The Role of the Two-Pore Domain Potassium Channel TREK-1 in the Therapeutic Effects of Escitalopram in a Rat Model of Poststroke Depression

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Keywords

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SUMMARY

Aim: Poststroke depression (PSD) is one of the most common neuropsychiatric complications after stroke. TREK-1, a two-pore-domain potassium channel, has been implicated in the pathogenesis of stroke and depression. The aim of this study was to investigate whether TREK-1 plays a role in the therapeutic effects of the selective serotonin reuptake inhibitor (SSRI) escitalopram in a rat PSD model. **Methods:** The whole-cell patch-clamp technique was performed to assess the effect of escitalopram on recombinant TREK-1 currents in HEK293 cells. The expression of TREK-1 mRNA and protein was measured in the hippocampus and prefrontal cortex (PFC), and neural stem cell (NSC) proliferation was detected in the hippocampal dentate gyrus (DG) in PSD rats after 3 weeks of escitalopram administration. **Results:** Escitalopram reversibly inhibited TREK-1 currents in a concentration-dependent manner. Chronic treatment with escitalopram significantly reversed the reductions in weight gain, locomotor activity, and sucrose preference in PSD rats. The expressions of TREK-1 mRNA and protein were significantly increased in hippocampal CA1, CA3, DG, and PFC in PSD rats, with the exception of TREK-1 mRNA in hippocampal CA1. NSC proliferation was significantly decreased in hippocampal DG of PSD rats. Escitalopram significantly reversed the regional increases of TREK-1 expression and the reduction of hippocampal NSC proliferation in PSD rats. **Conclusion:** TREK-1 plays an important role in the therapeutic effects of the SSRI escitalopram in PSD model, making TREK-1 an attractive candidate molecule for further understanding the pathophysiology and treatment of PSD.

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The first two authors contributed equally to the work.

Introduction

Stroke is a major cause of death and long-existing disability in adult populations around the world [1,2]. Poststroke depression (PSD), with a prevalence of 30–50%, is one of the most common neuropsychiatric complications after stroke [3]. PSD causes increased disability, more impaired cognitive function, reduced quality of life, and higher risk of suicide [4] and death [5]. The majority of PSD studies have focused on the epidemiological aspects of this condition. Our current understanding of the neurobiological mechanisms of PSD is primarily based on older studies of ischemic lesions and depression, including the lesions of fronto-subcortical circuit, the hyperactivation of hypothalamic-pituitary-adrenal (HPA) axis and neuroinflammation, and serotonergic dysfunction [6]. Meta-analyses have demonstrated the therapeutic effects of selective serotonin reuptake inhibitors (SSRIs) on PSD [7], which strengthen poststroke motor [8] and cognitive recovery [9] and reduce overall mortality [10]. The

mechanisms underlying the efficacy of SSRI antidepressants in PSD have not been clearly elucidated.

Recently, TREK-1 (TWIK-related potassium channel, type 1) has been implicated in the pathophysiological mechanisms of stroke and depression, resulting in it being an attractive candidate molecule for studying PSD. TREK-1 is one of the six types of K2p (two-pore domain background potassium) channels [11], and it is abundantly expressed in several brain regions involved in the emotional and cognitive aspects of depression, including the hippocampus, the prefrontal cortex (PFC), the amygdala, and the gyrus cinguli [11–13]. The STAR*D study found an association between four SNP variants in the TREK-1 gene and resistance to multiple antidepressants [14]. Moreover, TREK-1 knockout mice display a depression-resistant phenotype that mimics treatment with antidepressants [15]. The SSRI antidepressant fluoxetine and its metabolite norfluoxetine robustly inhibit TREK-1 channels [16]. These findings suggest that TREK-1 may play an important role in the etiology and treatment of depression.

