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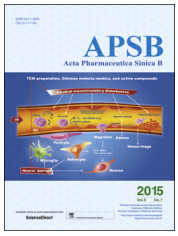

ORIGINAL ARTICLE

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

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KEY WORDS

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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.

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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

(25 cm × 25 cm) by white stripes. Rats were placed into the center sector of the open field under a dark light. The horizontal locomotion (numbers of crossing the sectors) and vertical locomotion (numbers of erection including rearing) during the following 5 min were assessed.

2.6. Western blot analysis

Total protein lysates from rats' frontal cortex and hippocampus were extracted with buffer lysis (1 mol/L Tris–HCl, pH 7.5, 150 mmol/L NaCl, 1% Nonidet P-40, 1 mmol/L EGTA, 10% Glycerin, 100 mmol/L Na_3VO_4 and 100 mmol/L NaF) supplemented with protease inhibitor cocktail (Roche). After incubation for 15 min at 4 °C, the lysates were clarified by centrifugation at 12,000 × *g* at 4 °C for 20 min. Protein concentration was determined by the Bradford method (Jiancheng Bioengineering Institute, Nanjing, China). Equal amounts of proteins (80 μg protein from each sample) were loaded on 8% (*w/v* ratio) SDS-polyacrylamide gel with molecular weight standards. Samples were then electrotransferred onto polyvinylidenefluoride membranes (Millipore, USA). The membranes were then blocked with 5% skimmed milk in TBST (10 mmol/L Tris–HCl, pH 7.5, 0.9% NaCl and 0.1% Tween 20) on an orbital shaker for 1 h at room temperature and incubated overnight with the indicated antibodies at 4 °C. The antibodies used were anti-Kv2.1 (1:200, APC-012, Alomone labs), anti-Kv3.1 (1:500, NBP1-42819, Novus Biologicals), anti-Kv4.2 (1:500, sc-11680, Santa Cruz Biotech), anti-TREK-1 (1:100, T6448, Sigma), and anti-*β*-actin (1:10000, A5441, Sigma). Following five TBST washes, the membranes were incubated for 1 h with horseradish peroxidase-conjugated anti-rabbit or anti-mouse antibody dissolved in TBST at room temperature. The membranes were developed by enhanced chemiluminescence, and the proteins detected by their specific antibodies were identified by their molecular sizes (Kv2.1 was located at 91 kDa, Kv3.1 was located at 110 kDa, Kv4.2 was located at 51 kDa, TREK-1 was located at 71 kDa and *β*-actin was located at 42 kDa). Immunoreactivities were quantified by optical density (OD) normalized against the corresponding *β*-actin band, and measured using Fujifilm imaging system (Fuji, Japan).

2.7. Statistical analysis

All data are expressed as mean ± SEM. Statistical comparisons among multiple groups were carried out by one-way ANOVA followed by *post-hoc* LSD test. In all cases, probability (*P*) values of less than 0.05 were considered significantly different.

3. Results

3.1. Establish the CMS rat model

CMS is one of the frequently used animal models for the study of depression. Rodents are exposed to a variety of unpredictable stressors and induced anhedonia, mimicking the symptoms that are seen in human depression patients[16]. Sucrose preference and locomotor activity are two important indicators of depression related behavior. In the present study, the sucrose preference and locomotor activity had no significant difference among the model and control groups (data not shown) at the beginning of the CMS. After 3 weeks of CMS induction, both indicators were not changed in the control group, but were significantly reduced in CMS rats compared to control rats (Table 2). Fluoxetine (2 mg/kg) increased the sucrose intake of rats significantly, and also increased the numbers of crossing and erection as shown in Table 2. A significant difference in body weight due to CMS process was also observed between control and CMS group. Fluoxetine can reverse this change (Table 2). All these data indicate that the depression rat model was successfully created by CMS.

3.2. Kv2.1 expression increased in cortex of CMS rats and was inhibited by fluoxetine

Kv2.1, a subtype of delayed rectifier potassium channels, is expressed at a high level on almost all neurons and has been suggested as the major subtype of delayed rectifier potassium ion channels in cortex and hippocampus[17]. Therefore, in our study, the expressions of Kv2.1 in rats with or without CMS were investigated. In CMS group, a significant increase of Kv2.1 expression was observed in frontal cortex when compared to the control group, and the enhanced expression was normalized after 3-week treatment of fluoxetine (Fig. 1A). However, same result was not observed in hippocampus, which also plays an important role in the depression disorder (Fig. 1B). There was no significant difference among three groups as shown in Fig. 1B.

