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Differential calcium dependence in basal and forskolin-potentiated spontaneous transmitter release in basolateral amygdala neurons

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HIGHLIGHTS

- Both basal and potentiated spontaneous release are regulated by extracellular Ca^{2+}.
- Basal, but not potentiated, spontaneous release is regulated by intracellular Ca^{2+}.
- CaSR inhibitor has no effect on basal spontaneous release.
- CaSR inhibitor increases the potentiated spontaneous release.

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ABSTRACT

Action potential-independent transmitter release, or spontaneous release, is postulated to produce multiple postsynaptic effects (e.g., maintenance of dendritic spines and suppression of local dendritic protein synthesis). Potentiation of spontaneous release may contribute to the precise modulation of synaptic function. However, the expression mechanism underlying potentiated spontaneous release remains unclear. In this study, we investigated the involvement of extracellular and intracellular calcium in basal and potentiated spontaneous release. Miniature excitatory postsynaptic currents (mEPSCs) of the basolateral amygdala neurons in acute brain slices were recorded. Forskolin, an adenylate cyclase activator, increased mEPSC frequency, and the increase lasted at least 25 min after washout. Removal of the extracellular calcium decreased mEPSC frequency in both naïve and forskolin-treated slices. On the other hand, chelation of intracellular calcium by BAPTA-AM decreased mEPSC frequency in naïve, but not in forskolin-treated slices. A blockade of the calcium-sensing receptor (CaSR) resulted in an increase in mEPSC frequency in forskolin-treated, but not in naïve slices. These findings indicate that forskolin-induced potentiation is accompanied by changes in the mechanisms underlying Ca^{2+}-dependent spontaneous release.

1. Introduction

Chemical synapses have 2 forms of transmitter release, action potential-dependent and -independent release. To date, most studies have focused on action potential-dependent release because this form of release is thought to be important for rapid information processing in the brain. However, recent observations indicate that action potential-independent (spontaneous) release also has physiological roles. For example, spontaneous neurotransmission maintains dendritic spines [11] and regulates dendritic protein synthesis [15].

Spontaneous release appears to involve different mechanisms than those involved in action potential-dependent release. It is well established that action potential-dependent release of neurotransmitters is dependent on Ca^{2+}, whereas spontaneous release is partly independent of extracellular and intracellular Ca^{2+} [7]. In addition, several molecules, such as Doc2b [6] and synaptotagmin-12 [10], are implicated in the selective regulation of spontaneous release.

Certain chemical or electrical stimulation triggers long-lasting enhancement in the efficacy of synaptic transmission (LTP), partly through presynaptic mechanisms [8,9]. Such stimulation potentiates both action potential-dependent and -independent transmitter release. In other words, LTP-inducing stimulation increases the probability of action potential-evoked transmitter release and the frequency of spontaneous release [2]. Although a recent study found that L-type voltage-dependent Ca^{2+} channels mediate the expression of the potentiation in action potential-dependent synaptic transmission in the amygdala [4], the expression mechanisms underlying potentiated spontaneous release remain totally unclear.

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The purpose of this study was to investigate the involvement of Ca^{2+} in spontaneous neurotransmitter release which was potentiated by forskolin in basolateral amygdala (BLA) synapses. Miniature excitatory postsynaptic currents (mEPSCs) of BLA neurons in acute murine brain slices were measured. Potentiation of spontaneous release was induced by the application of forskolin, an adenylate cyclase activator. Forskolin activates cAMP/PKA pathway which underlies formation of conditioned fear and long-term synaptic potentiation in BLA [5,13]. We examined whether or not both basal and forskolin-potentiated spontaneous release are sensitive to removal of extracellular calcium, chelation of intracellular calcium or inhibition of the calcium-sensing receptor.

2. Materials and methods

2.1. Slice preparations

All experiments were conducted according to the Guide for Care and Use of Laboratory Animals of the University of Tokyo. All efforts were made to minimize the number of animals used in this study. Postnatal 21- to 28-day-old male ICR (CD1) mice were deeply anesthetized with diethyl ether and immediately decapitated. The brains were quickly removed and immersed in ice-cold artificial cerebrospinal fluid (ACSF) containing: NaCl (127 mM), KCl (1.6 mM), KH_2PO_4 (1.24 mM), $MgSO_4$ (1.3 mM), $CaCl_2$ (2.4 mM), $NaHCO_3$ (26 mM), and glucose (10 mM), continuously aerated with 95% O_2/5% CO_2. Acute coronal slices containing amygdala (300 μm thick) were cut using a vibratome (Leica VT1200S, Leica Microsystems, Wetzlar, Germany) in ice-cold, sucrose-substituted, modified ACSF (mACSF) containing: sucrose (222.1 mM), $NaHCO_3$ (27 mM), NaH_2PO_4 (1.4 mM), KCl (2.5 mM), $MgSO_4$ (7.0 mM), $CaCl_2$ (1.0 mM), and ascorbic acid (0.5 mM), continuously aerated with 95% O_2/5% CO_2. Slices were maintained for 30 min at 37 °C, and then incubated for at least 30 min at room temperature before use.

