Psychoneuroendocrinology 70 (2016) 1–9

Contents lists available at ScienceDirect

Psychoneuroendocrinology

journal homepage: www.elsevier.com/locate/psyneuen

Enhanced noradrenergic activity in the amygdala contributes to hyperarousal in an animal model of PTSD

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ARTICLE INFO

Article history:
Received 30 October 2015
Received in revised form 29 March 2016
Accepted 22 April 2016

Keywords:
PTSD
Noradrenaline
Amygdala
Propranolol
Clenbuterol
Hyperarousal

ABSTRACT

Increased activity of the noradrenergic system in the amygdala has been suggested to contribute to the hyperarousal symptoms associated with post-traumatic stress disorder (PTSD). However, only two studies have examined the content of noradrenaline or its metabolites in the amygdala of rats previously exposed to traumatic stress showing inconsistent results. The aim of this study was to investigate the effects of an inescapable foot shock (IFS) procedure (1) on reactivity to novelty in an open-field (as an index of hyperarousal), and (2) on noradrenaline release in the amygdala during an acute stress. To test the role of noradrenaline in amygdala, we also investigated the effects of microinjections of propranolol, a β-adrenoreceptor antagonist, and clenbuterol, a β-adrenoreceptor agonist, into the amygdala of IFS and control animals. Finally, we evaluated the expression of mRNA levels of β-adrenoreceptors (β1 and β2) in the amygdala, the hippocampus and the prefrontal cortex. Male Wistar rats (3 months) were stereotaxically implanted with bilateral guide cannulae. After recovering from surgery, animals were exposed to IFS (10 shocks, 0.86 mA, and 6 s per shock) and seven days later either microdialysis or microinjections were performed in amygdala. Animals exposed to IFS showed a reduced locomotion compared to non-shocked animals during the first 5 min in the open-field. In the amygdala, IFS animals showed an enhanced increase of noradrenaline induced by stress compared to control animals. Bilateral microinjections of propranolol (0.5 μg) into the amygdala one hour before testing in the open-field normalized the decreased locomotion observed in IFS animals. On the other hand, bilateral microinjections of clenbuterol (30 ng) into the amygdala of control animals did not change the exploratory activity induced by novelty in the open field. IFS modified the mRNA expression of β1 and β2 adrenoreceptors in the prefrontal cortex and the hippocampus. These results suggest that an increased noradrenergic activity in the amygdala contributes to the expression of hyperarousal in an animal model of PTSD.

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1. Introduction

Post-traumatic stress disorder (PTSD) is an anxiety disorder that results from experiencing an extremely traumatic event and is defined as a long term, maladaptive stress response (Pitman et al., 2012; Yehuda et al., 2015). PTSD is characterized in part by symptoms of hyperarousal resulting from a non-associative general sensitization process (Dunsmoor and Paz, 2015; Pitman et al., 2012; Stam, 2007). Also, heightened heart rate reactivity to startling stimuli (loud tones) and larger skin conductance response to novel stimuli have been observed in PTSD patients (Pitman et al., 2012). In animal models, exaggerated acoustic startle response and reduced locomotor activity in a novel environment have been used as measures of hyperarousal after exposure to traumatic stressors (Hendriksen et al., 2010; Kinn Rød et al., 2012; Stam, 2007; van Dijken et al., 1992; Wang et al., 2012).

Noradrenaline has a special role in mediating arousal and emotional memories, and is also involved in fear responses (Roozendaal and McGaugh, 2011; Sara, 2009). It has been proposed that an altered noradrenergic activity may contribute to the hyperarousal symptoms associated with PTSD (Krystal and Neumeister, 2009; O'Donnell et al., 2004; Southwick et al., 1999; Strawn and Geracioti, 2008). This proposal is based on studies showing that the reduction and increase of noradrenergic activity attenuated and precipitated, respectively, some of the symptoms in PTSD patients (Boehnlein and Kinzie, 2007; Bremner et al., 1997; Raskind et al., 2007; Southwick et al., 1993; Taylor et al., 2008). Additionally, the systemic treatment with pharmacological agents that reduce

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http://dx.doi.org/10.1016/j.psyneuen.2016.04.018
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noradrenergic transmission normalized acoustic startle response in mice previously exposed to inescapable foot-shock (IFS) (Olson et al., 2011). Moreover, the evoked responses to a noxious event are higher in neurons of the locus coeruleus (primary source of noradrenaline in the forebrain) of rats previously exposed to single prolonged stress (George et al., 2013).

The amygdala is a key area involved in the recognition of dangerous stimuli and the coordination of fear response (LeDoux, 2007; Roozendaal and McGaugh, 2011). Indeed, it has been reported exaggerated amygdala activation in response to trauma-related stimuli as well as generic (neutral) stimuli in patients with PTSD compared to control subjects (Pitman et al., 2012; Rauch et al., 2006). Therefore, an increased stress-related noradrenergic activity in amygdala may contribute to the expression of hyperarousal symptoms in PTSD. Despite this, and to our knowledge, there are only two studies examining the content of noradrenaline or its metabolites in the amygdala of rats previously exposed to traumatic stress showing inconsistent results (Hendriksen et al., 2010; Tsuda et al., 1986). Moreover, no studies have monitored the *in vivo* release of noradrenaline in the amygdala or determined the causal relationship between these changes of noradrenaline outflow and the alterations of behavior in animals exposed to traumatic stress. Although the amygdala seems to be the main area upon which noradrenaline exerts its effects, other areas mediating fear processing such as prefrontal cortex and hippocampus may also be involved (Pitman et al., 2012).