Increasing evidence has demonstrated the possible role of TREK-1 in ischemic neuroprotection [17–19]. TREK-1 knockout mice are more sensitive to ischemia [20] and exhibit decreased vasodilation of the basilar artery in response to polyunsaturated fatty acids [21]. The hippocampus and prefrontal cortex receive the serotonergic neural projection from raphe nuclei and also have neural connections between each other, which are importantly implicated in the mood regulation and pathogenesis of depression. TREK-1 expression had been found to be increased in the rat cortex and hippocampus 2 h after middle cerebral artery occlusion (MCAO) [18]. The effect of increased TREK-1 expression lasted for up to 30 days in the rat hippocampus following permanent bilateral carotid artery ligation [22]. It is unclear whether TREK-1 expression is altered in the brain regions implicated in PSD, a disease that has characteristics of stroke and depression. The suppression of TREK-1 activity increases neuronal apoptosis [19] and inhibits the proliferation of astrocytes [17] in the cortex of rats exposed to hypoxia, suggesting that TREK-1 might be involved in neuronal cell renewal and survival. Opposite trends have been consistently reported regarding hippocampal neurogenesis following cerebral ischemia and depression. Neurogenesis is elevated following brain ischemia [23] and decreased in depression [24]. Our group recently reported that reduced hippocampal neurogenesis could be reversed by citalopram administration in a rat model of PSD [25]. The question of whether TREK-1 activity is related to changes in hippocampal neurogenesis following cerebral ischemia and PSD has not been studied. In this study, we conducted preliminary explorations into the role of TREK-1 in the pathophysiological mechanisms of PSD and SSRI therapeutic efficacy. We assessed the effect of escitalopram (ESC), a widely prescribed SSRI antidepressant, on the recombinant TREK-1 channel currents in human embryonic kidney cells (HEK293) by the whole-cell patch-clamp technique. Additionally, we investigated the effects of ESC on depressive behavior, hippocampal neurogenesis, and TREK-1 expression in the hippocampus and prefrontal cortex (PFC) of PSD rats, a model that we previously established using MCAO followed by a 21-day chronic unpredictable mild stress (CMS) paradigm combined with isolation rearing [26].

Methods

Electrophysiology

The electrophysiology experiments utilized HEK293 cells cultured at a density of 5×10^4 cells/35-mm plate 48 h after transfection with TREK-1-pEGFP-N1 plasmids. The HEK293 cells were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal calf serum (FBS) and antibiotics (ampicillin and streptomycin, 50 μ g/mL each) at 37°C in a 5% CO₂ incubator. TREK-1-pEGFP-N1 plasmids were transfected at a concentration of 565 ng/ μ L according to the Polyjet Reagent protocol. The HEK293 cells were visualized by fluorescence microscopy 48 h after transfection.

The whole-cell patch-clamp data were recorded using a 200B patch-clamp amplifier (Axon Instrument, Union City, CA, USA) and a 12-bit data acquisition system (Axon CNS Molecular Devices, Singapore city, Singapore). The TREK-1 currents were low-pass-filtered at 3 kHz and digitized by a 12-bit converter at

10 kHz (Axon CNS Molecular Devices). The patch-clamp pipettes were produced from borosilicate glass capillaries with a resistance of 3.0–4.0 M Ω . The patch-clamp electrode solution contained 1 mM CaCl₂, 2 mM MgCl₂, 140 mM KCl, 10 mM HEPES, and 10 mM EGTA, and the pH was adjusted to 7.2 with KOH. The external medium contained 150 mM NaCl, 5 mM KCl, 1.2 mM MgCl₂, 0.5 mM CaCl₂, and 10 mM HEPES, and the pH was adjusted to 7.4 with NaOH. The cells were clamped at -80 mV, and the incremental voltage was set from -100 to 80 mV, with a ramp of 20 mV and duration of 1 second. The seal resistance of the pipette was 1 G Ω in HEK293 cells. The TREK-1 potassium current was recorded at room temperature (20–24°C) at escitalopram concentrations of 10, 30, 50, 100, 500 μ M, and 1 mM ($n = 6$ at each concentration). Patch-clamp software (pCLAMP 10.2) was used to analyze the TREK-1 currents recorded by whole-cell patch clamping, and the data were expressed as the mean $\pm$ SD.

Experimental Design and Drug Administration in Animal Experiments

Fifty-two male Sprague-Dawley rats (Shanghai Laboratory Animal Center, Shanghai, China) weighing 210–250 g each were used. The rats were housed four per cage with free access to food and water and were maintained with a 12-h light/dark cycle (lights on at 7 am), temperature of $21 \pm 2^\circ\text{C}$, and humidity of 55%. The experiments were compliant with the animal experiment guidelines developed by the National Institutes of Health (Publication No. 85–23, revised 1985) and approved by the Animal Study Committee of Southeast University, China (NO 2011ZDLL015.2).

