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Enduring attenuation of norepinephrine synaptic availability and augmentation of the pharmacological and behavioral effects of desipramine by repeated immobilization stress

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ABSTRACT

Here we provide evidence that repeated immobilization stress (RIS) in rats induces a persistent increase in noradrenergic activity in the anterior aspects of the anterolateral bed nucleus of the stria terminalis (alBNST). This increase in noradrenergic activity results from both enhanced synthesis and reuptake of norepinephrine (NE). It leads to a decrease in the synaptic availability of NE, which elicits an augmented noradrenergic response to the inhibitors of NE reuptake (NRIs), such as desipramine (DMI), an antidepressant. The enduring depression-like behavior and the augmentation of the climbing behavior seen in repeatedly stressed rats following subchronic administration of DMI in the forced swimming test (FST) might be explained by a dysregulation of noradrenergic transmission observed in alBNST. Taken together, we propose that dysregulation of noradrenergic transmission such as the one described in the present work may represent a mechanism underlying major depressive disorders (MDD) with melancholic features in humans.

1. Introduction

The central noradrenergic system is involved in the regulation of stress response, and its dysregulation has been implicated in the pathogenesis of depression and anxiety disorders (Goddard et al., 2010; Kalk et al., 2011; Morilak and Frazer, 2004). However, the mechanisms underlying the dysregulation of the central noradrenergic system remain elusive. Animal models of chronic stress have been extensively used to asses the effects of stress on the brain in mood disorders such as anxiety and depression. The experiments using repeated immobilization stress (RIS) have shown that RIS elicits dendritic atrophy in hippocampal neurons also induces dendritic hypertrophy in basolateral amygdala (BLA) neurons and enhances anxiety-like behavior. In addition, RIS induces contrasting effects on norepinephrine (NE) synthesis and turnover in the central nucleus of the amygdala (CeA) and paraventricular nucleus of the hypothalamus (PVN) (Pacák et al., 1993, 1992). Further, RIS induces an enduring increase of corticotrophin releasing hormone (CRH) peptide expression in the CeA and the bed nucleus of the stria terminals (BNST) (Santibañez et al., 2006). Additionally, RIS decreased climbing, a noradrenergic-mediated active stress-coping behavior, in the forced swimming test (FST) and augmented the behavioral response to the subchronic administration of the antidepressant desipramine (DMI), a selective norepinephrine reuptake inhibitor (NRI) (Hadweh et al., 2010). Finally, neuronal remodeling, increase in CRH, and anxiety- and depression-like behavior induced by RIS are prevented by concomitant administration of antidepressants that modulate glutamatergic activity, such as tianeptine (Pillai et al., 2012), or by NRIs, such as DMI and reboxetine (Ampuero et al., 2015; Hadweh et al., 2010; Santibañez et al., 2006).

The BNST is a basal forebrain structure of the limbic system

Abbreviations: RIS, repeated immobilization stress; CSD, chronic social defeat; CUS, chronic unpredictable stress; DMI, desipramine; NE, norepinephrine; NRIs, norepinephrine reuptake inhibitors; SSRIs, Selective serotonin reuptake inhibitors.

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anatomically related to regions of the brain that are implicated in emotional and autonomic responses to stress and noxious stimuli (Alheid and Heimer, 1988; Deyama et al., 2007; Granjeiro et al., 2012; Roder and Ciriello, 1994). Additionally, the BNST is the brain region with the highest concentration of NE (Kilts and Anderson, 1986). The ventral area of the BNST is densely innervated by noradrenergic fibers arising mainly from the brainstem noradrenergic cell groups A1 and A2, and in a lesser extent from the locus coeruleus (LC) (Forray et al., 2000; Riche et al., 1990; Roder and Ciriello, 1994; Woulfe et al., 1990). The activation of the BNST noradrenergic system plays a major role in mediating behavioral responses to negative emotions such as fear, anxiety, and aversion (Deyama et al., 2011, 2009; Fendt et al., 2005; Hott et al., 2012; Naka et al., 2013). Finally, neuronal remodeling, increase in CRH, and anxiety- and depression-like behaviors induced by RIS are prevented by concomitant administration of antidepressants that modulate glutamatergic activity, such as tianeptine (Pillai et al., 2012), or by NRIs, such as DMI and reboxetine (Ampuero et al., 2015; Hadweh et al., 2010; Santibañez et al., 2006). While the electrolytic lesion of the BNST reduces the attempts to escape in the FST (Schulz and Canbeyli, 2000), the reversible inactivation of the BNST along with the blockade of α_1- and β-adrenergic receptors (AR) before the FST decreases the duration of immobility (Crestani et al., 2010; Nagai et al., 2013). However, the noradrenergic mechanisms underlying RIS-induced depressive-like behavior are presently unknown. Therefore, we studied the effect of RIS on the noradrenergic transmission focusing on the anterolateral region of the BNST (alBNST), particularly its anterior ventral aspects that exhibit the highest concentration of NE, as a model to unravel the mechanisms that could elucidate the depression-like behavior and the augmentation of behavioral effects of DMI in the FST.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats kept at the Pontificia Universidad Católica de Chile Animal Care Facility, weighing 220–250 g at the start of the experiment were used. Rats were maintained throughout the experimental procedures in the Animal Care Facility and housed in groups of three or four animals per cage on a 14 h light, 10 h dark schedule (lights on between 07:00 and 21:00, Eastern Standard Time). Food and water were available *ad libitum*. Rat body weight was monitored daily. All procedures were in strict accordance with international guidelines "NIH Guide for the Care and Use of Laboratory Animals" (8th Ed.).

