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Corticotropin releasing factor and catecholamines enhance glutamatergic neurotransmission in the lateral subdivision of the central amygdala

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ABSTRACT

Glutamatergic neurotransmission in the central nucleus of the amygdala (CeA) plays an important role in many behaviors including anxiety, memory consolidation and cardiovascular responses. While these behaviors can be modulated by corticotropin releasing factor (CRF) and catecholamine signaling, the mechanism(s) by which these signals modify CeA glutamatergic neurotransmission remains unclear. Utilizing whole-cell patch-clamp electrophysiology recordings from neurons in the lateral subdivision of the CeA (CeA_L), we show that CRF, dopamine (DA) and the β-adrenergic receptor agonist isoproterenol (ISO) all enhance the frequency of spontaneous excitatory postsynaptic currents (sEPSC) without altering sEPSC kinetics, suggesting they increase presynaptic glutamate release. The effect of CRF on sEPSCs was mediated by a combination of CRFR1 and CRFR2 receptors. While previous work from our lab suggests that CRFRs mediate the effect of catecholamines on excitatory transmission in other subregions of the extended amygdala, blockade of CRFRs in the CeA_L failed to significantly alter effects of DA and ISO on glutamatergic transmission. These findings suggest that catecholamine and CRF enhancement of glutamatergic transmission onto CeA_L neurons occurs via distinct mechanisms. While CRF increased spontaneous glutamate release in the CeA_L, CRF caused no significant changes to optogenetically evoked glutamate release in this region. The dissociable effects of CRF on different types of glutamatergic neurotransmission suggest that CRF may specifically regulate spontaneous excitatory transmission.

1. Introduction

Glutamatergic neurotransmission in the central nucleus of the amygdala (CeA) is important for many behaviors and physiologic processes. Extracellular glutamate levels increase in the CeA in response to acute stressors (Reznikov et al., 2007) and CeA glutamate activity has been suggested to play a critical role in the expression of anxiety-like behaviors (Kalin et al., 2004), fear conditioning (Samson and Pare, 2005), and conditioned place aversion (Watanabe et al., 2002). Furthermore, inactivation of the CeA is associated with disruptions to multiple forms of learning (Robledo et al., 1996; Lingawi and Balleine, 2012), cardiovascular regulation (Roozendaal et al., 1991; Saha, 2005), decreased pain sensitivity (Li and Neugebauer, 2004) and reductions in enhanced ethanol drinking during withdrawal (Roberts et al., 1996). While CeA glutamate signaling appears to be fundamentally important to a variety of functions, a clear understanding of the mechanisms regulating CeA glutamatergic transmission is currently lacking.

Corticotropin Releasing Factor (CRF) signaling plays an important role in many of the CeA-mediated behaviors described above (Fu and Neugebauer, 2008; Koob, 2009; Pitts et al., 2009; Skorzewska et al., 2009) and can modulate CeA excitability (Ji and Neugebauer, 2007; Liu et al., 2004). Furthermore, deletion of CRF type 1 receptors (CRFR1) specifically in forebrain glutamatergic neurons reduces anxiety-like behaviors (Refojo et al., 2011), suggesting a critical role of CRF in the regulation of glutamate transmission in the amygdala. In addition, catecholamine signaling may also play a role in the regulation of CeA glutamatergic transmission. For example, enhanced dopamine (DA) signaling within the CeA is associated with fear conditioning (Guarraci et al., 1999), drug preference/seeking (Rezayof et al., 2002; Thiel et al., 2010; Weiss

Abbreviations: ISO, isoproterenol; CRF, corticotropin releasing factor; CRFR1 and CRFR2, CRF receptor type 1 or type 2; CeA_L, lateral subdivision of the central nucleus of the amygdala; ChR2, channel rhodopsin; sEPSCs, spontaneous excitatory postsynaptic currents; oEPSCs, optically evoked excitatory postsynaptic currents.

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et al., 2000), and conditioned stress paradigms (Coco et al., 1992). Enhanced norepinephrine (NE) signaling has been shown to play a role in immobilization stress (Pacak et al., 1993) drug withdrawal and reinstatement (Watanabe et al., 2003; Yamada and Bruijnzeel, 2011), and pain sensitivity (Ortiz et al., 2007). CeA NE signaling, particularly via β-adrenergic receptor (β-AR) activation, is also important in drug-withdrawal induced conditioned place aversion (Watanabe et al., 2003) and in memory consolidation (Ellis and Kesner, 1983; Liang et al., 1986; Roozendaal et al., 1993). However, the mechanisms by which CRF and catecholamines may alter CeA glutamatergic neurotransmission have yet to be fully clarified.

