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Dopaminergic modulation of oscillatory network inhibition in the rat basolateral amygdala depends on initial activity state

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ABSTRACT

The amygdala receives dopaminergic innervation, and dopamine (DA) enhances various activities in cognitive and emotional behaviors. Periodic bursts of spontaneous inhibitory postsynaptic currents (IPSCs) with a low (<1 Hz) inter-event frequency have been observed in projection neurons of the basolateral nucleus of the amygdala (BL). Blockade of ionotropic glutamate receptors or $GABA_A$ receptors abolishes these oscillatory IPSC bursts in the BL, suggesting that the activity has a network origin. Here, we investigated dopaminergic modulation of the oscillatory network inhibition in rat brain slices. We evaluated the effects of DA receptor agonists and antagonists on the network inhibition; the resultant changes were quantified by integrated power spectral density (0.1–3.0 Hz). DA enhanced the power when its initial activity was low, but reduced it when the activity was initially robust. These changes in the power were accompanied by changes in burst IPSC amplitude. D1-like receptor agonist SKF 38393, or DA together with the D2-like receptor antagonist sulpiride, reproduced DA's facilitatory actions. D2-like receptor agonist quinpirole did not change the periodic IPSC burst activity of the high baseline power, though D_4 receptor agonist PD 168077, or DA together with the D1-like receptor antagonist SCH 23390, reduced its activity. These results suggest that: 1) dopaminergic modulation of the oscillatory network inhibition depends on its initial activity; and 2) facilitatory and suppressing effects of DA in the BL are mediated by D1-like receptors and D_4 receptors, respectively.

1. Introduction

The amygdala plays a fundamental role in the modulation of fear, anxiety, and memory consolidation (Davis, 1992; LeDoux, 2000; McGaugh, 2004). The network activity regulated by glutamatergic and GABAergic transmission is considered crucial for these processes (Ehrlich et al., 2009; Pape and Paré, 2010). Pyramidal cells and interneurons in the amygdala receive dopaminergic innervation (Asan, 1997; Brinley-Reed and McDonald, 1999; Muller et al., 2009; Pinard et al., 2008), and increases in dopamine (DA) levels are observed under stressful conditions, such as during handling or performance in a fear conditioning task (Inglis and Moghaddam, 1999; Yokoyama et al., 2005). Several behavioral studies have shown that modulation of dopaminergic activity by administration of DA receptor agonists and antagonists can affect amygdala-related behavior such as fear learning and expression of fear (Pérez de la Mora et al., 2010). *In vitro* studies have shown that DA increases the excitability of principal neurons (Kröner et al., 2005; Pickel et al., 2006; Yamamoto et al., 2007) as well as inhibitory interneurons in the basolateral complex of the amygdala (BLA) (Kröner et al., 2005; Lorétan et al., 2004), while GABAergic neurons in the intercalated cell groups are inhibited by DA (Marowsky et al., 2005).

The BLA, comprised of the lateral (LA), basolateral (BL), and basomedial nuclei, is a critical structure for consolidation of emotionally arousing memories (Paré, 2003; Paré et al., 2002; Pelletier and Paré, 2004). The synchrony of theta range (4–8 Hz) oscillatory activities between the CA1 subfield of the hippocampus and LA increases during consolidation (Seidenbecher et al., 2003) and reconsolidation (Narayanan et al., 2007) of fear memories. Another study showed that coherent theta oscillation between the

Abbreviations: aCSF, artificial cerebrospinal fluid; AMPA, α-amino-3-hydoroxy-5-methyl-4-isoxazolepropionic acid; BL, basolateral nucleus of amygdala; BLA, basolateral complex of amygdala; DA, dopamine; DMSO, dimethyl sulfoxide; EEG, electroencephalograph; EPSC, excitatory postsynaptic current; FFT, Fast Fourier Transformation; GABA, γ-aminobutyric acid; IPSC, inhibitory postsynaptic current; IPSP, inhibitory postsynaptic potential; LA, lateral nucleus of amygdala; NMDA, *N*-methyl-D-aspartic acid.

