P-Reticabine: An N-Propargyle Reticabine with Improved Brain Distribution and Enhanced Antiepileptic Activity

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ABSTRACT

Reticabine (RTG, [ethyl N-[2-amino-4-[(4-fluorophenyl)methyl] amino] phenyl] carbamate) is a first-in-class antiepileptic drug that acts by potentiating neuronal KCNQ potassium channels; however, it has less than optimal brain distribution. In this study, we report that P-RTG (ethyl N-[2-amino-4-][(4-fluorobenzyl)(prop-2-ynyl)amino]phenyl)carbamate), an RTG derivative that incorporates a propargyl group at the N position of the RTG linker, exhibits an inverted brain distribution compared with RTG. The brain-to-plasma concentration ratio of P-RTG increased to 2.30 compared with 0.16 for RTG. However, the structural modification did not change the drug’s potentiation potency, subtype selectivity, or RTG molecular determinants on KCNQ channels. In addition, in cultured hippocampal neurons, P-RTG exhibited a similar capability as RTG for suppressing both induced and spontaneous action potential firing. Notably, P-RTG antiepileptic activity in the maximal electroshock (MES)-induced mouse seizure model was significantly enhanced to a value 2.5 times greater than that of RTG. Additionally, the neurotoxicity of P-RTG in the rotarod test was comparable with that of RTG. Collectively, our results indicate that the incorporation of a propargyl group significantly improves the RTG brain distribution, supporting P-RTG as a promising antiepileptic drug candidate. The strategy for improving brain-to-plasma distribution of RTG might be applicable for the drug development of other central nervous system diseases.

Introduction

Epilepsy, one of the most common and serious neurologic disorders, affecting approximately 1% of the world’s population, is characterized by recurrent seizure attacks (Bialer et al., 2010; Bialer and White 2010). Epilepsy is typically managed with antiepileptic drugs (AEDs). Although there are over 20 AEDs in clinical use, approximately one-third of patients are not fully responsive and become refractory to drug treatment, despite trying multiple drugs (Brodie 2010). Consequently, it is necessary to perform further research to develop new AEDs. Reticabine (RTG, ethyl N-[2-amino-4-[(4-fluorophenyl)methyl] amino] phenyl] carbamate) is an AED that has a novel antiepileptic effect mechanism (Rundfeldt 1997; Main et al., 2000; Tatulian and Brown 2003; Gunthorpe et al., 2012). RTG was first reported in 1995 as a despydyl analog of flupirtine, a nonopioid analgesic (Kapetanovic et al., 1995). During preclinical animal testing, RTG displayed promising anticonvulsant activity in a variety of seizure models, including the maximal electroshock (MES) and pentylentetrazol-induced seizure models (Rostock et al., 1996; Bialer et al., 2010; Large et al., 2012). In 2011, RTG was approved by the U.S. Food and Drug Administration (FDA) for the treatment of adult epilepsy patients with partial-onset seizures.