To investigate whether an enhanced noradrenergic activity in the amygdala is involved in PTSD-like behavioral changes, we exposed male Wistar rats to an IFS procedure. This paradigm induces both long-lasting conditioned and non-conditioned anxiety (Chen et al., 2012; Daviu et al., 2010; Hendriksen et al., 2010; Hendriksen et al., 2012; Kinn Rød et al., 2012; van Dijken et al., 1992). In particular, IFS procedure produces a reduction in the activity in unknown environments 7 days after exposure to the shock (Daviu et al., 2010; Hendriksen et al., 2010; van Dijken et al., 1992, 1993). This effect of IFS has been considered an index of hypervigilance similar to the hyperarousal observed in PTSD patients (Pitman et al., 2012; Stam, 2007).

The specific aims of this study were to investigate: (1) the effect of IFS on the reactivity to novelty in an open-field (as an index of hyperarousal) and the noradrenaline release in the amygdala during an acute restraint stress; (2) the effect of the amygdala injection of the β-adrenoceptor antagonist propranolol on the changes in the reactivity to novelty produced by IFS; (3) the effect of the amygdala injection of the β-adrenoceptor agonist clenbuterol in control rats; and (4) the expression of mRNA levels of β-adrenergic (β1 and β2) receptors in the amygdala, the hippocampus and the prefrontal cortex of control and IFS rats.

2. Methods and materials

2.1. Animals

Young adult male Wistar rats (Harlan, The Netherlands) were 4 weeks old (125–150 g) upon arrival. Experimental procedures started 2 months later. Animals were housed (2 animals per cage) and provided with food and water *ad libitum* and maintained in a temperature-controlled room (22 ± 2 °C) under an inverted light/dark cycle (lights on at 20:00–8:00). The experiments were carried out during the dark phase of the cycle between 14:00 and 19:00. Three different sets of animals were used for (a) evaluation of the behavioral effects of IFS procedure and mRNA quantification, (b) microdialysis experiments, and (c) microinjection experiments (Fig. 1). All experiments carried out in our laboratory at the Universidad Complutense of Madrid followed the Spanish regulations for the protection of laboratory animals (RD53/2013).

2.2. Inescapable Foot-Shock (IFS) procedure

Rats were place in a shuttle-box divided into 2 compartments separated by a guillotine door. The starting compartment (light compartment, 50 × 50 × 20 cm) consisted on an open roof white plastic compartment illuminated by a 60 W bulb at 40 cm overhead. The shock compartment (dark compartment, 25 × 25 × 20 cm) was made of black plastic, a removable roof, no illumination, and an electrified grid floor. Rats were first place in the light compartment for 60 s and then the guillotine door was opened. Once the animal entered in the dark compartment the door was closed and 10 shocks (0.86 mA) of 6 s duration in 10 min were given. The conditions of the IFS procedure were based in previous studies (Hendriksen et al., 2010, 2012; van Dijken et al., 1992, 1993). Control animals remained 10 min in the dark compartment without receiving shocks. Rats were then placed back in their home cage during 7 days before experiments started. All animals were handled three times for 1 min before the exposure to the shuttle-box/IFS procedure in order to habituate them to the experimenter.

2.3. Open field test

Behavioral responses towards a novel context not associated with the IFS procedure were evaluated in open field arenas (MED Associates INC., St. Albans, USA). The open field apparatus consisted of a Plexiglas box (80 × 80 × 30 cm) equipped with two horizontal rows of eight infrared light sensitive photocell beams located at 5 and 15 cm, respectively, from the basement, allowing the detection of horizontal and vertical (rearing) motor activity. Interruptions of the photocell beams were registered automatically by computer software connected to the open field apparatus (MED Associates Inc., St. Albans, USA). Open field arenas were wiped with 70% ethanol between rats. Animals were placed in the center of the arena and activity was recorded every 5 min for a total time of 60 min. IFS-exposed rats spent more time in the center because of the reduction in the activity induced by the shocks (data not shown).

2.4. Implantation of guide cannulae

The procedures for the microinjections and microdialysis experiments were adapted from previous studies of the laboratory (del Arco et al., 2015; Ronzoni et al., 2016). Animals were anesthetized with equithesin (2.5 mg/kg i.p.) and received a subcutaneous dose of the local anesthetic lidocaine in the incision area (20 mg/ml) and the non-steroidal analgesic carprofen (4 mg/kg, i.m.) before been positioned in the stereotaxic apparatus (Kopf Instruments). Bilateral guide cannulae were implanted to reach the amygdala with the following coordinates according to Paxinos and Watson (1998): −3.1 mm caudal, ±5 mm medial and −5.5 or −6.5 mm (respectively for microdialysis and microinjection experiments) from the top of the skull, being the incisive bar set at −3.3 mm (Paxinos and Watson, 1998). Stainless-steel guide cannulae [made in our own workshop], for microdialysis experiments (10 mm; 20-gauge) or for microinjections (15 mm; 23-gauge) experiments were fixated to the skull with 2–3 anchoring screws (Angthoís, Sthockholm, Sweden) and dental acrylic cement. Stainless-steel dummy cannulae (24-gauge) were inserted into the guide to keep it clean and prevent occlusion. After surgery, the rats received a subcutaneous injection of 3 ml of saline to facilitate clearance of drugs and prevent dehydration. One day after surgery rats were again group-housed two per cage and allowed to recover for a minimum of 7 days before

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