Inhibition of G Protein-Activated Inwardly Rectifying K⁺ Channels by the Antidepressant Paroxetine

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Abstract. Paroxetine is commonly used as a selective serotonin reuptake inhibitor for the treatment of depression and other psychiatric disorders. However, the molecular mechanisms of the paroxetine effects have not yet been sufficiently clarified. Using Xenopus oocyte expression assays, we investigated the effects of paroxetine on G protein-activated inwardly rectifying K⁺ (GIRK) channels, which play an important role in reducing neuronal excitability in most brain regions and the heart rate. In oocytes injected with mRNAs for GIRK1/GIRK2, GIRK2, or GIRK1/GIRK4 subunits, paroxetine reversibly reduced inward currents through the expressed GIRK channels. The inhibition was concentration-dependent, but voltage-independent and time-independent during each voltage pulse. However, two structurally different antidepressants: milnacipran and trazodone, caused only a small inhibition of basal GIRK currents. Additionally, Kir1.1 and Kir2.1 channels were insensitive to all of the antidepressants. Furthermore, the GIRK currents induced by activation of A₁ adenosine receptors or by ethanol were inhibited by extracellularly applied paroxetine in a concentration-dependent manner, but not affected by intracellularly applied paroxetine. Our results suggest that inhibition of GIRK channels by paroxetine may contribute partly to some of its therapeutic effects and adverse side effects.

Keywords: paroxetine, antidepressant, G protein-activated inwardly rectifying K⁺ (GIRK) channel, ethanol, Xenopus oocyte

Introduction

Paroxetine has been widely used as a selective serotonin reuptake inhibitor (SSRI) for the treatment of depression and other psychiatric disorders such as panic and obsessive-compulsive disorders, social phobia, and premenstrual dysphoric disorder (1, 2). Inhibition of serotonin (5-hydroxytryptamine, 5-HT) transporters by paroxetine in the brain is generally thought to have important implications in its therapeutic effects (1). It has also been shown that paroxetine inhibits the functions of muscarinic cholinergic receptors (3), nicotinic acetylcholine receptors (4), volume-related anion channels (5), membrane steroid transporters (6), and nitric oxide synthase (7). These actions might also be involved in the molecular and cellular mechanisms underlying some of its therapeutic effects and side effects.

Recently, we demonstrated that the SSRI fluoxetine and some tricyclic antidepressants (TCAs) inhibit G protein-activated inwardly rectifying K⁺ (GIRK) channels (also known as Kir3 channels) (8, 9). Four GIRK channel subunits in mammals have been identified as members of a family of inwardly rectifying K⁺ (Kir) channels that includes seven subfamilies (10–14). Neuronal GIRK channels are predominantly heteromultimers composed of GIRK1 and GIRK2 subunits in most brain regions (12, 15–17) or homomultimers composed of GIRK2 subunits in the substantia nigra and ventral tegmental area (18), whereas atrial GIRK channels are heteromultimers composed of GIRK1 and GIRK4 subunits (19). Various G-protein-coupled receptors, such as M₂ muscarinic, α₂ adrenergic, D₂ dopaminergic, 5-HT₁A, opioid, nociceptin/orphanin FQ, and A₁ adenosine receptors, activate GIRK channels

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(20–23) through the direct action of G protein βγ subunits (24). In addition, GIRK channels are activated by ethanol independently of G-protein-coupled signaling pathways (25, 26). Activation of GIRK channels causes membrane hyperpolarization (20). Also, GIRK2 knockout mice show spontaneous seizures (27), whereas GIRK4 knockout mice show blunted heart rate regulation and mild tachycardia (28, 29). Thus the channels play an important role in the inhibitory regulation of neuronal excitability and heart rate (20, 27, 28). Therefore, modulators of GIRK channel activity may affect many brain and cardiac functions. In contrast to the inhibitory effects of fluoxetine on GIRK channels, we also showed that among SSRIs, fluvoxamine, zimelidine, and citalopram have slight or no significant effects on GIRK channels (9, 30). The distinct effects of these SSRIs on GIRK channels may be due to the diverse chemical structures, despite a common mode of action on 5-HT function (1). The structure of paroxetine is different from that of the SSRIs. In the present study, by conducting Xenopus oocyte expression assays, we examined whether paroxetine interacts with GIRK channels. Also, the effects of two different antidepressants: milnacipran, a serotonin and norepinephrine re-uptake inhibitor, and trazodone, an atypical depressant, on GIRK channels were examined.

Materials and Methods

Preparation of specific mRNAs

Plasmids containing the entire coding sequences for the mouse GIRK1, GIRK2, and GIRK4 channel subunits and the Xenopus A1 adenosine receptor (A1R) were obtained by using the polymerase chain reaction method as described previously (15, 31, 32). In addition, cDNAs for rat Kir1.1 in pSPORT (33) and mouse Kir2.1 in pcDNA1 (34) were provided by Dr. Steven C. Hebert (Yale University, USA) and Dr. Lily Y. Jan (University of California, San Francisco, USA), respectively. These plasmids were linearized by digestion with the appropriate enzyme as described previously (31, 33, 34), and the specific mRNAs were synthesized in vitro by using the mMESSAGE mMACHINETM In Vitro Transcription Kit (Ambion, Austin, TX, USA).