The primary mechanism of action of RTG is KCNQ potassium channel potentiation, which is a different mechanism of action than employed by all other AEDs, and RTG is the first neuronal potassium channel activator approved for epilepsy treatment. KCNQ channels, or Kv7, are the seventh subfamily of voltage-gated potassium channels (Jentsch 2000; Brown and Passmore 2009). To date, five members, referred to as KCNQ1 to KCNQ5, of this family have been identified, each with a specific tissue expression pattern. KCNQ1 is primarily expressed in cardiac tissues (Barhanin et al., 1996; Sanguinetti et al., 1996). KCNQ2 to KCNQ5 are predominantly distributed throughout the central nervous system (CNS) and peripheral nerves and are therefore referred to as neuronal KCNQs. The coassembly of KCNQ2 and KCNQ3...
was thought to mediate the neuronal M-current, which fundamentally controls neuron excitability (Wang et al., 1998; Wulff et al., 2009). However, neuronal KCNQ channels have also been observed in non-neuronal cells, such as KCNQ5 expression in bladder smooth muscle cells (Hurley et al., 2006; Mackie and Byron 2008; Jin et al., 2009; McCallum et al., 2009). Because neuronal KCNQ activation may dampen membrane excitability and suppress action potential firing, the potentiation of these channels by chemical ligands is believed to be an important strategy for treating epilepsy (Surti and Jan 2005; Lawson and McKay 2006). Currently, more than 20 KCNQ activators have been reported (Schoroder et al., 2001; Wu et al., 2003, 2004a,b; Peretz et al., 2005; Xiong et al., 2008; Mruk and Kobertz 2009; Padilla et al., 2009; Gao et al., 2010). The subtype selectivity of these activators is varied. For example, R-L3 potentiates KCNQ1 but not neuronal KCNQ (Salata et al., 1998; Seebohm et al., 2003). ICA-27243 is highly selective for KCNQ2/KCNQ3 rather than KCNQ3/ KCNQ5 (Wickenden et al., 2008). RTG can potentiate all neuronal KCNQ channels but not cardiac KCNQ1, which enhances its antiepileptic effects while avoiding cardiac toxicity (Tatulian et al., 2001). It is reasonable to deduce that the antiepileptic activity of RTG occurs by their activity on CNS KCNQ channels.

For a small molecule to be a CNS drug candidate, the ability to cross the blood-brain barrier (BBB) is essential (Partridge 1998). However, RTG does not easily cross the BBB. A preliminary pharmacokinetic (PK) study undertaken by our group indicated that the RTG brain-to-plasma ratio in mice is only 0.16 (Table 1), which indicates that the RTG concentration is 5 times higher in plasma than in brain tissue. This less than ideal RTG distribution may reduce its antiepileptic activity and neurotoxicity were evaluated using in vivo studies. Our results indicate that P-RTG is a potent activator of neuronal KCNQ channels and promising AED candidate drug.

### Materials and Methods

**Synthesis of P-RTG.** Compound P-RTG was prepared according to our previously published procedure (Nan et al., 2012), where P-RTG is named K21. Prior to use, P-RTG was further purified by following procedure. To a solution of P-RTG (13.00 g) in MeOH (260 ml) was added dropwise. The resulting precipitate was collected and dried to afford a pale yellow powder (12.12 g). 1H NMR (CDCl3, 300 MHz) δ 6.75(t, J = 8.1 Hz, 2H), 6.99(t, J = 8.7 Hz, 2H), 6.26–6.31 (m, 3H), 4.44(s, 2H), 4.17(q, J = 7.2 Hz, 2H), 3.93(s, 2H), 2.22(s, 1H), 1.27(t, J = 7.2 Hz, 3H); 13C NMR (CDCl3, 75 MHz) δ 163.8, 160.5, 148.3, 142.4, 134.1, 131.1, 128.9, 128.8, 127.5, 115.7, 115.4, 106.0, 103.0, 79.7, 72.5, 61.5, 54.5, 40.1, 14.7. ESIMS m/z 342.1 [M + H]+ (calculated for C19H27FN2O2 342.16); HPLC purity: 98.53%.

**Cell Culture and Transfection.** Chinese hamster ovary (CHO) cells were grown in 50/50 Dulbecco's modified Eagle's medium/Ham's F-12 (GIBCO/Life Technologies, Grand Island, NY) with 10% fetal bovine serum (FBS), and 2 mM l-glutamine (Invitrogen/Life Technologies, Carlsbad, CA). To express KCNQ channels and mutants, cells were split at 24 hours before transfection, plated in 60-mm dishes, and transfected with Lipofectamine 2000 reagent (Invitrogen), according to the manufacturer's instructions. To express KCNQ2/ KCNQ3 channels, the cDNA ratio is 1:1. At 24 hours after transfection, cells were split and replated onto coverslips coated with poly-t-lysine (Sigma-Aldrich, St. Louis, MO). A GFP cDNA (Amaxa, Gaithersburg, MD) was cotransfected to identify the transfected cells by fluorescence microscopy. Hippocampal neuronal cultures were prepared according to the protocol described previously elsewhere (Lin et al., 2009). Briefly, hippocampal tissue was dissected out from Sprague-Dawley rats on postnatal day 0. The tissue was digested with 0.25% trypsin-EDTA (30 minutes; Gibco) and resuspended into single cells. The single-cell suspension was plated onto a monolayer of glial cells that were growing on coated coverslips. The culture medium consisted of Neurobasal medium with B-27 supplement (GIBCO), penicillin, streptomycin, and 2 mM l-glutamine. All cells were maintained at 37°C with 5% CO2 before recording.

