antinociception, as demonstrated by a reduction in licking and flinches of the formalin-injected paw (Fig. 1D, Control: n = 5, Morphine: n = 8; Two-way ANOVA, p = 0.001; ($F_{(33, 297)} = 3.63$)). This finding suggests that the investigated nociceptive responses are modulated distinctly by the cholinergic and opioidergic neurotransmission systems in the ACC. In addition, prior microinjection of naloxone prevented morphine-induced antinociception in the formalin test (Fig. 1D). Figs. 3 and 4 shows a photomicrography and schematic representation of the microinjection sites of the drugs in the ACC.

3. Discussion

The present study demonstrated that intra-ACC injection of morphine induced antinociception in guinea pigs in the nociceptive test of vocalization evoked by peripheral noxious stimulus and in the formalin test. These effects were prevented by prior microinjection of intra-ACC naloxone, suggesting the involvement of μ-opioid receptors in the modulation of nociception in ACC. On the other hand, cholinergic stimulation in the ACC reduces the mean amplitude of vocalizations evoked by peripheral noxious stimuli but not the recuperative behaviors induced by subcutaneous formalin injection in the paw. In addition, the antinociception promoted by cholinergic stimulation in the ACC seems to be mediated by the activation of muscarinic receptors in opioid interneurons. In fact, our results suggest that the investigated nociceptive behaviors recruit these ACC neurotransmission systems and are modulated by them in a differentiated way (Harte et al., 2011; Fuchs et al., 2014).

Classically, opioid neurotransmission in the nervous system is associated with antinociception in animals and humans (Zubieta et al., 2003; Vogt and Vogt, 2009; Fuchs et al., 2014; Zubieta, 2009). In several species, ACC neurons have a high density of opioid receptors (Bozkurt et al., 2005; Vogt and Vogt, 2009). Accordingly,

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