After being screened with a sucrose preference test and an open field test (OFT), the animals were randomly divided into the sham surgery (SHAM) and MCAO groups. A left MCAO was performed in the ISCH rats, and neurological deficits were evaluated on a 5-point scale 24 h after surgery, as previously described [27,28]. MCAO rats with a score ≥ 1 and < 4 were further imaged with 7.0 T Magnetic Resonance Imaging (MRI) using a T2 protocol to confirm the area of cerebral infarction (Figure 1). The rate of infarction area of basal ganglia layer by T2, respectively, was analyzed to ensure the comparability of cerebral ischemia among the experimental groups (Supplementary Material). The overall mortality rate during the entire experiment was 35% in the rats undergoing MCAO and MRI scanning.

The survivors of MCAO were further divided into the following four subgroups: ischemic stroke (ISCH), escitalopram-treated ISCH (ISCH+escitalopram), PSD (ISCH+CMS), and escitalopram-treated PSD (PSD+escitalopram). Cerebral infarctions in the ISCH rats were confirmed by visual inspection after the animals were sacrificed on day 21. The rats in the SHAM group underwent a similar MCAO procedure except that a nylon thread was not inserted. The CMS procedure was performed on the PSD and PSD+escitalopram groups for 21 consecutive days beginning 48 h after the MCAO. The CMS, non-CMS, and control animals were kept in different rooms. The drug-treated rats received daily intraperitoneal injection of escitalopram (Lundbeck, Copenhagen, Denmark) at a dose of 10 mg/kg and a volume of 1.0 mL/kg for 21 days. This dose was selected according to previous animal studies of depression [29–31]. To label the proliferating cells, 5'-bromodeoxyuridine (BrdU, 50 mg/kg, Sigma, Saint Louis, MO, USA) was delivered i.p. twice per day at

Figure 1 Confirmation of permanent middle cerebral artery occlusion (MCAO). The rats in each group were scanned using 7.0-T micro-MRI according to the T2 protocol 24 h after surgery. MRI, magnetic resonance imaging; T2, transverse relaxation time.

8-h intervals for 3 days (days 19, 20, and 21 after ischemia). Sucrose preference tests and OFTs were performed at baseline and once per week over 3 consecutive weeks. The rats were sacrificed for sample collection 12 h after final drug administration.

CMS Regimen

The 21-day CMS regimen included eight stressors: water deprivation for 18 h (followed by a 1-h exposure to empty bottles), food and water deprivation for 20 h (followed by a sucrose preference test), 45° cage tilt for 17 h, overnight illumination (lights on for 36 h), swimming in 4°C water for 5 min, wet cage for 21 h (200 mL of water in 100 g of sawdust bedding), tail pinch for 1 min, and cage shaking for 2 h.

Body Weight

Body weight was recorded at 10:00 am 3 days before the end of the adaptive period (baseline) and then once per week for 3 consecutive weeks. The weight gain was calculated according to the following formula: weight gain (%) = [(body weight at measurement points (g) - baseline body weight (g)) / baseline body weight (g)] × 100%.

Open Field Test

To assess the general locomotor activity of the animals, OFT was performed using a plastic cube (75 cm on each side) with a black floor and white walls in a quiet and dusky room. Each rat was placed at the center of the floor, which was divided into 25 equal squares, including nine central squares and 16 peripheral squares. The rats were then allowed to move freely for 5 min, and their activity was subsequently recorded. Software (RD1112-IOF-R-1

v2.2.0; Mobile Datum, Shanghai, China) was used to track the total distance that the rats moved in the box.

Sucrose Preference Test

A sucrose preference test was used to operationally measure anhedonia in the animals. Prior to the baseline test, the animals were freely exposed to a training procedure of water and a 1% sucrose solution for 24 h. In the formal test, the rats were exposed to water and to a 1% sucrose solution for 1 h after a 24-h food and water deprivation period. The two bottles were randomly placed on the right/left side of the cage and switched after 30 min. Sucrose preference (SP %) was calculated using the following formula: [sucrose intake (g) / (sucrose intake (g) + water intake (g))] × 100%.

