Psychoneuroendocrinology (2014) 43, 114—125

Available online at www.sciencedirect.com

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Mineralocorticoid receptors in the ventral tegmental area regulate dopamine efflux in the basolateral amygdala during the expression of conditioned fear

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Received 18 November 2013; received in revised form 20 January 2014; accepted 10 February 2014

KEYWORDS
Fear conditioning;
Mineralocorticoid receptors;
Glucocorticoid receptors;
Ventral tegmental area;
Basolateral amygdala complex;
Dopamine

Summary Despite the recognized involvement of corticosteroids in the modulation of emotional behavior, the specific role of mineralocorticoid receptors (MRs) and glucocorticoid receptors (GRs) in the expression of conditioned fear responses is still open to investigation. The present study sought to clarify the involvement of both types of corticosteroid receptors in two different brain regions — the ventral tegmental area (VTA) and the basolateral amygdala complex (BLA) — on the expression of conditioned fear. The first experiment assessed the effects of intra-VTA or intra-BLA administration of spironolactone (MR antagonist) or mifepristone (GR antagonist) on the expression of conditioned freezing to a light-CS and on motor performance in the open-field test. Intra-VTA spironolactone, but not mifepristone, attenuated the expression of the conditioned freezing response whereas intra-BLA spironolactone or mifepristone had no significant effects. These treatments did not affect motor performance in the open-field test. Since dopamine is released in the BLA from the VTA during the expression of conditioned fear, the anxiolytic-like effect of decreased corticosteroid activity in the first experiment could be associated with changes in dopaminergic neurotransmission. The second experiment, using in vivo microdialysis, investigated the role of MRs in the VTA on dopamine levels in the BLA during the expression of conditioned fear. Blocking MRs locally in the VTA with spironolactone reduced dopamine efflux in the BLA and decreased the expression of conditioned freezing in response to the CS. Taken together, the data indicate that corticosterone, acting locally on MRs in the VTA, stimulates dopamine efflux in the BLA, which facilitates the expression of conditioned freezing to a light-CS.

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http://dx.doi.org/10.1016/j.psyneuen.2014.02.010
0306-4530/

1. Introduction

Considering the complexity of aversive information processing and defensive response expression, a combined action of several stress mediators may be required for optimal performance during threatening situations. With specific regard to fear conditioning, much research has been performed elucidating the involvement of distinct mediators during its acquisition and consolidation phases, but comparatively less is known about the retrieval and expression of conditioned fear memories (Lupien and McEwen, 1997; Rodrigues et al., 2009). Taking into account the adaptive importance of previous experience retrieval for the expression of appropriate defensive responses, studying the neural substrates and mediators involved in these processes is of great interest, especially because of their relevance to different aspects of human anxiety disorders.

The hypothalamic-pituitary-adrenocortical (HPA) axis activity, which leads to the release into the bloodstream of corticosteroids (cortisol in primates, corticosterone in rodents), has been considered a key part of the stress reaction and can be triggered either by innate or conditioned aversive stimuli (Cordero et al., 1998; Reis et al., 2012). Corticosteroids are hormones that can easily pass the blood—brain barrier, thus affecting a variety of fear-related brain areas (McEwen et al., 1969; Stevens et al., 1971). In the brain, corticosteroids bind to two types of receptors: mineralocorticoids (MRs) and glucocorticoids (GRs) (Reul and de Kloet, 1986; Lu et al., 2006). Despite the recognized involvement of corticosteroids in modulating emotional behavior, the specific role of MRs and GRs in the expression of conditioned fear responses is still open to investigation. The present study sought to clarify the possible involvement of both types of corticosteroid receptors in two different fear-related brain regions — the ventral tegmental area (VTA) and the basolateral amygdala complex (BLA) — on the expression of conditioned fear. Previous studies confirmed that both MRs and GRs are present in VTA and BLA neurons (Harfstrand et al., 1986; Ronken et al., 1994; Johnson et al., 2005). So, the first experiment assessed the effects of intra-VTA or intra-BLA administration of spironolactone (MR antagonist) or mifepristone (GR antagonist) on the expression of conditioned freezing to a light-CS and on motor performance in the open-field test.

Recently, several laboratories have shown great interest in the interaction between the activation of the HPA axis and dopaminergic neurotransmission during aversive states (Barr et al., 2009; Sapolsky, 2009). Dopamine, although more commonly associated with the reinforcing effects of various stimuli, is one of the most active neuromodulators of fear and anxiety (Reis et al., 2004; de Oliveira et al., 2006; Fadok et al., 2009; Zweifel et al., 2011). In fact, dopamine is released in the BLA (consisting of the lateral, basal and accessory basal nuclei) from neurons of the VTA during the expression of conditioned fear (de Oliveira et al., 2011, 2013). Furthermore, quinpirole (a dopamine D_2 receptor agonist) targeting autoreceptors in the VTA or microinjections of sulpiride (a dopamine D_2 receptor antagonist) into the BLA decrease the expression of conditioned fear responses (de Oliveira et al., 2009, 2011; de Souza Caetano et al., 2013). These findings suggest that reducing the activity of dopaminergic neurons in the VTA-BLA pathway reduces conditioned fear. However, the neurohumoral mechanisms involved in regulating the dopamine efflux in the VTA-BLA pathway triggered by a CS remain to be clarified.

In an attempt to determine the extent to which the combined action of the HPA axis and dopaminergic neurotransmission is important for the expression of conditioned fear responses, we observed in a previous study that systemic administration of metyrapone (a corticosterone synthesis blocker) prevented enhanced dopamine release in the BLA during a conditioned fear test and decreased the expression of conditioned freezing (de Oliveira et al., 2013). Thus, HPA axis activation seems to be an important step in an integrated neuroendocrine—neurochemical—behavioral response when the organism evaluates and interprets the threat associated with a specific environmental stimulus and subsequently triggers adaptive defense reactions to cope with this situation. To further clarify this issue, in the second part of the present study using in vivo microdialysis, we examined the influence of MRs in the VTA on modulating the release of dopamine in the BLA during the expression of conditioned fear.

2. Methods

2.1. Animals

One-hundred and forty naive male Wistar rats from the animal facility of the Campus of the University of São Paulo at Ribeirão Preto were used. The rats, weighing 270—290 g at the beginning of the experiments, were housed in groups of four in plastic boxes (40 cm × 33 cm × 26 cm) and maintained under controlled conditions (23 ± 1 °C; 12 h/12 h light/dark cycle, lights on at 0700 h) with food and water freely available. The experiments were carried out during the light phase of the cycle. All the procedures were approved by the Committee for Animal Care and Use of the University of São Paulo at Ribeirão Preto (No. 10.1.595.53.7), and were performed in compliance with the recommendations of the Brazilian Society of Neuroscience and Behavior, which are based on the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize animal suffering, to reduce the number of animals used, and to utilize alternatives to in vivo techniques, if available.

2.2. Surgery

The rats were anesthetized with ketamine/xylazine (100/7.5 mg/kg, intraperitoneal; Agener União, Embu-Guaçu, SP, Brazil) and fixed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA). The incisor bar was set at 3.0 mm below the interaural line, such that the skull was horizontal between bregma and lambda. For experiment 1, bilateral guide cannulae for drug injections (0.6 mm outer diameter, 0.4 mm inner diameter) were stereotaxically implanted over the VTA or the BLA. For experiment 2, unilateral VTA cannulation for drug injection and unilateral BLA cannulation for the microdialysis probe (CMA/12; CMA/Microdialysis AB, Solna, Sweden) were performed in the right hemisphere. In previous studies, we showed that microdialysis with a