Electrophysiological analysis

Adult female Xenopus laevis frogs were purchased from Copacetic (Soma, Aomori) and maintained in the laboratory until used. The frogs were anesthetized by immersion in water containing 0.15% tricaine (Sigma Chemical Co., St. Louis, MO, USA). A small incision was made on the abdomen to remove several ovarian lobes from the frogs, which were humanely killed after the final collection. Oocytes (Stages V and VI) were isolated manually from the ovary and maintained in Barth’s solution (32). Xenopus laevis oocytes were injected with mRNA(s) for GIRK1/GIRK2 or GIRK1/GIRK4 combinations (each approximately 0.3 ng), GIRK2 (approximately 2 ng), Kir1.1 (approximately 2 ng), or Kir2.1 (approximately 0.3 ng) and/or A1R (approximately 10 ng). The oocytes were incubated at 19°C in Barth’s solution, and defolliculated manually after treatment with 0.8 mg·ml−1 collagenase as described previously (32). Whole-cell currents of the oocytes were recorded from 3 to 10 days after the injection with a conventional two-electrode voltage clamp (25, 35). The membrane potential was held at −70 mV, unless otherwise specified. The micro-electrodes were filled with 3 M KCl. The oocytes were placed in a 0.05 ml narrow chamber and superfused continuously with a high-potassium (hK) solution (composition: 96 mM KCl, 2 mM NaCl, 1 mM MgCl2, 1.5 mM CaCl2, and 5 mM HEPES, pH 7.4 with KOH) or a K+-free high-sodium (ND98) solution (composition: 98 mM NaCl, 1 mM MgCl2, 1.5 mM CaCl2, and 5 mM HEPES, pH 7.4 with NaOH) at a flow rate of 2.5 ml·min−1. The stock solutions of the compounds tested were added to a perfusion solution in appropriate amounts immediately before the experiments. The magnitude of Kir currents in normal extracellular solution was very small for analyzing in the Xenopus oocyte expression assay as previously shown (10, 12, 34). Because Kir channels allow K+ ions to enter the cells much more readily than does K+ permeation in the outward direction (34), the magnitude of Kir currents is enhanced by utilizing the driving force for potassium by raising extracellular K+ to 96 mM. In the hK solution used to readily analyze Kir currents, the K+ equilibrium potential was close to 0 mV, and inward K+ current flow through Kir channels was observed at negative holding potentials, as previously shown (12, 33, 34, 36). For examining the effect of intracellular paroxetine, 23 nl of 5 mM paroxetine dissolved in distilled water was injected into an oocyte by using a Nanoliter injector (World Precision Instruments, Sarasota, FL, USA) as described previously (8), and the oocyte currents were continuously recorded for approximately 30–40 min. As the volume of the Xenopus oocytes used was approximately 1 μl, the intracellular concentration of paroxetine was estimated to be approximately 112 μM. Data were fitted to a standard logistic equation by using KaleidaGraph (Synergy Software, Reading, PA, USA) for analysis of concentration-response relationships. The EC50 value, which is the concentration of a drug that produces 50% of the maximal current response for that drug; the IC25 and IC50 values, which are the concentra-
tions of a drug that reduces control current responses by 25% and 50%, respectively; and the Hill coefficient (n_H) were obtained from the concentration-response relationships.

Statistical analyses of results
The values obtained are expressed as the mean ± S.E.M., and n indicates the number of oocytes tested. Statistical analysis of differences between groups was carried out by using the paired t-test, one-way ANOVA, or two-way factorial ANOVA followed by the Tukey-Kramer post hoc test. A probability of 0.05 was taken as the level of statistical significance.

Compounds
Paroxetine maleate and adenosine were purchased from Tocris Cookson (Bristol, UK) and Research Biochemicals International (Natick, MA, USA), respectively. Milnacipran hydrochloride and trazodone hydrochloride were purchased from Sigma. Paroxetine was dissolved in dimethyl sulfoxide (DMSO) or distilled water, and milnacipran and adenosine were dissolved in distilled water. Trazodone was dissolved in DMSO. The stock solutions of all of the compounds were stored at −30°C until used. Ethanol was purchased from Wako Pure Chemical Industries (Osaka).

