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STIMULATION OF α_1-ADRENOCEPTORS FACILITATES GABAergic TRANSMISSION ONTO PYRAMIDAL NEURONS IN THE MEDIAL PREFRONTAL CORTEX

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Abstract—Whereas activation of α_1-adrenoceptors (α_1-ARs) modulates glutamatergic transmission, the roles of α_1-ARs in GABAergic transmission in the medial prefrontal cortex (mPFC) are elusive. Here, we examined the effects of the α_1-AR agonist phenylephrine (Phe) on GABAergic transmission onto pyramidal neurons in the deep layers of the mPFC. We found that bath application of Phe dose-dependently increased the amplitude of evoked IPSCs (eIPSCs). Phe increased the frequency but not the amplitude of miniature IPSCs (mIPSCs). Ca^{2+} influx through T-type voltage-gated calcium channels is required for Phe-induced increases in GABA release. Phe increases GABA release probability and the number of releasable vesicles. Phe depolarizes the fast-spiking (FS) interneurons without effects on the firing rate of action potentials (APs) of interneurons. Phe-induced depolarization is independent of extracellular Na^+, Ca^{2+} and T-type calcium channels, but requires inward rectifier K^+ channels (Kirs). The present study demonstrates that Phe enhances GABAergic transmission onto mPFC pyramidal neurons through inhibiting interneurons Kirs, which further depolarizes interneurons leading to increase in Ca^{2+} influx via T-type calcium channels. Our results may provide a cellular and molecular mechanism that helps explain α_1-AR-induced PFC dysfunction. © 2015 Published by Elsevier Ltd. on behalf of IBRO.

Key words: α_1-adrenoceptor, GABAergic transmission, medial prefrontal cortex, rat.

INTRODUCTION

The prefrontal cortex (PFC) is involved in a large number of advanced cognitive functions such as working memory, attention, executive function, and so forth (Goldman-Rakic, 1995, Bodner et al., 2005; Morgane et al., 2005). Neurobiological study reveals that dysfunction of the PFC contributes to neuropsychiatric disorders such as attention deficit disorder and attention-deficit/hyperactivity disorder (ADHD) (Arnsten and Li, 2005; McKenna and Eyler, 2012).

At the behavioral level, activation of α_1-adrenoceptors (α_1-ARs) could influence cognitive functions including working memory (Arnsten et al., 1999; Birnbaum et al., 1999). For example, stimulation of α_1-ARs by infusions of the α_1-AR agonist phenylephrine (Phe) into the rat PFC significantly impairs working memory, and co-infusion of the α_1-ARs antagonist can reverse this detrimental effect (Arnsten et al., 1999). Similar effects have been observed in monkeys, where infusions of Phe into the dorsolateral PFC cause a prominent delay-related impairment in working memory performance (Mao et al., 1999). Moreover, overstimulation of α_1-ARs is observed in patients with anxiety-related syndromes including PTSD (Krystal et al., 1996; Kaouane et al., 2012).

α_1-AR stimulation has been demonstrated to enhance glutamatergic processes in the PFC. A previous study demonstrates that activation of α_1-ARs increases glutamate release onto PFC pyramidal neurons from glutamatergic terminals (Marek and Aghajanian, 1999). Moreover, a recent study in our laboratory reveals that α_1-ARs activation augments glutamatergic transmission via both pre- and postsynaptic mechanisms in rat medial prefrontal cortex (mPFC) (Luo et al., 2014b). However, the effects of α_1-ARs on the modulation of GABAergic transmission onto pyramidal neurons in the PFC have not been determined completely yet.

Recently, Santana et al. (2013) examines the cellular expression of α_1-ARs in GABAergic fast-spiking (FS) interneurons in rat PFC using double *in situ* hybridization, and they find that α_1-ARs are expressed by a high proportion of GABAergic FS interneurons, suggesting that α_1-AR modulates functions of GABAergic interneurons in the PFC (Santana et al., 2013). Indeed, the PFC is sensitive and its functions are easily influenced by changes of neurochemical environment (Arnsten et al., 1997), and the event-related activity of the PFC is regulated by GABA in the PFC (Kritzer and Goldman-Rakic, 1995). The deep layers (layer V/VI) of PFC pyramidal neurons can integrate multiple glutamatergic and GABAergic inputs and send projections to many other brain regions (Heidbreder and Groenewegen, 2003), and through this

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Abbreviations: ACSF, artificial cerebrospinal fluid; AP-5, DL-2-amino-5-phophonovaleric acid; APs, action potentials; DNQX, 6,7-dinitroquinoxaline-2,3-dione; EGTA, ethylene glycol tetraacetic acid; eIPSCs, evoked IPSCs; FS, fast-spiking; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; mIPSCs, miniature IPSCs; mPFC, medial prefrontal cortex; NMDA, N-methyl-D-aspartic acid; PFC, prefrontal cortex; Phe, phenylephrine; PPF, paired-pulse facilitation; PTX, picrotoxin; RMPs, resting membrane potentials; sIPSCs, spontaneous IPSCs; TTX, tetrodotoxin; α_1-ARs, α_1-adrenoceptors.

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neural network, the PFC guides complex cognitive responses, such as working memory and execution of goal-directed behaviors (Fuster et al., 2000). Therefore, understanding how α_1-ARs modulates GABAergic transmission onto pyramidal neurons would provide important insights into its role in cognitive functions associated with the PFC. In the current study, we examined the effects of Phe on GABAergic transmission in the PFC using whole-cell patch-clamp recordings. Our results demonstrate that activation of α_1-ARs enhances GABAergic transmission via potentiation of both GABA release probability and the number of releasable vesicles. We also find that Phe-induced increase in GABA release onto pyramidal neurons is mediated by depolarization of GABAergic interneurons through inhibition of Kirs resulting in increase in Ca^{2+} influx through T-type calcium channels.