2.2. Stress protocol

RIS consisted of a daily session of immobilization (15 d, 2 h/d, 10:00–12:00 h) in rodent bags without access to either food or water as described previously (Hadweh et al., 2010; Pillai et al., 2012; Suvrathan et al., 2010).

2.3. Experimental procedures

2.3.1. Experimental procedure 1

Rats were randomly distributed into one of two experimental groups: repeatedly stressed rats (RIS) or unstressed control rats (Naïve). Naïve unstressed animals were daily manipulated for 15 d to avoid stress induced by handling. On day 17, 2 d following the termination of stress sessions, both groups of rats were subjected to *in vivo* microdialysis. This procedure was used to evaluate the effect of RIS, DMI (Sigma Chemical Co., St. Louis, MO), and amphetamine- (Laboratorio Chile, Santiago, Chile) perfusion on alBNST NE extracellular levels.

2.3.2. Experimental procedure 2

The rats subjected to RIS remained in the Animal Care Facility for an additional stress-free period (SFP) of 22 d, and on day 38 (RIS SFP), were subjected to *in vivo* microdialysis study. This procedure was used to evaluate whether RIS induces enduring effects on the alBNST noradrenergic system.

2.3.3. Experimental procedure 3

The rats subjected to RIS and their unstressed naïve littermates remained in the Animal Care Facility for an additional SFP of 22 d. During SFP, rat body weight was measured every alternate day. On day 37, both experimental groups (naïve and RIS) were subjected to the behavioral test elevated plus maze (EPM), followed by the swim pre-test of the FST on day 38. Then, 10 mg/kg of DMI (Sigma Chemical Co., St. Louis, MO) or vehicle (deionized water) was administered at 23.5, 5, and 1 h before the 5 min swim test (Detke et al., 1995; Hadweh et al., 2010; Porsolt et al., 1978).

2.4. In vivo microdialysis studies

2.4.1. Microdialysis procedure

The microdialysis studies were performed as previously described (Forray et al., 1997; Fuentealba et al., 2000; Santibañez et al., 2005). Briefly, rats subjected to experimental protocols 1 or 2, weighing 275–400 g were anesthetized with chloral hydrate (400 mg/kg, intraperitoneally [i.p.]) and placed on a stereotaxic apparatus (Stoelting; Wood Dale, IL). The skull was exposed, and a small hole was drilled in the area overlying the BNST. A CMA 12 microdialysis probe of 2 mm in length, 0.5 mm in outer diameter, and a cutoff value 100 kDa (CMA Microdialysis; Acton, MA), was lowered into the BNST. The following coordinates were used: 0.12 mm posterior to and 1.5 mm lateral to bregma, and 7.6 mm below the dura, according to the Atlas by Paxinos and Watson (2007). Body temperature was maintained at 37.3°C–37.6 °C and supplemental chloral hydrate was given as needed to maintain anesthesia.

The probe was continuously perfused at a rate of 2 μL/min with a Krebs-Ringer-Phosphate (KRP) buffer with or without 10 μM DMI using a Harvard infusion pump (Model 22; Dover, MA). The composition of the KRP buffer was 120 mM NaCl, 2.4 mM KCl, 1.2 mM $CaCl_2$, 0.9 mM NaH_2PO_4, and 1.4 mM Na_2HPO_4 (pH 7.4). To stimulate NE release, high K^+-KRP (110 mM) was perfused through the probe for 10 min. KCl replaced equimolar amounts of NaCl to maintain an isosmotic condition. *In vitro* recovery for potassium was measured as an index of probe permeability to K^+. The percentage of K^+ recovery was 27% for 110 mM that corresponds to 30 mM. After 90 min of stabilization, three samples were collected following 10-min perfusion intervals, and NE extracellular levels were analyzed in each sample. The stimulation of the BNST was performed by perfusion with 110 mM K^+ solution through the microdialysis probe during a period of 10 min. After the stimulation period, KRP was perfused during the rest of the experiment in which four 10-min post-stimulation samples were collected, and NE extracellular levels were analyzed.

After 90 min of stabilization, 10 min interval perfusion samples collected in 4 μL of 0.2 M perchloric acid were maintained on ice during the experiment until analysis. At the end of the experiment, the animals were euthanatized, and their brains were withdrawn and stored in formalin. Twenty-five micron-thick brain sections were cut using a microtome (Vibratome 3000: Technical Products International, USA) and stained with cresyl violet to verify the location of the probe.