3.3. Expression of Kv3.1 in CMS rats

Kv3.1 is also a delayed rectifier-type potassium ion channel with fast activation and deactivation kinetics. Based on a high activation threshold of −20 mV, Kv3.1 is suggested to act a key role of fast spiking in neurons[18]. In the present study, the expression of Kv3.1 was decreased after CMS in hippocampus (Fig. 2B). A slightly increase was seen in fluoxetine treatment group, although the level was still lower than the control rats (Fig. 2B). Meanwhile, no significant difference was seen in frontal cortex among three groups (Fig. 2A).

Table 2 Effects of CMS induction and treatment with fluoxetine on body weight, locomotor activity (crossing number and erection number) and sucrose preference.

Group	Body weight (g)	Crossing number	Erection number	Sucrose preference
Control	400.5 ± 6.3	106.2 ± 11.6	25.8 ± 1.5	0.80 ± 0.04
CMS+saline	353.9 ± 8.1$^{\#\#}$	40.6 ± 8.9$^{\#\#}$	9.4 ± 1.7$^{\#\#}$	0.56 ± 0.04$^{\#\#}$
CMS+fluoxetine	380.0 ± 5.6*	75.8 ± 6.9*	21.1 ± 2.4**	0.71 ± 0.04*

Data are expressed as mean ± SEM, $n = 10$.
$^{\#\#}P < 0.01$ *vs.* control group.
$^{*}P < 0.05$, $^{**}P < 0.01$ *vs.* CMS group.

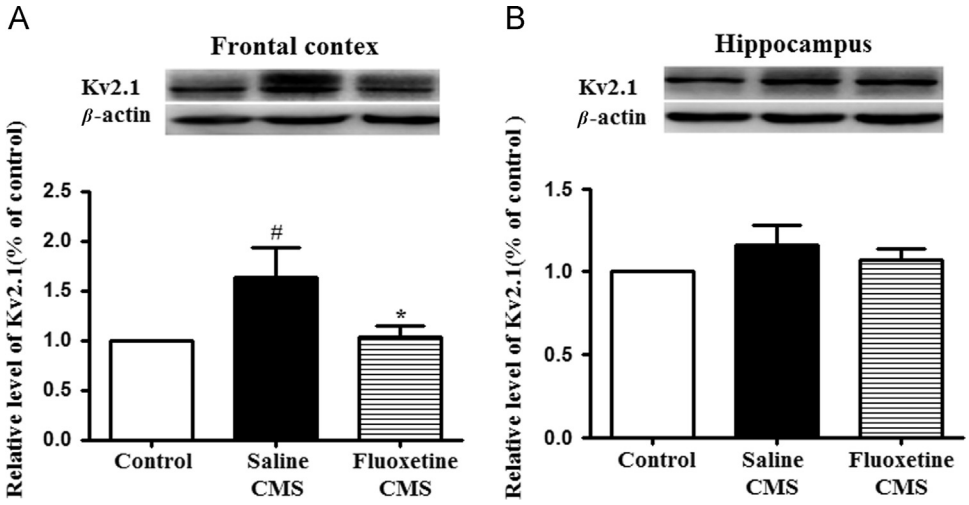

Figure 1 Effects of CMS and fluoxetine treatment on Kv2.1 protein expression in frontal cortex and hippocampus of rat brains. Expression of Kv2.1 protein was obviously increased in frontal cortex (A) of CMS rats and could be reversed by fluoxetine treatment (2 mg/kg/day *p.o.* for 3 weeks). But no significant changes could be detected in hippocampus (B). $^{\#}P<0.05$ *vs.* control group, $^{*}P<0.05$ *vs.* CMS+saline group, $n=4$.

