Neuroscience 182 (2011) 193–202

α_2-ADRENOCEPTOR REGULATES THE SPONTANEOUS AND THE GABA/GLUTAMATE MODULATED FIRING ACTIVITY OF THE RAT MEDIAL PREFRONTAL CORTEX PYRAMIDAL NEURONS

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Abstract—The spontaneous and event-related firing activity of the medial prefrontal cortex (mPFC) pyramidal neurons are modulated mainly by glutamatergic inputs and GABAergic afferents. Substantial data demonstrate that α_2-adrenoceptors also play specific roles in the regulation of the firing of these pyramidal neurons. In the present study, the effects of α_2-adrenoceptor agents on spontaneous, GABA- and glutamate-mediated firing of mPFC pyramidal neurons were examined in anaesthetized rats. Microiontophoresis of norepinephrine (NE, 30 nA) decreased the spontaneous firing rate in the majority of the pyramidal neurons (25/36) and induced unchanged (six out of 36) or excitatory (five out of 36) effects in a minority of the pyramidal neurons. The inhibitory effect of NE was reproduced by α_2-adrenoceptor agonist clonidine (40 nA) and blocked by α_2-adrenoceptor antagonist idazoxan (15 nA). Clonidine application (2–5 nA) enhanced the inhibitory responses to GABA administration in the most of the pyramidal neurons examined (seven out of 12). Clonidine with low current intensity (2–5 nA) did not significantly modulate the excitatory effect of glutamate ejection on firing rate of the pyramidal neurons for both the absolute effect and the percentage of excitation. In contrast, the absolute excitatory effect of glutamate was not significantly strengthened in the presence of clonidine with high current intensity (20–40 nA) but the percentage of excitation by glutamate was increased. These results indicate that the inhibitory effects of NE on spontaneous firing of the mPFC pyramidal neurons are mediated by α_2-adrenoceptors, whereas α_2-adrenoceptors stimulation enhanced GABA-mediated inhibition and play a specific part in modulation of glutamate-mediated excitation on the neurons.

Key words: α_2-adrenoceptor, medial prefrontal cortex, pyramidal neuron, microiontophoresis, extracellular recording.

The mammalian prefrontal cortex (PFC) is involved in a large number of advanced cognitive functions such as working memory, attention, emotion, executive control, and so forth (Heidbreder and Groenewegen, 2003; Nyberg et al., 2003; Morgane et al., 2005; Rajah and McIntosh, 2006; Ramos and Arnsten, 2007; Rouillon et al., 2008). Neurobiological and imaging studies indicate that dysfunction of the PFC contributes to attention-deficit/hyperactivity disorder (ADHD) and depression (Amen and Carmichael, 1997; Arnsten and Li, 2005). Pyramidal neurons and several types of interneurons are populated in the PFC. Pyramidal neurons use excitatory glutamate (Glu) as neurotransmitter. In contrast, the interneurons utilize inhibitory GABA as neurotransmitter and are divided into several subpopulations based on peptide co-transmitters, calcium binding proteins and electrophysiological characteristics (Goldman-Rakic, 1995; Sherwood et al., 2010). In the microcircuitry of the PFC, pyramidal-interneuronal interactions are a key neuronal substrate of the PFC functions (Wilson et al., 1994). The persistent activity of PFC pyramidal neurons is created by mutual excitation between glutamatergic pyramidal neurons and regulated by inhibitory GABAergic interneurons (Kritzer and Goldman-Rakic, 1995). The PFC receives thalamic inputs and connects with associative cortical areas, and is innervated by the ascending monoaminergic systems (Groenewegen, 1988; Pandya and Yeterian, 1990; Arnsten, 1997; Heidbreder and Groenewegen, 2003). The PFC is sensitive and its functions are easily disturbed by changes of neurochemical environment (Arnsten, 1997). When the animal is performing specific tasks, the event-related activity of the PFC is regulated by GABA from intracortical interneurons, and Glu from the cortex and thalamus (Goldman-Rakic, 1995; Kritzer and Goldman-Rakic, 1995).

