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Research Report

μ- and κ-Opioid receptor activation in the dorsal periaqueductal grey matter differentially modulates panic-like behaviours induced by electrical and chemical stimulation of the inferior colliculus

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ABSTRACT

It has been shown that electrical stimulation of the mesencephalic tectum (MT) provokes defensive responses in both humans and rodents. During an emotional aversive state, some convergent studies have also demonstrated the existence of a complex interaction between endogenous opioid peptide- and γ-aminobutyric acid (GABA)-containing connections during fear-induced responses. It has been proposed that opioid neurons exert an influence on GABAergic interneurons, which, in turn, exert inhibitory tonic control on the mesencephalic excitatory pathways. Thus, opioid peptides can disinhibit neurons that are tonically inhibited by GABA, therefore, modulating the expression of defensive behavioural reactions. In the present work, we used both electric stimulation and microinjections of the $GABA_A$ receptor antagonist bicuculline in the inferior colliculus (IC) of Wistar rats in combination with microinjections of μ- and κ-opioid receptor selective agonists into the dorsal columns of periaqueductal grey matter (dPAG) to evaluate the effects on panic-like behaviours elicited by IC electrical and chemical stimulation. The present results showed that neurochemical lesions of the dPAG caused a significant impairment in the organisation of defensive responses by IC neurons, reducing the duration [$t_{(14)}=3.0$; $p<0.01$] of defensive immobility and the duration [$t_{(14)}=2.8$; $p<0.05$] and frequency [$t_{(14)}=2.5$; $p<0.05$] of escape. Paradoxically, treating the dPAG with the μ-opioid receptor agonist met-enkephalin caused a significant reduction of panic-like behaviours induced by both electrical and chemical stimulation of the IC, increasing the escape behaviour threshold [$F_{(2,23)}=13.5$; $p<0.001$] and decreasing the frequency [$F_{(3,36)}=11.7$; $p<0.001$] and duration [$F_{(3,36)}=11.6$; $p<0.001$] of escape and the duration of defensive immobility [$F_{(3,36)}=16.1$; $p<0.05$]. In contrast, treating the dPAG with the κ-opioid receptor agonist salvinorin-A

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increased the frequency [$F_{(3,36)}=12.4$; $p<0.01$] and duration [$F_{(3,34)}=16.1$; $p<0.01$] of defensive immobility induced by $GABA_A$ receptor blockade in the IC. The present results suggest the existence of a complex neuronal network in the MT in which endogenous opioid peptides and GABAergic pathways interact in the control of fear-related behavioural responses.

1. Introduction

Microinjections of pharmacologic agents that block GABA-A receptors or stimulate NMDA receptors in dorsal midbrain structures, such as the superior and inferior colliculus and the dorsal periaqueductal grey matter (dPAG), elicit defensive behavioural responses (Brandão et al., 1988, Melo et al., 1992, Coimbra and Brandão, 1993, Moreira et al., 2003, Borelli et al., 2006, Coimbra et al., 2006). It is also known that gradual increases in the intensity of electric stimulation of dorsal columns of the periaqueductal grey matter (dPAG), deep layers of the superior colliculus and inferior colliculus (IC) central and pericentral nuclei evoke defensive behaviour, characterised by alertness (defensive attention), freezing (defensive immobility) and escape, followed by autonomic reactions and antinociception (Nashold et al., 1969, Coimbra et al., 1992, Coimbra and Brandão, 1997, Castilho and Brandão, 2001, Coimbra et al., 2006).

