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Research report

Dorsomedial hypothalamus CRF type 1 receptors selectively modulate inhibitory avoidance responses in the elevated T-maze

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HIGHLIGHTS

- CRF intra-dorsomedial hypothalamus is anxiogenic.
- Antalarmin intra-dorsomedial hypothalamus is anxiolytic.
- Antalarmin couteracts the anxiogenic effects of CRF.
- Panic-related responses and locomotor activity are not altered.

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ABSTRACT

Corticotropin-releasing factor (CRF) plays a critical role in the mediation of physiological and behavioral responses to stressors. In the present study, we investigated the role played by the CRF system within the dorsomedial hypothalamus (DMH) in the modulation of anxiety- and panic-related responses. Male Wistar rats were administered into the DMH with CRF (125 and 250 ng/0.2 μl, experiment 1) or with the CRFR1 antagonist antalarmin (25 ng/0.2 μl, experiment 2) and 10 min later tested in the elevated T-maze (ETM) for inhibitory avoidance and escape measurements. In clinical terms, these responses have been respectively related to generalized anxiety and panic disorder. To further verify if the anxiogenic effects of CRF were mediated by CRFR1 activation, we also investigated the effects of the combined treatment with CRF (250 ng/0.2 μl) and antalarmin (25 ng/0.2 μl) (experiment 3). All animals were tested in an open field, immediately after the ETM, for locomotor activity assessment. Results showed that 250 ng/0.2 μl of CRF facilitated ETM avoidance, an anxiogenic response. Antalarmin significantly decreased avoidance latencies, an anxiolytic effect, and was able to counteract the anxiogenic effects of CRF. None of the compounds administered altered escape responses or locomotor activity measurements. These results suggest that CRF in the DMH exerts anxiogenic effects by activating type 1 receptors, which might be of relevance to the physiopathology of generalized anxiety disorder.

1. Introduction

Stress is a complex phenomenon that refers to the observation that any environmental change can disrupt the maintenance of homeostasis, causing a series of physiological and behavioral modifications that together encompass the so-called "stress response" [1]. One of the main physiological alterations that accompany the stress reaction is the activation of the hypothalamic–pituitary–adrenocortical (HPA) axis by corticotropin-releasing factor (CRF) release from the paraventricular nucleus of the hypothalamus. CRF activates the HPA axis by stimulating adrenocorticotropic hormone (ACTH) release from the anterior pituitary. ACTH, in turn, triggers the release of glucocorticoids from the adrenal cortex.

Since it regulates the HPA axis activity, CRF is a critical element for the maintenance of an organism's homeostasis. Nevertheless, nowadays CRF has also been considered for its role within the central nervous system (CNS) [2,3], outside the HPA axis [4]. In fact, CRF neurons have been identified in several different brain regions, i.e.,

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the neocortex, amygdala, medial septal nucleus, thalamus, other hypothalamic nuclei, cerebellum, and in autonomic midbrain and hindbrain nuclei [5,6].

CRF produces its biological effects binding to two major protein-coupled receptors, CRF type 1 (CRFR1) and CRF type 2 (CRFR2). The activation of CRFR1 has been associated with an increased reaction to stress and anxiety-like behavior [7–10]. On the other hand, the involvement of CRFR2 with stress and anxiety-related responses is still a matter of debate [7–10].

The medial hypothalamus has been implicated in a series of different behavioral and physiological functions, including feeding and metabolism, reproduction and stress/anxiety [11,12]. The region is composed of a number of well-circumscribed neuronal groups [12] – i.e., the dorsomedial hypothalamus (DMH), the anterior hypothalamus, the ventromedial hypothalamus and the premamillary nucleus – that are highly interconnected. The medial hypothalamus seems to be particularly involved with the integration of innate stress/anxiety-related responses to environmental threats [12–17]. In fact, the electrical stimulation of the DMH induces escape behavior and autonomic arousal that resemble the ones presented by animals when facing natural threats [18–20]. Administration of glutamate agonists [13,21] and of GABA antagonists into the structure [22,23] also evokes a similar defense pattern.

CRF positive neurons have been shown to be present in large numbers in the DMH [24]. Furthermore, the region possesses a substantial concentration in particular of CRF type 1 receptors [25]. It has been previously demonstrated that intracerebroventricular administration of CRF and corticosterone induces neurochemical changes in the DMH, i.e., increases in dopamine and serotonin, that parallel changes observed in the region following exposure to a variety of physical and psychological stress-related stimuli [26]. Also, intraperitoneal injection of JNJ19567470/CRA5626, a CRFR1 antagonist, prevents the sodium lactate-induced panic-like behavioral and cardiovascular responses observed in adult male rats with chronic reduction of GABA levels in the region [27].