Tissue Processing

The animals were sacrificed by deep anesthesia using 10% chloral hydrate (30 mL/kg, i.p.) 12 h after the final escitalopram treatment. For immunostaining experiments, the rats were successively perfused with 0.1 M phosphate-buffered saline (PBS, 300 mL) and 4% paraformaldehyde (PFA, 300 mL). The brains were removed and fixed overnight in 4% PFA followed by dehydration in 30% sucrose at 4°C overnight. A freezing cryostat was used to cut coronal frozen sections (30 μm) from the hippocampus (bregma -3.14 to -4.16 mm) and the PFC (bregma 3.70 to 2.70 mm), as determined by the rat brain atlas of Paxinos and Watson and then stored at -20°C in cryoprotectant. For real-time PCR and Western blotting analyses, the rats were decapitated after deep anesthesia. The brains were quickly removed and stored in isopentane at -50°C. The brains were cut into 1-mm-thick coronal sections at -20°C using a freezing cryostat. Then, bilateral

tissues containing the PFC and dentate gyrus (DG), the cornu ammonis 1 (CA1), and the cornu ammonis 3 (CA3) of the hippocampus were separated using a 16-gauge needle. The tissues were immediately frozen in liquid nitrogen and stored at -80°C .

Immunofluorescence

BrdU and TREK-1 were co-labeled in the hippocampal sections. To detect the BrdU-labeled nuclei, the DNA was denatured as follows: incubation for 2 h in 50% formamide and $2\times$ SSC (0.3 M NaCl, 0.03 M sodium citrate) at 65°C , wash with $2\times$ SSC for 5 min, wash with 2 N HCl for 30 min at 37°C , and incubation in 0.1 M boric acid, pH 8.5, for 10 min. The sections were blocked for 2 h in 3% normal goat serum/1% BSA/0.3% Triton X-100 in PBS at room temperature. The sections were then incubated with a mouse monoclonal anti-BrdU antibody (1:300; IVGN, B35128) and a TREK-1 (C-20) goat anti-rat antibody (1:100, sc-11557; Santa Cruz Biotechnology, Inc., Dallas, Texas, USA) for 24 h at 4°C . Subsequently, the sections were washed and incubated for 2 h with Alexa Fluor 488 donkey anti-goat IgG (1:500; IVGN, A21206) and Alexa Fluor 546 donkey anti-mouse IgG (1:500; IVGN, A10036) at room temperature. To label TREK-1 in the PFC, the sections were incubated with antibodies as described above but were not subjected to the DNA denaturation steps. Images of the DG, CA1, CA3, and PFC were captured using an OLYMPUS

FV1000 laser-scanning microscope (Melville, NY, USA). The number of BrdU and TREK-1 double-labeled cells in three sections ($300\ \mu\text{m}$ apart) of each DG was analyzed by a designated blinded person using the manual counting option of the Image proplus software (version 4.1; Diagnostic, Inc., New York, NY, USA).

Real-time PCR

Total RNA was isolated from micropunches of the DG, CA1, and CA3 tissues using the TRIzol reagent (TaKaRa, Dalian, Liaoning, China). RNA ($1\ \mu\text{g}$) was converted to first-strand cDNA using reverse transcriptase (TaKaRa), and the mRNA expression was determined using an ABI 7300 PCR instrument (Applied Biosystems, Oceanside, CA, USA). The $20\text{-}\mu\text{L}$ total-volume reactions contained the following: $10\ \mu\text{L}$ SYBR Premix Ex TaqTM (TaKaRa), $1\ \mu\text{L}$ cDNA, $0.5\ \mu\text{L}$ each forward and reverse primer ($0.25\ \mu\text{M}$ each), and $8\ \mu\text{L}$ ddH₂O. The synthesized primer pairs were as follows: TREK-1 sense, 5'-TCA TCA CTC TGA CCA CCA TTG-3'; TREK-1 antisense, 5'-ACC TCT TCC TTC GTC TTC TTA G-3'; GAPDH sense, 5'-ACA GCA ACA GGG TGG TGG AC-3'; and GAPDH antisense, 5'-TTT GAG GGT GCA GCG A AC TT-3'. The PCR included an initial denaturation at 95°C for 5 min, followed by 40 cycles of denaturation at 95°C for 5 second and annealing/extension at 60°C for 1 min. The expression of the TREK-1 mRNA was normalized to that of GAPDH based on the standard curve method.

