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DIFFERENTIAL MODULATION OF PHASIC AND TONIC INHIBITION UNDERLIES SEROTONERGIC SUPPRESSION OF LONG-TERM POTENTIATION IN THE RAT VISUAL CORTEX

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Abstract—GABA receptor type A ($GABA_AR$)-mediated inhibition is divided into phasic and tonic inhibition. $GABA_ARs$ mediating the two inhibitory modalities exhibit differences in subcellular localization and subunit composition. We previously demonstrated that phasic and tonic inhibition are independently regulated by Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) and protein kinase A (PKA), respectively. Since modulation of $GABA_ARs$ by phosphorylation differs depending on subunit composition and protein kinases, phasic and tonic inhibition might be differentially regulated by a single neuromodulator activating multiple protein kinases. However, the neuromodulatory control for phasic and tonic inhibition is largely unknown. Thus, in the present study, we concurrently investigated the serotonin (5-HT) regulation of phasic and tonic inhibition and its functional implication in the pyramidal neurons of the rat visual cortex. Interestingly, 5-HT enhanced phasic inhibition but suppressed tonic inhibition. Increase in phasic inhibition was mediated by $5\text{-}HT_2$ receptor and CaMKII, whereas decrease in tonic inhibition depended on $5\text{-}HT_{1A}$ receptor and PKA. Thus, phasic and tonic inhibition might be independently regulated even by a single neuromodulator. Functionally, the opposite modulation of phasic and tonic inhibition decreased the summation of consecutive excitatory postsynaptic potentials (EPSPs) without affecting the shape of single EPSPs, which might underlie the suppression of the induction of long-term potentiation by 5-HT. These results suggest that the integrative regulation of phasic and tonic inhibition provides mechanisms for elaborate modulation of shape and summation of EPSPs and long-term synaptic plasticity.

Key words: 5-HT, $GABA_A$ receptor, CaMKII, PKA, LTP, summation.

INTRODUCTION

Ionotropic γ-aminobutyric acid receptor type A ($GABA_AR$)-mediated inhibition is largely divided into phasic and tonic inhibition depending on the subcellular localization of receptors, sources of GABA, and the timing of receptor activation (Farrant and Nusser, 2005). Phasic inhibition is mediated by a brief increase in synaptically released GABA, which acts on the $GABA_ARs$ located in the synapses. Tonic inhibition depends on ambient GABA, which consistently activates extra- and perisynaptic receptors. It is generally speculated that phasic inhibition controls the flow of specific signals while tonic inhibition regulates overall excitability (Vogels and Abbott, 2009; Connelly et al., 2013a). Furthermore, the subunit compositions of $GABA_ARs$ mediating phasic and tonic inhibition are believed to be different (Belelli et al., 2009). Since $GABA_ARs$ composed of different subunits exhibit different properties and pharmacologic responses (Mohler, 2006), phasic and tonic inhibition might be differentially regulated by various modulators of $GABA_ARs$. In addition, phasic and tonic inhibition could be selectively modulated by subunit-specific phosphorylation of $GABA_ARs$ (Kittler and Moss, 2003; Connelly et al., 2013a). In a previous study, we demonstrated that phasic and tonic inhibition are specifically modulated by Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) and protein kinase A (PKA), respectively (Joo et al., 2014). These results suggest that neuromodulators could independently regulate phasic and tonic inhibition depending on their signaling pathways. However, little is known about the differential regulation of phasic and tonic inhibition by neuromodulators.

Serotonin [5-hydroxytryptamine (5-HT)] is one of the major neuromodulators in the brain. In the visual cortex, 5-HT regulates the development of phasic inhibition (Jang et al., 2010), and the induction of long-term synaptic plasticity (Kim et al., 2006; Moreau et al., 2013) and ocular dominance (OD) plasticity (Gu and Singer, 1995).

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Abbreviations: ACSF, artificial cerebrospinal fluid; CaMKII, Ca^{2+}/calmodulin-dependent protein kinase II; D-AP5, D-(−)-2-amino-5-phosphonopentanoic acid; DOI, 2,5-dimethoxy-4-iodoamphetamine; DPAT, 8-hydroxy-*N,N*-dipropyl-2-aminotetralin; E–I, excitation–inhibition; EPSP, excitatory postsynaptic potential; fEPSP, field EPSP; $GABA_AR$, GABA receptor type A; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; LTP, long-term potentiation; NMDA, N-methyl-D-aspartic acid; OD, ocular dominance; PKA, protein kinase A; PKI, PKA inhibitor 6–22 amide; PPR, paired-pulse ratio; sIPSC, spontaneous inhibitory postsynaptic current; TBS, theta-burst stimulation; THDOC, tetrahydrodeoxycorticosterone.

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Since GABAergic inhibition is also critically involved in the regulation of long-term synaptic plasticity (Jang et al., 2009) and OD plasticity (Hensch et al., 1998), 5-HT signaling on GABAergic inhibition has been of particular interest. Although we previously reported that 5-HT enhances phasic inhibition (Jang et al., 2012), it is largely unknown whether 5-HT affects tonic inhibition in the visual cortex and in other brain regions. Because phasic and tonic inhibition might regulate cellular excitability cooperatively, the action of neuromodulators on phasic and tonic inhibition should be studied concurrently to understand the functional implications of the two modes of inhibition in the regulation of neuronal activity.

