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Activation of Neuronal Voltage-Gated Potassium Kv7/KCNQ/M-Current by a Novel Channel Opener SCR2682 for Alleviation of Chronic Pain

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ABSTRACT

Treatment of chronic pain remains an unmet medical need. The neuronal voltage-gated potassium Kv7/KCNQ/M channel has been implicated as a therapeutic target for chronic pain. However, whether pharmacological activation of the Kv7 channel can alleviate pain remains elusive. In this study, we show that selective activation of native M-currents by a novel channel opener SCR2682 reduces repetitive firings of dorsal root ganglia (DRG) sensory neurons. Intraperitoneal administration of SCR2682 relieves mechanical allodynia and thermal hyperalgesia in rat models of pain induced by complete Freund's adjuvant (CFA) or spared nerve injury (SNI) in a dose-dependent manner without affecting locomotor activity. The antinociceptive efficacy of SCR2682 can be reversed by the channel-specific blocker XE991. Furthermore, SCR2682 increases Kv7.2/KCNQ2 mRNA and protein expression in DRG neurons from rats in the SNI model of neuropathic pain. Taken together, pharmacological activation of neuronal Kv7 channels by opener SCR2682 can alleviate pain in rats, thus possessing therapeutic potential for chronic pain or hyperexcitability-related neurologic disorders.

SIGNIFICANCE STATEMENT

A novel voltage-gated potassium Kv7 channel opener SCR2682 inhibits action potential firings in dorsal root ganglia sensory neurons and exhibits efficacy in antinociception, thus possessing a developmental potential for treatment of chronic pain or epilepsy.

Introduction

Neuronal hyperexcitability defines the fundamental mechanism of neurologic diseases such as chronic pain and epilepsy (Snowball and Schorge, 2015). Therefore, pharmacological inhibition of repetitive neuronal firing represents an attractive strategy for therapy of chronic pain (Payne et al., 2015). The most commonly prescribed medications for chronic pain are nonsteroidal anti-inflammatory drugs, skeletal muscle relaxants, and opioid analgesics (van Hecke et al., 2013; Tsantoulas and McMahon, 2014; Hsu, 2017). However, clinical use of these drugs for treatment of pain renders either limited efficacy or serious side effects (Gordon, 2003; Hu et al., 2016; Volkow et al., 2018). Therefore, the treatment of chronic pain still remains an unmet medical need.

Potassium channels are considered to be key targets for controlling membrane excitability and treatment of chronic pain (Liu and Wang, 2019). The voltage-gated Kv7 potassium channels encoded by the *KCNQ* gene subfamily include five members of Kv7.1–Kv7.5 (Cooper and Jan, 2003; Brown and Passmore, 2009). Neuronal Kv7 channels formed by Kv7.2, Kv7.3, and Kv7.5 subunits are mainly expressed in the central nervous system or peripheral sensory system, such as dorsal root ganglia (DRG) sensory neurons and dorsal horn neurons (Passmore et al., 2003; Wickenden and McNaughton-Smith, 2009; Wang and Li, 2016). Kv7 channels elicit slow activating, non-inactivating, and voltage-dependent K^+ currents that exert an inhibitory control on neuronal excitability (Wang et al., 1998; Cooper and Jan, 2003; Wulff et al., 2009). Loss of function or inhibition of Kv7 channels increases cell excitability through depolarizing resting membrane potential and increasing firings of nociceptive neurons (Barkai et al., 2017) and is involved in the pathogenesis of epilepsy, migraine, and

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ABBREVIATIONS: CFA, complete Freund's adjuvant; DRG, dorsal root ganglia; GAPDH, Glyceraldehyde-3-phosphate Dehydrogenase; Kv, voltage-gated potassium; KCNQ, voltage-gated potasium 7; PGB, pregabalin; PWMT, paw withdrawal mechanical threshold; PWTL, paw withdrawal thermal latency; RMP, resting membrane potential; RTG, retigabine; RT-PCR, reverse-transcription polymerase chain reaction; SD, Sprague-Dawley; SNI, spared nerve injury.

neuropathic pain (Biervert et al., 1998; Charlier et al., 1998; Schroeder et al., 1998; Blackburn-Munro and Jensen, 2003; Gribkoff, 2003). Therefore, enhancing Kv7 function in nociceptors may provide a promising treatment of chronic pain (Passmore et al., 2003; Wulff et al., 2009).

Kv7 opener retigabine, or ezogabine, was the first drug approved by the US Food and Drug Administration for the adjuvant treatment of partial seizures (https://www.gsksource.com/gskprm/htdocs/documents/POTIGA-PI-MG.PDF). In preclinical studies, retigabine exhibits antinociceptive effects on inflammatory, neuropathic, and bone cancer pain in rodent models (Hayashi et al., 2014; Cai et al., 2015; Pottabathini et al., 2015). Flupirtine, a structural analog of retigabine, was used for decades as a centrally acting, nonopioid analgesic for treatment of a variety of acute and chronic pain (Puls et al., 2011; Szelenyi, 2013). However, both retigabine and flupirtine have recently been discontinued because of their safety issues associated with retina and skin discoloration and liver toxicity (Puls et al., 2011; Garin Shkolnik et al., 2014). Therefore, it is necessary to identify more selective and potent Kv7 channel openers with fewer side effects for therapy of chronic pain.

In this study, we evaluated the antinociceptive effect of a novel Kv7/KCNQ/M channel opener SCR2682 on neuronal firing and nociception in rat models of chronic pain. SCR2682 potently activates Kv7.2–Kv7.5 channels in a dose-dependent manner with an EC_{50} of 9.8 ± 0.4 nM on Kv7.2/7.3 (Zhang et al., 2019), which is 100-fold more potent than retigabine in activating Kv7.2/Kv7.3 channel.

Materials and Methods

Animals

The 8-week-old male Sprague-Dawley (SD) rats and 7-day-old SD rats were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. Animals were housed three to five per cage and received food and water ad libitum. Before behavioral experiments, rats were acclimated for 1 week in a quiet animal breeding room with a 12-hour light/dark cycle (7:00 AM to 7:00 PM) under controlled temperature (23 ± 2°C) and humidity (50% ± 5%). All in vivo experiments were conducted from 9:00 AM to 5:00 PM in a double-blind manner. All experimental procedures were approved by the Animal Ethics Committee of Qingdao University College of Medicine and complied with the ethical guidelines of the International Association for the Study of Pain.

