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KCNQ/Kv7 CHANNEL ACTIVATOR FLUPIRTINE PROTECTS AGAINST ACUTE STRESS-INDUCED IMPAIRMENTS OF SPATIAL MEMORY RETRIEVAL AND HIPPOCAMPAL LTP IN RATS

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Abstract—Spatial memory retrieval and hippocampal long-term potentiation (LTP) are impaired by stress. KCNQ/Kv7 channels are closely associated with memory and the KCNQ/Kv7 channel activator flupirtine represents neuroprotective effects. This study aims to test whether KCNQ/Kv7 channel activation prevents acute stress-induced impairments of spatial memory retrieval and hippocampal LTP. Rats were placed on an elevated platform in the middle of a bright room for 30 min to evoke acute stress. The expression of KCNQ/Kv7 subunits was analyzed at 1, 3 and 12 h after stress by Western blotting. Spatial memory was examined by the Morris water maze (MWM) and the field excitatory postsynaptic potential (fEPSP) in the hippocampal CA1 area was recorded *in vivo*. Acute stress transiently decreased the expression of KCNQ2 and KCNQ3 in the hippocampus. Acute stress impaired the spatial memory retrieval and hippocampal LTP, the KCNQ/Kv7 channel activator flupirtine prevented the impairments, and the protective effects of flupirtine were blocked by XE-991 (10,10-*bis*(4-Pyridinylmethyl)-9(10*H*)-anthracenone), a selective KCNQ channel blocker. Furthermore, acute stress decreased the phosphorylation of glycogen synthase kinase-3β (GSK-3β) at Ser9 in the hippocampus, and flupirtine inhibited the reduction. These results suggest that the KCNQ/Kv7 channels may be a potential target for protecting both hippocampal synaptic plasticity and spatial memory retrieval from acute stress influences.

Key words: acute stress, KCNQ/Kv7 channels, flupirtine, spatial memory retrieval, long-term potentiation (LTP).

INTRODUCTION

The hippocampus is a region that plays a crucial role in learning and memory and is an area also exquisitely sensitive to stress. Extensive research has shown that acute stress can exert a profound influence on hippocampal function. Acute stress not only disrupts the hippocampus-dependent spatial memory (de Quervain et al., 1998; Li et al., 2008), but also dramatically affects the synaptic plasticity of the hippocampal CA1 region, either by suppressing long-term potentiation (LTP) or by facilitating long-term depression (LTD) (Shors et al., 1989; Xu et al., 1997; Yang et al., 2005; Wong et al., 2007), both of which are putative cellular substrates for learning and memory.

Voltage-gated KCNQ/Kv7 potassium channels are formed by a homomeric or heteromeric complex of five different Kv7 subunits (Kv7.1–5, encoded by the KCNQ1–5 genes). Unlike Kv7.1, all Kv7.2–5 subunits are expressed in the central nervous system (Jentsch, 2000). KCNQ/Kv7 channels mediate the non-inactivation M current, and the slowly activating and deactivating channels are usually activated at subthreshold membrane potentials. These features enable KCNQ/Kv7 channels to play important roles in regulating neuronal excitability, spike generation, hippocampal theta oscillation and neurotransmitter release (Hu et al., 2002; Yue and Yaari, 2004; Peters et al., 2005; Vervaeke et al., 2006). Thus, it is not difficult to understand that KCNQ/Kv7 channels are involved in synaptic plasticity, learning and memory (Peters et al., 2005; Fontan-Lozano et al., 2011; Petrovic et al., 2012; Cavaliere et al., 2013). It has been postulated that the inhibition of KCNQ/Kv7 channels participates in the regulation of synaptic plasticity and subsequently facilitates the memory process in certain circumstances (Fontan-Lozano et al., 2011). Conversely, some reports have demonstrated that dysfunction of KCNQ/Kv7 channels is implicated in memory deficit. Reduction of KCNQ/Kv7 channels mediates age-dependent memory decline (Wang et al., 2011; Cavaliere et al., 2013). Suppression of M currents in mice causes impairment of hippocampus-dependent spatial memory (Peters et al., 2005). On the other hand, memory retrieval is especially vulnerable to acute stress. In rodents, acute stress disrupts the hippocampus-dependent spatial memory retrieval (Wong et al., 2007; Li et al., 2008). Exposure to acute stress has been reported to increase 5-hydroxytryptamine (5-HT) output in the hippocampus (Amat et al., 1998; Fujino et al., 2002), and 5-HT has been

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Abbreviations: ANOVA, analysis of variance; DMSO, dimethyl sulfoxide; fEPSP, field excitatory postsynaptic potential; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GSK-3β, glycogen synthase kinase-3β; HFS, High-frequency stimulation; i.p., intraperitoneally; LTP, long-term potentiation; MWM, Morris water maze; SEM, standard error of mean; XE-991, 10,10-*bis*(4-Pyridinylmethyl)-9(10*H*)-anthracenone.

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shown to inhibit M currents in mammalian neurons (Roepke et al., 2012). Therefore, we wondered whether KCNQ/Kv7 channels involve in acute-stress induced impairments of spatial memory retrieval and synaptic plasticity.

