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Tianeptine modulates amygdalar glutamate neurochemistry and synaptic proteins in rats subjected to repeated stress

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ABSTRACT

Stress is a common environmental factor associated with depressive illness and the amygdala is thought to be integral for this association. For example, repeated stress impairs amygdalar neuroplasticity in rodents and these defects parallel amygdalar deficits in depressive illness patients. Because the excitatory neurotransmitter glutamate is important in neuroplasticity, we hypothesized that alterations in amygdalar glutamatergic systems may serve as key players in depressive illness. Moreover, restoration of amygdalar glutamatergic systems may serve as important therapeutic targets in the successful management of multiple stress-related mood disorders. To address these hypotheses, we measured glutamate efflux in the basolateral and central amygdalar complexes via in vivo microdialysis, as well as the expression of synaptic proteins that regulate vesicular glutamate packaging and release, in rats subjected to repeated stress and treated daily with saline or the antidepressant tianeptine. Glutamate efflux was significantly reduced in the central amygdalar complex of animals subjected to repeated stress. In addition, repeated stress nearly eliminated amygdalar vGLUT2 expression, thereby proving a potential mechanism through which repeated stress impairs amygdalar glutamate neurochemistry. These stress-induced changes in glutamate efflux and vGLUT2 expression were inhibited by daily tianeptine administration. Moreover, tianeptine administration increased the vesicular localization of SNAP-25, which could account for the ability of tianeptine to modify glutamatergic tone in non-stressed control rats. Collectively, these results demonstrate that repeated stress differentially affects amygdalar glutamate systems and further supports our previous studies indicating that tianeptine's antidepressant efficacy may involve targeting amygdalar glutatamatergic systems.

Introduction

Major depressive illness is one of the most common psychiatric disorders, affecting an estimated 12–15% of the general population (Kessler et al., 1994). Beyond the monoamine hypothesis of depression, which predicts that decreases in synaptic concentrations of neurotransmitters such as norepinephrine, dopamine and serotonin represent an etiological mechanism for the development of major depressive disorder (Charney, 1998; Heninger et al., 1996), clinical studies also support a role for the glutamatergic system in the pathology of major depressive illness. For example, administration of the NMDA receptor antagonist ketamine produces rapid antidepressant effects in treatment resistant patients (Berman et al., 2000; Price et al., 2009; Zarate et al., 2006) and recent preclinical studies have begun to identify the mechanisms through which ketamine produces these effects (Autry et al., 2011; Li et al., 2011). From a neurochemical perspective, plasma and cerebrospinal fluid (CSF) glutamate levels and glutamate/glutamine ratios are modulated in affective disorders (Altamura et al., 1993, 1995; Kim et al., 1982; Levine et al., 2000). Proton magnetic resonance spectroscopic analyses support these findings and identified specific neuronal populations where changes in glutamatergic neurotransmission may take place (Auer et al., 2000; Mirza et al., 2004; Sanacora et al., 2004). Decreases in glial cell densities observed in depressive illness patients (Cotter et al., 2001; Hamidi et al., 2004; Ongur et al., 1998; Rajkowska et al., 1999) may result in decreased glial glutamate transporter expression, thereby reducing the capacity to regulate synaptic concentrations of glutamate. These clinical observations support the hypothesis that modulation of the glutamatergic system participates in the pathophysiology of major depressive disorder.

The amygdalar complex is a key component of the physiological and behavioral responses to stressful stimuli (McEwen, 2003) and plays a central role in elucidating the well-established connection between stress and psychopathology. Imaging studies have revealed that amygdala volumes may be increased (Bremner et al., 2000; Frodl et al., 2002) or decreased (Sheline et al., 1998, 1999; von Gunten et al., 2000) in major depression patients. While these

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Table 1
List of anatomical abbreviations.

BLA	basolateral amygdaloid nucleus anterior
BLC	basolateral amygdalar complex
BLP	basolateral amygdaloid nucleus posterior
BLV	basolateral amygdaloid nucleus ventral
BMA	basomedial amygdaloid nucleus anterior
BMP	basomedial amygdaloid nucleus posterior
CeA	central amygdalar complex
CeC	central amygdaloid nucleus capsular
CeL	central amygdaloid nucleus lateral division
CeM	central amygdaloid nucleus medial division
LaDL	lateral amygdaloid nucleus dorsolateral
LaVL	lateral amygdaloid nucleus ventrolateral
LaVM	lateral amygdaloid nucleus ventromedial
opt	optic tract
Pir	piriform cortex

disparities may be related to illness duration and/or therapeutic interventions (Campbell et al., 2004), the results demonstrate that the amygdala is a site for neuroanatomical alterations in depressive illness. The amygdalar complex receives glutamatergic afferents from several extrinsic sources, including cortical and thalamic regions (LeDoux et al., 1990; McDonald et al., 1999; Turner and Herkenham, 1991). An additional source of neuronal glutamate in the amygdala is from intra-amygdalar projections of excitatory amygdalar output neurons that also project to cortical and limbic regions (Pitkanen et al., 1997). Stress has been shown to impact virtually all of these regions, and it is likely that stress-related increases in amygdalar glutamate reflect a summed neurochemical correlate of activity in these stress-responsive circuits.