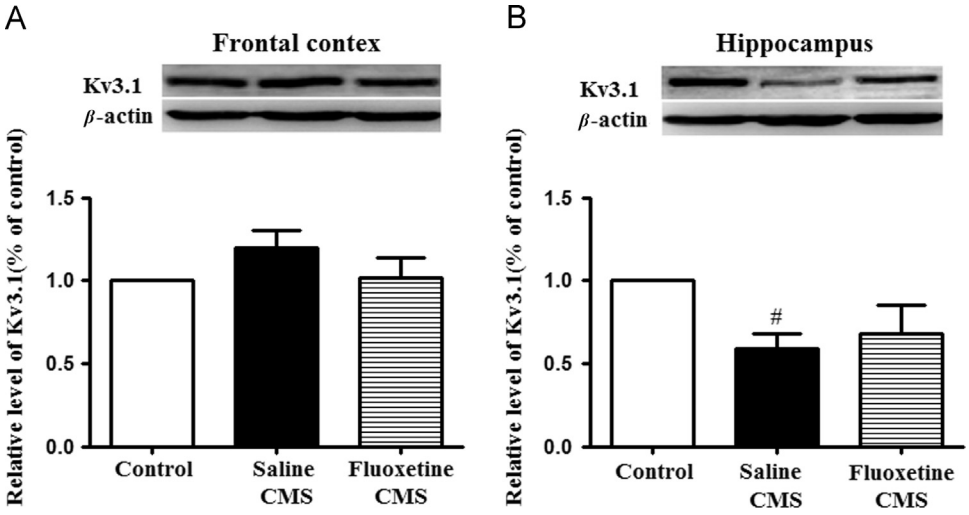

Figure 2 Effects of CMS and fluoxetine treatment on Kv3.1 protein expression in frontal cortex and hippocampus of rat brains. Expression of Kv3.1 protein was not affected by CMS and fluoxetine in frontal cortex (A) but was significantly reduced in hippocampus (B) of CMS rats. $^{\#}P<0.05$ *vs.* control group, $n=4$.

3.4. Expression of Kv4.2 in CMS rats

We examined Kv4.2, a critical contributor to A-type voltage-gated potassium channels, which is also largely expressed in central nervous system[19]. In CMS group, the rats showed a considerably down-regulation of Kv4.2 levels in both frontal cortex and hippocampus compared with control group (Fig. 3). However, the changes were not affected by fluoxetine treatment. It indicated that the pharmacological effects of fluoxetine may not be mediated by Kv4.2.

3.5. TREK-1 expression changed in CMS rats and was reversed by fluoxetine

Recent study showed that the two pore potassium ion channel TREK-1 play a critical role in depression[15]. But there is no direct evidence that the channel can be modulated by fluoxetine during pathological condition till today. Therefore, it was investigated in our study. As shown in Fig. 4A, the expression of TREK-1 in frontal cortex was significantly increased after 3 weeks of CMS, and it was reversed after fluoxetine treatment. However, the expression level of TREK-1 in hippocampus was not significantly changed after CMS and fluoxetine treatment (Fig. 4B).

4. Discussion

CMS is one of the most commonly used animal models of depression[16]. In the present study, CMS induced depression-like symptoms in rats were shown, including decrease of sucrose consumption, reduction of the locomotor activity and drop of the body weight. We also found the expressions of Kv2.1 and TREK-1 in frontal cortex of CMS rats were increased, and fluoxetine inhibited the up-regulation of these ion channel proteins. Meanwhile, Kv3.1 and Kv4.2 were down-regulated in hippocampus and frontal cortex, but fluoxetine could not inhibit the changes of both the channels in CMS rats.

