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Research report

Similar effect of CRF_1 and CRF_2 receptor in the basolateral or central nuclei of the amygdala on tonic immobility behavior

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ABSTRACT

Studies have used paradigms based on animal models to understand human emotional behavior because they appear to be correlated with fear- and anxiety-related defensive patterns in non-human mammals. In this context, tonic immobility (TI) behavior is an innate response associated with extreme threat situations, such as predator attack. Some reports have demonstrated the involvement of corticotropin-releasing factor (CRF) in regulation of the endocrine system, defensive behaviors and behavioral responses to stress. Particularly, a previous study showed that the activation of CRF receptors in the basolateral (BLA) or central (CeA) nuclei of the amygdala increased TI responses, whereas treatment with a non-selective CRF antagonist, alpha-helical-CRF_{9-41}, decreased this innate fear response. However, while CRF_1 receptors have pronounced effects in stress-induced anxiety, CRF_2 receptors appear be involved in the expression of both stress-induced anxiety and spontaneous anxiety behavior. In this study, we investigated the effects of specific CRF receptors, CRF_1 and CRF_2, in the BLA and CeA on the duration of TI in guinea pigs. The results show that blockade of CRF_1 and CRF_2 receptors in the BLA and CeA produces a decrease in fear and/or anxiety, as suggested by a decrease in TI duration in the guinea pigs. Additionally, the specific antagonists for CRF_1 and CRF_2 receptors were able to prevent the increase in TI duration induced by CRF administration at the same sites. These results suggest that the modulation of fear and anxiety by the CRF system in the BLA and CeA occurs through concomitant effects on CRF_1 and CRF_2 receptors.

1. Introduction

The neurobiology of stress-related mood disorders and related anxiety disorders has been extensively studied over the last decades to develop new strategies and effective therapeutic approaches to psychiatric diseases (Catena-Dell'Osso et al., 2013; Kormos and Gaszner, 2013). However, limited understanding of the pathophysiology of emotional disorders affects the development of novel therapeutic drugs (Catena-Dell'Osso et al., 2013). Thus, the progression of basic research with respect to the neurobiological mechanisms involved in mood disorders and anxiety is critical to support the development of reliable approaches.

Since corticotropin-releasing factor (CRF) was isolated and characterized as the major physiological regulator of the hypothalamus-pituitary-adrenal gland axis and was shown to be responsible for the coordination of the endocrine, autonomic and behavioral responses associated with stress (Vale et al., 1981), this neuropeptide has become a potential target for the treatment of stress-related mood disorders (Kormos and Gaszner, 2013). Although the highest concentration of CRF is found in the hypothalamus (Bittencourt and Sawchenko, 2000), this peptide is widely distributed within the central nervous system, including limbic areas (Cummings et al., 1983), which reinforces its involvement in the modulation of emotional responses (Anthony et al., 2014; Donatti and Leite-Panissi, 2011; Henry et al., 2006; Klemm, 2001; Radulovic et al., 1999). In particular, the central nucleus of the amygdala has one of the highest densities of CRF-immunoreactive neurons in the brain (Cummings et al., 1983; Palkovits et al., 1985; Swanson et al., 1983). The CRF neuropeptide exerts its biological activity by binding to two types of CRF receptors, CRF_1 and CRF_2 receptors (Hauger et al., 2006). More specifically, whereas a high density of CRF_1 receptors is found in the anterior lobe of the pituitary, neocortex, hippocampus, basolateral nucleus of the amygdala and brainstem (Potter et al., 1994; Sánchez et al., 1999), CRF_2 receptor expression is more restricted to subcortical structures, including the hypothalamus, amygdala, and brainstem (Lovenberg et al., 1995).

Fear-like behaviors are produced by intracerebroventricular CRF administration (Meloni et al., 2006; Radulovic et al., 1999), as well its administration into specific brain areas such as the amygdala (Daniels

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et al., 2004; Donatti and Leite-Panissi, 2011), the periaqueductal gray matter (Martins et al., 1997), the hippocampus (Radulovic et al., 1999), and the lateral septum (Bakshi et al., 2002). In this way, while antagonist CRF_1 receptors have pronounced effects in normalizing stress-induced anxiety when CRF is released, CRF_2 receptors appear be involved in the expression of both stress-induced anxiety and spontaneous anxiety behavior (Takahashi, 2001). Moreover, although preclinical data using CRF_1 receptor antagonists in experimental animal models were unclear concerning their antidepressant activity (Nielsen, 2006), this receptor is considered to be possible target for the treatment of psychiatric diseases (Arborelius et al., 1999; Heinrichs et al., 1997; Reul and Holsboer, 2002). Additionally, previous study has shown that the activation of neurons that express CRF_2 in the lateral septum promotes persistent anxious behavior, as evaluated by the light-dark box test, the open field test and the novel object test in mice (Anthony et al., 2014).

Many studies have used paradigms based on animal models to understand human emotional behavior because it appears to be correlated with fear- and anxiety-related defensive patterns in non-human mammals (Blanchard et al., 2001). In this case, defensive reactions are used to study the neural substrate of the modulation of innate fear and anxiety responses (Canteras, 2003). Defensive reactions are triggered based on prey-predator distance (Ratner, 1967) and on the degree of threat posed by the situation (Blanchard and Blanchard, 1988). Therefore, when a predator or other dangerous stimulus is very close to the prey and physical contact is possible, behavioral responses such as fight or flight are exhibited. However, when physical contact is prolonged and there is no chance to escape, the prey's last attempt to survive is the tonic immobility (TI) response, or "death feigning" (Klemm, 2001).

