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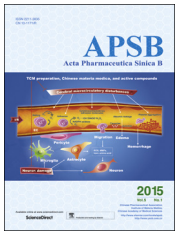

ORIGINAL ARTICLE

Effects of fluoxetine on protein expression of potassium ion channels in the brain of chronic mild stress rats

Chunlin Chen[1], Ling Wang, Xianfang Rong, Weiping Wang, Xiaoliang Wang*

State Key Laboratory of Bioactive Substances and Functions of Natural Medicines, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, China

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CMS;
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Abstract The purpose of this study is to investigate the expression of major potassium channel subtypes in the brain of chronical mild stress (CMS) rats and reveal the effects of fluoxetine on the expression of these channels. Rats were exposed to a variety of unpredictable stress for three weeks and induced anhedonia, lower sucrose preference, locomotor activity and lower body weight. The protein expressions were determined by Western blot. CMS significantly increased the expression of Kv2.1 channel in frontal cortex but not in hippocampus, and the expression level was normalized after fluoxetine treatment. The expression of TREK-1 channel was also obviously increased in frontal cortex in CMS rats. Fluoxetine treatment might prevent this increase. However, the expression of Kv3.1 and Kv4.2 channels was considerably decreased in hippocampus after CMS, and was not affected by fluoxetine. These results suggest that different subtypes of potassium channels are associated with the pathophysiology of depression and that the therapeutical effects of fluoxetine may relate to Kv2.1 and TREK-1 potassium channels.

*Corresponding author. Tel.: +86 10 63165330; fax: +86 10 63017757.
E-mail address: wangxl@imm.ac.cn (Xiaoliang Wang).
[1]Current address: Key Laboratory of Jiangxi Province Natural Active Pharmaceutical Ingredients, Yichun University, Yichun 336000, China.
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1. Introduction

Depression is a serious disease and becomes more and more prevalent in aged people. Although it is well known that the level of serotonin (5-HT) decreases in brain, other mechanisms affecting neuronal excitability may also be involved. For example, the activities of potassium channels were suggested to be changed during depression. In general, when stimulated, open potassium channels may inhibit excitability of cells and lower the effectiveness of excitatory inputs by hyperpolarizing cell membrane potential. Various potassium channels with different electrophysiological characteristics have been identified in neurons[1], including delayed rectifier potassium channels, A-type potassium channels, background potassium channels (such as two-pore domain potassium channels), and so on[2,3]. Blockade of these potassium channels may potentially exert therapeutic effects in the treatment of certain clinical central nervous system disorders, such as epilepsy, multiple sclerosis, dementia, anxiety, depression and stroke[4].

Antidepressant drugs may modulate neuronal excitability *via* potassium channel inhibition, which has been suggested by several preclinical studies. In fact, different types of K^+ channel blockers such as tetraethylammonium (TEA), apamin, charybdotoxin, gliquidone and glibenclamide were able to produce an antidepressant-like effect in the mouse forced swimming test (FST)[5–7]. However, K^+ channel openers such as minoxidil or cromakalim increased the immobility time, indicating the induction of a depressant-like behavior[5]. Recent studies suggested that fluoxetine, a selective serotonin reuptake inhibitor, acted as a potent blocker of different tapes of K^+ channels, including that TWIK (tandem P-domain weak inward rectifying K^+)-related K^+ channel 1 (TREK-1) currents expressed in tsA 201 cells, delayed rectifier potassium currents and A-type potassium currents in neurons[8–10]. Moreover, evidence indicates that other kinds of antidepressant drugs also produce an inhibition of K^+ currents, such as desipramine, amitriptyline, imipramine and paroxetine[11–14]. In addition, it was demonstrated that the TREK-1 knock out mice showed antidepressant behavior in several tests[15].

Although many *in vitro* and *in vivo* studies have shown that several types of K^+ channels are involved in the pathology of depression and even act as a pathway of pharmacological action of some antidepressants, it is still in short of direct evidence about the expression of these potassium channels in depression animal models and in depression patients. To our knowledge, some antidepressants such as fluoxetine could inhibit potassium channel (Kv and TREK-1) currents[8–10], but the effects of fluoxetine on expression of these channels, especially during depression, were not reported. Therefore, the purpose of the present study is to observe the expressional changes of major K^+ channels, such as Kv2.1, Kv3.1, Kv4.2 and TREK-1, in the brain of depression-like symptoms rat model and to reveal the effects of fluoxetine on the expression of the above channels. We further demonstrate certain K^+ channels as potential anti-depression drug targets.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley (SD) rats weighing 250–280 g were obtained from Vital Rital Laboratories (Beijing, China), and housed in an air-conditioned room with a constant temperature (22 °C ± 1 °C), humidity (50%–70%), and a 12-h light/dark cycle for one week for habituation. Food and water were available *ad libitum* until the beginning of the chronic mild stress (CMS) test. All procedures and tests were approved by the Institutional Animal Care Committee of Peking Union Medical College.

2.2. Drugs and experimental groups

Thirty rats were randomly assigned to 3 groups: control group, CMS+Saline group and CMS+fluoxetine group. Fluoxetine hydrochloride (Lilly, France) was dissolved in physiological saline (0.9%) and administered *p.o.* daily at a dose of 2 mg/kg for 3 weeks of CMS. Animals in saline group were administered with same volume of saline.

2.3. CMS procedure

All animals except control group were treated with a fixed weekly schedule of unpredictable stress following the reported protocol with some modification[16]. The protocol included nine different kinds of stress such as food and water deprivation, grouped housing, cold stress (10 °C) and heat stress (45 °C), and performed in the following order shown in Table 1. The CMS procedure lasted for 21 days and started from 1st day after the sucrose test in the adaptation period. The CMS groups of rats were housed separately in different cage in the duration of the CMS procedures, and 5 animals per cage were housed for the control group rats.

2.4. Sucrose preference test

Sucrose preference test was applied before and after 1st day of CMS procedure. All rats were trained to adapt to 1% sucrose solution during the 7-day adaptation period. Before test, rats were deprived of water and food for 14 h, followed by 200 mL 1% sucrose solution and 200 mL water for 1 h. The bottles of 1% sucrose solution and water were weighted before and after the test. The sucrose preference was calculated as sucrose intake (g) / (sucrose intake (g) + water intake (g)).

2.5. Open field test

The open field apparatus consisted of a square box with black wall and black base, and was divided into 25 identical sectors

Table 1 Schedule of applied stressors during 1 week.

Day	Duration/start	Stressor
Monday	12 h (start at 9 am)	Tilting the cage
Tuesday	24 h (start at 9 am)	Water deprivation
Wednesday	12 h (start at 8 pm)	Pairing
Thursday	5 min	Swimming in 10 °C water
	24 h (start at 5:30 pm)	Food and water deprivation
Friday	12 h (start at 9 pm)	Wet bedding
Saturday	5 min	Heat stress (45 °C)
	24 h (start at 8 pm)	Reversal of light/dark cycle
Sunday	30 min	Lever shaking