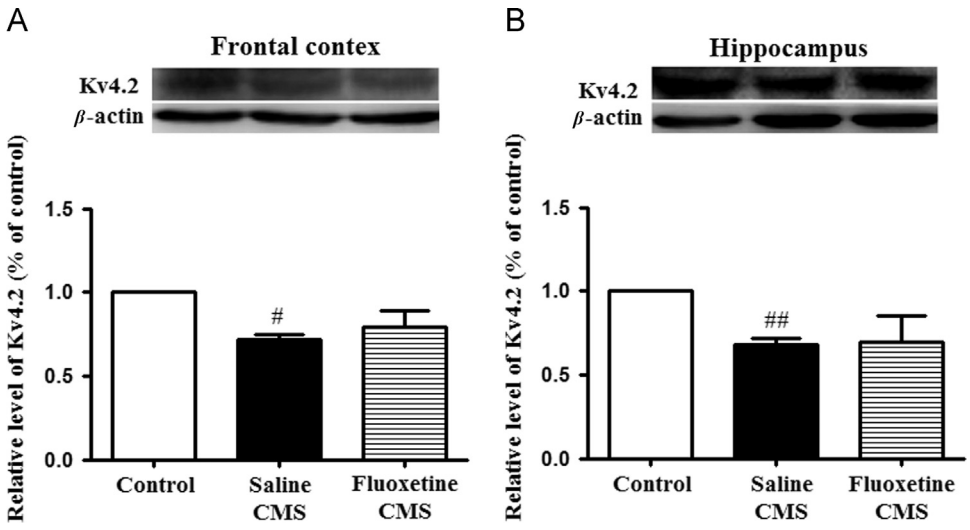

Figure 3 Effects of CMS and fluoxetine treatment on Kv4.2 protein expression in frontal cortex and hippocampus of rat brains. In both of frontal cortex (A) and hippocampus (B), the expression of Kv4.2 protein was decreased in CMS rats. Fluoxetine was unable to restore the decrease. $^{\#}P<0.05$, $^{\#\#}P<0.01$ *vs*. control group, $n=4$.

It has been known that potassium ion channels are related to the pathogenesis and therapeutics of depression[15]. Generally, inhibition of potassium channel may increase excitability of neurons or brain. Fluoxetine, a commonly used antidepressant, was reported to block the currents of several potassium channels, which are mediated by Kv1.1, Kv1.3, Kv1.4, Kv1.5, Kv3.1, Kv4.3, hERG and TREK-1[10,11,20–26]. However, the changes of potassium channels and the modulation of fluoxetine on potassium channel expression during depression were rarely reported.

Kv2.1, which shapes the action potential repolarization, is one of the important regulators of excitability in neurons and the process of synaptic integration in the central nervous systems[27,28]. Deletion of Kv2.1 in rat neurons enhanced somatodendirtic excitability, particularly during a high-frequency synaptic transmission[27]. Our results indicated that the expression of Kv2.1 in frontal cortex was significantly increased by CMS. In addition, up-regulation of Kv2.1 level in neuron has been suggested to promote cell apoptosis[29]. Moreover, previous studies demonstrated that fluoxetine, a selective 5-HT reuptake inhibitor, was an effective blocker of delayed rectifier potassium channel in cerebellar granule neurons at the therapeutic concentration[9]. In this study, our results demonstrated that the expression of Kv2.1 was significantly down-regulated by fluoxetine compared to the CMS control group. This result not only validates the relationship between delayed rectifier potassium channel and antidepressant effect of fluoxetine, it also supports the view that the effects of antidepressant drugs on neuronal excitability *via* the inhibition of potassium channels may represent the common pathway of their pharmacological action.

Different from Kv2.1 channel, the main role of Kv3.1 channel is rapid repolarization of the action potential and shortening of the action potential duration; therefore it significantly contributes to the ability of neuronal cells to fire brief action potentials at high frequencies[18]. GABAergic neurons play an important role in hippocampal θ rhythm and basal ganglia motor control circuit, due to its particular neurophysiological properties of firing sustained high-frequency, short-duration spikes[30]. Recent evidences confirmed that due to their fast activation and slow inactivation kinetics, Kv3.1 channels, in particular, are essential to high-frequency GABAergic neurons firing[31]. Deficits in GABAergic inhibition and a decrease in Kv3.1 currents have been suggested to be related with Alzheimer's disease[32]. Interestingly, decreased GABAergic function was also observed in major depression patients[33], and we reported again that the expression of Kv3.1 channel was significantly decreased in hippocampus of depression rats and fluoxetine had no significant effect on it. Our results further suggested that the decreased expression of Kv3.1 channel in depression rats may affect functions of hippocampus. However, more studies are needed to further confirm this inference.

Previous studies have shown that Kv4.2 relatively locates in central nervous system and is important in regulating neuron plasticity and synaptic function[34,35]. Recently, the relationship between Kv4.2 channel and behavioral depression was also reported[36]. Our results showed that the expression of Kv4.2 subunit was decreased significantly both in frontal cortex and hippocampus in CMS model and was not affected by fluoxetine. These observations were in accordance with that in *Kv4.2* knockout mice[36], suggesting that Kv4.2 plays an important role in depression and the antidepressant effect of fluoxetine may not act through Kv4.2.