Figure 2 The inhibition of TREK-1 channel currents by escitalopram. (A) The record of typical TREK-1 background potassium currents by the whole-cell patch-clamp technique in HEK293 cells transfected with the TREK-1-pEGFP-N1 plasmid. (B) The significant inhibition of the TREK-1 current by escitalopram (at 0 mV with 10, 30, 50, 100, and 500 μM) as compared with the vehicle ($n = 6$ each, all $*P < 0.05$). (C) The concentration response curve for the escitalopram-mediated inhibition of the TREK-1 current. The IC_{50} value at 0 mV is 81.94 μM . (D) There is no significant change in the percentage inhibition of the TREK-1 current within the voltage range from -40 to $+40$ mV. All of the data are represented as the mean $\pm$ SD.

Western Blotting Analysis

The DG, CA1, and CA3 micropunch tissues were homogenized in ice-cold lysis buffer, and the protein concentration was detected by the BCA method. In total, 50 μ g of proteins was separated by SDS-PAGE using 10% polyacrylamide gels and then transferred to PVDF membrane (Millipore, Billerica, MA, USA). The membranes were blocked using 5% nonfat milk for 2 h at room temperature and then incubated overnight at 4°C with a TREK-1 (C-20) goat anti-rat antibody (polyclonal, unconjugated; 1:200; Santa Cruz). The blots were incubated with donkey anti-goat IgG-HRP secondary antibody (1:5000; Santa Cruz) at room temperature for 2 h, and the protein bands were detected using the enhanced chemiluminescence (ECL) method. The density of each TREK-1 band was normalized against a β -actin control.

Statistical Analyses

All of the data were expressed as the mean $\pm$ SEM, except that the electrophysiology data were expressed as the means $\pm$ SD. The behavioral index and body weight data were all examined using repeated-measures analysis of variance (ANOVA), with the different drug groupings and time as the independent variables. Subsequent group comparisons of the behavioral index, electrophysiology, protein, and mRNA data were performed using one-way ANOVA and *post hoc* Bonferroni analyses. Statistical significance was set at $P < 0.05$.

Results

The Effect of Escitalopram on TREK-1 Channel Currents

The whole-cell patch-clamp technique showed that the TREK-1 background current was an outward rectification that reversed the K⁺ current in HEK293 cells transfected with the TREK-1-pEGFP-N1 plasmids (Figure 2A). Bath application of escitalopram inhibited the TREK-1 currents in a concentration-dependent manner, and this could be clearly reversed following washing.

The TREK-1 current at 0 mV was decreased on average by $12.42 \pm 0.47\%$, $25.21 \pm 0.23\%$, $37.98 \pm 0.22\%$, $54.02 \pm 0.87\%$, $61.72 \pm 0.93\%$, and $62.12 \pm 0.92\%$ with 10, 30, 50, 100, and 500 μ M of escitalopram, respectively (Figure 2A). Escitalopram significantly inhibited the TREK-1 currents at 0 mV at 50, 100, and 500 μ M compared with vehicle treatment ($n = 6$ each, all $P < 0.05$) (Figure 2A,B). The concentration response curve indicated an IC₅₀ value of 81.94μ M for escitalopram at 0 mV, with a Hill coefficient of 2.49 ± 0.31 (Figure 2C). Escitalopram (50 μ M) voltage-dependently inhibited the TREK-1 current compared with a control ramp trace (Figure 2D). However, no significant change in the percent inhibition of the TREK-1 current was found in the voltage range from -40 to $+40$ mV, indicating that blockage by escitalopram is not voltage dependent.

The Effect of PSD and Escitalopram on Body Weight

Repeated-measures ANOVA demonstrated a significant effect [$F(5,227) = 74.70$, $P < 0.001$] of ISCH, CMS, and escitalopram on body weight at the 3 weekly time points (Figure 3A). Bonferroni *post hoc* tests showed a significant reduction in body weight in the PSD group at day 7 ($P < 0.05$), day 14 ($P < 0.05$), and day 21 ($P < 0.001$) compared with the SHAM group. The body weight in the PSD rats was significantly decreased relative to the ISCH group on day 14 ($P < 0.05$) and day 21 ($P < 0.001$). Three weeks of escitalopram treatment significantly reversed the reduction of body weight of the PSD rats ($P < 0.05$).

