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OPIOID NEUROTRANSMISSION MODULATES DEFENSIVE BEHAVIOR AND FEAR-INDUCED ANTINOCICEPTION IN DANGEROUS ENVIRONMENTS

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Abstract—The effects of endogenous opioid peptide antagonists on panic-related responses are controversial. Using elevated mazes and a prey-versus-predator paradigm, we investigated the involvement of the endogenous opioid peptide-mediated system in the modulation of anxiety- and panic attack-induced responses and innate fear-induced antinociception in the present work. Wistar rats were intraperitoneally pretreated with either physiological saline or naloxone at different doses and were subjected to either the elevated plus- or T-maze test or confronted by *Crotalus durissus terrificus*. The defensive behaviors of the rats were recorded in the presence of the predator and at 24 h after the confrontation, when the animals were placed in the experimental enclosure without the rattlesnake. The peripheral non-specific blockade of opioid receptors had a clear anxiolytic-like effect on the rats subjected to the elevated plus-maze but not on those subjected to the elevated T-maze; however, a clear panicolytic-like effect was observed, i.e., the defensive behaviors decreased, and the prey-versus-predator interaction responses evoked by the presence of the rattlesnakes increased. A similar effect was noted when the rats were exposed to the experimental context in the absence of the venomous snake. After completing all tests, the naloxone-treated groups exhibited less anxiety/fear-induced antinociception than the control group, as measured by the tail-flick test. These findings demonstrate the anxiolytic and panicolytic-like effects of opioid receptor blockade. In addition, the fearlessness behavior displayed by preys treated with naloxone at higher doses enhanced the defensive behavioral responses of venomous snakes.

Key words: innate fear, conditioned fear/anticipatory anxiety, panic attacks, endogenous opioid peptide-mediated neural system, prey-versus-rattlesnake pit viper paradigm, instinctive fear-induced antinociception.

INTRODUCTION

Polygonal arenas and complex labyrinths with snakes have been used as experimental models of panic attacks (Coimbra et al., 2017b) and are suitable for the study of the antiaversive effects of new potential panicolytic drugs (Uribe-Mariño et al., 2012; Twardowschy et al., 2013). Although both constrictor (Guimarães-Costa et al., 2007; Lobão-Soares et al., 2008) and Viperidae venomous (Almada and Coimbra, 2015) serpents have been used as threatening stimuli to elicit defensive responses in small rodents in these behavioral paradigms, rattlesnakes have not been consistently used as a source of innate aversion-inducing stimuli. However, some animals use sonorous clues to evaluate a dangerous environment. For example, rattlesnakes signal an imminent attack by vibrating their rattles, and some mammals can mimic these rattle sounds to repel other predators from their territory (Owings et al., 2002). Rodents

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Abbreviations: ANOVA, analysis of variance; EAE, end-arm exploration; EPM, elevated plus-maze; ETM, elevated T-maze; FBA, flat-back approach; SAP, stretched attend posture.

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assume a biped posture and hesitate to engage in exploratory behavior or approach an area associated with a snake-produced audible signal (Swaisgood et al., 1999a,b). Therefore, the use of rattlesnakes in prey-versus-predator paradigms may be suitable because rattlesnakes provide threatening visual, olfactory and auditory clues that result in aversion. An instinctive or learned stimulus signaling danger activates the encephalic circuits responsible for generating and elaborating these aversion-inducing states (Blanchard and Blanchard, 1972; Blanchard et al., 1991; Fanselow and Kim, 1994), which are interpreted as a motivational state of fear in humans (Nashold et al., 1969).

Defensive behaviors may consequently be expressed as behavioral inhibition and defensive attention (Hilton, 1982; Coimbra and Brandão, 1993; Guimarães-Costa et al., 2007; Lobão-Soares et al., 2008; Uribe-Mariño et al., 2012), defensive immobility or freezing (Uribe-Mariño et al., 2012; Almada et al., 2015a), escape behavior (Coimbra and Brandão, 1993; Uribe-Mariño et al., 2012; Almada et al., 2015b), or tonic immobility (Leite-Panissi et al., 2003, 2012). Affective defense, which is a set of threatening postures, vocalizations, and eventual attacks (when escape is impossible) (Hess and Bruger, 1943), can also be elicited by preys in a dangerous situation.