The tonic immobility (TI) response is an innate and reversible defensive response characterized by profound physical inactivity and relative lack of responsivity to environmental stimuli (Klemm, 2001; Ratner, 1967). This behavior is shown during situations of extreme, inescapable threat (Gallup, 1977) and is observed in many species of invertebrate and vertebrate animals (Klemm, 2001; Ratner, 1967) including humans (Volchan et al., 2011). In fact, previous studies have suggested that tonic immobility can predict the severity of posttraumatic stress disorder (PTSD) symptoms (Fiszman et al., 2008; Lima et al., 2010; Rocha-Rego et al., 2009).

During the TI response, neurovegetative and behavioral alterations are observed, including vocalizations, intermittent eye closure, muscular stiffness, parkinsonism-like tremors (Jones, 1986), and alterations in neural activity (Rusinova and Davydov, 2010) as well as changes in heart rate, breathing (Giannico et al., 2014), and body temperature (Eddy and Gallup, 1990). The neurophysiological events that occur while TI is exhibited have been observed in aversive emotional states and resemble innate fear (Nash et al., 1976). Recently, Alves and colleagues (Alves et al., 2014) have demonstrated a positive correlation between heart rate changes after viewing trauma-related pictures and tonic immobility scores in individuals exposed to a traumatic event. This innate fear response can be induced in the laboratory by a manual inversion and restriction of animal movements; tactile and proprioceptive sensations are essential to trigger TI behavior (Gallup, 1977; Klemm, 2001). Regarding the neural substrates involved in TI modulation, previous studies have shown that distinct structures of the central nervous system, such as the periaqueductal gray matter (Vieira et al., 2011), hypothalamus (De Oliveira et al., 1997), and amygdaloid complex (Donatti and Leite-Panissi, 2011, 2009; Leite-Panissi et al., 2006, 2003; Leite-Panissi and Menescal-de-Oliveira, 2002). In particular, Donatti and Leite-Panissi (Donatti and Leite-Panissi, 2011) showed that the activation of CRF receptors in the BLA or CeA increased the TI response, whereas treatment with a non-selective CRF antagonist, alpha-helical-CRF_{9-41}, decreased this innate fear response. So, these data support the role of the amygdaloid CRF system in the control of emotional responses; however, further experiments are required to understand the interplay between CRF receptors and the TI response to provide support for the development of new drugs to treat emotional disorders in humans.

Based on the finding described above, the present study was designed to investigate the effects of specific CRF receptors, CRF_1 and CRF_2, in the BLA and CeA on the duration of TI in guinea pigs (*Cavia porcellus*). To this end, we evaluated whether administration of the CRF_1 receptor antagonist CP-376395 or the CRF_2 receptor antagonist Astressin 2B into the BLA or CeA could alter TI duration. Additionally, we investigated whether previous microinjection of CP-376395 or Astressin 2B into the BLA or CeA could modify the CRF-induced increase in TI behavior.

2. Materials and methods

2.1. Ethics statement

The experiments were carried out in compliance with the recommendations of international guides for animal use with the approval of the Animal Care and Use Committee of the University of São Paulo-Brazil, Campus of Ribeirão Preto (Protocol number 12.1.1393.53.0). All efforts were made to minimize animal suffering.

2.2. Animals

Adult male guinea pigs (*Cavia porcellus*, from the University of São Paulo, *Campus* of Ribeirão Preto, Brazil) weighing 400–500 g (n = 114) were kept in Plexiglas wall cages (56 cm × 17 cm × 39 cm, five guinea pigs per cage) in a room maintained at 24 ± 1° C, on a 12 h light cycle, with free access to water and food throughout the experimental period.

2.3. Tonic immobility recording

Each guinea pig was submitted to five maneuvers of TI induction and the duration of each episode was recorded. Induction of TI was attempted by holding the guinea pig around the thorax with the hands, then quickly inverting the animal and pressing the animal down into a shaped plywood trough (25 cm long × 15 cm high). The pressure applied by the experimenter was proportional to the resistance offered by the guinea pig during the restraining maneuver. When the guinea pig stopped moving, the experimenter slowly withdrew his hand and a chronometer was activated to measure the duration of the response (in seconds). The response was considered to be finished when the guinea pig resumed an upright position. If the guinea pig did not become motionless within 60 s, the duration of the episode was recorded as zero. For group analysis, the mean of each guinea piǵs five episodes was used.

2.4. Surgical procedures

One day after the control TI experiment, the guinea pigs were anesthetized by an intramuscular injection of ketamine (100 mg/kg) plus xylazine (14 mg/kg) and placed in a stereotaxic apparatus (David-Kopf Instruments, USA) with the buccal piece 21.4 mm below the interauricular line. One guide cannula (14 mm in length and 0.6 mm in diameter, prepared from a hypodermic needle) was implanted into the left hemisphere toward the BLA or the CeA nuclei. According to the Rössner (Rössner, 1965) atlas for guinea pig, the stereotaxic coordinates for the placement of the guide cannula implanted toward the BLA were 3.4 mm caudal to the bregma, 6.0 mm lateral to the midline,