**Electrophysiology.** To record KCNQ current in CHO cells, standard whole-cell recording was used. Pipettes were pulled from borosilicate glass capillaries (World Precision Instruments, Sarasota, FL). When filled with the intracellular solution, the pipettes have resistances of 3–5 MΩ. During the recording, constant perfusion of the extracellular solution was maintained using a bath perfusion system (ALA Scientific Instruments; Westbury, NY). The pipette solution contained (in mM) 145 KCl, 1 MgCl2, 5 EGTA, 10 HEPES, and 5 MgATP (pH 7.3); the extracellular solution contained (in mM) 140 NaCl, 3 KCl, 2 CaCl2, 1.5 MgCl2, 10 HEPES, and 10 glucose (pH 7.4). Current and voltage were recorded using an Axopatch-200B amplifier, filtered at 2 kHz, and digitized using a DigiData 1440A.

### Table 1

<table>
<thead>
<tr>
<th>Compound</th>
<th>Sample</th>
<th>Tmax (h)</th>
<th>Cmax (ng/ml/g)</th>
<th>AUC0–t (ng·h/ml/g)</th>
<th>AUC0–∞ (ng·h/ml/g)</th>
<th>MRT (h)</th>
<th>f1/2 (h)</th>
<th>Ratio of AUC0–t</th>
<th>Brain/Plasma</th>
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<td>RTG</td>
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<td>1589</td>
<td>1935</td>
<td>1943</td>
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<td>0.89</td>
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<tr>
<td></td>
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<td>7635</td>
<td>4466</td>
<td>4839</td>
<td>1.95</td>
<td>2.31</td>
<td>1.63</td>
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<td>2788</td>
<td>21088</td>
<td>34353</td>
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<td>6.99</td>
<td>3.60</td>
<td></td>
</tr>
<tr>
<td></td>
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<td>849</td>
<td>3460</td>
<td>3849</td>
<td>5.20</td>
<td>3.02</td>
<td>0.16</td>
<td></td>
</tr>
</tbody>
</table>

AUC0–∞, area under the plasma concentration-time curve from time zero to the last measurable concentrations calculated by the trapezoidal method; AUC0–t, area under the plasma concentration-time curve from time zero to infinity; Cmax, maximum observed plasma concentration; Tmax, time to reach Cmax; MRT, the mean residence time; f1/2, half-life.
with pClamp 10.2 software (Axon Instruments/Molecular Devices, Sunnyvale, CA). Series resistance compensation was also used and set to 60–80%.

For recording in hippocampus neurons, whole-cell current clamp was used. Current was injected to patched hippocampal neurons to elicit the action potential. The intracellular and extracellular solutions were the same as those used in the CHO cells.

**Mouse MES-Induced Seizure Assays.** Male KM mice weighing 20 ± 2 g were obtained from the Shanghai Slac Laboratory Animal (Shanghai, People’s Republic of China). All animal procedures were performed in accordance with the National Institutes of Health’s guide for the Care and Use of Laboratory Animals,” strictly following protocols that were approved by the institutional animal care and use committees. Male KM mice were tested in the MES assay using a physiologic and pharmacologic electronic stimulator (Jinan, Shandong, People’s Republic of China). The shock level was set at 160 V, and the duration was set at 5.4 seconds with continuous wave under the eighth configuration. The day before the experiment, the mice were prepared for the examination of the anticonvulsant effects of P-RTG and RTG by inducing generalized tonic-clonic seizures using MES. P-RTG or RTG was administered intraperitoneally or orally 30 minutes before electroshock application.