Growing evidences indicate that TREK-1 channel may be a new antidepressant drug target and may improve antidepressant treatment. Heurteaux et al.[15] showed that *TREK-1* knockout mice were resistant to depression-like behavior. Consistent with this finding, fluoxetine have been shown to inhibit TREK1 currents, and variation in *TREK1* gene (*KCNK2*) was linked to the depression as well as antidepressant response in humans[10,37,38]. We report that the protein expression of TREK-1 channel in frontal cortex is much higher in depression-like rat model than normal ones, and is normalized after chronic treatment with fluoxetine. But we did not see the change of this channel and the effect of flouxetine in hippocampus. Our results are consistent with the study of Xi et al.[39] which showed that fluoxetine could attenuate the up-regulation of TREK-1 expression in cultured neural stem cells. These findings suggested that the enhancement effect of 5-HT on neurons excitability and transmission caused by fluoxetine may

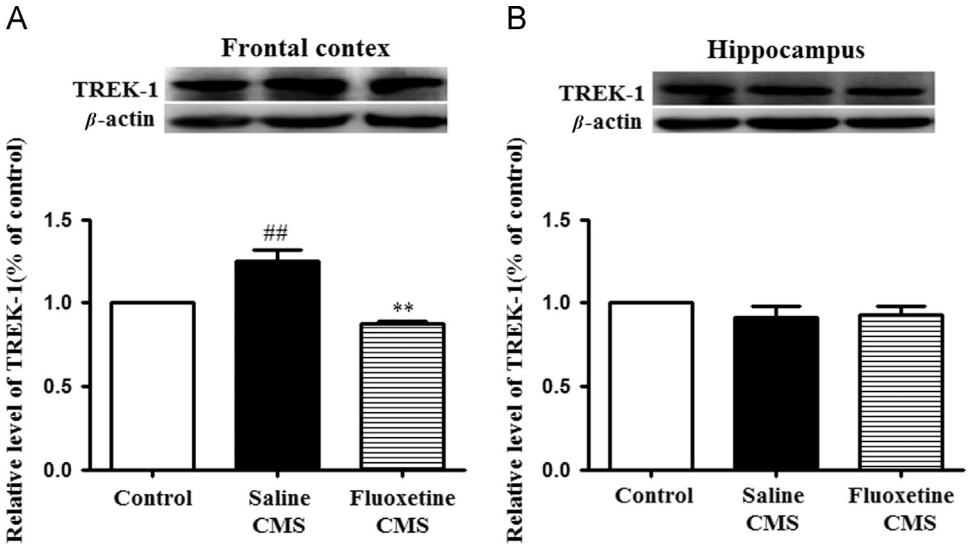

Figure 4 Effects of CMS and fluoxetine treatment on TREK-1 protein expression in frontal cortex and hippocampus of rat brains. Expression of TREK-1 protein was obviously enhanced in frontal cortex (A) of CMS rats and could be reversed by fluoxetine. While no obvious changes could be observed in hippocampus (B). $^{\#\#}P<0.01$ *vs*. control group, $^{**}P<0.05$ *vs*. CMS+saline group, $n=4$.

be through both of direct inhibition of TREK-1 currents and down-regulation of its protein. Moreover, the relationship between TREK-1 and 5-HT system is still unclear. Gordon et al.[40] suggested that TREK-1 might be involved in the 5-HT feedback system, which need to be confirmed further.

Although our study provides some information about expressions of major potassium channels in the brain of CMS rats and the regulation of fluoxetine, we did not detect the mRNA expression after CMS because the protein expression reflects the function of ion channels more directly. In addition, we did not measure the activities of the four channels due to the lack of available selective blockers for Kv2.1, Kv3.1, Kv4.2 and TREK-1 till now, and it is also difficult to isolate the four native potassium channel currents from the ion channel currents in neurons. Moreover, because TSAK-3 was also reported to be related to depression[41] and fluoxetine did not significantly inhibit TASK-3[42], we did not observe this potassium channel subtype in the present study.

5. Conclusions

In conclusion, our results suggest that different subtypes of potassium channels are associated with the pathogenesis of depression. Kv2.1 and TREK-1 channels are related to the function of frontal cortex and they might be regulated by fluoxetine, while Kv3.1 and Kv4.2 are relatively related to the functions of hippocampus and can not be regulated by fluoxetine. Therefore, Kv2.1 and TREK-1 channels might be new drug targets for antidepressants.

Acknowledgments

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