Nevertheless, apart from this indirect evidence implicating the DMH CRF system with stress/anxiety, to our knowledge no previous study has investigated the effects of the direct infusion of CRF or CRF-related compounds into the region in the modulation of stress/anxiety-related responses. The present study addresses this question by investigating the effects of intra-DMH CRF and antalarmin – a CRFR1 antagonist [28] – in an animal model of anxiety, the elevated T-maze (ETM). The model, composed of one arm enclosed by walls disposed perpendicularly to two opposed open arms, allows the measurement of an anxiety and a panic-related response.

Although the distinction between anxiety and panic is often unclear, the ethopharmacological analysis of the rodent defensive repertoire has provided a sound theoretical framework [29]. According to this view, anxiety is an emotion related to behavioral inhibition and risk assessment, reactions performed in situations of potential danger, either because the context is new or because the aversive stimulus was once present in the past. These responses also seem to be performed when an animal is faced with a threat, which involves a conflict between approach and avoidance (e.g. seeking for food in an area where a predator was once present) [30,31]. On the other hand, panic corresponds to vigorous escape or flight reactions evoked by proximal danger [29]. In terms of psychopathology, it has been proposed that the neurobiological substrates that regulate anxiety are disrupted in generalized anxiety, while dysfunction of the brain circuitry controlling proximal defense reactions has been related to panic disorder [32].

The ETM was developed on the basis of the ideas presented above [33]. The model is composed of one arm surrounded by 40-cm high walls, disposed perpendicularly to two opposed open arms. ETM inhibitory avoidance is an anxiety-related response, measured by placing the animal for three consecutive times at the distal end of the closed arm of the maze and registering the latency to leave this arm with the four paws. Escape, on the other hand, is a panic-related response, measured by placing the animals directly in one of the open arms of the maze and measuring the latency to leave this arm with the four paws.

The pharmacological validation of the ETM has shown that compounds representative of three classes of anxiolytics – namely the agonist of benzodiazepine receptors diazepam, the serotonin 1A agonist buspirone, and the nonselective serotonin type 2 antagonist ritanserin – selectively impair inhibitory avoidance while leaving one-way escape unchanged [34–36]. These results are compatible with the view that inhibitory avoidance relates to generalized anxiety. In contrast the escape task is impaired by chronic, but not acute administration of imipramine [37], clomipramine and fluoxetine [38], drugs that are used to treat panic. As a result, ETM escape has been used as an animal model of panic disorder.

To further verify if the anxiogenic effects of CRF were in fact mediated by CRFR1 activation, in the present study we also investigated the effects of the combined treatment with CRF and antalarmin.

2. Materials and methods

2.1. Subjects

Male Wistar rats (CEDEME, Universidade Federal de São Paulo, Campus Santos, Brazil), weighing 280–320 g at the beginning of the experiment, were housed in groups of 5–6 per cage. After surgery, animals were housed in pairs in Plexiglas-walled cages until testing. Room temperature was controlled ($22 \pm 1\,^{\circ}C$) and a light–dark cycle was maintained on a 12-h on–off cycle (0700–1900 h lights on). Food and water were available all throughout the experiments. The study was approved by the Ethical Committee for Animal Research of the Federal University of São Paulo and was performed in compliance with the recommendations of the Brazilian Society of Neuroscience and Behavior, which are based on the conditions stated by the "Guide for the Care and Use of Laboratory Animals" (Institute of Laboratory Animal Resources on Life Sciences, National Research Council, 1996).

2.2. Apparatus

The elevated T-maze was made of wood and had 3 arms of equal dimensions (50×12 cm). One of the arms was enclosed by 40 cm high walls and was oriented perpendicularly to two opposed open arms. The whole apparatus was elevated 50 cm above the floor. To avoid falls, a 1 cm high Plexiglas rim surrounded the open arms.

An open field, composed of a round arena (60×60 cm), with the floor divided into 12 parts, and walls 50 cm high, was used to evaluate locomotor activity.

Luminosity at the level of the T-maze arms or at the open field center was 60 lx. After the experimental sessions, each experimental apparatus was cleaned with a 10% ethanol solution.

2.3. Compounds

Rat/human CRF (r/h CRF no. 102-282-15 – PBL, The Salk Institute for Biological Studies, USA) was initially dissolved in a solution of 0.04% acetic acid and 0.02 M KPBS and diluted 1:1 in sterile saline (0.9%). Control animals were administered with a solution of 0.04% acetic acid and 0.02 M KPBS in sterile saline (0.9%) (1:1). The CRF type 1 antagonist antalarmin (no. A8727, Sigma, USA) was dissolved in sterile saline (0.9%) with 2% Tween 80. Control animals were injected with a solution of sterile saline (0.9%) with 2% Tween 80.