The Effect of PSD and Escitalopram on Sucrose Preference

Repeated-measures ANOVA demonstrated significant effects [$F(5,227) = 97.52$, $P < 0.001$] of ISCH, CMS, and escitalopram on the sucrose preference at the 3 weekly time points (Figure 3B). Bonferroni *post hoc* tests showed significant reductions in the sucrose preference at day 7, day 14, and day 21 in the PSD group relative to the SHAM group and the ISCH group ($P < 0.05$ and

Figure 3 The effect of escitalopram administration on body weight (A), sucrose preference (B), and locomotor activity (C). * $P < 0.05$ and ** $P < 0.001$ versus PSD group. Eight animals were included in the SHAM and ISCH groups, and 7 animals were included in the ISCH+ESC, PSD, and PSD+ESC groups. All of the data are shown as the mean $\pm$ SE. PSD, poststroke depression; SHAM, sham surgery; ISCH, ischemia; ESC, escitalopram.

$P < 0.001$, respectively). The reduction of sucrose preference in the PSD group was significantly reversed following 3 weeks treatment by escitalopram ($P < 0.05$).

The Effect of PSD and Escitalopram on Locomotor Activity

Repeated-measures ANOVA demonstrated robust effects [$F(5,227) = 103.96$, $P < 0.001$] of ISCH, CMS, and escitalopram on locomotor activity at the 3 weekly time points (Figure 3C). *Post hoc* tests showed a significant reduction of locomotor activity in the PSD group at day 7, day 14, and day 21 relative to the SHAM group (all $P < 0.001$). Locomotor activity was significantly decreased in the PSD group compared to the ISCH group at day 14 and day 21 (both $P < 0.05$). The reduction of locomotor activity was significantly reversed by escitalopram in the PSD group after 3-week administration ($P < 0.05$).

The Effect of Escitalopram on TREK-1 Expression in the PFC and Hippocampus of PSD Rats

Immunofluorescence staining revealed a broad distribution of TREK-1-positive cells in hippocampal DG, CA1, and CA3 and in the PFC (Figure 4A–D). No significant differences in TREK-1 mRNA or protein expression were observed in the PFC, CA3, or DG regions between the ISCH and SHAM groups. The expression of TREK-1 mRNA and protein was significantly higher in the PFC, CA3, and DG regions of the PSD group than in those of the ISCH and SHAM groups (all $P < 0.05$). The TREK-1 protein level was also increased significantly in the CA1 of the ISCH ($P < 0.05$) and PSD ($P < 0.05$) groups relative to the SHAM group (Figure 4J). Escitalopram significantly reversed the increases of the expression of TREK-1 mRNA and protein in these regions of the PSD rats after 3 weeks of administration (all $P < 0.05$) (Figure 4E–L, except 4F).

Figure 4 The distribution of TREK-1 in the rat hippocampal CA1, CA3, DG, and PFC regions (A–D). The images shown as 60× magnification (E–H). The expression of TREK-1 mRNA in the four brain regions ($n = 8$ each) (I–L). The expression of TREK-1 protein in the four brain regions ($n = 7$ each). * $P < 0.05$ versus PSD group; # $P < 0.05$ versus ISCH group. PFC, prefrontal cortex; CA1, cornu ammonis 1; CA3, cornu ammonis 3; DG, dentate gyrus.

Figure 5 The double staining of TREK-1 and newly proliferated cells in the subgranular zone (SGZ) of the hippocampal dentate gyrus (mean ± SEM). **(A)** TREK-1 (green) co-localized with BrdU-labeled granule cells (red) in the SGZ of the dentate gyrus (60×). **(B)** A representative image of the newly proliferated cells in the SGZ of the dentate gyrus. **(C)** Group comparisons of the numbers of BrdU-positive cells in the hippocampal dentate gyrus. * $P < 0.05$ versus ISCH group; # $P < 0.05$ versus PSD group. Eight animals were included in the SHAM and ISCH groups, and 7 animals were included in the ISCH+escitalopram, PSD, and PSD+escitalopram groups. PSD, poststroke depression; SHAM, sham surgery; ISCH, ischemia; ESC, escitalopram.