Reagents

Compounds SCR2682 [4-(2-bromo-6,7-dihydrothieno[3,2-c]pyridin-5(4H)-yl)-2,6-dimethylphenyl)-3,3 dimethylbutanamide], XE991, and retigabine (RTG) were synthesized with above 95% purity by Shanghai Zhimeng Biopharma Co., Ltd. (Shanghai, China). Pregabalin (PGB) was purchased from Beijing Solarbio Science & Technology Co, Ltd. (Beijing, China). Glyceraldehyde-3-phosphate Dehydrogenase (GAPDH) antibody and secondary antibody were purchased from Abcam (Shanghai, China). KCNQ2 antibody was purchased from Santa Cruz Biotechnology. The primers for KCNQ2 and β-actin were synthesized by Invitrogen (Shanghai, China). All drugs were dissolved in DMSO stock solutions and stored at −20°C before use. The stock solutions were diluted to working concentrations with saline or electrophysiological bath solution on the day of experiment.

Culture of Rat DRG Neurons

DRG neurons were isolated from the 7-day-old SD rats as previously described (Zhang et al., 2013). Briefly, DRG neurons acutely dissected from the intervertebral foramina were digested at 37°C with 1 mg/ml collagenase for 30 minutes, followed by another 30-minute digestion with 2.5 mg/ml trypsin, before subsequently being suspended at least twice in Dulbecco's modified Eagle's medium (DMEM) plus 10% fetal calf serum (FBS) to stop the digestion. Thereafter, the acutely dissociated neurons from DRGs were plated on poly(D-lysine)–coated glass coverslips and cultured for 4 days before electrophysiological recordings within 48 hours.

Electrophysiology

For current recordings of DRG neurons, the perforated patch-clamp recording technique was used with amphotericin B (250 μg/ml; Sigma) in the pipette (Zhang et al., 2013) and signals were amplified using an EPC10 patch-clamp amplifier (HEKA, Harvard Bioscience, USA). The acquisition rate of data was at 10 kHz before being filtered at 2.5 kHz. Patch electrodes were pulled using a micropipette puller (Sutter Instruments, Novato, CA) and fire-polished to a final resistance of 1 to 2 MΩ. Series resistance was compensated by 60%–80%. The internal solution for recordings of DRG neurons was as follows (in millimolars): KCl 150, $MgCl_2$ 5, HEPES 10, and pH 7.4 adjusted with KOH; and the external solution (in millimolars) contained NaCl 160, KCl 2.5, $MgCl_2$ 1, $CaCl_2$ 2, glucose 10, HEPES 20, and pH 7.4 adjusted with NaOH.

Locomotor Activity

The open field test was used to measure the general locomotor activity. SD rats were randomly divided into groups consisting of vehicle control (10% Tween-80-saline), RTG (7 mg/kg), and SCR2682 (0.5, 1, 2, 4, and 8 mg/kg). After 1 hour of intraperitoneal administration, each rat was placed in an open field box (40 × 40 cm) for 5 minutes, and its total distance and the mean travel speed in the apparatus were recorded and analyzed using SMART 3.0 (Panlab, Barcelona, Spain). The apparatus was wiped clean with 75% alcohol before the next testing.

Chronic Pain Models

Complete Freund's Adjuvant–Induced Inflammatory Pain Model. Adult male SD rats were anesthetized with 10% chloral hydrate (intraperitoneally, i.p.) before being injected with 50% complete Freund's adjuvant (CFA) (in a 100-μl injection volume; Sigma) into the planter surface of the right hind paw (Peng et al., 2012). Rats in the sham control group underwent the same procedures, except saline was injected into the right hind paw (Teng et al., 2016). SCR2682 (0.5, 1, and 2 mg/kg, i.p.) or RTG (7 mg/kg, i.p.) was administered after CFA injection for 24 hours, and rats in the sham control and CFA model group were injected with 10% Tween-80-saline. The paw withdrawal mechanical threshold (PWMT) and paw withdrawal thermal latency (PWTL) were performed at time points 0, 0.5, 1, 2, and 3 hours after compound administration.

The Spared Nerve Injury–Induced Model of Persistent Peripheral Neuropathic Pain. Adult male SD rats were anesthetized by intraperitoneal injection with 10% chloral hydrate. The rat spared nerve injury (SNI) model was generated based on previous descriptions (Decosterd and Woolf, 2000). Briefly, the skin of the left posterior leg was incised, and the biceps femoris muscles were separated for the exposure of the three branches of sciatic nerve (tibial, common peroneal, and sural nerves). The tibial and common peroneal nerves and surrounding tissues were isolated and ligated with removal of about 2 to 3 mm of distal nerve stump to avoid regeneration while ensuring the sural nerve stayed intact. The same procedure was performed for sham-operated rats without nerve ligation and cutting. After the operation, muscles and skin were closed with absorbable sutures. After the establishment of the SNI model, rats were divided into eight groups: sham, SNI + vehicle, SNI + SCR2682 (0.5 mg/kg), SNI + SCR2682 (1 mg/kg), SNI + SCR2682 (2 mg/kg), SNI + SCR2682 (2 mg/kg) + XE991 (3 mg/kg), SNI + RTG (7 mg/kg), and SNI + PGB (10 mg/kg). The rats from the sham and SNI

+ vehicle groups were injected with 10% Tween-80-saline. Drugs were injected (i.p.) for nine consecutive days (Chen et al., 2015), and the PWMT was determined after 1 hour of administration on days 1, 3, 5, 7, and 9. After the behavioral experiments, the animals were decapitated for isolation of DRGs from the L4 and L5 spinal segment. The isolated DRGs were stored at −80°C until further use for detection of Kv7.2 mRNA and protein levels in quantitative RT-PCR and Western blot assays, respectively.

