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Enhanced MK-801-induced locomotion in Kir6.2 knockout mice

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ABSTRACT

ATP-sensitive K^+ (K-ATP) channels provide a unique link between cellular energetics and electrical excitability, and also act as a unifying molecular coordinator of the body's response to stress. Although the body's response to stress is implicated in the worsening or relapse of psychotic symptoms in schizophrenia, the role of K-ATP channels remains unclear. Therefore, the aim of the current study was to investigated the effect of K-ATP channels on schizophrenia-like symptoms induced by MK-801 using Kir6.2 (one pore-forming subunit of K-ATP) knockout mice. We demonstrated that Kir6.2 knockout enhanced locomotor activity significantly compared to the wild-type mice after MK-801 administration. Moreover, we found that depletion of Kir6.2 significantly increased the numbers of Arc-positive cells in cortex, hippocampus and striatum in basal state. MK-801 augmented the Arc expression in wild-type mice. Collectively, our findings in this study indicate that K-ATP channels are involved in the regulation of MK-801-induced acute symptoms of schizophrenia, which is associated with the neural excitability. In addition, our results may provide valuable information for the development of new treatments for schizophrenia.

1. Introduction

Schizophrenia is a chronic and progressive mental disorder characterized by the disintegration of thought processes and emotional responsiveness (Tamminga and Holcomb, 2005; Gur, 2011). For years, schizophrenia has been attributed to dopamine overabundance in the brain. Conventional antipsychotics such as dopamine-D2 receptor antagonists have been considered the most effective treatment for patients with Schizophrenia (Guillin et al., 2007; Seeman, 2011). Based on their action through the dopaminergic mechanism, conventional neuroleptics effective are effective for the treatment of the positive symptoms of schizophrenia but not for the negative symptoms of the disease. Later, it is recognized that serotonin also plays an important role in the pathogenesis of schizophrenia (Shin et al., 2011). By blocking the dopamine and serotonin receptors, the newer antipsychotic agents such as clozapine, risperidone, and olanzapine (San et al., 2008) are considered more effective to treat both the positive and negative symptoms of schizophrenia and are less likely to cause extrapyramidal symptoms compared to conventional neuroleptics. Although the newer antipsychotics are better tolerated than the conventional neuroleptics, each of these newer drugs is associated with some adverse effects, such as sedation, weight gain, and postural hypotension (De Oliveira and Juruena, 2006). Thus, neuroreceptors should not be considered as the only therapeutic target, when it comes to identify and develop new approaches to treat schizophrenia.

Recently, it has been reported that iptakalim, a putative adenosine triphosphate (ATP)-sensitive potassium (K-ATP) channel opener, has demonstrated a potential antipsychotic effect in several preclinical tests (Sun et al., 2010). It effectively reduced amphetamine- and phencyclidine-induced hyperlocomotion as well as selectively disrupted conditioned avoidance response. Furthermore, the treatment of iptakalim also produced a reduction on prepulse inhibition of acoustic startle. In fact, diazoxide, another ATP-sensitive potassium channel opener, has been tested in clinics as an adjunctive treatment together with haloperidol (Akhondzadeh et al., 2002). Since coupling cell metabolism with electrical activity by regulating membrane K^+ fluxes (Nichols, 2006) and acting as a unifying molecular coordinator of the body's response to stress (Zingman et al., 2003), K-ATP channels support execution of the general adaptation syndrome under stress and allocation of resources to balance the need for confrontation with prevention of metabolic exhaustion. In schizophrenia, stress seems to be associated with the worsening or relapse of psychotic symptoms (Corcoran et al., 2003). In addition, since dopamine receptors have been shown to modulate the opening of K-ATP channels (Wu et al., 2001; Kawano et al., 2008), the K-ATP channel activators may be beneficial for the treatment of schizophrenia.

In the present study, a Kir6.2 (a pore-forming subunit of neuronal K-ATP channel) knockout mouse model was used to investigate the roles of K-ATP channels in the MK-801-induced schizophrenia. Our findings indicate that K-ATP channels with the depletion of Kir6.2 increases the responsibility to MK-801-induced

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acute symptoms in schizophrenia, which is associated with the regulation of neural excitability.

2. Materials and methods

2.1. Animals

Kir6.2 knockout mice were a gift from Professor Miki (Kobe University, Japan) (Miki et al., 1998). Wild-type (WT) mice were bred and maintained in the Animal Resource Centre of the Faculty of Medicine, Nanjing Medical University. Littermates generated from the same heterozygous breeding pairs were used as WT controls. Knockout of the Kir6.2 gene was confirmed by RT-PCR. All mice had free access to food and were maintained under room temperature and on a 12:12 h light/dark cycle. 12-week male mice were used. All experimental procedures were conducted in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (publication no. 85-23, revised 1985), and all animals were treated according to protocols approved by IACUC (Institutional Animal Care and Use Committee of Nanjing Medical University).