The ascending projections from pontine noradrenergic neurons provide important influence on PFC functions (Arnsten and Goldman-Rakic, 1985; Arnsten, 1997; Winterer and Weinberger, 2003). The PFC receives dense noradrenergic innervations mainly from the ipsilateral locus coeruleus (LC) and norepinephrine (NE) was measured at high levels in the PFC (Versteeg et al., 1976; Jones and Moore, 1977; Waterhouse et al., 1983). It has been reported that α_2-adrenoceptor agonists are effective for treatment of human psychiatric disorders that involve PFC dysfunctions, such as ADHD and defect in working memory (Riekkinen et al., 1999; Scahill et al., 2001). Several types of adrenoceptors are expressed in the PFC, while the presynaptic and postsynaptic α_2-adrenoceptors are regarded as playing a critical role in the regulation of spontaneous firing activity and activity related to some specific events of the PFC (Arnsten and Goldman-Rakic, 1985; Sara and Hervé-Minvielle, 1995; Jodo et al., 1998; Li et al., 1999; Franowicz et al., 2002; Ramos and Arnsten, 2007). In previous reports, the spontaneous firing activity

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Abbreviations: ADHD, attention-deficit/hyperactivity disorder; cAMP, cyclic AMP; CLO, clonidine hydrochloride; Glu, glutamate; HCN, hyperpolarization-activated cyclic nucleotide-gate; IDA, idazoxan hydrochloride; LC, locus coeruleus; mPFC, medial prefrontal cortex; NE, norepinephrine; PFC, prefrontal cortex.

0306-4522/11 $ - see front matter
doi:10.1016/j.neuroscience.2011.03.016

of medial PFC (mPFC) neurons in anaesthetized rats was inhibited by selective α_2-adrenoceptor agonists and noradrenergic lesions in the LC. In addition, α_2-adrenoceptor activation has been shown to increase the prefrontal cortical event-related activity in monkeys which implied that NE may also interact with Glu and GABA on activity of the PFC (Li et al., 1999; Wang et al., 2010). However, the specific roles of noradrenergic neurotransmitter NE on the spontaneous firing and α_2-adrenoceptor agents on GABA- and Glu-mediated firing activity of mPFC neurons have rarely illuminated. Therefore, the purpose of the present study was to investigate the effects of α_2-adrenoceptor agents on spontaneous, GABA- and Glu-mediated firing activity of pyramidal neurons in the mPFC in anaesthetized rats by using electrophysiological and microiontophoretic techniques *in vivo*.

EXPERIMENTAL PROCEDURES

Animal preparation

The experiments were performed on 42 adult male Sprague-Dawley rats weighing 270–350 g, provided by the Experimental Animal Center of Shaanxi Province (Xi'an, Shaanxi, China). All procedures used in this study were performed in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised in 1996, and according to the guideline of the Institutional Animal Care and Use Committee of Xi'an Jiaotong University. All efforts were made to minimize the number of animals used and their suffering. Experiments were made in rats anaesthetized with 4% chloral hydrate (400 mg/kg, i.p.) and mounted in a stereotaxic frame as described previously (Wang et al., 2010). No recordings were made until the former anesthesia began to wear off, as indicated by sporadic whisker movement, then animals remained anaesthetized to a moderate but stable level throughout the experiments by i.v. administration of additional doses of chloral hydrate (90 mg/kg. h). Loss of toe-pinch reflex was used to indicate the moderate level of anesthesia (Díaz-Mataix et al., 2006; Di Mauro et al., 2008). Body temperature was maintained at 37±0.5 °C, and heart rate was monitored throughout the experiment.