Measurement of Pain Threshold

Paw Withdrawal Mechanical Threshold. The PWMT induced by von Frey hair was measured by an up-and-down method for evaluation of mechanical allodynia. Rats were individually placed in a transparent plexiglass cover on a metal mesh for 20–30 minutes until the disappearance of basic combing and exploring activities. A series of von Frey hairs with different forces were applied to stimulate the plantar of rat hind paws until a bend in a slight S-shape was observed for 5 to 6 seconds. If rats showed paw withdrawal reaction, the result was positive; otherwise, it was negative. The PWMT was calculated by the following formula: PWMT (g) = $10^{[Xf+K\delta]}$, where Xf is the log value of the last von Frey hair force, K is the value looked up from the standardized table based on the up-and-down pattern, and δ is the average of the difference between the log values of the adjacent von Frey hair force.

Paw Withdrawal Thermal Latency. The PWTL of thermal hyperalgesia was measured using a fully automatic plantar analgesia meter (BME-410C). An individual rat was placed on a glass plate with a transparent plexiglass cover. After 15 minutes of adaptation, the plantar of the rat's hind paw was heated, and the time from contact with the thermal radiation source to paw withdrawal was recorded as the PWTL, with cutoff latency of 35 seconds to prevent potential tissue damage. Each rat was repeatedly measured five times at an interval of 5 minutes, and an average PWTL was calculated.

Quantitative RT-PCR and Western Blot. The total RNA was extracted from DRG neurons of the L4 and L5 spinal segment using Trizol reagent. The FastKing RT Kit (With gDNase) was used to remove genomic DNA contamination and synthetize cDNA. The real-time polymerase chain reaction was performed using Taq PCR Mastermix in a Bio-Rad iCycler Thermal Cycler and using SYBR Green as a reporter. The primer sequences used were as follows: KCNQ2, sense 5′-CAGTGCGGATCAGAGTCTCG-3′, antisense 5′-CTTGCTTCTTCTGAGTTCTGCC-3′; β-actin, sense 5′-CATTGCTGACAGGATGCAGAAGG-3′, antisense 5′-TGCTGGAAGGTGGACAGTGAGG-3′. Expression levels were normalized to the β-actin. Data were analyzed using the $2^{-\Delta\Delta CT}$ method.

The total protein was extracted using radio immunoprecipitation assay (RIPA lysis buffer containing 1% protease and phosphatase inhibitor cocktail (Thermo Fisher Scientific). The bicinchoninic acid (BCA) protein assay kit (Thermo Fisher Scientific) was used to determine the total protein concentration. An equal amount of proteins was denatured and separated with 10% SDS-PAGE before being transferred to the polyvinylidene fluoride (PVDF) membrane. The PVDF membranes were blocked with 5% nonfat milk in 0.5% Tween-20-TBS for 2 hours at room temperature before incubation with primary antibodies of mouse anti-KCNQ2 (1:500) and rabbit anti-GAPDH (1:10,000) overnight at 4°C. The horseradish peroxidase–conjugated secondary antibody (1:10,000) was incubated at room temperature for 1 hour after a wash of primary antibody with Tween-20-TBS. The membrane was placed in chemiluminescence reagent for 2 to 3 minutes and imaged under dark conditions. The bands were quantified using Image Laboratory software.

Statistical Analysis

Statistical analysis was performed using GraphPad Prism 5 software. All data were expressed as the means ± S.E.M. Statistical analysis for differences between groups in electrophysiological experiments was carried out using paired Student's t tests. Statistical differences among groups for in vivo behavioral tests were compared by one-way or two-way ANOVA, followed by Bonferroni post hoc test. The Bonferroni post hoc tests were conducted only if F in ANOVA achieved $P < 0.05$ and there was no significant variance inhomogeneity. When $P < 0.05$, the difference was considered to be statistically significant.

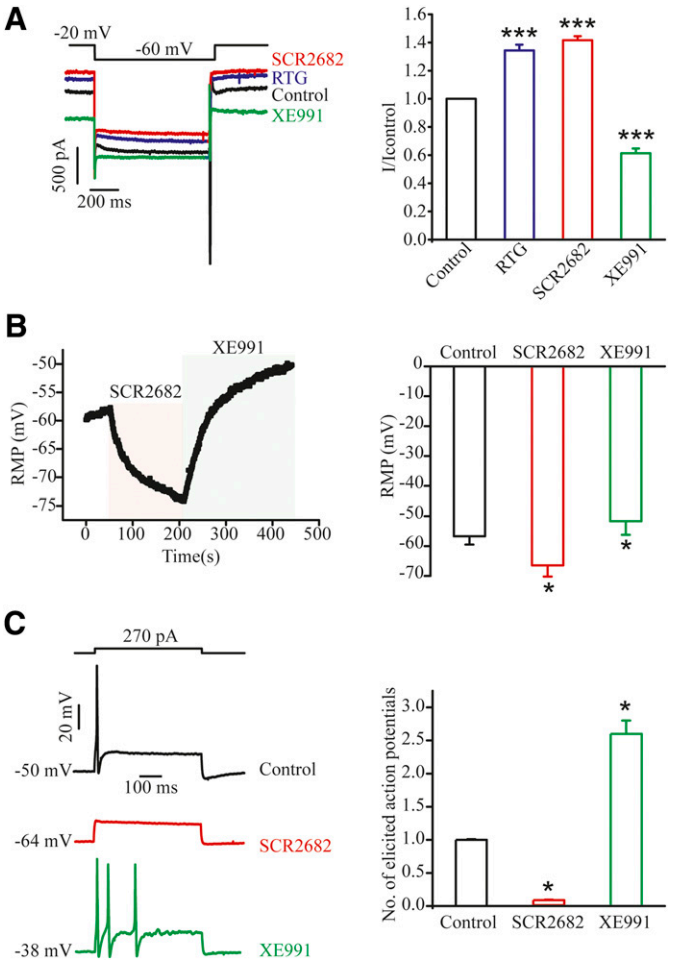

Fig. 1. Enhancement of native M-currents in DRG neurons and attenuation of neuronal firings by SCR2682. (A) Left panel: representative M-current traces were recorded in DRG neurons with holding potential at −20 mV for activation of M-current and −60 mV for deactivation of M-current. SCR2682 (0.1 μM) or RTG (10 μM) as a positive control enhanced the amplitude of M-current. A specific blocker, XE991 (3.0 μM), was used to inhibit the M-current. A compound effect was washed out before addition of another compound. Right panel: a summary for effect of SCR2682 (0.1 μM), RTG (10 μM), or XE991 (3.0 μM) on M-current measured at −20 mV; $n = 9$. (B) Left panel: representative RMP before and after application of SCR2682 at 0.1 μM or XE991 at 3.0 μM. Right panel: a summary for RMP with −56.7 ± 2.8 mV for control, −66.4 ± 3.7 mV for SCR2682, and −51.7 ± 4.6 mV for XE991. (C) Left panel: representative traces for action potentials in the absence or presence of SCR2682 (0.1 μM), RTG (10 μM), or XE991 (3.0 μM). The action potential was evoked by injection of 270 pA depolarizing current as indicated at the top of the panel. Right panel: a summary for the numbers of the evoked action potential recorded in cultured DRG neurons in the presence and absence of M channel modulators. *$P < 0.05$; ***$P < 0.001$, indicates statistical significance in comparison with the control.

