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Research report

CaMKIIα knockdown decreases anxiety in the open field and low serotonin-induced upregulation of GluA1 in the basolateral amygdala

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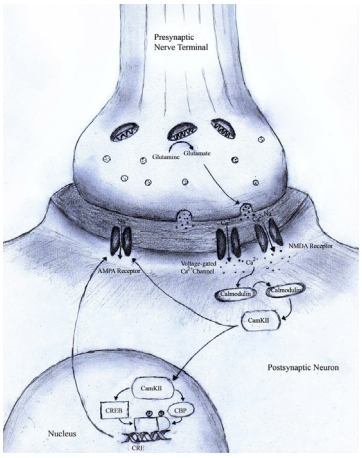
HIGHLIGHTS

- Low serotonin (5-HT) increased anxiety and glutamate receptor (GluA1) expression.
- Depletion of 5-HT in the basolateral amygdala (BLA) increased CaMKII activation.
- CaMKII activation correlated with anxiety and GluA1 expression.
- Knockdown of CaMKII in the BLA attenuated anxiety and decreased GluA1 expression.

GRAPHICAL ABSTRACT



Molecular model of the putative relationship between 5-HT depletion, the CaMKII signaling pathway, and GluA expression in the BLA. Decreased 5-HT removes the tonic inhibition in the BLA, increasing glutamate signaling. The sustained increase in neurotransmission elevates postsynaptic intracellular calcium and activates CaMKII. Activation of CaMKII then increases transcription and membrane expression of GluAs.

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ABSTRACT

Hyperactivation of the amygdala is implicated in anxiety and mood disorders, but the precise underlying mechanisms are unclear. We previously reported that depletion of serotonin (5-hydroxytryptamine, 5-HT) in the basolateral nucleus of the amygdala (BLA) using the serotonergic neurotoxin 5,7-dihydroxytryptamine (5,7-DHT) potentiated learned fear and increased glutamate receptor (Glu) expression in BLA. Here we investigated the hypothesis that CaMKII facilitates anxiety-like behavior and increased Glu/AMPA receptor subunit A1 (GluA1) expression following depletion of 5-HT in the BLA. Infusion of 5,7-DHT into the BLA resulted in anxiety-like behavior in the open field test (OFT) and increased the phosphorylation of CaMKIIα (Thr-286) in the BLA. Knockdown of the CaMKIIα subunit using adeno-associated virus (AAV)-delivered shRNAi concomitantly attenuated anxiety-like behavior in the OFT and decreased GluA1 expression in the BLA. Our results suggest that the CaMKII signaling plays a key role in low 5-HT-induced anxiety and mood disturbances, potentially through regulation of GluA1 expression in the BLA.

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1. Introduction

Previous studies have suggested that anxiety and mood disorders may be linked to sub-seizure hyperexcitability in the amygdala [1]. Moreover, the mechanisms of hyperexcitability, which involve increased glutamatergic activity [2,3], may be caused by the serotonin (5-hydroxytryptamine, 5-HT) dysfunction associated with abnormal emotional behavior [4–6]. In support, we recently showed that targeted depletion of 5-HT the rat basolateral nucleus of the amygdala (BLA) using bilateral stereotaxic infusions of the serotonergic neurotoxin 5′7-dihydroxytryptamine (DHT) increased fear potentiated startle and expression of the ionotropic glutamate receptor subunit (GluA1) [7]. Although evidence suggested low 5-HT facilitates neuronal excitability through alteration of the glutamatergic system [1,7–10], the molecular mechanisms facilitating this process are unclear.

The calcium/calmodulin-dependent protein kinase II (CaMKII) signaling pathway has been implicated in the molecular pathophysiology of mood and anxiety disorders [11]. Behavioral deficits have been observed in correlation with abnormal CaMKII expression and activation [12]. Specifically, preclinical studies have reported increased expression of CaMKII in animals with exaggerated anxiety-like behaviors [13]. Increased CaMKII expression has also been reported in the postmortem brain tissue of patients with temporal lobe epilepsy [14], suggesting CaMKII is important for glutamatergic plasticity and signaling in the amygdala [15]. Thus, CaMKII may be a putative intermediate facilitating low 5-HT-induced upregulation of GluA1 implicated in mood and anxiety disorders.

In the present study, we investigated the role of CaMKII in low 5-HT-induced increase in anxiety-like behavior and GluA1 expression. We observed an increase in phosphorylated CaMKIIα (Thr-286) expression following 5,7-DHT microinfusion into the BLA. Using an adeno-associated virus (AAV) vector to deliver specific shRNAi targeting CaMKIIα, we subsequently show that CaMKIIα knockdown has anxiolytic effects on the open field test concomitant with a reduction of GluA1 expression in the BLA. Taken together, our data suggests a possible mechanism linking low 5-HT with changes in fear and anxiety-like behavior and pathophysiology, and further suggests CaMKII may be an effective target for treatment of emotional disorders.