Anatomical (Asan et al., 2005; Rudoy et al., 2009) and behavioral (Li et al., 1998) evidence suggests that catecholamines may directly influence the activity of CRF producing neurons in the CeA, which are mainly found in the lateral subdivision of the CeA (CeA_L) (Asan et al., 2005; Eliava et al., 2003; Swanson et al., 1983; Treweek et al., 2009). These findings may suggest that catecholamine actions in the CeA_L could require CRF signaling to enhance glutamatergic activity, a mechanism similar to that shown in a related subregion of the extended amygdala, the bed nucleus of the stria terminalis (BNST) (Kash et al., 2008; Nobis et al., 2011; Silberman et al., 2013). Therefore, we sought to determine if catecholamine and CRF signaling mechanisms interact to enhance CeA_L glutamatergic transmission. Surprisingly, our findings indicate that DA, β-AR and CRF agonists all enhance spontaneous glutamatergic transmission in the CeA_L through non-overlapping mechanisms. Furthermore, we also show that the effect of CRF on spontaneous glutamatergic transmission is distinct from that of evoked transmission in this brain region.

2. Methods

2.1. Animals and brain slice preparation

Seven-to-14 week old, male wild-type C57BL/6J mice (Jackson Laboratories) were used for most studies. In a subset of studies, 7–14 week old, male Thy1-ChR2 mice [B6.Cg-Tg(Thy1-COP4/EYFP)18Gfng/J; Jackson Laboratories] were used for optogenetic stimulation of glutamatergic afferents in the CeA_L. In this transgenic mouse line, the light activated channel rhodopsin receptor (ChR2) is expressed in neurons under the control of the mouse thymus cell antigen 1 (Thy1) promoter. Expression of the transgenic ChR2 protein is detected predominantly in layer 5 cortical neurons, CA1 and CA3 pyramidal neurons of the hippocampus, cerebellar mossy fibers, and neurons in the thalamus, midbrain and brainstem (Wang et al., 2007). All mice were group housed throughout these studies. Food and water were available *ad libitum*. All procedures were approved by the Animal Care and Use Committee at Vanderbilt University. Brain slices (300 μm) containing the CeA_L were prepared as previously described (Silberman et al., 2013). Following dissection, slices were transferred to a holding chamber where they were heated (27°–30 °C) and were allowed to equilibrate for at least 1 h before being transferred to a submerged perfusion chamber (also heated to 27°–30 °C) for electrophysiology studies.

2.2. Electrophysiology

All electrophysiology recordings were made using Clampex 9.2 and analyzed using Clampfit 10.2 (Molecular Devices, Sunnyvale, California). Whole-cell voltage-clamp recordings of alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor-mediated spontaneous excitatory postsynaptic currents (sEPSCs) and optically-evoked excitatory postsynaptic currents (oEPSCs) were made at −70 mV and pharmacologically isolated by the addition of 25 μM picrotoxin to the artificial cerebrospinal fluid (ACSF) containing (in mM): NaCl (124), KCl (4.4), $CaCl_2$ (2), $MgSO_4$ (1.2), 1 NaH_2PO_4 (1), glucose (10), and $NaHCO_3$ (26). Electrode placement was limited to be within the CeA_L. Cells were allowed to equilibrate to whole-cell configuration for 3–5 min before recordings began. Recording electrodes (3–6 MΩ) were pulled on a Flaming/Brown Micropipette Puller (Sutter Instruments, Novato, CA) using thin-walled borosilicate glass capillaries and filled with (in mM): CsOH (118), D-gluconic acid (117), NaCl (5), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES, 10), ethylene glycol tetra-acetic acid (EGTA, 0.4), $MgCl_2$ (2), Tetraethylammonium chloride (5), adenosine triphosphate (ATP, 4), guanosine triphosphate (GTP, 0.3), pH 7.2–7.3, 280–290 mOsmol. sEPSC recordings were acquired and analyzed in 2-min gap-free blocks. Access resistance was monitored between blocks of sEPSC recordings. oEPSCs were evoked every 30 s by a 1msec TTL pulse to activate a LED light driver (Thorlabs, Newton, NJ) passed through a EN-GFP filter cube (Olympus) to produce blue wavelength light. Access resistance was monitored continuously. Experiments in which access resistance changed by more than 20% were not included in the data analyses.