**Rotarod Test.** The rotarod test was used to compare the neurotoxic effects of RTG and P-RTG on motor coordination using a previously described method (Dunham and Miya, 1957). Mice were placed on a rotarod apparatus (Biowill, Shanghai, People’s Republic of China), which consisted of a circular rod (3-cm diameter) separated into five sections (9.5-cm width) by plastic dividers (43-cm diameter). The rod allowed five mice to be tested simultaneously. The mouse was first trained to remain on the rod while the apparatus rotated at a fixed speed of 20 rpm. Only those mice remaining on the apparatus for 300 seconds without falling were used for the experiment the next day. On the day of the experiment, 30 minutes after treatment with RTG or P-RTG, the mice were placed on the rotarod apparatus (20 rpm), and their falling latency was measured. The rotarod test was repeated 3 times for each mouse, and the average amount of time that the mice remained on the rotarod in each group was recorded.

**PK studies.** Male KM mice weighing 20 ± 2 g were used in the PK study. Animals were fasted 12 hours before and 2 hours after drug administration. In the PK studies, three mice were euthanized at each time point, and blood samples were obtained via cardiac puncture under deep isoflurane anesthesia. P-RTG or RTG was administered intraperitoneally (20 mg/kg). Blood samples were collected at 0.25, 0.5, 1, 2, 3, 5, 7, 9, and 24 hours after dosing. The heparinized test tubes with collected blood were immediately centrifuged at 3000 g for 10 minutes, and the plasma was separated and stored at −20°C until analyzed. The whole brain was anatomically removed after the animals had been euthanized, and the brain was stored at −20°C after an iced saline wash to remove residual blood. The plasma and brain levels of each compound were analyzed using liquid chromatography with magnetic spectrometry. The PK data were processed using MassLynx V4.1 and analyzed using MetaboLynx and MassFragment software (all Waters Corporation, Milford, MA).

**Fig. 1.** Molecular structures of RTG and P-RTG.

**Fig. 2.** P-RTG and RTG activation of KCNQ2 channels. Representative traces of KCNQ2 before (left) and after (right) the administration of 10 μM P-RTG (A) or RTG (B). The holding potential is −100 mV, followed by a series of depolarization steps from −90 to +50 mV with a 10-mV increment. To elicit the tail currents, a 1000-millisecond hypopolarization step to −120 mV was applied after the step stimulations. The middle panel shows the potentiation on the outward current of KCNQ2 by 10 μM P-RTG or RTG (C). (D) Voltage activation curves of KCNQ2 in the absence or presence of 10 μM P-RTG (left) or RTG (right). (D) Histograms show the ΔV1/2 of KCNQ2 before and after 10 μM P-RTG or RTG.
Data Analysis. Patch-clamp data were processed using Clampfit 10.2 (Molecular Devices, Sunnyvale, CA) and then analyzed in GraphPad Prism 5 (GraphPad Software, San Diego, CA). Voltage-dependent activation curves were fitted with the Boltzmann equation,

\[ G = G_{\text{min}} + \frac{G_{\text{max}} - G_{\text{min}}}{1 + \exp \left( V - V_{1/2} \right) / S} \]

where \( G_{\text{max}} \) is the maximum conductance, \( G_{\text{min}} \) is the minimum conductance, \( V_{1/2} \) is the voltage for reaching 50% of maximum conductance, and \( S \) is the slope factor. Dose-response curves were fitted with the Hill equation,

\[ E = E_{\text{max}} \left( \frac{C}{C_{50}} \right)^P \]

where \( E_{50} \) is the drug concentration producing half of the maximum response, and \( P \) is the Hill coefficient. Data are presented as mean ± S.E.M. Statistical significance was estimated using paired two-tailed Student’s t tests. For animal experiments, results are expressed as mean ± S.E.M. The half maximal effective dose (ED_{50}) or half maximal toxic dose (TD_{50}) in animal experiments was also determined by Hill equation. Statistical analysis was performed using one-way analysis of variance or Fisher’s exact probability test. In all cases, \( P < 0.05 \) was considered statistically significant.