2. Methods

2.1. Animals

All experimental animal procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals and conform to a protocol approved by the Baylor University Institutional Animal Care and Use Committee (IACUC). Male Sprague-Dawley rats ($n = 32$; Harlan, Houston, TX) were group housed in a light controlled 12-h light/dark cycle (lights on at 5 AM) and temperature controlled (23 °C) room. Commercial rodent pellets and water were provided *ad libitum*. Animals were approximately 45 days old (~270 g) at the start of the experiments.

2.2. Stereotaxic surgeries

Stereotaxic infusions of 5,7-DHT were performed as previously described [7]. Animals were anesthetized with intraperitoneal (i.p.) injections of equithesin (35 mg/kg sodium pentobarbital; 145 mg/kg chloral hydrate) and mounted on a stereotaxic frame. Approximately 30 min prior to stereotaxic infusions, all animals were injected with desipramine (30 mg/kg; i.p.) to protect norepinephrine axon terminals from the neurotoxic effects of 5,7-DHT. A midline incision was made to expose the skull, and two small holes were drilled through the skull 2.7 mm posterior to bregma and 4.7 mm bilateral from the midline. The tip of a 1 μl, 22-gauage Hamilton microsyringe was lowered 6.5 mm from skull surface into the BLA. The 5,7-DHT (16 μg/μl) or vehicle (VEH, 0.9% saline, 0.02% ascorbic acid) was administered at a rate of 0.5 μl over 2.5 min. The needle remained in place for an additional 5 min. following infusion. Following infusion of 5,7-DHT, the same stereotaxic procedure was repeated to deliver 0.5 μl of AAV containing one of three shRNAi (8×10^{11} GC/mL), as described below. Animals recovered for two weeks prior to experimentation. Histological verification of 5,7-DHT lesion was described previously [7], and infusion locations are described in Fig. 1A.

2.3. Vector construction and virus production

The AAV vectors were designed to express GFP under a CBA promoter and a U6 promoter-driven shRNA as outlined by Babcock et al. [16]. The purpose of the GFP transgene was to confirm infection as depicted in Fig. 1B. Only animals that were concordant in needle placement, 5,7-DHT infusion, and viral infection were included in this study. Three different shRNAs were generated: two sequences specific for CAMKIIα (shCAM1 & shCAM2) and a scrambled (negative control) sequence (shSCR). Complementary oligonucleotides were custom synthesized (Integrated DNA Technologies, Coralville, IA) to create the shRNA sequences. Each hairpin contained an ApaI-compatible overhang at the 5′ end, a KpnI restriction site, and an EcoRI compatible overhang at the 3′ end. The three sets of complementary oligonucleotide sets were as follows:

shCAM1

sense: 5′-TCC TCT GAG AGC ACC AAC ATT CAA GAG ATG TTG GTG CTC TCA GAG GAT TTT TTG GTA CC-3′

antisense: 5′ ATT TGG TAC CAA AAA ATC CTC TGA GAG CAC CAA CAT CTC TTG AAT GTT GGT GCT CTC AGA GGA GGC C-3′

shCAM2

sense: 5′-TCA GTC CGT CTG TGA AGT TTT CAA GAG AAA CTT CAC AGA CGG ACT GAT TTT TTG GTA CC-3′

antisense: 5′-AAT TGG TAC CAA AAA ATC AGT CCG TCT GTG AAG TTT CTC TTG AAA ACT TCA CAG ACG GAC TGA GGC C-3′

shSCR

sense: 5′-ATC ATA AAC GGC CCA TCG CTT CAA GAG AGC GAT GGG CCG TTT ATG ATT TTT TTG GTA CC-3′

antisense: 5′-AAT TGG TAC CAA AAA AAT CAT AAA CGG CCC ATC GCT CTC TTG AAG CGA TGG GCC GTT TAT GAT GGC C-3′

Oligonucleotides were annealed and ligated into the ApaI and ecoRI sites downstream of the U6 promotor in a pSilencer plasmid (Ambion, Austin, TX). A 373-bp KpnI fragment containing the U6 promotor-siRNA hairpin sequence was then excised from agarose gel electrophoresis and subcloned into the KpnI site of the AAV vector pAM-CBA-GFP (a gift from D. Poulson, University of Montana). Plasmids were sequenced for verification using the Big Dye Terminator kit (Applied Biosystems, Foster City, CA) and primers targeting the upstream LTR.

The recombinant AAV1 plasmids were packaged into HEK-293T cells. Approximately 1.5×10^7 cells were seeded into 100-mm dishes in complete DMEM/F-12 supplemented with 10% fetal bovine serum, and 0.05% penicillin-streptomycin (5000 units/ml). Approximately 24 hr after seeding, the cells were transferred to culture medium containing 5% fetal bovine serum and transfected with three separate plasmids: an adeno-helper plasmid (pFΔ6), an AAV helper (H21), and the AAV transgene vector containing one of the three shRNA constructs. The helper plasmids contained the CAP and REP proteins and adenovirus transcription proteins required for viral replication. The plasmids were transfected into HEK-293T cells using Fugene 6 (Roche Molecular Biochemicals), and incubated in 5% CO_2 for ~72 h at 37 °C. The cells were then