EXPERIMENTAL PROCEDURES

Ethics statement

The present study was strictly in compliance with Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. All the experimental protocols used were approved by the Committee on the Ethics of Animal Experiments of the Nanchang University (Permit Number: 2007–0002).

Preparation of brain slices

Horizontal Brain slices were obtained from Sprague–Dawley rats (14–23 days old) according to a procedure described previously (Luo et al., 2015). In brief, after being deeply anesthetized with sodium pentobarbital (40 mg/kg, i.p.), rats were decapitated and their brains were dissected out in ice-cold artificial cerebrospinal fluid (ACSF), containing (in mM) 124 NaCl, 1.5 $MgSO_4$, 3 KCl, 2.5 $CaCl_2$, 25 $NaHCO_3$, 0.4 ascorbic acid and 10 glucose, pH 7.4 with 95% O_2/5% CO_2, 310–320 m Ω. Brains were cut into coronal slices (350 μm in thickness) containing the PFC, and slices were incubated at 30 °C for 1 h in the above ACSF solution for recovery and then maintained at room temperature (25 °C) until use.

Identification of pyramidal neurons and FS interneurons

A slice was viewed using an upright microscope (Axioskop Fsmot, Zeiss, Germany) equipped with infrared-differential interference contrast (IR-DIC) optics. The image was observed with an infrared-sensitive CCD (IR 1000, DAGE-MTI, USA) and displayed on a video monitor. Layer V/VI is the largest layer in the PFC, and layer V/VI pyramidal cells are easily identified under DIC optics because they possess much larger pyramidal-shaped somas than in other layers. FS interneurons were characterized by a round or oval cell body and the lack of a visible apical dendrite. Moreover, the typical firing pattern of pyramidal neurons showed significant firing frequency adaptation, whereas FS interneurons displayed FS action potentials followed by pronounced hyperpolarization (Cauli et al., 1997).

Whole-cell patch-clamp recordings

Whole-cell patch-clamp recordings utilizing an Axopatch 200B amplifiers (Molecular Devices, Sunnyvale, CA, USA) in current- or voltage-clamp were performed from PFC pyramidal neurons or interneurons. The tip resistance of the recording pipettes was 3–7 M Ω after being filled with the following intracellular solution (in mM): 130 cesium gluconate, 10 Na_2phosphocreatine, 10 HEPES, 8 NaCl, 0.4 EGTA, 2 ATP. Mg, 0.1 GTP·Na_3^+ (PH was adjusted to 7.4). Action potentials (APs) and resting membrane potentials (RMPs) are recorded from interneurons under the current-clamp mode, and 130 cesium gluconate was replaced by 130 K^+ gluconate in the pipette solution. As dialysis of K^+ into recorded neurons can influence APs firing and RMPs, recording start ~10 min after the RMPs is stable. Recordings of spontaneous IPSCs (sIPSCs), miniature IPSCs (mIPSCs), evoked IPSCs (eIPSCs) and postsynaptic $GABA_A$ receptor-mediated currents from pyramidal cells were performed under the voltage-clamp mode at the holding potential of +30 mV. Electrical signals were low-pass-filtered at 2 kHz, digitized at 10 kHz. For individual neurons, only neurons demonstrating series resistance (R_s) < 25 M Ω and <20% change during recordings were used in these experiments. Cell input resistance was assessed by applying a 10-pA, 500-ms hyperpolarizing pulse.

For recordings of eIPSCs, the extracellular solution was supplemented with AMPA receptor antagonist 6,7-dinitroquinoxaline-2,3-dione (DNQX) (20 μM) to block AMPA receptor-mediated response and N-methyl-D-aspartic acid (NMDA) receptor antagonist DL-2-amino-5-phophonovaleric acid (AP-5) (50 μM) to abolish NMDA receptor-mediated response. A custom-made bipolar stimulation electrode was placed approximately 100–200 μm away from the apical dendrites of recorded neurons. Stimulations were delivered at 0.033 Hz using Master-8 (A.M.P. Instruments Ltd, Jerusalem, Israel). mIPSCs were recorded by additionally including tetrodotoxin (TTX) (1 μM) in the above extracellular solution for recordings of eIPSCs. To record postsynaptic $GABA_A$ receptor-mediated currents, glass pipettes (the same as recording pipettes) were filled with $GABA_A$ receptor agonist muscimol (50 μM), and positioned a point perpendicular to the soma of recorded cell in the extracellular presence of DNQX, AP-5 and TTX. The distance between the tip of the pipette and cell body of recorded neuron was ~30 μm. Muscimol (50 μM) was applied by pressure ejection (10–15 psi, 10–50 ms) using a pneumatic PicoPump (PV820, World Precision Instruments, USA). In some experiments, unitary IPSCs were evoked by minimal stimulation as described previously (Yoshimura et al., 2000). Briefly, Brief (100 μs) stimulation pulses were evoked at 0.033 Hz, the stimulation intensity was increased gradually until failure rates of IPSCs were 40–60%. Minimal stimulation was set to meet the standard of single-fiber stimulation including all-or-none synaptic response and absence of change in the shapes or latencies of IPSCs. Under this condition, it was likely that only a single fiber was activated by minimal stimulation. Stronger stimulation probably activates many

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