2.2. Treatment

Dizocilpine maleate (MK-801) purchased from Sigma–Aldrich Corporation (St. Louis, MO, USA) was dissolved in 0.9% saline solution. A stock solution of 0.5 mg/ml was prepared. The final concentration of 0.05 mg/ml was used. All animals were injected intraperitoneally with 0.5 mg/kg of MK-801. The same amount of 0.9% saline was used as a control.

2.3. Measurement of locomotor activity

The ZIL-2 mouse locomotor activity analysis system is developed by Chinese Academy of Medical Sciences, including four spontaneous activity observation box (20 cm × 20 cm × 15 cm/each). Total locomotor frequencies of a mouse collected in 10 min was used as an index to assess the spontaneous activity. Increased locomotor frequencies suggests increased spontaneous activity. Mice were randomly divided into four groups based on their weights. Two groups of WT mice and two groups of KO mice were placed into the four activity monitor chambers for pre-adaption, respectively. MK-801 or saline control was administrated after 10 min, then their locomotor activities were detected by using ZIL-2 activity meter comprising an infrared beam sensor (Chinese Academy of Medical Sciences). Activities were defined as the number of interruptions of the infrared light beam with a 10-min interval for 2 h.

2.4. Arc immunohistochemistry staining and evaluation

At the end of each experiment, all animals (8 mice per group) were killed and immediately perfused with 4% paraformaldehyde (PFA). Brain samples were collected and kept in 4% PFA at 4 °C overnight. The following day, samples were transferred into 20% sucrose in phosphate-buffered saline (PBS). After 72 h, the samples were transferred into 30% sucrose. After gradient dehydration, the brain tissues were then sectioned by Leica freezing microtome at 30 μm. The immunostaining experiments were preformed as described previously (West, 1993). Briefly, the brain tissue sections were rinsed with PBS and then incubated in 3% H_2O_2 in PBS for 30 min. After being blocked with 5% bovine serum albumin for 1 h, sections were incubated with mouse anti-Arc first antibody (1:100; Chemicon) overnight at 4 °C, while the secondary antibody (goat anti-mouse-HRP, 1:800; Chemicon) was used for 1 h. KO and WT tissues are placed on the same slide glass during staining procedures, and the optical intensity of KO and WT tissues is to be compared for each slide. Arc-positive cells were visualized with DAB (Vector Laboratories, CA, USA). Twelve slices of every mouse were counted for Arc-positive cells in the medial Prefrontal Cortex (mPFC), dentate gyrus (DG), hippocampal layer (CA), and striatum using Optical Fractionator method with Microbrightfield Stereo Investigator software (Stereo Investigator software, Microbrightfield, Williston, VT, USA) (West, 1993) on a Z-series of sections 240 μM apart with an overlay grid of 100 μm × 100 μm with a counting frame of 100 μm × 100 μm.

2.5. Statistical analysis

All values were presented as means ± SEM. A three-way mixed design analysis of variance (ANOVA) was used to analyze data collected for locomotor activities and a two-way ANOVA with factors of genotype and drug was used to analyze data collected for arc staining, followed by the *post hoc* Student–Newman–Keuls test. Differences were considered significant at a P value of <0.05.

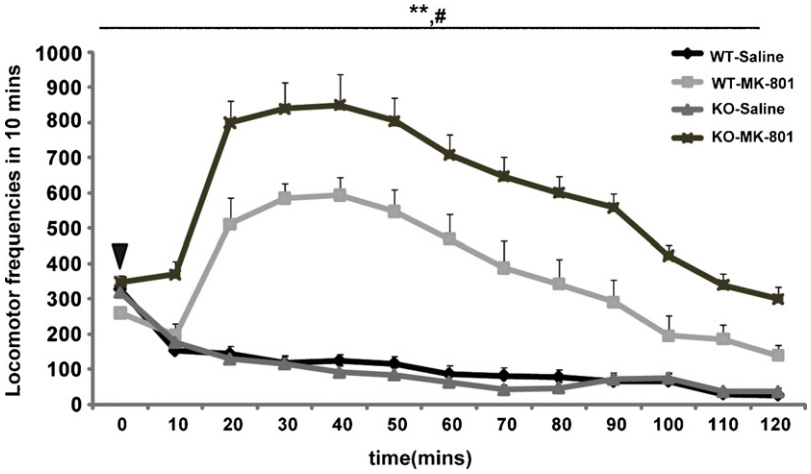

Fig. 1. Acute effect of MK-801 on locomotor activity in wild type (WT) and Kir6.2 knockout (KO) mice. Time-course of locomotor activities were shown [WT mice treated with saline, WT mice treated with MK-801, KO mice treated with saline and KO mice treated with MK-801, n = 8/each group]. The arrows indicate the time when MK-801 was administered i.p. (0.5 mg/kg). Kir6.2 knockout increased MK-801-induced hyper-locomotion. $^{**}P < 0.01$ *vs.* corresponding saline-treated groups, $^{\#}P < 0.05$ *vs.* MK-801-treated WT mice.