Several reports demonstrated the involvement of GABAergic (Coimbra et al., 1989, Coimbra and Brandão, 1993, Brandão et al., 2005), serotonergic (Coimbra and Brandão, 1997, Castilho and Brandão, 2001, Kishimoto et al., 2001, Borelli et al., 2004) and opioid (Jenck et al., 1986, 1988, Blanchard et al., 1997, Kishimoto et al., 2001, Eichenberger et al., 2002, Ribeiro et al., 2005, Castellan-Baldan et al., 2006) mechanisms in the modulation of the neural substrates involved with the organisation of the defence behaviour in the mesencephalic tectum. The role played by endogenous opioid peptides in anxiety and innate fear is not yet completely understood, even though the interaction between the opioid and serotonergic mechanisms in the modulation of panic-related behavioural responses has been receiving increased attention (Roncon et al., 2013). The amygdaloid complex, the hypothalamus and midbrain structures constitute the main neural substrates for the integration of aversive states and defensive behaviour in the brain and brainstem (Graeff, 1993, Brandão et al., 1999, LeDoux, 2003). The correlation between defensive behaviour, fear and anxiety is consistent with many behavioural, electrophysiological and immunohistochemical studies that also show activation of mesencephalic structures by threatening stimuli or conditions (Brandão et al., 1999, 2005, Coimbra et al., 2006, Colasanti et al., 2010).

Opioid modulatory pathways establish reciprocal connections between the central nucleus of the inferior colliculus with the lateral and ventrolateral columns of PAG (Osaki et al., 2003) and between intratectal outputs with nigrotectal GABAergic terminals (Eichenberger et al., 2002, Kirouac et al., 2004). These integrated pathways exert a significant influence on the elaboration of the panic-like defensive reactions (Ribeiro et al., 2005; Castellan-Baldan et al., 2006).

It has been reported that microinjections of low doses of morphine into the midbrain tectum (dPAG and IC) attenuates the aversive consequences of stimulation of dorsal mesencephalon structures in a dose-dependent manner (Jenck et al., 1986, Cardoso et al., 1992). However, high doses of morphine, when locally injected into this region, elicit behavioural activation together with a vigorous defensive response, which is similar to the reaction observed following either the electrical stimulation of this area or local microinjections of GABA-A receptor antagonists (Jenck et al., 1988, Motta and Brandão, 1993). The opposite effect of decreasing fear-induced behaviours is recorded after pretreatment of the inferior colliculus with naloxone, an opioid antagonist, followed by intra-collicular administration of bicuculline (Calvo and Coimbra, 2006).

However, there is controversial evidence on the effects of opioids on panic-like behaviours. Multidisciplinary approaches have suggested that the interaction between endogenous opioid peptides and GABA-A-receptor-mediated inhibitory neural links may represent the neural bases for the antiaversive or antipanic effects of either peripheral or central administration of endogenous opioid peptide receptor antagonists (Coimbra et al., 1996, 2000, Tongjaroenbungam et al., 2004, 2006). In addition, anatomical findings suggest that the opioid and GABAergic systems are closely linked, and the activity of the same neuron may be directly regulated by both GABA and endogenous opioid peptides (Kalyuzhny et al., 2000, Tongjaroenbuangam et al., 2004, 2006).

The study of GABAergic pathways and endogenous opioid peptide-containing projections must be focused on the investigation of the neural substrates of instinctive fear, panic and phobia. Therefore, the aim of the present study was to investigate (a) the effects of neurotoxic lesions of dPAG neurons on the organisation of defensive responses and (b) the effects of microinjection of μ- and κ-opioid receptor selective agonists in the dPAG on the expression of defensive behaviours evoked by electrical stimulation or by $GABA_A$ receptor blockade in the IC.

2. Results

Activation of the IC neurons elicited alertness, freezing and escape defensive behavioural responses in all animals studied here. Non-defensive behaviours such as grooming and rearing, an exploratory behaviour, were also displayed by rodents before and after the aversive stimulation of the dorsal midbrain at the caudal level.

2.1. *Effects of ibotenic acid lesions of the dPAG on defensive behaviours elicited by GABA-A receptor blockade in the IC*

The neurotoxic lesion of dPAG neurons with ibotenic acid microinjections significantly reduced the duration [$t_{(14)}=3.0$;