Results

P-RTG Is a KCNQ2 Activator with an Improved Brain-to-Plasma Ratio. The chemical structure of P-RTG is slightly different from that of RTG. A propargyl group is incorporated at the \( N \) position of the RTG linker (Fig. 1). However, the P-RTG brain-to-plasma ratio increased to 2.30 versus 0.16 for RTG (Table 1). Subsequently, the effect of P-RTG on KCNQ2 channels was examined to evaluate its potentiation activity. Two parameters were measured: first the potentiation on outward current (\( I/I_0 \)), and second the shift of half voltage of maximal activation (\( \Delta V_{1/2} \)). Representative traces with and without P-RTG are shown in Fig. 2. We determined that 10 \( \mu \)M P-RTG effectively augmented KCNQ2 channels for both parameters. After P-RTG application, the values of \( I/I_0 \) and \( \Delta V_{1/2} \) were 1.79 and 47.1 mV, respectively. In contrast, the RTG values were 1.54 and 34.9 mV, respectively (Fig. 2, C and D). These results indicate that P-RTG is a potent KCNQ2 activator that has an improved brain distribution compared with RTG.

The P-RTG Molecular Mechanism Is the Same as RTG. RTG potentiates the homotetramers of all KCNQs, except KCNQ1 (Tatulian et al., 2001). To determine the subtype specificity of P-RTG, we individually expressed KCNQ1, KCNQ3, and KCNQ4 in CHO cells to determine their sensitivity to P-RTG. The effect of P-RTG on KCNQ5 is not presented because the KCNQ5 current we evaluated was not robust. Similar to RTG, P-RTG (10 \( \mu \)M) potentiated all tested neuronal isoforms, KCNQ3 and KCNQ4, but did not potentiate the cardiac isoform, KCNQ1 (Fig. 3 and Table 2).

Previous studies had revealed that a tryptophan residue in S5 (W236) is crucial for RTG activity and that KCNQ2 is not sensitive to RTG when this tryptophan is mutated to a leucine residue (Schenzer et al., 2005; Lange et al., 2009). Therefore, we tested the effect of P-RTG on W236L and determined that in the presence of 10 \( \mu \)M P-RTG, the outward current of W236L was no longer potentiated (Fig. 4A). The voltage-dependent activation of W236L was also not affected (Fig. 4B).

![Fig. 3. P-RTG subtype specificity for KCNQ channels. (A) Representative traces of KCNQ1, KCNQ3, and KCNQ4 in the absence (left) and presence (right) of 10 \( \mu \)M P-RTG. (B) Histograms summarize the potentiation on the outward current (\( I/I_0 \)) and \( \Delta V_{1/2} \) of KCNQ1, KCNQ3, and KCNQ4 by 10 \( \mu \)M P-RTG.](attachment:image.png)
The loss of P-RTG sensitivity in the W236L protein indicates that P-RTG requires the same molecular determinants as RTG.

We further tested this assumption by the simultaneous application of P-RTG and RTG. The preapplication of 30 μM RTG, the saturated concentration for KCNQ2 channels, caused a 1.46 ± 0.15-fold augmentation of the outward current and a −35.4 mV left shift of the activation curve. However, in the presence of 30 μM RTG, the additional application of 10 μM P-RTG did not further potentiate the outward current or shift the voltage-dependent activation curve (Fig. 4, C and D). Therefore, our data indicate that P-RTG potentiates KCNQ channels through the same molecular mechanism as RTG.