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BL and medial prefrontal cortex promotes fear memory consolidation during sleep (Popa et al., 2010). BL neurons have also been reported to express delta (1–4 Hz) oscillations during slow wave sleep (Pape et al., 1998; Paré and Gaudreau, 1996). In the thalamocortical circuitry, EEG-synchronized sleep is considered to be based on the network oscillation composed of ensemble repetitive burst discharges of thalamocortical neurons observed *in vivo* as well as *in vitro* (McCormick and Bal, 1997). In the slice experiments of the amygdala, previous studies observed spontaneous and periodic bursts of inhibitory postsynaptic potentials/currents (IPSP/Cs) in BL principal neurons (Popescu and Paré, 2010; Rainnie, 1999). These periodic IPSP/C bursts are characterized by a low frequency (0.3–1 Hz and *ca.* 0.8 Hz) and sensitivity to blockade not only of $GABA_A$ receptors but also of AMPA/kainate glutamate receptors (Popescu and Paré, 2010; Rainnie, 1999). This sensitivity implies that the periodic bursts need network activity involving excitatory synapses. Indeed, Popescu and Paré (2010) focused on the mechanism of this "slow periodic inhibitory oscillation" and reported the presence of atypical BL projection cells which generate action potential bursts periodically. These spontaneous burst events shortly preceded the periodic IPSP bursts observed in other standard principal cells, suggesting that the IPSC/P bursts represent consequence of the whole synaptic activities involved in the network oscillation. Therefore, we call the periodic IPSP/C bursts in the acute slice preparation as oscillatory network inhibition hereafter.

Although this slow periodic activity, *i.e.*, oscillatory network inhibition, may play a role in memory consolidation, there have been few studies examining its mechanism, particularly with respect to its modulation by DA. Therefore, in this study, we investigated dopaminergic modulation of the oscillatory network inhibition in rat brain slices to provide detailed analyses of dopaminergic actions and of participation of DA receptor subtypes.

2. Materials and methods

All experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and were approved by the Institutional Animal Care and Use Committee at The University of Tokyo (approval ID #: 19-21) and Saitama Medical University (approval ID #: 624).

2.1. Slice preparation

Coronal brain slices (400 μm thick) containing the amygdala were obtained from male and female Wistar rats (10–40 days old). After halothane (Takeda) or enflurane (Abbott) anesthesia was administered, the animals were decapitated, and their brains were rapidly removed and placed in an ice-cold cutting solution, oxygenated with a mixture of 95% O_2 and 5% CO_2, containing the following (in mM): 120 choline chloride, 3 KCl, 28 $NaHCO_3$, 1.25 NaH_2PO_4, 22 glucose, and 8 $MgCl_2$. Brain slices were cut using a vibrating microtome (Leica, VT 1000S) and incubated for at least 1 h in an oxygenated interface chamber at room temperature before being subjected to electrophysiological experiments.

2.2. Whole-cell patch clamp recording

Microelectrodes were pulled from fiber-filled capillary tubing of thin wall borosilicate glass (World Precision Instruments, type 1B150F-4) with a Flaming/Brown type micropipette puller (Sutter Instruments, model P-97) and filled with an internal solution containing the following (in mM): 150 K-methanesulphonate, 5 KCl, 0.1 K-EGTA, 5 Na-HEPES, 3 Mg-ATP, and 0.4 Na-GTP (pH = 7.4). The resistance of the microelectrodes was 3–5 MΩ. Recordings were made with an amplifier (HEKA, EPC-8) using pClamp 7.0 software and a Digidata 1200 A–D interface (Axon Instruments). Whole-cell patch clamp recordings were obtained under visual control using differential interference contrast and infrared video microscopy (Zeiss, Axioskop 2 and Hamamatsu Photonics, IR-CCD and Argus-10). Recordings were made from individual principal neurons in the BL under voltage-clamp mode at −45 mV. During recording, slices were perfused with warmed artificial CSF (aCSF) containing the following (in mM): 120 NaCl, 3 KCl, 26 $NaHCO_3$, 1.25 NaH_2PO_4, 15 glucose, 2.5 $CaCl_2$, and 1.3 $MgCl_2$. The temperature of the perfusing solution was maintained at 34–35 °C by a temperature regulating unit (Warner Instruments TC-324B). Signals were acquired at 5 kHz after filtering through an amplifier buried filter at 1 kHz.