The Effect of Escitalopram on Cell Proliferation in the DG

Immunofluorescence staining showed that the TREK-1 protein co-localized with the BrdU staining in the cells of the DG (Figure 5A). The BrdU-labeled cells frequently formed clusters and were nearly exclusively located near the subgranular zone (SGZ) (Figure 5B). The number of BrdU-positive cells in the SGZ was significantly higher in the PSD and ISCH groups than in the SHAM group (Figure 5C). The number of BrdU-positive cells in the SGZ was significantly lower in the PSD rats than in the ISCH rats. Escitalopram significantly increased the BrdU-positive cells in the SGZ of the PSD rats ($P < 0.05$), but not in the ISCH or SHAM rats (Figure 5C), after 3 weeks of administration.

Discussion

To our knowledge, this is the first study to provide the evidence of the reversible inhibition of escitalopram on TREK-1 currents in a concentration-dependent manner, and the TREK-1 elevation in the hippocampus and PFC in a rat model of PSD. The elevation of TREK-1 levels was also associated with decreased cellular proliferation in the DG. The results further showed that chronic escitalopram administration could attenuate the upregulation of TREK-1 expression and increase proliferation in the DG as well as improve weight gain, locomotor activity, and sucrose preference in PSD rats. Although the exact mechanisms by which TREK-1 influences PSD pathogenesis and escitalopram regulates TREK-1 expression

remain unclear, these studies imply that TREK-1 may be an important molecular mediator involved in the pathophysiology of PSD and the influence of SSRIs on PSD.

This study had performed a PSD-like animal model that uses MCAO, CMS, and isolation rearing to mimic the complex clinical conditions of PSD patients by replicating stroke, stress, and psychosocial conditions [26]. The results show a broad spectrum of behavioral alterations, including reduced sucrose preference, reduced motor activity, and reduced body weight, in PSD rats 1 week after MCAO surgery. These changes are similar to the core depression-like symptoms. The chronic treatment by escitalopram for 3 weeks significantly reversed these behavioral changes in the PSD rats. These findings demonstrate that escitalopram has a significant therapeutic effect on depressive symptoms in PSD animals, which is consistent with the clinical efficacy of SSRI antidepressants [32–34], in PSD patients. Combined with our previous reports [35], this study provides evidence showing that this animal model is suitable for mimicking PSD in the clinical setting.

The presented study revealed dose-dependent inhibition by escitalopram on the current mediated by recombinant TREK-1 channels in HEK293 cells. The blockage by escitalopram was reversible by washing and was voltage independent, which is consistent with a previous report of the inhibition of the SSRI antidepressant fluoxetine on TREK-1 currents [16]. The mechanisms by which antidepressants inhibit TREK-1 channels are poorly understood. It was previously suggested that fluoxetine does not bind to the pore region or the cytosolic C-terminal domain of TREK-1

channels [16]. Recently, TREK-1 has been importantly implicated in mood regulation, in which suppression of TREK-1 channels may have an antidepressant efficacy. Both TREK-1 knockout mice [15] and wild-type mice treated with spadin, a peptide that blocks TREK-1 channels [36], displayed depression-resistant responses in five behavioral models. These responses were similar to the responses observed in antidepressant-treated, wild-type mice. It has been assumed that opening of the TREK-1 channels leads to hyperpolarization of the cell, reduction of the firing rate, and a decrease in the release of serotonin from the dorsal raphe neurons (DRN) [37,38]. Consistently, TREK-1 knockout mice and spadin treatment were found to increase the firing rate of the DRN 5-HT neuron *in vivo* [36]. The inhibition of TREK-1 by the SSRI escitalopram that was shown in this study might increase DRN excitability and 5-HT release, which could further contribute to its therapeutic efficacy for poststroke depression.