KCNQ/Kv7 channel activators are of great interest as anticonvulsants, analgesics and anti-psychiatry drugs (Fritch et al., 2010; Yu et al., 2011; Kristensen et al., 2012). For example, flupirtine, which is characterized as a KCNQ/Kv7 channel activator, is clinically used as a non-opioid analgesic. Several studies have demonstrated that flupirtine has significant neuroprotective functions either *in vitro* or *in vivo* (Seyfried et al., 2000; Yu et al., 2011). Flupirtine prevents learning and memory impairment induced by repetitive hyperthermic seizures (Yu et al., 2011). It is known that, glycogen synthase kinase-3β (GSK-3β), a ubiquitous cellular serine/threonine protein kinase, plays a role in various essential physiological processes in the mammalian brain, such as development, cell cycle, or apoptosis (Medina and Wandosell, 2011). Recently, a link between GSK-3β and memory has been proposed. Inactivation of GSK-3β by phosphorylation at serine 9 facilitates the induction of LTP and contributes to hippocampus-dependent memory formation (Hooper et al., 2007; Dewachter et al., 2009). GSK-3β is activated in the hippocampus of stressed rat (Yoshida et al., 2006; Fang et al., 2013), and inhibition of GSK-3β activity produces antidepressant-like effect in the forced-swimming test (Kaidanovich-Beilin et al., 2004). Previous studies indicate that there is an interaction between KCNQ/Kv7 channels and GSK-3β (Borsotto et al., 2007; Kapfhamer et al., 2010). KCNQ/Kv7 channel activator retigabine or ICA-27243 can inhibit the activation of GSK-3β in the hippocampus of mice used to model mania (Kristensen et al., 2012). In the present study, we examined the impact of acute stress on the expression of KCNQ/Kv7 channels and observed the effects and mechanisms of KCNQ/Kv7 channel activator flupirtine on acute stress-induced impairments of spatial memory retrieval and hippocampal LTP.

EXPERIMENTAL PROCEDURES

Animals

Adult male Sprague–Dawley rats (250–350 g) aged 10–12 weeks were obtained from the Animal Center of the Tongji Medical College. Animals were housed in groups of four or five with free access to food and water *ad libitum*. They were maintained at a constant temperature of 23 ± 1 °C, humidity at 55 ± 5% and under a 12:12 light/dark cycle (lights on at 8:00 A.M.) Animals were allowed to acclimate to the laboratory at least 1 week before the beginning of the experiments. All experiments were conducted in accordance with the institutional and National Research Council's guideline for animal experiments, which comply with international rules and policies.

Drugs

Flupirtine was purchased from Targsense scientific Co., Ltd (Shanghai, China). Retigabine was purchased from Melone Pharmaceutical Co., Ltd (Dalian, China). Both were freshly dissolved in normal saline containing 0.3% dimethyl sulfoxide (DMSO) for the experimental doses. XE-991 dihydrochloride (10,10-*bis*(4-Pyridinylmethyl)-9(10*H*)-anthracenone dihydrochloride) was obtained from Tocris (Bristol, United Kingdom) and dissolved in normal saline.

Stress protocol

Acute stress was evoked as described previously (Wong et al., 2007; Li et al., 2008; Qi et al., 2009). Rats were placed on an elevated and unsteady platform (1 m tall, 21 × 21 cm) in the middle of a bright room for 30 min. The rats showed behavioral "freezing" (piloerection, immobility for up to 10 min, defecation, urination) while on the platform.

Experimental design

Experiment 1 was designed to detect the influences of acute stress on the expression of KCNQ/Kv7 subunits and phosphorylation of GSK-3β in non-anesthetized and anesthetized rats. Rats were sacrificed for western blotting analysis. Control, unstressed rats remained in their home cage. Anesthetized stressed rats were injected with urethane (supplemented when necessary throughout the experiment) at the end of stress and placed in their home cage until being sacrificed. Non-anaesthetized stressed rats were put back in their home cage without being anesthetized after stress. Another group of unstressed rats anesthetized with urethane was used to study the influence of urethane anesthesia. The body temperature was maintained at 37 °C by a homeothermic warming blanket during the anesthesia process.

Experiment 2 was designed to explore the effects of flupirtine on acute stress-induced spatial memory retrieval impairment and acute stress-induced change in GSK-3β phosphorylation. Rats were subjected to the Morris water maze (MWM) test, and some rats were sacrificed immediately after the probe test for western blotting analysis. Rats were injected with flupirtine, retigabine, vehicle (0.3% DMSO) or saline (0.9%) 1 h before the probe test. Rats treated with saline served as the control.

Experiment 3 was designed to investigate the effect of flupirtine on acute stress-induced hippocampal LTP impairment. Electrophysiological recordings of LTP were performed *in vivo* in rats under anesthesia. Stressed rats were anaesthetized with urethane at the end of stress and placed in a stereotaxic frame. Unstressed rats were anaesthetized immediately after transfer from the animal cage. The LTP was induced within 180 min after the end of stress, due to the time spent in surgery, electrode implantation and baseline recording. Rats were injected with flupirtine, XE-991, vehicle or saline 30 min prior to the induction of LTP. Rats treated with saline served as the control.

In these experiments, flupirtine (5-mg/kg), retigabine (8-mg/kg), XE-991 (0.3-mg/kg), vehicle or saline was administered intraperitoneally (i.p.) in an injection