Archival Report

Biological Psychiatry

Delta Subunit-Containing Gamma-Aminobutyric Acid A Receptor Disinhibits Lateral Amygdala and Facilitates Fear Expression in Mice

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ABSTRACT

BACKGROUND: Maintaining gamma-aminobutyric acidergic (GABAergic) inhibition in the amygdala within a physiological range is critical for the appropriate expression of emotions such as fear and anxiety. The synaptic GABA type A receptor ($GABA_AR$) is generally known to mediate the primary component of amygdala inhibition and prevent inappropriate expression of fear. However, little is known about the contribution of the extrasynaptic $GABA_AR$ to amygdala inhibition and fear.

METHODS: By using mice expressing green fluorescent protein in interneurons (INs) and lacking the δ subunit-containing $GABA_AR$ ($GABA_A(\delta)R$), which is exclusively situated in the extrasynaptic membrane, we systematically investigated the role of $GABA_A(\delta)R$ in regulating inhibition in the lateral amygdala (LA) and fear learning using the combined approaches of immunohistochemistry, electrophysiology, and behavior.

RESULTS: In sharp contrast to the established role of synaptic $GABA_AR$ in mediating LA inhibition, we found that either pharmacological or physiological recruitment of $GABA_A(\delta)R$ resulted in the weakening of GABAergic transmission onto projection neurons in LA while leaving the glutamatergic transmission unaltered, suggesting disinhibition by $GABA_A(\delta)R$. The disinhibition arose from IN-specific expression of $GABA_A(\delta)R$ with its activation decreasing the input resistance of local INs and suppressing their activation. Genetic deletion of $GABA_A(\delta)R$ attenuated its role in suppressing LA INs and disinhibiting LA. Importantly, the $GABA_A(\delta)R$ facilitated long-term potentiation in sensory afferents to LA and permitted the expression of learned fear.

CONCLUSIONS: Our findings suggest that $GABA_A(\delta)R$ serves as a brake rather than a mediator of GABAergic inhibition in LA. The disinhibition by $GABA_A(\delta)R$ may help to prevent excessive suppression of amygdala activity and thus ensure the expression of emotion.

Keywords: Disinhibition, Fear, GABA, Interneuron, Lateral amygdala, LTP

http://dx.doi.org/10.1016/j.biopsych.2016.06.022

The amygdala is one of the kernel brain regions subserving emotion expression such as fear and anxiety (1–3). Proper function of the amygdala requires the dynamic regulation of gamma-aminobutyric acidergic (GABAergic) inhibition within a physiological range (4,5). Under resting conditions, the inhibitory tone in amygdala is much higher relative to its neighboring areas (6). However, when emotionally charged information arrives at the amygdala, this highly inhibitory tone is quickly removed, thereby allowing the information to pass down to the nuclei in the hypothalamus and brainstem to elicit an appropriate response (5,7). Dysregulated GABAergic inhibition in the amygdala has been shown to be closely associated with the development of a spectrum of neuropsychiatric diseases such as anxiety and mood disorders (8–10), and many anxiolytic drugs act through modulating GABAergic transmission in the amygdala and other regions (11).

Being the primary mediator of brain inhibition, GABA is known to act mainly through the postsynaptic GABA type A receptors ($GABA_ARs$). In the past two decades, growing evidence has shown that the ambient GABA activates perisynaptic and extrasynaptic $GABA_AR$ to generate a tonic form of inhibition in various brain regions (12–15). Among the $GABA_AR$ subtypes mediating tonic inhibition in the brain (16,17), the δ subunit-containing $GABA_AR$ ($GABA_A(\delta)R$) is regarded as the ideal one primarily due to its exclusively extrasynaptic location, higher affinity toward GABA, and lower desensitization to continuous GABA exposure (14,18). Thus, it has been repeatedly implicated in mediating tonic inhibition in numerous brain regions such as the cerebellum and hippocampus (14,16,18). This receptor was also found in the intercalated cells (ITCs) surrounding the amygdala and the lateral part of the central amygdala, with its activation greatly affecting the neuronal activity in these areas (18,19). However, it remains unclear whether $GABA_A(\delta)R$ is also present in the lateral amygdala (LA), a region serving to receive and integrate multiple modes of sensory afferents entering the amygdala,

 ISSN: 0006-3223

and if it is present, how this receptor contributes to the inhibition in the microcircuit of the LA. We observed that in the LA, GABA$_A$(δ)R was preferentially expressed in interneurons (INs) but not projection neurons (PNs). Surprisingly, in sharp contrast to the synaptic GABA$_A$R, which dominates inhibition in brain, GABA$_A$(δ)R activation decreased instead of augmenting LA inhibition.

METHODS AND MATERIALS

Animals

Experiments were performed on male mice ages 35–40 days. The mice were bred and raised under the care of the Division of Laboratory Animals, Nanchang University. All animals had ad libitum access to water and food and were housed under a 12-hour light/dark cycle. Experimental procedures were approved by the Animal Care and Use Committee of Nanchang University. More detailed information about the animal breeding and use was shown in Supplemental Methods and Materials.