2.2. Electrophysiology

Slices were transferred to a recording chamber and superfused ACSF saturated with 95% O_2/5% CO_2 (30–32 °C, 1–2 mL/min). Whole cell recordings were made from visually identified, pyramidal neurons in the basolateral nucleus of the amygdala using infrared differential interference contrast (IR/DIC) techniques. Patch pipettes (2.5–5 MΩ) were fabricated from borosilicate glass and filled with a solution containing K-gluconate (120 mM), KCl (5 mM), HEPES (10 mM), $MgCl_2$ (1 mM), phosphocreatine–Na_2 (10 mM), MgATP (2 mM), Na_2GTP (0.2 mM), and EGTA (0.2 mM), pH 7.2–7.3, 280–295 mOsm. All recordings were made in the presence of tetrodotoxin (1 μM, TOCRIS), picrotoxin (0.1 mM, Nacalai Tesque), and 0.1% DMSO to block action potentials and GABA-activated currents. Forskolin (50 μM, Sigma), 1,2-bis(o-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid tetra(acetoxymethyl) ester (BAPTA-AM; 50 μM, Nacalai Tesque) and NPS-2143 hydrochloride (1 μM, TOCRIS) were added in ACSF, as required by the type of the experiment being conducted. NPS-2143 blocks the calcium-sensing receptor (CaSR) with an IC_{50} of 43 nM, and is expected to block >95% of CaSR at 1 μM. ACSF, which was added EGTA (0.5 mM), but not $CaCl_2$, was used as nominally Ca^{2+}-free ACSF. BAPTA-AM was loaded by bath application.

Data were sampled at 20 kHz and filtered at 2 kHz using an Axopatch 700B amplifer (Axon Instruments, Foster, CA), DIGIDATA1440A (Axon Instruments, Foster, CA) and pClamp 10.2 (Molecular Devices, Sunnyvale, CA). Series resistance was monitored throughout the experiments, and if it changed by more than 25% or exceeded 30 MΩ, the data were discarded.

Medium after hyperpolarization (mAHP) was recorded in current clamp mode. Cells were held at −70 mV, and injected with 800 ms current pulses to evoke 2 action potentials. The amplitude of mAHP was defined as the difference between the negative peak of the membrane potential after the end of current injection and the resting membrane potential (−70 mV).

mEPSCs were detected using a homemade MATLAB program, and defined as inward currents with amplitudes larger than 7 pA. In detail, this program calculates the difference between successive local maximums and minimums after filtering, and events with amplitudes larger than 7 pA were treated as putative mEPSCs. Putative mEPSCs with rise times larger than their decay times were regarded as false positives and omitted. The amplitude and standard deviation of recording noise was 1.1 pA and 0.9 pA, respectively. This program can distinguish mEPSCs occurring with intervals <10 ms. All values are given as mean ± SEM. Paired *t*-test after one factor repeated measures ANOVA, Student's *t*-test, or Kolmogorov–Smirnov (K–S) test was used, as appropriate, for comparisons.

3. Results

The effects of forskolin on mEPSCs of basolateral amygdala neurons were examined. The basal mEPSC frequency, amplitude, decay time, and rise time were 4.6 ± 1.3 Hz, 10.9 ± 0.4 pA, 4.4 ± 0.2 ms, 0.93 ± 0.04 ms, respectively. Forskolin (50 μM) was subsequently applied for 10 min, and washed out. The mEPSC frequency increased after forskolin application (Fig. 1A). The degree of the increase was stabilized within 15 min (10 min vs. 15 min after the end of forskolin, $P = 0.91$, paired *t*-test), and the increase lasted for at least 25 min (Fig. 1E). The mEPSC frequency, 20–25 min after forskolin washout (9.2 ± 3.3 Hz), was significantly higher than before its application (4.6 ± 1.3 Hz) (Fig. 1E; $n = 5$ neurons; $P = 0.013$, paired *t*-test after one-way repeated measures ANOVA), and there was a significant difference between cumulative distributions of interevent intervals (IEIs) before and after forskolin application (Fig. 1F, $P < 0.01$, K–S test). However, the mEPSC decay time, rise time, and amplitude were unchanged (Fig. 1B–D; decay time, 4.7 ± 0.4 ms; rise time, 0.91 ± 0.04 ms; amplitude, 11.3 ± 0.5 pA; all cases, $P > 0.05$, paired *t*-test after ANOVA). Without forskolin application, all parameters were unchanged over a 40 min period (Fig. 1B–E, $P > 0.05$).

The involvement of extracellular Ca^{2+} in naïve and potentiated spontaneous release in basolateral amygdala neurons was also examined. In the naïve neurons, superfusing the slices with nominally Ca^{2+}-free ACSF significantly decreased the mEPSC frequency (Fig. 2A and B; $n = 5$ neurons; 0–5 min, 7.9 ± 2.7 Hz; 25–30 min, 2.4 ± 0.75 Hz; 0–5 min vs. 10–30 min, $P < 0.05$, paired *t*-test after one-way repeated measures ANOVA). Consistent with this, there was a significant difference between the cumulative distributions of IEIs before and after the superfusion of Ca^{2+}-free ACSF (Fig. 2C, $P < 0.01$, K–S test). Next, forskolin was applied, washed out, and the slices were superfused with Ca^{2+}-free ACSF. Superfusing nominally Ca^{2+}-free ACSF significantly decreased the mEPSC frequency (Fig. 2D and E; $n = 5$ neurons; 25–30 min, 22.8 ± 8.7 Hz; 50–55 min, 8.2 ± 2.5 Hz; 25–30 min vs. 40–55 min, $P < 0.05$, paired *t*-test after one-way repeated measures ANOVA), and there was a significant difference between the cumulative distributions of IEIs before and after the superfusion of Ca^{2+}-free ACSF (Fig. 2F, $P < 0.01$, K–S test). These findings indicate that, in basolateral amygdala neurons, both naïve and potentiated spontaneous release are, at least in part, regulated by extracellular Ca^{2+}.

In some preparations, the intracellular Ca^{2+} rise underlies spontaneous transmitter release. In hippocampal neurons,