Results

Compound SCR2682 Enhances Native M-Current in Rat DRG Neurons. To examine the effect of SCR2682 on the activation of native M-current, we carried out recordings of

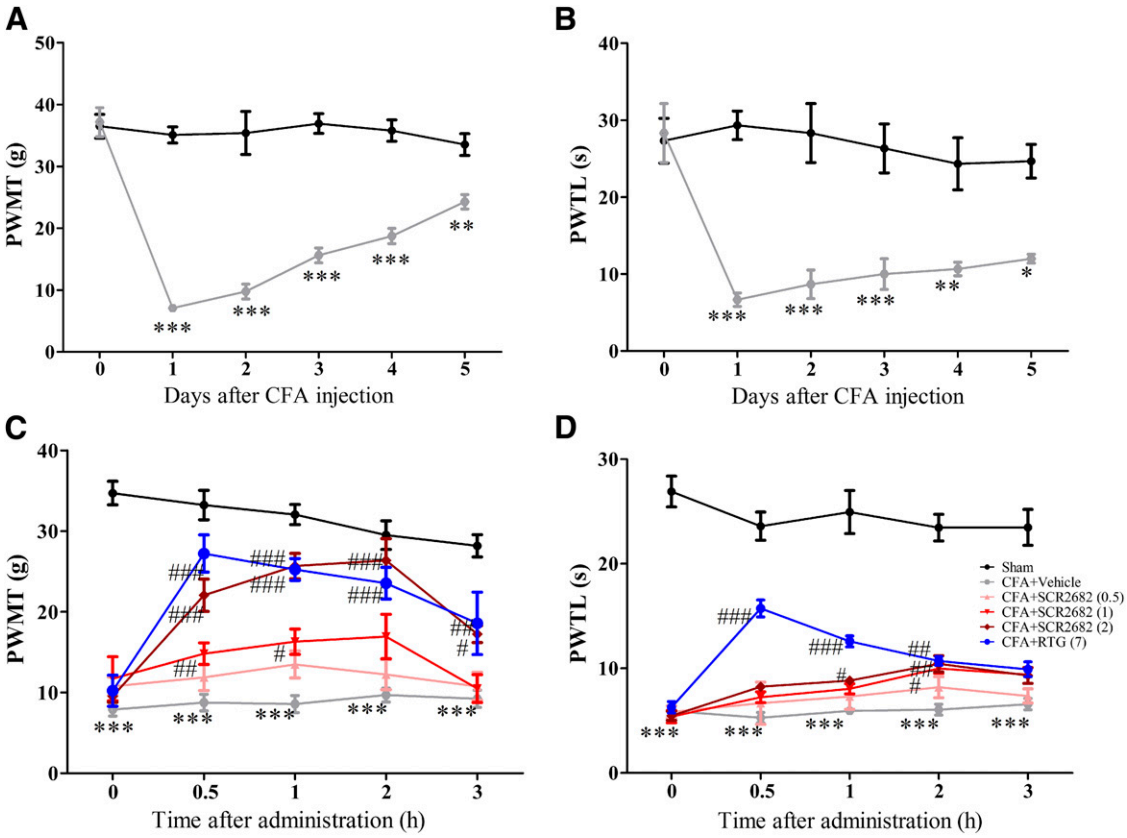

Fig. 2. Antinociceptive effects of SCR2682 on inflammatory pain induced by CFA. (A) The change of PWMT between 0 and 5 days after CFA injection in rats; $n = 3$. (B) The change of PWTL between 0 and 5 days after CFA injection; $n = 3$. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. sham control. (C) Effect of SCR2682 (0.5, 1, and 2 mg/kg, i.p.) on mechanical allodynia in rat model of CFA-induced inflammatory pain; $n = 6$–8. (D) Effect of SCR2682 (0.5, 1, and 2 mg/kg, i.p.) on thermal hyperalgesia in a rat model of CFA-induced inflammatory pain; $n = 6$–8. $^{**}P < 0.01$ vs. sham; $^{\#}P < 0.5$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. CFA + vehicle; $^{\$}P < 0.5$, $^{\$\$\$}P < 0.001$ vs. CFA + SCR2682 (2 mg/kg). Data were expressed as the means ± S.E.M. Comparison was made by two-way ANOVA, followed by post hoc Bonferroni's test.

acutely isolated DRG neurons from rats. The M-current was activated by a depolarizing voltage at −20 mV, and the tail current was observed at −60 mV (Fig. 1A). Application of SCR2682 at 0.1 μM or RTG at 10 μM as a positive control increased M-current by approximately 30% measured at −20 mV, which was blocked by the channel-specific inhibitor XE991 (3 μM) (Fig. 1A). SCR2682 also induced a significant hyperpolarization of the resting membrane potential (RMP), with a leftward shift of about 10 mV to −66.4 ± 3.7 from −56.7 ± 2.8 mV (Fig. 1B). Similarly, perfusing SCR2682 at 0.1 μM also abolished the firing spikes of DRG neurons, whereas M-channel–specific blocker XE991 significantly increased the number of action potentials elicited by injection of 270 pA inward current (Fig. 1C). These results indicate that activation of native M-current by SCR2682 significantly suppresses the firing of DRG neurons, suggesting that SCR2682 may have antinociceptive activity.