The expression of defensive behavior is followed by neurovegetative and endocrine alterations, which have been extensively studied (Mancia and Zanchetti, 1981; Hilton, 1982; Carrive, 1993), as well as by antinociception (Fanselow and Bolles, 1979; Fanselow, 1986; Coimbra et al., 2006), a phenomenon in which both opioid (Terman et al., 1986; Nichols et al., 1989) and non-opioid (Coimbra et al., 1992; Coimbra and Brandão, 1997) mechanisms have been implicated. Interestingly, both opioid (Miczek et al., 1982; Thompson et al., 1988) and non-opioid (Griesel et al., 1993) antinociception have been implicated in behavioral responses evoked during resident-intruder interactions.

Some animals assume appeasing postures of submission to avoid ulterior offensive behaviors by dominant opponents (Miczek and Thompson, 1984). However, the behavioral responses displayed by preys confronted by a potential predator are completely different and are rich in risk assessment behavior, defensive immobility and oriented and non-oriented escape behaviors (Almada et al., 2015b; Almada and Coimbra, 2015; Coimbra et al., 2017b). However, the threatening/defensive and offensive responses displayed by predators in snake-versus-rodent confrontation paradigms must be more carefully ethologically assessed in laboratory environments.

Recently, a new controversy has emerged in the literature regarding the proposal of two new coadjutant treatments for panic syndrome using either opioid agonists at low doses (Roncon et al., 2015, 2017; Maraschin et al., 2016) or opioid receptors antagonists at high concentrations (Eichenberger et al., 2002; Ribeiro et al., 2005; Castellan-Baldan et al., 2006). Endogenous opioid peptides, which act presynaptically on GABAergic terminals, have been proposed to modulate the activity of the dorsal mesencephalon neural networks involved in the organization of defensive behavior (Eichenberger et al., 2002; Osaki et al., 2003; Ribeiro et al., 2005; Castellan-Baldan et al., 2006; Twardowschy and Coimbra, 2015). There is evidence that endogenous opioid peptides inhibit the GABA-mediated synaptic transmission in the periaqueductal gray matter and other encephalic regions by reducing the probability of presynaptic neurotransmitter release (Vaughan et al., 1997; Kishimoto et al., 2001; Tongjaroenbungam et al., 2004).

Considering these findings, a study of the GABAergic and opioid peptidergic pathways is required to identify the neural substrates responsible for fear-induced behavior, panic-like reactions and anticipatory anxiety. Several investigations in the behavioral neurosciences have focused on the neurochemical mechanisms evoked by instinctive or learned stimuli that signal danger (new environments, silhouettes of predators, emotional expressions indicating rage and imminent attacks, odors or sounds of a given predator, threatening vocalizations of intra-specific dominants in a social conflict, or any other factor that may indicate the occurrence of noxious or painful stimuli) (Blanchard et al., 1989; Blanchard et al., 1991, 2003a,b; Griebel et al., 1996; Guimarães-Costa et al., 2007; Almada et al., 2015b).

Most studies focusing on the neurophysiological bases of behavior use invasive techniques in experimental animal models in which only segmental divisions of the limbic system are targeted. These structures are related to the elaboration of emotional states and are usually accessed either by local microinjections of pharmacologic agents or by induction of specific neurotoxic lesions in nuclei rich with a given neurotransmitter (Coimbra and Brandão, 1993; Ribeiro et al., 2005; Biagioni et al., 2013; Almada et al., 2015a, b). However, the entire limbic system is commonly activated when animals are faced with situations that threaten their survival, such as those characterized by the presence of a natural predator.

The neural bases of attentional behavior related to aversion-inducing events, fear and antinociception must be clarified. In controlled environments, these behaviors are usually displayed by experimental animals exposed to imminent danger or elicited by stimulation of the brainstem and the pathways that elaborate or modulate panic-like reactions (Coimbra and Brandão, 1993; Borelli et al., 2005; Ribeiro et al., 2005; Coimbra et al., 2017a), and their underlying neural bases must be clarified. Furthermore, the precise role played by the opioid system in the modulation of panic-like responses, which has been studied using preclinical and clinical approaches, remains controversial (Colasanti et al., 2011), and the neural bases of the antinociception that follows defensive behavior requires further characterization. The aim of this work was to investigate the possible anxiolytic and panicolytic effects of systemic opioid peptide receptor inhibition using two classical models of unconditioned fear-induced responses, including the elevated plus- and T-maze (EPM and ETM, respectively) tests, and an experimental setup based on confrontations between rodents and rattlesnakes (Coimbra et al., 2017b). The