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Fear conditioning suppresses large-conductance calcium-activated potassium channels in lateral amygdala neurons

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HIGHLIGHTS

- Fear conditioning increases the excitability of lateral amygdala principal cells.
- This is attributed to suppression of a class of potassium channels.
- The large-conductance calcium-activated potassium channel is suppressed.
- Suppression of this channel may be involved in emotional disorder in general.

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ABSTRACT

It was previously shown that depression-like behavior is accompanied with suppression of the large-conductance calcium activated potassium (BK) channel in cingulate cortex pyramidal cells. To test whether BK channels are also involved in fear conditioning, we studied neuronal properties of amygdala principal cells in fear conditioned mice. After behavior, we made brain slices containing the amygdala, the structure critically relevant to fear memory. The resting membrane potential in lateral amygdala (LA) neurons obtained from fear conditioned mice (FC group) was more depolarized than in neurons from naïve controls. The frequencies of spikes evoked by current injections were higher in neurons from FC mice, demonstrating that excitability of LA neurons was elevated by fear conditioning. The depolarization in neurons from FC mice was shown to depend on BK channels by using the BK channel blocker charybdotoxin. Suppression of BK channels in LA neurons from the FC group was further confirmed on the basis of the spike width, since BK channels affect the descending phase of spikes. Spikes were broader in the FC group than those in the naïve control in a manner dependent on BK channels. Consistently, quantitative real-time PCR revealed a decreased expression of BK channel mRNA. The present findings suggest that emotional disorder manifested in the forms of fear conditioning is accompanied with BK channel suppression in the amygdala, the brain structure critical to this emotional disorder.

1. Introduction

Fear learning is accompanied by synaptic modification in the amygdala, such as long-term potentiation (LTP) [1–3]. We have recently demonstrated a positive-feedback regulation of neuronal excitability in the lateral amygdala (LA) in the presence of monoaminergic neuromodulation, whereby a preceding activity of principal neurons increases their own excitability, and thereby augments responses to following synaptic volleys that come in with a short delay [4,5]. It turned out that the cellular underpinnings of this positive-feedback mechanism are reduction of spike after-hyperpolarization, induction of after-depolarization and closure of potassium channels [5,6]. These cellular phenomena have been known to foster LTP [7–10], and therefore this positive-feedback mechanism for regulating LA neuron excitability may facilitate synaptic modification in amygdala neural circuits, thereby playing a pivotal role in fear learning. The particular potassium channel class involved in this regulation was G protein-coupled inwardly-rectifying potassium (GIRK) channels [6]. Consistently, mice lacking GIRK channels are known to be defective in taste aversion, an amygdala-dependent learning with emotional influence [11].

Notably, there is another class of potassium channel that is involved in regulating excitability and LTP in the amygdala, and hence fear memory as well, which is the small conductance calcium-activated potassium channel (SK channel) [12,13]. It is also demonstrated that

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SK channels are suppressed in LA neurons by chronic stress, thereby increasing LA neuron excitability [14].

We have recently shown that another major member of the calcium-activated potassium channel, i.e., the large-conductance calcium-activated potassium channel (BK channel) is suppressed in cingulate cortex neurons in a mouse model of depression and increases excitability of these neurons [15]. This BK channel suppression and the accompanying increase in excitability are recovered in harmony with reversal of depression-like behavior [15]. The possibility thus arises that BK channels might be involved in a more wide variety of emotional systems. In particular, BK channel might be a third class of potassium channel, besides GIRK and SK channels, that has relevance to fear learning. To test this possibility, we set out to evaluate activity of BK channels in LA principal neurons by using fear-conditioned mice.

2. Materials and methods

2.1. Animals

All experiments were approved by the Animal Care Committee of Kanazawa Medical University and performed in accordance with the guiding principles of the Physiological Society of Japan. C57BL/6 male mice (15–20 weeks old; 25–37 g) were kept under an automatic day–night control (12:12 h) and allowed free access to food and water.

2.2. Behavioral studies

Fear conditioning was performed in a dark-colored plastic chamber (Panlab s.l.u., Cornella, Spain; 25 W × 25 D × 27 cm H) located within a tone-proof box (66 W × 54 D × 54 cm H). The whole chamber is positioned on a PC-controlled gravity sensor that can quantify the freezing. Rats were fear-conditioned with the two day paradigm. On day 1, after a 3-min habituation, the animals were exposed to white tone (80 dB) and illumination for 28 s, then to the same tone and illumination combined with electrical shock (0.4 mA) for 2 s, which was repeated twice at the interval of 30 s. On day 2, the animals were placed in the same chamber as on day 1, initially with no tone given. Freezing was assessed for 5 min by a PC using the FREEZING software (Panlab). One hour later, the animals were placed in a white-colored chamber of the same size to assess the baseline activity for 3 min, and then the same tone as on day 1 was given for 3 min to assess tone-dependent fear conditioning. For evaluation of context- and tone-dependent conditioning, the freezing scores obtained by the FREEZING software was expressed as percent of the baseline activity.