Antinociceptive Effect of SCR2682 on CFA-Induced Inflammatory Pain in Rats. We further examined the effect of SCR2682 on mechanical allodynia and thermal hyperalgesia in CFA-induced inflammatory pain in rats. As shown in Fig. 2, A and B, subcutaneous injection of CFA resulted in development of hypersensitivity to both mechanical and thermal stimuli at 24 hours after the injection, and the hyperalgesic effect was observed and maintained for 5 days before a slow recovery over time to baseline, consistent with our previous observations (Teng et al., 2016). In contrast, administrations of SCR2682 at different concentrations (0.5, 1, and 2 mg/kg, i.p.) caused an increase of both PWMT and PWTL in a dose-dependent manner with maximal effect 2 hours after injection (Fig. 2, C and D). As a positive control, RTG (7 mg/kg, i.p.) also increased the PWMT and PWTL with maximum effect at 0.5 hours (Fig. 2, C and D). The effects of the highest doses of SCR2862 and RTG were comparable in the PWMT procedure, with RTG at 0.5 hours producing greater effect in PWTL compared with PWMT. These results demonstrate that SCR2682 can alleviate the mechanical allodynia and thermal hyperalgesia induced by CFA.

SCR2682 Alleviates SNI-Induced Neuropathic Pain. To further evaluate the effect of SCR2682 on chronic pain, we generated the neuropathic pain model of SNI in rats. Compared with the sham group, rats in SNI vehicle groups showed hypersensitivity responses to von Frey hair stimuli on day 9 after surgery, and the mechanical allodynia was observed and maintained during the 10-day period of experiments. As shown in Fig. 3 and Table 1, there was no difference in the baseline threshold for mechanical pain response determined in von Frey hair assays among the different groups before surgical operation. In contrast, administration of SCR2682 (i.p.) at different doses (0.5, 1, and 2 mg/kg) resulted in a dose-dependent increase of ipsilateral PWMT, as compared with the vehicle group

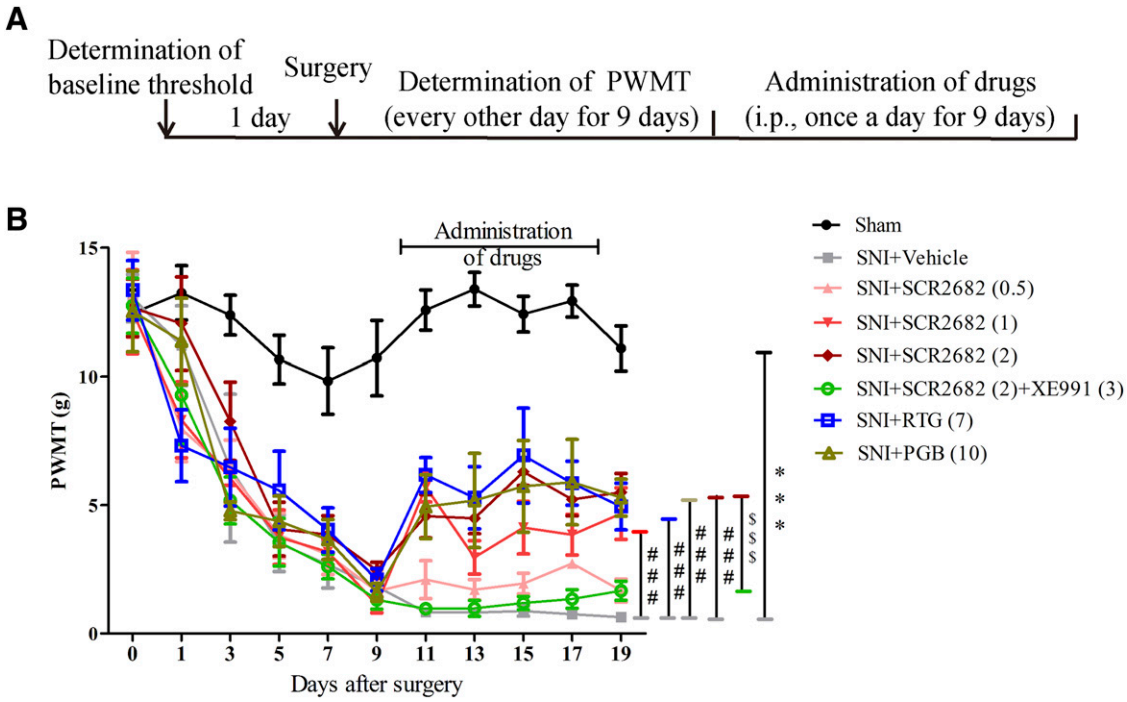

Fig. 3. Effect of SCR2682 on alleviation of SNI-induced neuropathic pain. (A) A schematic drawing of timeline and treatment in a rat SNI model of rats. (B) Intraperitoneal injections of SCR2682 at different concentrations of 0.5, 1, and 2 mg/kg were made for 9 days, and the PWMT was determined after 1 hour of administration of compounds on days 1, 3, 5, 7, and 9. RTG at 7 mg/kg and PGB at 10 mg/kg were used as positive controls. $^{***}P < 0.001$ vs. sham; $^{\#\#\#}P < 0.001$ vs. SNI + vehicle; $^{\$\$\$}P < 0.001$ vs. SNI + SCR2682 (2 mg/kg). Data were expressed as the means ± S.E.M.; $n = 6–8$. Comparison was made by one-way ANOVA, followed by post hoc Bonferroni's test.

(Fig. 3; Table 1). Cotreatment of Kv7 channel blocker XE991 (3 mg/kg, i.p.) and SCR2682 (2 mg/kg, i.p.) resulted in a decrease of PWMT, indicating the antagonism of XE991 on antinociception of SCR2682 through the inhibition of Kv7 (Fig. 3; Table 1). As positive controls, RTG at 7 mg/kg (i.p.) or PGB (10 mg/kg, i.p.) also exhibited a significant increase of PWMT in SNI rats (Fig. 3; Table 1). These results demonstrate that selective activation of Kv7 by SCR2682 can alleviate neuropathic pain induced by SNI.