Thus, in the present study, we concurrently investigated 5-HT modulation of phasic and tonic inhibition and elucidated their roles in the regulation of long-term synaptic plasticity. In layer 2/3 pyramidal neurons of the rat visual cortex, 5-HT enhanced phasic inhibition via 5-HT_2 receptor and CaMKII. On the contrary, tonic inhibition was diminished by 5-HT_{1A} receptor and depended on the activity of PKA. The opposite regulation appeared to have a compensatory effect on the shape of single excitatory postsynaptic potential (EPSP). However, increased phasic inhibition resulted in decreased summation of consecutive EPSPs and suppressed the induction of long-term potentiation (LTP). These results suggest that phasic and tonic inhibition could be independently regulated even by a neuromodulator in a single cell type and the changes might integratively regulate neuronal excitability and the induction of long-term synaptic plasticity.

EXPERIMENTAL PROCEDURES

Animal and slice preparation

Coronal slices of the visual cortex were obtained from 5-week-old Sprague–Dawley rats of either sex (Orientbio Inc., Seoul, Korea), raised under standard conditions (23 ± 1 °C, 12/12-h light/dark cycle). We chose 5-week-old rats since it is well known that 5-HT suppresses long-term synaptic plasticity at this age (Edagawa et al., 2001; Kim et al., 2006; Jang et al., 2010). Animal care and surgical procedures were conducted with the approval of the Ethics Committee of the Catholic University of Korea, and were consistent with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*. The animals were sedated with chloral hydrate (400 mg/kg, i.p.) and decapitated after the eye blink reflex disappeared. The brains were quickly placed in ice-cold dissection medium consisting of (in mM) 125 NaCl, 2.5 KCl, 1 $CaCl_2$, 2 $MgSO_4$, 1.25 NaH_2PO_4, 25 $NaHCO_3$, and 10 D-glucose, bubbled with 95% O_2/5% CO_2. Afterward the occipital cortexes were sliced to 300-μm-thick coronal slices on a vibratome (Campden Instruments, Leics, UK). The slices were recovered at 37 °C for 40 min in a submerging chamber with carbogenated dissection medium and maintained at room temperature before recording.

Whole-cell patch-clamp recording

The slices were perfused with carbogenated artificial cerebrospinal fluid (ACSF, 1.5–2 ml/min of flow rate) in a recording chamber. The temperature was maintained at 32–33 °C. ACSF consisted of (in mM) 125 NaCl, 2.5 KCl, 2 $CaCl_2$, 1 $MgSO_4$, 1.25 NaH_2PO_4, 25 $NaHCO_3$, and 10 D-glucose. Cells were identified under infrared-DIC video-microscopy with an upright microscope (BX50-WI fitted with a 40×/0.80 NA water immersion objective, Olympus, Tokyo, Japan). Pyramidal neurons in layer 2/3 of the primary visual cortex were identified as pyramid-shaped cells located 250–500 μm from the pial surface, with a prominent apical dendrite toward the pia. An EPC8 amplifier (HEKA Elektronik, Lambrecht, Germany) and pClamp 9.0 software (Axon Instruments, Foster City, CA, USA) were used for the whole-cell recording. Data were low-pass filtered at 5 kHz and sampled at 10 kHz. EPSPs were recorded with K-gluconate-based pipette solution containing (in mM) 130 K-gluconate, 10 KCl, 4 Mg-ATP, 10 Na_2-phosphocreatine, 0.3 Na_3-GTP and 10 HEPES (pH 7.25 with KOH). Inhibitory currents were measured with CsCl-based pipette solution containing (in mM) 145 CsCl, 4 Mg-ATP, 10 Na_2-phosphocreatine, 0.3 Na_3-GTP, 10 HEPES and 3 QX-314 (pH 7.25 with CsOH). Recording electrodes (3–5 MΩ) were pulled from borosilicate glass pipettes (1B150F-4, World Precision Instruments, Inc., Sarasota, FL, USA) using a micropipette puller (P-97, Sutter instrument Co., Novato, CA, USA). Typical access resistance was about 15–20 MΩ.

Measurement of inhibitory currents

Inhibitory currents were recorded with a CsCl-based pipette solution. To isolate $GABA_A$R-mediated currents, the alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor antagonist 6,7-dinitroquinoxaline-2,3-dione (DNQX, 20 μM), the N-methyl-D-aspartic acid (NMDA) receptor antagonist D-(−)-2-amino-5-phosphonopentanoic acid (D-AP5, 50 μM) and the $GABA_B$ receptor antagonist CGP 52432 (1 μM) were added to ACSF. After whole-cell current was stabilized at a −70-mV holding potential, various drugs including 5-HT were applied into the bath to measure the changes in phasic and tonic inhibition. Spontaneous inhibitory postsynaptic current (sIPSC) was regarded as representing phasic inhibition. The averages of sIPSCs for at least 80 s were compared to measure the changes in phasic inhibition. The amplitude of the tonic $GABA_A$ current was analyzed as the difference between the holding currents measured before and after the application of the $GABA_A$ receptor antagonist bicuculline (10 μM). The holding current was calculated from 100-ms epochs, containing no obvious spontaneous synaptic events, taken every four seconds over an 80-s period as in our previous study (Jang et al., 2013). To enhance inhibition, the endogenous neurosteroid tetrahydrodeoxycorticosterone (THDOC) (3α,21-dihydroxy-5α-pregnan-20-one; 500 nM), GABA