**Suppression of Neuronal Excitability by P-RTG.** The neuronal M-current is primarily mediated by heterotetramers of KCNQ2 and KCNQ3 (Wang et al., 1998). We characterized the effects of P-RTG on recombinant KCNQ2/KCNQ3 channels in CHO cells. Similar to its effect on KCNQ2, P-RTG (10 μM) significantly potentiated the outward current of KCNQ2/KCNQ3 and left-shifted the voltage-dependent activation curve (Fig. 5A). A dose-response curve analysis determined that the EC_{50} of P-RTG was 0.99 ± 0.74 μM, which is comparable with the RTG EC_{50} (0.64 ± 0.49 μM; Fig. 5B).

The loss of P-RTG sensitivity in the W236L protein indicates that P-RTG requires the same molecular determinants as RTG. The train of discharge evoked by injecting constant depolarizing current pulses (+20 pA, 200 milliseconds) in isolated pyramidal neurons was abolished after application of 10 μM P-RTG, and the activity was restored after removing the drug (Fig. 5C). Some of the recorded neurons that formed the neuronal network exhibited spontaneous firing. These firings were largely suppressed by 10 μM P-RTG application (Fig. 5D). Therefore, P-RTG dampens neuronal excitability by potentiating KCNQ channels.

**P-RTG Antiepileptic Effects.** The antiepileptic effect of P-RTG was initially evaluated in a MES-induced seizure mouse model after drug administration via intraperitoneal injection. At a dose of 10 mg/kg, the protection rate of P-RTG was 80% (n = 10, Fisher’s exact probability test, **P < 0.001). In contrast, the protection rate of RTG was only 64% (n = 10, Fisher’s exact probability test, ***P < 0.001).

The antiepileptic activity of P-RTG was further evaluated after oral administration. Protection from MES-induced seizures was achieved in 0, 10, 60, 80, and 100% of mice treated with vehicle, 2.5, 7.5, 15, and 30 mg/kg P-RTG, respectively, with statistically significant differences from the vehicle at doses of 2.5 mg/kg and higher (Fisher’s exact probability test, ***P < 0.001). The ED_{50} of P-RTG was calculated to be 6.5 mg/kg (Fig. 6A). In parallel experiments under the same conditions, the antiepileptic effects of RTG were evaluated and revealed an ED_{50} value of 15.0 mg/kg (Fig. 6A). These data suggest that P-RTG is significantly more potent in terms of antiepileptic activity than RTG.

**The Neurotoxicity of P-RTG and RTG Are Comparable in the Rotarod Test.** The rotarod test is often used to evaluate the neurotoxic activity of drugs on motor coordination.

![Fig. 4. Molecular determinants of P-RTG on KCNQ channels. (A) Representative traces of the KCNQ2 mutant W236L before and after 10 μM P-RTG administration. The holding potential is −100 mV, and the depolarization is −10 mV. (B) G-V curves of W236L with and without 10 μM P-RTG as indicated. (C) Representative traces of KCNQ2 in the presence of 30 μM RTG and 30 μM RTG and 10 μM P-RTG. The holding potential is −100 mV, and the depolarization is −10 mV. (D) Histograms showing the ΔV_{1/2} of KCNQ2 after 30 μM RTG and 30 μM RTG and 10 μM P-RTG.](image-url)
At 10 mg/kg (oral), the ED$_{50}$ concentration of P-RTG in the MES test, P-RTG did not produce any significant effects on motor coordination. The TD$_{50}$ of P-RTG was 103.4 mg/kg, which was higher than that of RTG (74.2 mg/kg; Fig. 6B). Accordingly, the protective index of P-RTG was much higher than that of RTG (Table 3).

Discussion

The field of AED discovery is at an impasse. Although there has been a profusion of new-generation AEDs and remarkable progress in the understanding of pathophysiologic processes over the past two decades, approximately one-third of patients remain refractory to the current therapies (Brodie 2010). These patients often require novel and more efficacious treatment regimens to prevent the occurrence of seizure attacks.