Effect of SCR2682 on Locomotor Activity. Locomotor activity is commonly used for evaluation of psychostimulative effect or sedative activity. We next tested the effect of SCR2682 on the locomotor activity by assessing total travel distance and average speed in the open field test. As depicted in Fig. 4, rats treated with different doses of SCR2682 (0.5, 1, and 2 mg/kg, i.p.) or RTG (7 mg/kg, i.p.) had no significant differences in the total travel distance and the average speed, as compared with the control group. However, dosing SCR2682 at higher concentrations of 4 and 8 mg/kg caused the reduction of total travel distance and the mean travel speed (Fig. 4), suggesting that there is a sedative effect of SCR2682 at high doses.

Increased Expressions of Kv7.2/KCNQ2 by SCR2682 in Isolated DRG Neurons from Rats with Spared Nerve Injury. To investigate the effect of SCR2682 on Kv7.2/KCNQ2 channel subunit expressions in DRG neurons from rats with SNI, we examined the mRNA and protein expression levels of KCNQ2 using the quantitative RT-PCR and Western blot analysis. As illustrated in Fig. 5, the expression levels of KCNQ2 mRNAs and proteins in DRG neurons from the SNI group were significantly lower than that of the sham control group. In contrast, treatment with SCR2682 (2 mg/kg, i.p.) or RTG (7 mg/kg, i.p.) significantly increased KCNQ2 mRNA and protein expression levels as compared with the SNI + vehicle group (Fig. 5). To further confirm the increased expression of KCNQ2 in DRG neurons, we also coinjected SCR2682 with XE991 (3 mg/kg, i.p.), which caused the reduction of KCNQ2 mRNA and protein expression levels (Fig. 5). These results show that SCR2682 upregulates KCNQ2 expression in DRG neurons, which can be reversed by blocker XE991.

Discussion

Neuronal Kv7/KCNQ/M channels serve as promising therapeutic targets for chronic pain and epilepsy (Wulff et al., 2009). Our recent identification of small-molecule SCR2682 shows potent and selective activation of the Kv7/KCNQ/M channel with nanomolar range of IC_{50} (9.8 nM) (Zhang et al., 2019). In the present study, we aimed at investigating whether this novel SCR2682 could alleviate chronic pain. We find that in vitro SCR2682 activates the native M-current and suppresses action potential firing of DRG neurons. In in vivo behavioral tests, SCR2682 also alleviates inflammatory and neuropathic pain in rats without affecting locomotor activity at doses of 2 mg/kg or lower, thus demonstrating the therapeutic potential of SCR2682 for chronic pain without sedative activity.

Over the past years, many small molecules, including retigabine, flupirtine, ICA-27243, and QO-58, have been reported to exhibit antinociceptive activities in various pain models through activation of Kv7/KCNQ/M channels (Munro

TABLE 1

Effect of SCR2682 on alleviation of SNI-induced neuropathic pain

SCR2682, RTG, and PGB were administrated (intraperitoneally, once a day) from day 11 to 19 in rats after SNI surgery. The PWMT (grams) was determined 1 h post–compound administration. Data were expressed as the means ± S.E.M.; n = 6–8. Comparison was made by two-way ANOVA, followed by post hoc Bonferroni's test.

Days after SNI	Paw Withdrawal Mechanical Threshold							
	Sham	Vehicle	SCR2682 (0.5 mg/kg)	SCR2682 (1 mg/kg)	SCR2682 (2 mg/kg)	SCR2682 + XE991[a]	RTG (7 mg/kg)	PGB (10 mg/k)
					g			
0	12.45 ± 0.89	13.03 ± 0.93	13.16 ± 1.65	12.50 ± 1.63	12.65 ± 1.12	12.74 ± 1.07	13.34 ± 1.16	12.52 ± 1.57
1	13.24 ± 1.06	11.19 ± 1.54	7.96 ± 1.26	8.31 ± 1.48	12.05 ± 1.82	9.28 ± 1.93	7.31 ± 1.40	11.36 ± 1.67
3	12.37 ± 0.77	6.44 ± 2.88	6.28 ± 1.25	6.04 ± 0.27	8.25 ± 1.53	5.178 ± 0.91	6.47 ± 1.52	4.77 ± 0.35
5	10.65 ± 0.94	3.51 ± 1.10	3.83 ± 0.87	3.75 ± 1.06	4.06 ± 1.05	3.55 ± 0.92	5.57 ± 1.53	4.37 ± 0.98
7	9.82 ± 1.29	2.68 ± 0.91	3.07 ± 0.78	3.18 ± 0.49	3.85 ± 0.73	2.61 ± 0.48	4.03 ± 0.86	3.66 ± 0.78
9	10.71 ± 1.46	1.83 ± 0.55	1.65 ± 0.80	1.13 ± 0.32	2.46 ± 0.31	1.31 ± 0.36	2.09 ± 0.44	1.61 ± 0.34
11	12.57 ± 0.78	0.83 ± 0.13***	2.10 ± 0.73	5.71 ± 0.59$^{\#\#\#}$	4.57 ± 0.84$^{\#\#\#}$	0.97 ± 0.07$^{\$\$}$	6.17 ± 0.68$^{\#\#\#}$	4.94 ± 1.24$^{\#\#}$
13	13.38 ± 0.66	0.82 ± 0.12***	1.71 ± 0.39	2.96 ± 0.65	4.49 ± 0.60$^{\#\#\#}$	0.98 ± 0.31$^{\$\$}$	5.28 ± 1.21$^{\#\#\#}$	5.18 ± 1.83$^{\#\#\#}$
15	12.41 ± 0.70	0.88 ± 0.20***	1.94 ± 0.40	4.12 ± 1.02$^{\#}$	6.30 ± 0.53$^{\#\#\#}$	1.19 ± 0.26$^{\$\$\$}$	6.93 ± 1.84$^{\#\#\#}$	5.73 ± 1.79$^{\#\#\#}$
17	12.92 ± 0.62	0.76 ± 0.14***	2.73 ± 0.17	3.84 ± 0.79$^{\#}$	5.22 ± 0.64$^{\#\#\#}$	1.35 ± 0.36$^{\$\$\$}$	5.85 ± 0.85$^{\#\#\#}$	5.89 ± 1.66$^{\#\#\#}$
19	11.28 ± 0.89	0.63 ± 0.13***	1.67 ± 0.45	4.67 ± 1.002$^{\#\#}$	5.50 ± 0.73$^{\#\#\#}$	1.66 ± 0.37$^{\$\$\$}$	4.94 ± 0.91$^{\#\#\#}$	5.29 ± 0.72$^{\#\#\#}$

[a]Administration of SCR2682 (2 mg/kg) plus XE991 (3 mg/kg).