The open field test was performed in a plastic cylindrical arena (80 cm ∅) surrounded by a wall of 45 cm high. The intensity of room illumination was adjusted to about 10 lx. The mice were allowed to walk around for 5 min, and the trajectory of walking was analyzed off-line (SMART, Panlab). On the PC display, the bottom of the arena was virtually divided into the two concentric regions with the equal floor areas, which were defined as the inner and outer zones. Preference to the inner and outer zone would point to less and more anxiety-like tendency, respectively.

2.3. Slice experiments

Mice used for behavioral studies were sacrificed by decapitation under ether anesthesia. The brain was dissected out and soaked into a medium (pH 7.4; 2–5 °C) containing (in mM): NaCl 124, KCl 3.3, KH_2PO_4 1.3, $NaHCO_3$ 26, $CaCl_2$ 2.5, $MgSO_4$ 2.0, and glucose 10. Slices of the amygdala were cut with a slicer at 200 μm (Zero-1, Dosaka, Kyoto, Japan). Frontal sections were made from the region containing the amygdala (approximately 1.00 to 2.50 mm posterior from the Bregma) according to a standard atlas [16]. Slices were placed in a recording chamber on the stage of an upright microscope (Eclipse E600FN, Nikon) with a ×40 water immersion objective (Fluor 40 × 0.80 W, Nikon). The chamber was continuously perfused with medium (25 °C) bubbled with a mixture of 95% O_2 and 5% CO_2. For recording, we used patch pipettes (resistance, 4–10 MΩ) filled with a solution (pH 7.3) containing (in mM) KCl 7, K-gluconate 144, KOH 10, HEPES 10. Whole-cell recordings were made from lateral amygdala principal cells that had sufficiently negative resting membrane potentials (<− 55 mV) without spontaneous action potentials. Principal cells were visually selected initially on the basis of their pyramidal shape as we did in our previous experiments [4–6]. This selection was confirmed by examining whether these neurons showed electrophysiological characteristics consistent with the previous reports [17,18], showing the regular-spiking pattern of firing. Neurons exhibiting the fast-spiking pattern were excluded. To verify our visual separation of glutamatergic principal neurons and GABAergic interneurons, microscopic observation was done in amygdala slices obtained from GFP-GAD67 transgenic mice (obtained from Dr. Yanagawa, Gunma University, Maebashi, Japan), in which GABAergic but not glutamatergic neurons express GFP [19]. Membrane potentials were recorded in the current-clamp mode (Axoclamp 200A and B, Molecular Devices, California, USA) and digitized at 10 kHz (Digidata 1322 and pCLAMP10, Molecular Devices).

To assess membrane excitability of recorded neurons, depolarizing currents (0.05–0.5 nA for 500 ms) were injected through the patch pipette. Currents of varying intensities were injected every 1 min, and spikes were elicited repetitively. To assess the spike width, which is used as an index for BK channel suppression [20], a train of five pulses of depolarizing currents were injected at 100 Hz. The duration (4–5 ms) and intensity of injected current were adjusted in such a way that each pulse evoked a single spike. The time point of spike initiation was easily determined because of a clear deflection of the membrane potential at the transition from the passive to active phase of voltage rising. Based on the voltage at this spike initiation and that at the peak, the spike half height was calculated. On the depolarizing and repolarizing phases of the spike, the two time points corresponding to this spike half height were determined. The spike half width was defined as the time length between these two time points. Charybdotoxin (CTx, 50 nM; Alomone, Israel), a blocker of BK channels, and their activator isopimaric acid (10 μM, Alomone) were applied into the bath.

2.4. Quantitative real-time PCR (qRT-PCR)

After the behavioral test, forebrain tissue containing the whole amygdala was rapidly dissected out, so that tissue outside the amygdala could be involved as minimally as possible. One hemisphere was used for electrophysiology, and the other for RT-PCR. The tissue was then placed into liquid nitrogen, and stored at −80 °C until the time of mRNA extraction with an RNeasy lipid tissue kit (QIAGEN-Japan K.K., Tokyo). Reverse transcription was done with SuperScript III First-Strand Synthesis SuperMix for qRT-PCR (Life Technologies Japan Ltd., Tokyo). We used an Applied Biosystems 7900HT Real-Time PCR System with TaqMan probes and the other requisite chemicals following the manufacturer's indications (Applied Biosystems, Foster City, CA). Measurement was based on the ΔΔCt method. The probes and primers used were: Mm00607939_s1 (β-actin) and Mm00516078_m1 (BK channel; potassium large conductance calcium-activated channel, subfamily M, alpha member 1). Expression of BK channel was standardized by expression of β-actin in the same sample. For each experimental group, samples obtained from 7 individuals were subjected to RT-PCR in duplicate or triplicate, and then 7 β-actin-standardized data were averaged.

2.5. Data analysis

Data are expressed as averages ± s.e.m. For statistics, pairwise or unpaired *t*-tests, and repeated measure or one-way ANOVA followed by Tukey HSD test were used (SPSS v18, Japan IBM Ltd., Tokyo, Japan). The significance level was set at $P < 0.05$.