Previous studies determined that acute stress increases glutamate efflux in the rat amygdala (Singewald et al., 2000). Indeed, our previous studies determined that acute restraint stress increases extracellular glutamate levels in the rat basolateral amygdala and central amygdala complexes, and that these increases are inhibited by the antidepressant tianeptine (Reznikov et al., 2007). Moreover, tianeptine administration alleviates stress-associated alterations in amygdalar function and plasticity (McEwen and Chattarji, 2004; Vouimba et al., 2006). Unlike responses to acute stress, which appear to be adaptive in nature, less is known regarding how repeated stress affects glutamate neurochemistry in the amygdala. Accordingly, the aims of the current study were: 1) to determine the effects of repeated restraint stress (RRS) on glutamate efflux in the rat basolateral (BLC) and central amygdalar (CeA) complexes; 2) to determine the effects of RRS upon synaptic proteins that regulate vesicular packaging, release and reuptake of glutamate in the amygdala; and 3) to determine whether tianeptine administration mitigated RRS-mediated alterations in these systems.

Materials and methods

Animals

Eight week old male Sprague Dawley rats (CD strain, Charles River) weighing approximately 225–275 g were singly housed and provided ad libitum access to standard Purina rat chow and water. Animals were maintained in a temperature-controlled room, with a light/dark cycle of 12/12 h (lights on at 0700 h) and handled daily for 5 to 7 days prior to experimentation. All experiments were conducted during the light phase, beginning at least 1 h after light phase onset and concluding at least 1 h prior to the beginning of the dark phase. Animal care and use procedures were carried out in accordance with protocols written under the guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by The University of South Carolina Animal Care and Use Committee.

Repeated restraint stress (RRS) and antidepressant treatment

Rationale

While investigators employ a variety of stress paradigms, including chronic variable stress or chronic unpredictable stress paradigms, we have chosen to use repeated restraint stress (RRS) for a number of reasons. To begin, our previous studies demonstrated that 10 days of repeated restraint stress reduces the acute stress activation of glutamatergic neurons in the basolateral nucleus of the amygdala (BLA; Reznikov et al., 2008). Moreover, we have also reported that the effects of acute stress upon amygdalar GABA neurochemistry are fundamentally different in naive rats (i.e. non-stress controls) when compared to rats subjected to RRS (Reznikov et al., 2009). Moreover, additional studies indicate that stress paradigms of this duration (i.e. 6 h/day for 10 days) represent an important transition period in which the effects of stress-induced pathological changes emerge in the amygdala [see (Reznikov et al., 2008) for extensive discussion]. For these reasons, we examined the effects of acute stress on amygdalar glutamate efflux in non-stress control (NSC) rats and rats subjected to RRS. Specifically, rats were placed in flexible wire mesh restrainers with protective rubberized edges and were restrained 6 h/day for 10 days in their home cages as described previously (Reznikov et al., 2008). Prior to initiation of restraint stress, rats received a daily i.p injection of sterile saline or 10 mg/kg tianeptine (Servier, France) for the entire duration (10 days) of the RRS paradigm. Tianeptine was dissolved in sterile saline with dose chosen based upon previous studies (McEwen et al., 2002; McEwen et al., 2010; Reagan et al., 2004, 2007; Watanabe et al., 1992). NSC animals were handled daily and returned to their home cage and received either saline vehicle or 10 mg/kg tianeptine daily for 10 days. Therefore, experimental groups were as follows: non-stressed controls given saline (NSC + S), rats subjected to repeated restraint stress and given saline (RRS + S), non-stressed controls given tianeptine (NSC + T) and rats subjected to repeated restraint stress and given tianeptine (RRS + T).

Surgery

On the subsequent day immediately following experimental conditions (day 11), under sodium pentobarbital anesthesia (70 mg/kg, i.p.) animals received dual microdialysis guide cannula surgery (BAS, West Lafayette, IN, USA) with placement targeted to the CeA and contralateral BLC as previously described (Reznikov et al., 2007) (Fig. 1, Panels A and B). Target coordinates were calculated according to Paxinos and Watson (Paxinos and Watson, 1998) relative to bregma: for CeA: AP −2.0 mm, L ±3.9 mm, DV −7.0 mm from skull surface; for BLC: AP −3.1 mm, L ±5.0 mm; DV −7.0 mm. Animals were allowed 2 recovery days following surgery, during which daily handling continued and habituation to microdialysis chambers occurred.

In vivo microdialysis

On the morning of microdialysis, stylets were removed and replaced with concentric microdialysis probes (BAS, West Lafayette, IN) with a semipermeable membrane (nominal molecular weight cutoff of 30 kDa) extending 2.0 mm beyond the ventral tip of the guide cannulas. Probes were continuously perfused (2.0 μl/min) with an artificial cerebrospinal fluid (pH 6.5) composed of the following in mM concentrations: NaCl 150, KCl 3.0, $CaCl_2$ 1.7, $MgCl_2$ 0.9, D-glucose 4.9. Collection of dialysates (in 15 min intervals) from both probes began 3 h following probe insertion. Following four baseline collections, all animals were exposed to an hour-long acute restraint stress stimulus, an event which we previously described reliably increases glutamate efflux in the BLC and CeA (Reznikov et al., 2007). One hour after onset of acute restraint stress, animals were released from the restrainers and four final post-restraint stress collections occurred. Thus, a total of 12 collections were made: four baseline, four stress and four post-stress collections. Basal glutamate concentrations were uncorrected for in vitro probe recovery.