$^{***}P < 0.001$ vs. sham; $^{\#}P < 0.05$; $^{\#\#}P < 0.01$; $^{\#\#\#}P < 0.001$ vs. SNI + vehicle; $^{\$\$}P < 0.01$; $^{\$\$\$}P < 0.001$ vs. SNI + SCR2682 (2 mg/kg).

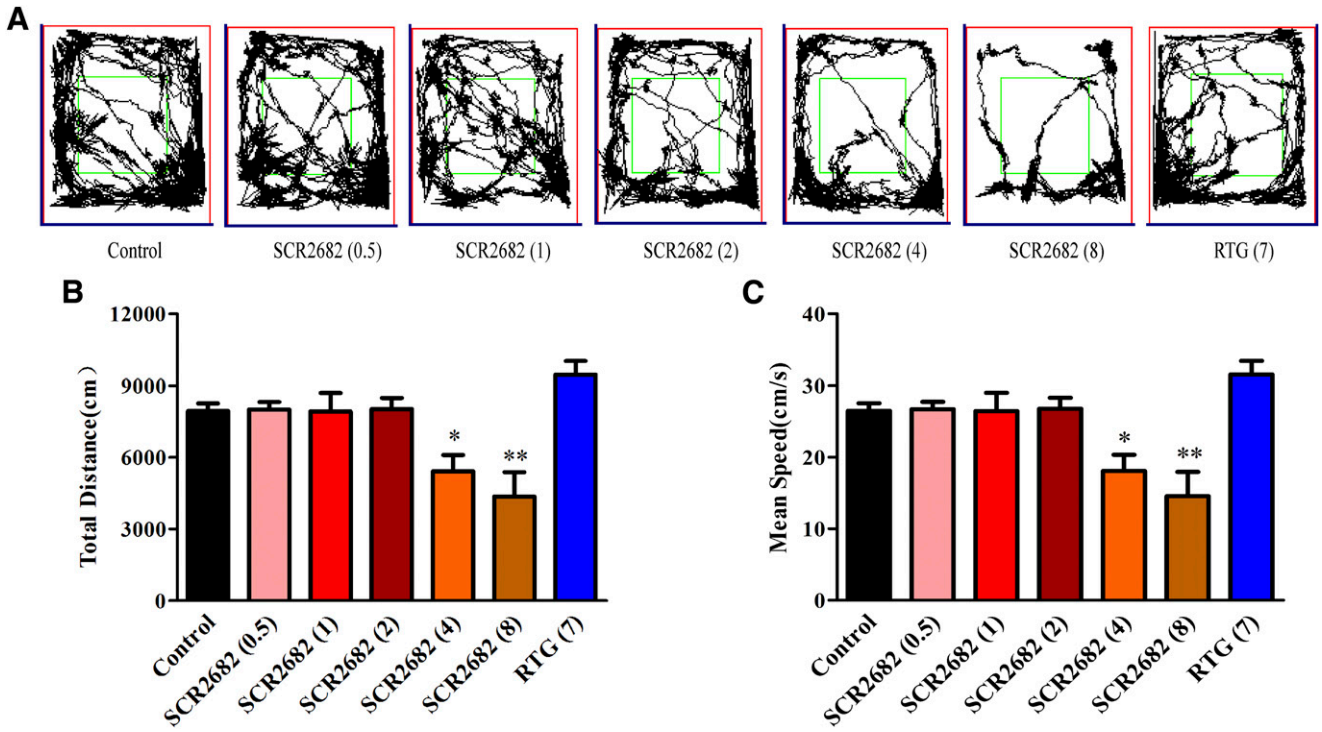

Fig. 4. Effect of SCR2682 on locomotor activity. (A) Representative traces of mice in the open field test after injections of SCR2682 (0.5, 1, 2, 4, and 8 mg/kg, i.p.) and RTG (7 mg/kg, i.p.). (B) The total distance traveled in the open field for 5 minutes after injections of RTG (7 mg/kg) or SCR2682 (0.5, 1, 2, 4, and 8 mg/kg, i.p.). (C) The mean travel speed in open field for 5 minutes after injection of RTG (7 mg/kg, i.p.) or SCR2682 (0.5, 1, 2, 4, and 8 mg/kg, i.p.). $^{*}P < 0.05$, $^{**}P < 0.01$ vs. vehicle. Data were expressed as the means ± S.E.M.; $n = 6$ to 7. Comparison was made by one-way ANOVA, followed by post hoc Bonferroni's test.

and Dalby-Brown, 2007; Zhang et al., 2013; Hayashi et al., 2014). Our SCR2682 is effective in relieving mechanical allodynia and/or thermal hyperalgesia in inflammatory or neuropathic pain in a dose-dependent manner. The antinociceptive effect of SCR2682 can be reversed by M-channel inhibitor XE991, indicating that SCR2682-mediated antinociception is directly involved in activation of Kv7 channel (Blackburn-Munro and Jensen, 2003; Hayashi et al., 2014; Cai et al., 2015; Zhang et al., 2015; Di Cesare Mannelli et al., 2017). The efficacy of SCR2682 on chronic pain is consistent with our previous report that SCR2682 is more potent in activating neuronal homomeric Kv7.2, Kv7.3, and Kv7.4 and heteromeric Kv7.2/7.3 and Kv7.3/7.5 channels than RTG (Zhang et al., 2019). The antinociceptive effect of SCR2682 can be reversed by M-channel inhibitor XE991, indicating that SCR2682-mediated antinociception is directly involved in activation of Kv7 channel (Blackburn-Munro and Jensen, 2003; Hayashi et al., 2014; Cai et al., 2015; Zhang et al., 2015; Di Cesare Mannelli et al., 2017).