Electrophysiological recordings

Five-barrel glass microiontophoretic recording electrodes (5B120F-4, World Precision Instruments, Sarasota, FL, USA) were constructed using a glass microelectrode puller (PE-22, Narishige, Tokyo, Japan), broken back to yield electrodes with a central recording barrel impedance of 5–10 MΩ. The firing activity of individual pyramidal neurons in the mPFC was recorded extracellularly using the central barrel of the five-barrel glass microiontophoretic recording electrodes, which was filled with 2% Pontamine Sky Blue in 2 M NaCl. These electrodes were directed stereotaxically to the mPFC (AP 2.8–3.4 mm, L 0.6–1.0 mm, D 1.5–5.0 mm; Paxinos and Watson, 2005) by means of a high precision digital micromanipulator with a stepping motor (SM-21, Narishige, Tokyo, Japan). The recording coordinates are in accordance with recording area described by previous report (Puig et al., 2005). In the area, recordings were made mainly in layer V neurons. The neuronal firings were amplified, bandpass-filtered using a preamplifier (AVB-11A, Nihon Kohden, Tokyo, Japan), displayed on an oscilloscope (VC-11, Nihon Kohden, Tokyo, Japan) and stored in a computer equipped with PowerLab digital data acquisition system (ML846, AD Instruments, Castle Hill, New South Wales, Australia) for off-line analysis. Single-spike neuronal activity with a good signal-to-noise ratio (>3:1) and stable discharge were investigated. The mPFC pyramidal neuron recorded typically had a broad action potential (>1 ms) and an irregular firing pattern with burst activity (Tseng et al., 2006). Only those neurons whose spontaneous firing rate remained stable for at least 2 min during the recording were considered for pharmacological investigation.

Drug microiontophoresis

One of the outer barrels of five-barrel glass microiontophoretic recording electrodes was filled with 3 M NaCl for automatic current balancing, and each of the remaining outer barrels was filled with one of the following substances: noradrenergic neurotransmitter norepinephrine bitartrate salt (NE, Sigma-Aldrich, 200 mM, pH 4.0), α_2-adrenoceptor agonist clonidine hydrochloride (CLO, Sigma-Aldrich, 100 mM, pH 4.0), α_2-adrenoceptor antagonist idazoxan hydrochloride (IDA, Sigma-Aldrich, 20 mM, pH 4.0), GABA (Sigma-Aldrich, 200 mM, pH 3.0) and L-glutamic acid monosodium salt hydrate (Glu, Sigma-Aldrich, 1000 mM, pH 7.5) (Feldman and Moises, 1988; Zhao and Duggan, 1988; Levine and Jacobs, 1992). All drugs were dissolved in distilled water. The microiontophoretic system (6400 ADVANCED, Dagan Corp., Minneapolis, MN, USA) balanced all currents automatically through the barrel filled with 3 M NaCl to neutralize voltage shift due to any applied currents. Retaining currents were applied constantly to each micropipette barrel to prevent drug leakage from the pipette tip. Glu was retained using positive current that ranged between 5 and 10 nA, and other drugs were retained by negative currents (−10 nA). All drugs were ejected by applying ejecting currents. GABA, NE, CLO and IDA were applied with positive current pulses. In contrast, Glu was ejected with brief negative current pulses (15 s). We searched for an optimal ejection current that induced consistent responses among mPFC pyramidal neurons for each drug. NE, CLO, GABA and Glu were ejected at 30 nA, 40 nA, 10 nA and −5 nA, respectively, to test their influence on the spontaneous firing of mPFC pyramidal neurons. In order to test the effect of α_2-adrenoceptor stimulation on Glu and GABA modulated neuronal activity, GABA or Glu were ejected at least three times before and during the continuous ejection of α_2-adrenoceptor agonists, respectively.

Histology

At the end of each experiment, the recording site was marked by the ejection of Pontamine Sky Blue (−20 μA, 15 min). The rat was given an overdose of urethane, and perfused with 150 ml normal saline followed by 200 ml of 4% paraformaldehyde, the brain was immediately removed and postfixed in the same fixative for 4 h. They were then placed in phosphate buffered saline containing 25% sucrose overnight. The brains were frozen and cut into 40 μm thick coronal sections using a cryostat. Cresyl Violet staining of the sections mounted on gelatin-coated slides was used to determine the location of recording sites. Only rats with electrode placements localized within the mPFC were included in the analysis.

Data treatment and statistical analysis

Off-line analysis was performed using the Spike Histogram software (LabChart V6, PowerLab, AD Instruments). The firing rate meter over 1 s bins for each recorded unit was calculated. The neuronal firing recorded in 1 min before any drug administration was defined as the baseline activity. Responses to each ejection were assessed during microiontophoresis and within 60 s after the termination of drug administration. The effect of each drug on the firing rate of neuron was labeled as a significant excitatory or inhibitory event if the change in the mean firing rate in 30 s increased or decreased by 20% or more from the average firing rate before drug ejection (in 60 s) (Kovács and Hernádi, 2006). In case of short lasting application of Glu (15 s), mean firing rate