The results demonstrated a robust increase in the TREK-1 protein in hippocampal CA1 after 21 days of left unilateral ISCH, which is partially consistent with prior findings indicating increased TREK-1 expression in the cortex and hippocampal CA1 of MACO rats 3, 7, and 30 days after reperfusion [17]. A previous *in situ* hybridization study reported that there were more TREK-1-positive neurons in the cortex and hippocampus at 3 and 30 days after bilateral carotid artery ligation [22]. This increased TREK-1 expression was expected to hyperpolarize the membrane potential, decrease neuronal excitability, and rescue homeostatic dysfunctions under ischemic conditions [17,22,39]. The present study showed, for the first time, that TREK-1 expression is significantly increased in hippocampal CA1 and CA3 and in the PFC of PSD rats, and this could be reversed by chronic escitalopram administration. Chronic, but not acute, SSRI administration has been found to inhibit the firing rate of pyramidal neurons in rat hippocampal CA1 and PFC regions, and this is paralleled by the amelioration of depressive symptoms [15,40]. However, the reduction in TREK-1 expression following escitalopram treatment that was found in this study might seemingly increase neuronal excitability, which is opposite to the effects of chronic escitalopram on the regional firing rates in depressed animals. Interestingly, TREK-1 channels could also preferentially pass an outward current at depolarized potentials to facilitate recovery and repetitive firing when C-terminal serine S333 is phosphorylated by protein kinase A [41]. Therefore, TREK-1 theoretically possesses two distinct functional modes to fine-tune neuronal excitability. The reduced TREK-1 expression in the hippocampal CA1 and PFC regions might have a different role than that in the DRN and might decrease the firing rate of pyramidal neurons following chronic escitalopram treatment. The present results demonstrated that changes in TREK-1 expression in the hippocampal CA1 and PFC regions were largely consistent with behavioral changes before and after escitalopram treatment. Therefore, these results support our hypothesis and emphasize the importance of future research into the mechanisms of the aforementioned effects.

This research was consistent with previous reports showing increased neural stem cell (NSC) proliferation in the SGZ of ischemic rats compared with SHAM rats [35,42]. Ischemia has been shown to promote neurogenesis in the germinative zones of the neostriatum and in the hippocampal DG, which contributes to brain recovery. These results present several potential therapeutic

targets for PSD [43,44]. Our results showed a robust reduction of NSC proliferation in the SGZ of the PSD rats compared to the ischemic rats, and this could be ameliorated by chronic escitalopram treatment. Along with our previous report [35], the results of this study indicate a potential association between the deficits of adult hippocampal neurogenesis and the development of stroke-induced depression, which are consistent with the well-documented involvement of hippocampal neurogenesis in the etiology and therapy of endogenous depression [24,45–47]. This study is the first to find that TREK-1 channels are implicated in the decreased hippocampal neurogenesis in PSD, showing that TREK-1 expression in the hippocampal DG of PSD rats was significantly higher compared with that of ischemic rats, and this increased expression could be reversed by continual escitalopram administration. Interestingly, TREK-1 blockage had been demonstrated by the antidepressant effects through hippocampal neurogenesis [36,48]. The TREK-1 channel blocker spadin enhanced both hippocampal CREB phosphorylation and neurogenesis in mice, which have been regarded as key indicators of antidepressant action following continuous SSRI treatment [36]. We previously demonstrated that the proliferation of embryonic NSCs was significantly inhibited by transfection of hTREK-1 *in vitro*, and this proliferation was reversible following fluoxetine administration [49]. Fluoxetine (3 mg/kg) robustly elevated NSC proliferation in the SGZ of TREK-1 knockout mice compared to wild-type mice [15]. Therefore, TREK-1 might be an important target in the regulation of antidepressants on hippocampal neurogenesis in PSD rats.

The following limitations should be particularly paid attention in the present study. Firstly, the Brdu-labeled cells had not been double-stained with a neural stem cell marker, which was further helpful to illuminate the effect of escitalopram on hippocampal neurogenesis and the relationship with TREK-1 channel. A previous double staining study indicated that Brdu-positive cells increased approximately sixfold in the subgranular zone of the hippocampal DG 7 days after MCAO, in which more than 80% of Brdu-positive cells were the newly generated neural cells in the hippocampus [50]. Secondly, we acknowledge that the present findings should be accepted with caution due to the difference between animal models and human syndrome. The current studies of PSD-like rat model do not necessarily extrapolate to the PSD clinical symptoms in the human.

In summary, this study demonstrates that the SSRI escitalopram can reversibly inhibit the TREK-1 current concentration-dependently. Continuous escitalopram treatment decreased the TREK-1 overexpression in the hippocampus and prefrontal cortex and elevated hippocampal NSC proliferation in PSD rats, which was accompanied by the amelioration of depressive symptoms. These data indicate that the K2p channel TREK-1 plays an important role in the pathophysiology of PSD and might represent a potential therapeutic target for PSD.

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Conflict of Interests

The authors declare no conflict of interest.

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Supporting Information

The following supplementary material is available for this article:

Table S1. The rate of infarction area of basal ganglia layer by T2 24 h after MCAO surgery.