Our spontaneous locomotion tests show that SCR2682 at low doses of 0.5, 1, or 2 mg/kg has no effect on altering the total travel distance. However, dosing at higher concentrations, such as 4 and 8 mg/kg, starts to reduce total travel distance and mean speed, suggesting that a sedative effect is induced by SCR2682 at high doses. It has been reported that retigabine at high doses can increase inhibitory neurotransmission through potentiation of $GABA_A$ receptor response (Otto et al., 2002), which may help explain the reduction of total travel distance caused by SCR2682 at higher doses likely through increasing GABA activity. For cardiac safety evaluation, SCR2682 exhibits a weak inhibition on cardiac hERG (human *ether-a-go-go* related gene) channels in comparison with retigabine (Zhang et al., 2019), suggesting that SCR2682 is less likely to cause liability concerns, as seen in retigabine for corrected QT interval prolongation and urinary retention (Daniluk et al., 2016).

Previous studies indicate that Kv7.2 expressions or Kv7.2-positive neurons are significantly decreased in DRG from the SNI model (Cisneros et al., 2015) or bone cancer model in rats (Rose et al., 2011; Zheng et al., 2013). The relative expression of KCNQ2 mRNA is approximately 4-fold greater than KCNQ3 or 180-fold greater than KCNQ5 (Rose et al., 2011). In line with these observations, our findings show that expression of KCNQ2 mRNA and protein in DRGs of the SNI model group is significantly lower than that of the sham group, and KCNQ2 mRNA and protein expressions are significantly increased after administration of SCR2682 or retigabine, which can be reversed by XE991. These observations suggest that SCR2682 attenuates neuropathic pain by upregulating Kv7.2 channel expression and increasing channel activity in the DRG neurons.

In conclusion, our findings show that the novel opener SCR2682 potently activates neuronal Kv7 currents and reduces excitability of DRG neurons in vitro. SCR2682 also alleviates mechanical allodynia and/or thermal hyperalgesia in models of inflammatory and persistent peripheral neuropathic pain. Therefore, Kv7 channel activator SCR2682 may have therapeutic potential for chronic pain.

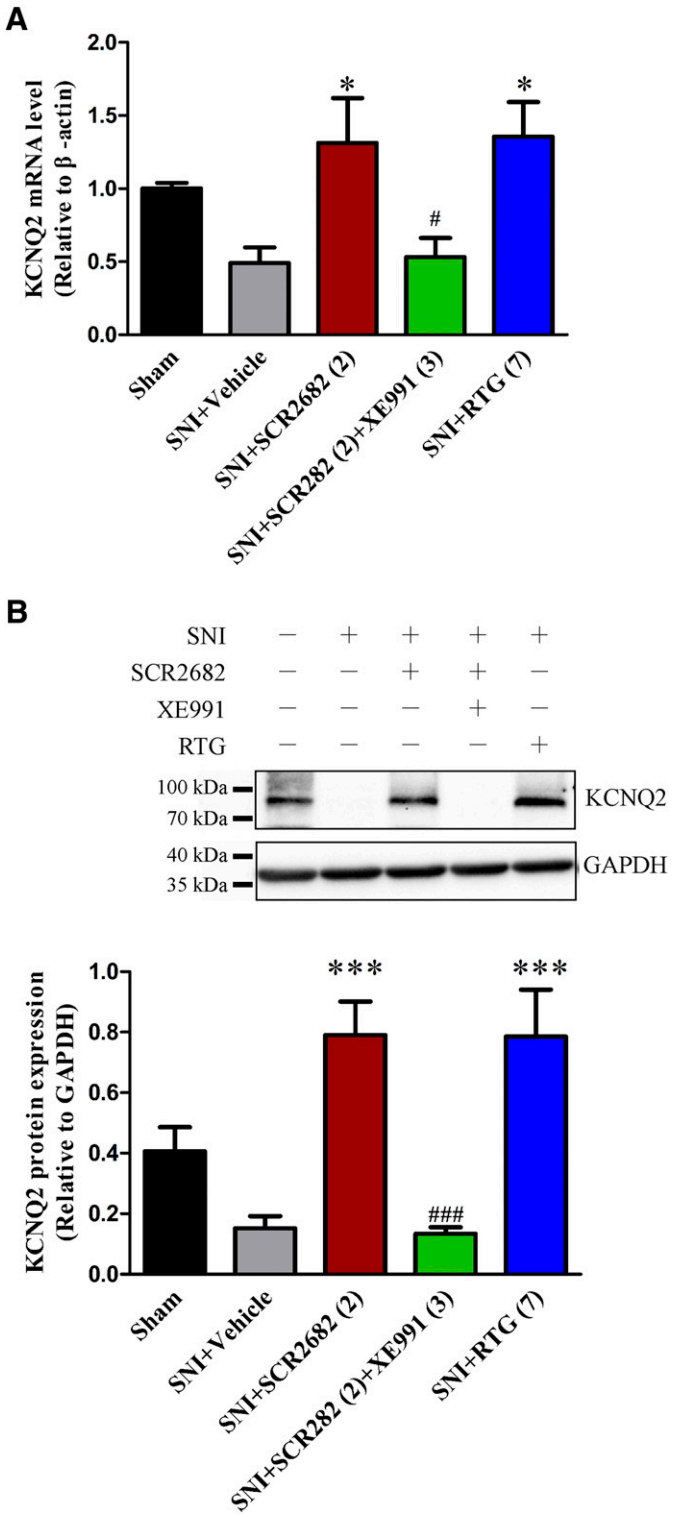

Fig. 5. Effect of SCR2682 on the expression levels of KCNQ2 in DRG of rats with neuropathic pain induced by SNI. (A) Quantification of the relative changes in KCNQ2 mRNA expression. (B) Top panel: representative Western blots of KCNQ2 and GAPDH in ipsilateral L4 and L5 DRG from 9 days after saline- and compound-injected rats. Bottom panel: quantification of the relative changes in KCNQ2 protein expression. $^{*}P < 0.05$ vs. SNI + vehicle, $^{\#}P < 0.05$ vs. SNI + SCR2682 (2 mg/kg). Data were expressed as the means ± S.E.M.; n = 6. Comparison was made by one-way ANOVA, followed by post hoc Bonferroni's test.

Authorship Contributions

Participated in research design: J. Wang, Liu, Ju, K. Wang.
Conducted experiments: J. Wang, Liu, Hu, Yang, Guo, Hou.
Performed data analysis: J. Wang, Liu.
Wrote or contributed to the writing of the manuscript: J. Wang, Liu, Ju, K. Wang.

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