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Acute stress enhances learning and memory by activating acid-sensing ion channels in rats

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ABSTRACT

Acute stress has been shown to enhance learning and memory ability, predominantly through the action of corticosteroid stress hormones. However, the valuable targets for promoting learning and memory induced by acute stress and the underlying molecular mechanisms remain unclear. Acid-sensing ion channels (ASICs) play an important role in central neuronal systems and involves in depression, synaptic plasticity and learning and memory. In the current study, we used a combination of electrophysiological and behavioral approaches in an effort to explore the effects of acute stress on ASICs. We found that corticosterone (CORT) induced by acute stress caused a potentiation of ASICs current via glucocorticoid receptors (GRs) not mineralocorticoid receptors (MRs). Meanwhile, CORT did not produce an increase of ASICs current by pretreated with GF109203X, an antagonist of protein kinase C (PKC), whereas CORT did result in a markedly enhancement of ASICs current by bryostatin 1, an agonist of PKC, suggesting that potentiation of ASICs function may be depended on PKC activating. More importantly, an antagonist of ASICs, amiloride (10 μM) reduced the performance of learning and memory induced by acute stress, which is further suggesting that ASICs as the key components involves in cognitive processes induced by acute stress. These results indicate that acute stress causes the enhancement of ASICs function by activating PKC signaling pathway, which leads to potentiated learning and memory.

1. Introduction

Acute stress is important for adaptation and maintenance of homeostasis, producing a boost that provides the drive and energy to help people get through situations like exams or work deadlines [1]. Some evidences prove that acute stress facilitates memory formation. For example, histone deacetylase 6 (HDAC6), as a key controller, involves in regulating the synaptic effects of acute stress in the prefrontal cortex (PFC). Targeted knockdown of HDAC6 blocks the enhancing effect of acute stress on learning and memory [2]. Although the mechanism of learning and memory under acute stress has been researched broadly, the new target or signaling pathway is still interesting and attracting us. Therefore, investigation of these mechanisms is being continued.

ASICs, members of degenerin/epithelial sodium channel superfamily (DEG/ENaC), are highly expressed in peripheral sensory and central neurons and activated by application a drop of acid solution applied extracellularly [3,4]. Mounting findings support that ASICs are involved in many physiological processes and pathologic conditions, including depression, synaptic plasticity and learning and memory [5,6]. In our previous study, we found that the rapid increase of ASIC1a (one of the six ASICs subunits) current might be caused by the activation of corticosteroid receptors found on the cell membranes of hippocampal neurons [7]. However, we are not sure whether ASICs impair or facilitate learning and memory when acute stress is applied.

It is well known that acute stress induces the activation of hypothalamic-pituitary-adrenocortical (HPA) axis in order to adapt to the new change on the body [8]. Naturally, CORT is secreted by the adrenal glands in high amounts after acute stress occurs and enters the brain and binds to intracellular receptors [9]. Although

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we found that CORT increased the current of ASIC1a by the activation of corticosteroid receptors on the cell membranes, we did not excluded the effects of CORT on AISCs through intracellular receptors of corticosteroid under a long time stress. Thus, these interesting proposals strongly attract us to explore the effects of ASICs on learning and memory induced by acute stress. To this end, we observed animals' behavior (escape latency and time in quadrant), CORT level of blood plasma, and ASICs current recording in hippocampal neurons during acute stress.

2. Materials and methods

2.1. Animals and reagents

Adult male Sprague-Dawley (SD) rats (160–180 g) were obtained from the Experimental Animals Center of Tongji Medical College, Huazhong University of Science and Technology. All animals were housed in groups under standard conditions (12 h light–dark cycle; light on 7 a.m.-7 p.m; temperature of 22 ± 1 °C) with free access to water and food, and allowed to acclimate a week before starting the experiment. The experimental protocols were approved by the Committee of Animal Care of Jianghan University. The acute stress was conducted as described previously [2,10]. Animals were exposed to a 30 min forced swim stress (in 25 °C water).

CORT, amiloride, RU38486 and RU28318 were obtained all from Sigma–Aldrich (St. Louis, MO,USA). Dimethyl Sulphoxide (DMSO), RU38486, Amiloride and Bryostatin 1 were obtained from Sigma (St. Louis, MO, USA). Bisindolylmaleinide I (GF109203X) was purchased from Calbiochem (San Diego, CA). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum and B27 supplement were purchased from Gibco Invitrogen (Carlsbad, CA, USA). CORT and amiloride were initially dissolved in DMSO as a stock solution and then in extracellular or intracellular solution (final concentration of DMSO 0.1%).