Whole-cell voltage-clamp recordings were made from 167 BL neurons in rat amygdala. BL principal neurons were accessed by preferentially aiming the recording electrode at cells with large somata and a pyramidal shape using differential interference contrast and infrared video microscopy. Cells were identified electrophysiologically according to their broad action potentials (half-width 0.95 ± 0.01 ms, $n = 165$) and accommodating spiking pattern during stimulation with a prolonged (1200 ms) depolarizing current pulse, as is consistent with previously reported criteria of principal (*i.e.*, pyramidal) neurons in the BL (Faber et al., 2001; Faulkner and Brown, 1999; Rainnie et al., 1993; Sah et al., 2003; Washburn and Moises, 1992). Under the experimental conditions with the present composition of internal solution and holding membrane potential of −45 mV, inhibitory postsynaptic current (IPSC) mediated by $GABA_A$ receptors was recorded as an outward current, whereas excitatory postsynaptic current (EPSC) mediated by ionotropic glutamate receptors was recorded as an inward current.

2.3. Drugs

Drugs were prepared from frozen, highly concentrated stock solutions, diluted directly in aCSF to obtain the appropriate concentration, and were applied to the slices via bath application. The drugs used in this study were: dopamine hydrochloride (DA); the D1-like receptor agonist SKF 38393 hydrobromide; the D2-like receptor agonist (−)-quinpirole hydrochloride; the D1-like receptor antagonist SCH 23390 hydrochloride; the D2-like receptor antagonist (*S*)-(−)-sulpiride; the D_4 receptor agonist PD 168077 maleate; the D_4 receptor antagonist FAUC 213 and L-745,870 tryhydrochloride. Sulpiride and FAUC 213 were initially dissolved in DMSO and then diluted to their final concentrations. DMSO concentration in aCSF was <0.1%. All drugs were obtained from Tocris, with the exception of DA and FAUC 213 (Sigma–Aldrich).

2.4. Data analysis

Off-line analyses were performed with pClamp 9.2 (Axon Instruments) and Mini Analysis 4.0.1 (Synaptosoft Inc.). Power spectral analysis using FFT was also performed with pClamp 9.2. Significance of the differences was determined with Dunnett's test and significance of correlation coefficients was determined with Student's *t*-test. All values are expressed as means ± SEM, and *p*-values < 0.05 were considered significant.

3. Results

3.1. Properties of oscillatory network inhibition

Oscillatory inhibitory synaptic activities, *i.e.*, periodic spontaneous IPSC bursts, were observed in many but not all recordings (Fig. 1A). When observed, they continued to occur stably for long periods of time (1–2 h) with relatively constant frequencies in the range of 0.1–3 Hz (for further analysis, see below). The amplitudes of the periodic IPSC bursts were substantially larger (usually several hundreds pA) than those of non-rhythmic spontaneous IPSCs (<100 pA), which were observed in inter-event intervals of the periodic bursts or in the burst negative cells (Fig. 1A2).

In order to quantify oscillatory activity level of the IPSC bursts, we first utilized power spectral analysis. As illustrated in Fig. 1B, a spectrogram of power spectral density was constructed from a 30-s chunk of the recording trace that represented a typical and stable state of interest, and an integral value between 0.1 and 3.0 Hz was used as an index of the power of the oscillatory activity. Accordingly, distribution of this index for the oscillatory power from all recorded neurons was plotted in Fig. 1C. The distribution suggests the presence of two groups separated by a trough at 100 pA^2 in terms of low frequency periodic activities of the burst IPSCs. Thereafter, we regarded the recordings with power greater than 100 pA^2 as oscillatory IPSC burst positive and the recordings with power less than 100 pA^2 as oscillatory burst negative. When sorted in accordance with this criterion, oscillatory IPSC bursts were observed in 84 out of 167 cells (50.3%) in the BL.

We further analyzed amplitude and frequency of the bursting IPSC events within each 30-s recording period from the oscillatory IPSC burst positive cells. We detected IPSCs having a peak amplitude > 2 SD (108.1 pA, $n = 54$) of the collective data from these cells as burst IPSC events, and then averaged their amplitude and inter-event frequency within the single chunk of data. Their