Electrophysiology

Mice were anesthetized with diethyl ether and sacrificed by decapitation. The brains were quickly removed to ice-cold oxygenated artificial cerebrospinal fluid. Coronal amygdala slices were cut with a tissue slicer (Leica VT 1000S; Leica Biosystems, Nussloch, Germany) and transferred to a recording chamber continuously superfused with artificial cerebrospinal fluid. The major components of the pipette solution are shown in the Supplement. The pipette resistance was around 4-7 MΩ.

To measure single channel activity of GABA$_A$(δ)R, outside-out patches were used. After obtaining stable whole-cell recordings, we slowly pulled the recording pipette back above the surface of the slice until a stable giga seal was re-established. In experiments measuring the neuronal excitability of INs and its modulation by GABA$_A$(δ)R, current clamps were employed and the holding currents were set at 0 pA. To determine the resting membrane potential using the non-invasive cell-attached recording, a pipette solution with high K^+ was used to yield a symmetric K^+ gradient between the two sides of a membrane patch. Thus, the K^+ current through this patch reverses when the potential of the pipette is equal to the membrane potential (Supplemental Figure S1).

To measure the biphasic evoked excitatory postsynaptic currents/inhibitory postsynaptic currents (eEPSCs/eIPSCs) in LA PNs, the electric stimuli were delivered through a bipolar electrode placed in either the external or internal capsule. We routinely verified the disynaptic nature of eIPSCs by testing the attenuation of eIPSCs by 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; Tocris Bioscience, Bristol, UK) upon the cessation of the experiments. In experiments comparing the eIPSCs evoked by train stimuli in wild-type (WT) and knockout (KO) mice, the stimulus intensity was adjusted to evoke eIPSCs of 200–400 pA in response to a single stimulus before the train stimuli were used. In the long-term potentiation (LTP) experiment, stimulus intensity was adjusted to produce eEPSCs of 100–150 pA. A pairing protocol was used to induce LTP and included 100 presynaptic stimuli at 2 Hz delivered to either the cortical or the thalamic pathway while the recorded PNs were held at +30 mV. LTP was quantified by normalizing the data collected in the last 5 minutes to the mean value of the amplitude of baseline eEPSCs, which were recorded for ≥6 minutes before LTP induction. More detailed information on the electrophysiological experiments and analysis of the tonic currents are provided in the Supplement.

Immunohistochemistry, Drug Infusion, and Fear Conditioning

Immunohistochemistry, drug infusion, and cued fear conditioning were performed as we described previously (20–22). A detailed description is provided in the Supplement.

Data Analysis

In all cases, a Kolmogorov-Smirnov test was applied to test the normality of the distribution. Appropriate statistical approaches, including the paired or unpaired *t* test, and the one-way or two-way analysis of variance (ANOVA) followed by comparisons using Bonferroni-corrected *t* test, were used. The specific tests used for each experiment are provided in the respective figure legend. Data are presented as means ± SEM.

RESULTS

GABA$_A$(δ)R Is Selectively Expressed and Functionally Active in INs of the LA

We investigated the functional role of extrasynaptic GABA$_A$(δ)R in tuning LA inhibition by starting to explore its expression profile inside the LA. The immunostaining results showed that in mice expressing green fluorescent protein under the control of the glutamic acid decarboxylase 67 promoter (designated as WT mice; see the Supplement), the δ subunit of GABA$_A$R was primarily coexpressed with green fluorescent protein but not Ca^{2+}/calmodulin-dependent kinase II, a marker for glutamatergic PNs (Figure 1A). The majority of LA INs (87 ± 4%, n = 4 mice) exhibited clear staining of the δ subunit. By contrast, very few PNs (4 ± 1%) had positive staining for the δ subunit. As expected, when we repeated the staining in mice selectively expressing green fluorescent protein in their INs in which δ subunit had been genetically deleted (designated as KO mice), considerable staining of the δ subunit was not observed in either neuron type. We also examined the coexpression of the δ subunit with the molecular markers of INs including parvalbumin, cholecystokinin, somatostatin, and found that this subunit existed in the majority of INs positive for any of the markers (Supplemental Figure S2), demonstrating the ubiquitous presence of the δ subunit in distinct subsets of LA INs.

In line with its IN-specific expression, we observed that GABA$_A$(δ)R was also preferentially active in INs but not PNs. By employing outside-out patches from the soma of both cell types, we measured the single-channel activity of GABA$_A$(δ)R in WT mice. We found that tetrahydroisoxazolopyridinol (THIP) (2 μmol/L), which was shown to preferentially target GABA$_A$(δ)R (23), evoked activation of single channels characteristic of GABA$_A$(δ)R in seven of 12 patches from INs (n = 5 mice) (Figure 1B–D). First, the channel opening was sensitive to a low concentration of THIP and completely blocked by 100 μM picrotoxin (PTX; Sigma-Aldrich, St. Louis, MO), a GABA$_A$R