2.3. Statistical analyses

Statistical analyses were performed using Microsoft Excel 2010 and GraphPad Prism 5, while figures were finalized in Coreldraw 12. Specifically, when determining if a compound had a significant effect, a Student's paired *t* test was used, comparing the baseline value to the experimental value. One-way ANOVA was used to compare the effects of drugs between groups, followed by Tukey's post-test to determine the significance of specific comparisons. All values given for drug effects throughout the study are presented as average ± SEM typically expressed as a normalized percentage of baseline where baseline levels are set as 100%.

2.4. Drugs

Isoproterenol, CRF, Stressin, Astressin-2B and NBI27914 were purchased from Tocris. All other compounds and experimental drugs were purchased from Sigma-Aldrich unless otherwise noted in the text. All experimental drugs were bath applied at their final concentrations as noted in the text. Dimethylsulfoxide (DMSO) was the solvent used for stock solutions of NBI27914 and picrotoxin where the maximum final concentration of DMSO in ACSF was 0.02% by volume.

3. Results

3.1. Effect of CRF receptor activation on sEPSCs in the CeA_L

We first assessed whether CRF can enhance sEPSCs in the CeA_L. A 6 min bath application of 300 nM CRF significantly enhanced sEPSC frequency from baseline levels (175.3 ± 21.3%, $n = 11$, $p < 0.05$, Fig. 1), without causing any significant changes to sEPSC amplitude (102.5 ± 7.8%, $p > 0.05$), area (101.6 ± 9.2%, $p > 0.05$), rise time (101.0 ± 6.8%, $p > 0.05$) or decay time (100.1 ± 4.2%, $p > 0.05$). To determine the CRF receptor subtype required for CRF mediated enhancement of CeA_L glutamatergic activity, we next assessed the effect of Stressin, a CRFR1 selective agonist (Rivier et al., 2007), on sEPSCs. Bath application of 100 nM Stressin for 9 min significantly increased sEPSC frequency (148.2 ± 17.6%, $n = 6$, $p < 0.05$; Fig. 2) without causing significant changes to sEPSC amplitude (94.2 ± 7.0%, $p > 0.05$), area (95.9 ± 7.3%, $p > 0.05$), rise time (104.9 ± 7.0%, $p > 0.05$) or decay time (98.8 ± 3.9%, $p > 0.05$). Previous work from our lab has shown that CRFR1 activation increases sEPSC frequency in the BNST (Kash et al., 2008; Nobis et al., 2011; Silberman et al., 2013), a brain region closely related to the CeA. Therefore, as a positive control, we tested the effect of 100 nM Stressin on sEPSCs in the BNST and found that Stressin enhanced sEPSC frequency (157.7 ± 14.5%, $n = 7$, $p < 0.05$) without altering sEPSC kinetics. Together, these data suggest that the effect of CRFR1 activation on glutamatergic neurotransmission is similar in the CeA_L compared to the BNST.

3.2. Effect of CRF on evoked glutamatergic neurotransmission in the CeA_L

The above findings suggest that CRF can enhance spontaneous glutamatergic neurotransmission in the CeA_L via a presynaptic mechanism. However, these studies cannot determine the presynaptic source of glutamate that is altered by CRF. To begin to address this question, we recorded optically evoked EPSCs (oEPSCs) in CeA_L neurons from Thy1-ChR2 mice. These mice harbor ChR2 predominantly in glutamatergic neurons in the cortex and hippocampus, which are known to send projections to the CeA, as well as in neurons of the thalamus, midbrain, brain stem and cerebellum (Wang et al., 2007). Therefore, many of the glutamatergic afferents to the CeA that were enhanced in sEPSC experiments may also be activated by light stimulation of the CeA in Thy1-ChR2 mice. oEPSCs in CeA_L were not modulated by picrotoxin but had a reversal potential near 0 mV (data not shown) and were almost completely