2.2. Intracerebroventricular surgeries and injections

For drug (vehicle or amiloride) delivery to hippocampus, anesthetized rats were implanted with double guide cannulas using a stereotaxic apparatus (Stoelting Instrument, USA). The hippocampus coordinates were 3.0 mm anterior to bregma; 2.0 mm lateral to midline; and 3.5 mm ventral to skull surface. The injection cannula extended 1.5 mm beyond the guide. After the implantation surgery, animals were returned to the home cage and recover for 7 days. Drugs were dissolved in artificial CSF (ACSF) (in mM: NaCl 124, KCl 3, NaH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 2, $NaHCO_3$ 26) and injected (1 h before animal's behavior testing) via the cannula bilaterally into hippocampus using a Hamilton syringe connected to a 22-gauge stainless steel injector. Animals with misplaced or plugged cannula were excluded from the analyses.

2.3. Morris water maze (MWM) test

The spatial learning performance of rat was evaluated by MWM. The experiment protocols were described in our previous study [11,12]. The hidden platform was placed in one of the zones. Each rat was allowed to circumnavigate the pool in search of the escape platform at 4 trials (120 s per trial) per day from 1st to 4th day. On 5th day, escape latency was recorded as final behavior performance. Then the platform was removed from the pool and each rat received one 60 s swimming probe trial. The time spent in the target quadrant (where the platform was located) was recorded.

2.4. CORT assay

According to our previous methods, to measure CORT, the rats were decapitated immediately after MWM testing and their trunk blood were collected in tubes with EDTA, centrifuged (3500 × g, 15 min) at 4 °C and the plasma was stored at −80 °C until used for the CORT assay similar with our previous studies [12,13]. CORT levels were measured using a commercially available RIA kit (ICN Biomedicals, Costa Mesa, CA, USA).

2.5. Whole cell patch clamp recordings

Hippocampal neurons were obtained from neonatal Sprague–Dawley rats (day 0–3) of both sexes and cultured for 8–14 d as described previously [7,13]. According to the previous study, the whole cell patch-clamp techniques were performed in a voltage-clamp mode and membrane currents were recorded with HEKA EPC-10 patch-clamp amplifier (HEKA) connected to a compatible computer via D/A and A/D converter [9,14]. Borosilicate glass pipettes (1.5-mm diameter) were pulled with P-97 microelectrode puller (Sutter Instruments Company, USA), and the resistance between the recording electrode filled with pipette solution (140 mM KCl, 10 mM NaCl, 1 mM $MgCl_2 \cdot 6H_2O$, 5 mM EGTA, 2 mM MgATP, 10 mM HEPES, pH 7.4 with Tris-OH) was 2–5 MΩ. Hippocampal neurons tested were voltage clamped at −70 mV throughout the experiments. A multibarrel perfusion system was used to achieve a rapid exchange of extracellular solutions (150 mM NaCl, 5 mM KCl, 1 mM $MgCl_2 \cdot 6H_2O$, 2 mM $CaCl_2$, 10 mM glucose, 10 mM HEPES, pH 7.4, or 6.0 with Tris-OH. The duration of the pH changes applications were 5 s.

2.6. Statistical analysis

All analyses were performed using SPSS 13.0 software and data which are presented as mean ± SE. Student's *t*-test was used to assess significance in experiments comparing 2-groups and comparisons among multiple groups were made using one-way ANOVA or two-way ANOVA, followed by Bonferroni for post hoc comparison of the means according to our previous study [7,12,13]. Differences were considered statistically significant at $p < 0.05$.

3. Results

3.1. Acute stress improves the performance of learning and memory and increases CORT level in blood plasma of rats

Acute stress was conducted as described previously [2,10] and we chose three time points (1 h, 2 h and 6 h) to observe the effect of acute stress on learning and memory. Meanwhile, animals' escape latency was measured by MWM test. On 5th day, the model groups at 1 h, 2 h, and 6 h after acute stress spent less time reaching the platform compared with control groups (in Fig. 1A). (1 h: Con: 27.33 ± 2.87 s, Model: 22.67 ± 2.84 s vs Con, $p < 0.05$, *t*-test, n = 12; 2 h: Con: 24.00 ± 2.73 s, Model: 19.00 ± 2.48 s vs Con, $p < 0.05$, *t*-test, n = 12; 6 h: Con: 26.33 ± 1.84 s, Model: 20.67 ± 2.65 s vs Con, $p < 0.05$, *t*-test, n = 12) Meanwhile, to explore animals' memory ability, time spent in the target quadrant of the pool was measured. We found the model group at 2 h and 6 h spent much more time staying in the target quadrant of the pool (in Fig. 1A). (1 h: Con: 29.50 ± 3.56 s, Model: 30.17 ± 3.75 s vs Con, $p > 0.05$, *t*-test, n = 12; 2 h: Con: 32.83 ± 3.42 s, Model: 41.33 ± 4.02 s vs Con, $p < 0.05$, *t*-test, n = 12; 6 h: Con: 33.50 ± 2.54 s, Model: 44.50 ± 5.27 s vs Con, $p < 0.05$, *t*-test, n = 12). To observe the change of CORT in acute stress, the level of CORT in blood plasma was measured. CORT of the model groups at 1 h, 2 h, and 6 h after acute stress was higher than