P-RTG was rationally designed to improve its brain-to-plasma distribution. A propargyl group was incorporated at the N position of the RTG linker. The PK parameters of P-RTG indicate that our goal was achieved (Table 1). The P-RTG blood-to-plasma concentration ratio increased to 2.30 from 0.16 for RTG. Although the half-life ($t_{1/2}$) of P-RTG in the blood is only 0.89 hour, the rapid blood clearance rate of P-RTG does not significantly affect its half-life in the brain. The $t_{1/2}$ values in the brain are 2.66 hours and 3.02 hours for P-RTG and RTG, respectively.

The propargyl group is also found in other drugs used to treat nervous system diseases, such as rasagiline, selegiline, pargyline, ladostigil, and clorgyline (Potashman and Duggan 2009; Song et al., 2013). However, the role of the propargyl group in their distribution profiles has never been discussed. We assume the incorporation of the propargyl group may improve the ability of RTG to penetrate the BBB because this group would increase the lipophilicity of the compound (Pardridge 1998).

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P-RTG potentiates all examined neuronal isoforms, KCNQ2, KCNQ3, and KCNQ4, but not the cardiac isoform KCNQ1, which indicates the same subtype selectivity as RTG (Padilla et al., 2009; Tatulian et al., 2001; Wuttke et al., 2005). The P-RTG potentiation efficacy is also similar to that of RTG. The EC$_{50}$ for the KCNQ2/3 channels is 0.99 $\mu$M, which is
similar to that of RTG (Fig. 5). A conserved tryptophan (W236) in the N-terminal region of S5 has been identified as the residue responsible for the actions of RTG on the KCNQ2 channel (Wutkie et al., 2005). The loss of sensitivity of W236L to P-RTG indicates that P-RTG requires this essential residue as well. The competitive effects of P-RTG and RTG that we observed indicate that P-RTG binds to the same sites as RTG. In the presence of saturated RTG concentrations, P-RTG did not further increase the effects on the KCNQ2 channel (Fig. 4). These results indicate that P-RTG potentiates KCNQ channels via the same mechanism as RTG.

P-RTG is more potent than RTG in terms of antiepileptic activity. In the MES-induced seizure mouse model, when the drugs were orally administered, the ED₅₀ of P-RTG was only half of the ED₅₀ of RTG. Although the $T_{\text{max}}$ of P-RTG and RTG in the brain are the same, the $C_{\text{max}}$ and AUC₀₋₅₀ of P-RTG were much higher than those of RTG. Specifically, the $C_{\text{max}}$ of P-RTG was approximately 9-fold higher than that of RTG. However, the high distribution of P-RTG in the brain did not cause more severe side effects on the nervous system. The $T_{\text{50}}$ of P-RTG in the rotator test was the same level as that of RTG. Accordingly, the protective index (PI = ED₅₀/T₅₀) of P-RTG was much higher than that of RTG, which suggests a large safety window for P-RTG (Table 3). Therefore, the increased distribution of P-RTG in the brain benefits its antiepileptic activity and may decrease potential side effects.

In summary, the less than ideal RTG brain-to-plasma distribution may reduce its antiepileptic efficacy and increase the potential for non-CNS side effects. We have developed a novel KCNQ channel activator, P-RTG, by incorporating a propargyl group into RTG. This novel activator potentiates KCNQ channels via the same underlying molecular mechanism as RTG but exhibits significantly enhanced antiepileptic activity, which may result from its improved brain-to-plasma distribution. The inversion of the brain-to-plasma distribution of a CNS drug by the incorporation of a propargyl group has been reported for the first time. Therefore, our study provides an effective strategy for enhancing the antiepileptic activity of lead compounds through improved brain-to-plasma distribution profiles. This strategy could be applied to the drug development of other CNS diseases.

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Authorship Contributions

Participated in research design: Nan, Gao.


Performed data analysis: Zhou, Zhang, Nan, Gao.

Wrote or contributed to the writing of the manuscript: Zhou, Zhang, Nan, Gao.

References


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