Results
Concentration-dependent inhibition of GIRK channels by paroxetine
To investigate whether paroxetine interacts with GIRK channels, we conducted Xenopus oocyte expression assays. In oocytes co-injected with GIRK1 and GIRK2 mRNAs, basal GIRK currents (9), which are known to depend on free G protein \( \beta\gamma \) subunits present in the oocytes because of the inherent activity of G proteins (37), were observed under the conditions of a hK solution containing 96 mM K+ and negative membrane potentials (1680.7 ± 396.6 nA at −70 mV, n = 20, Fig. 1A). Application of 10 \( \mu \)M paroxetine immediately and reversibly caused a reduction of the inward currents through the expressed GIRK channels in the hK solution (Fig. 1A). The current responses to additional paroxetine at 100 \( \mu \)M during application of 3 mM Ba\( ^{2+} \), which blocks the Kir channel family including GIRK channels, were not significantly observed (reduction of inward currents by 3.5 ± 2.9 nA, n = 4). The 3 mM Ba\( ^{2+} \)-sensitive current components in oocytes expressing GIRK channels are considered to correspond to the magnitude of GIRK1/2 currents (9). Paroxetine produced no significant response in the K+‐free ND98 perfusion solution containing 98 mM Na+ instead of the hK solution (n = 4; data not shown), suggesting that paroxetine‐sensitive current components showed K+ selectivity. However, in oocytes expressing Kir1.1 channels, an ATP‐regulated Kir channel, or Kir2.1 channels, a constitutively active Kir channel, which belong to other Kir channel subfamilies, application of paroxetine at 300 \( \mu \)M had no significant effect on the inward currents through the channels in the hK solution (Ba\( ^{2+} \)-sensitive current components at −70 mV: 483.3 ± 49.7 nA for Kir1.1, n = 4 and 753.8 ± 182.4 nA for Kir2.1, n = 7, respectively; Figs. 1B and 2). In un‐injected oocytes, paroxetine, even at the highest concentrations tested, as well as 3 mM Ba\( ^{2+} \), caused no significant response (Fig. 1C; n = 4 and 8, respectively), suggesting no effect of paroxetine and Ba\( ^{2+} \) on intrinsic oocyte channels. In addition, application of DMSO, the solvent vehicle, at the highest concentration (0.3%) used, induced no significant current response in oocytes co‐injected with GIRK1 and GIRK2 mRNAs (n = 4, data not shown). These results suggest that paroxetine inhibited GIRK1/2 channels, but not Kir1.1 and Kir2.1
channels. Similarly, in oocytes injected with GIRK1 and GIRK4 mRNAs or GIRK2 mRNA, basal GIRK currents were observed under the same conditions; and the current components sensitive to 3 mM Ba\(^{2+}\) were 1261.3 ± 152.3 nA (n = 9) or 1079.6 ± 298.6 nA (n = 9) at −70 mV, respectively. Paroxetine also inhibited these basal GIRK1/4 and GIRK2 currents (Fig. 2), suggesting that it also inhibited GIRK1/4 channels and GIRK2 channels.

We next investigated the concentration-response relationships of the inhibitory effects of paroxetine on GIRK channels expressed in Xenopus oocytes, compared with the current components sensitive to 3 mM Ba\(^{2+}\), which fully blocked basal GIRK currents (9). Figure 2 shows that inhibition of GIRK1/2, GIRK2, and GIRK1/4 channels by paroxetine was concentration-dependent. Interestingly, GIRK1/2 heteromeric channels were inhibited by paroxetine at submicromolar concentrations or more, while GIRK2 homomeric channels and GIRK1/4 heteromeric channels were inhibited by its micromolar concentrations. Paroxetine inhibited these types of GIRK channels to a limited extent even at the high concentrations tested. Table 1 shows the EC\(_{50}\) and n\(_H\) values obtained from the concentration-response relationships and the percentage inhibition of the GIRK currents by paroxetine at the highest concentrations tested. In addition, because of the incomplete blockade of GIRK channels by paroxetine, we also calculated the drug concentrations required to inhibit the GIRK currents by 25% or 50% to further compare the effects of paroxetine on GIRK channels (Table 1). The inhibition by paroxetine of GIRK1/2 channels was more effective than those of GIRK1/4 and GIRK2 channels (P < 0.05, significant interaction between the channel effect and the effect of paroxetine, two-way factorial ANOVA; and P < 0.05, significant differences between the effects of paroxetine on GIRK1/2 channels and those on GIRK2 and GIRK1/4 channels at 3 to 30 µM, and between the effects of paroxetine on GIRK1/2 channels and those on GIRK1/4 channels at 100 and 300 µM, Tukey-Kramer post hoc test; Fig. 2 and Table 1). On the other hand, the effect of paroxetine on GIRK2 channels was statistically similar to that on GIRK1/4 channels (P > 0.05 at each concentration, Tukey-Kramer post hoc test), although inhibition of GIRK2 channels by paroxetine was less effective at 1 to 10 µM and more effective at 100 to 300 µM than that of GIRK1/4 channels.

In addition, the effects of chemically different antidepressants: milnacipran and trazodone, on GIRK channels were examined. However, milnacipran and trazodone slightly inhibited GIRK channels even at 100 µM (n = 4, respectively, Table 2). Also, Kir1.1 and

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**Table 1. Inhibitory effects of paroxetine on GIRK channels**

<table>
<thead>
<tr>
<th></th>
<th>GIRK1/2</th>
<th>GIRK2</th>
<th>GIRK1/4</th>
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<tbody>
<tr>
<td>EC(_{50})</td>
<td>9.48 ± 1.99</td>
<td>26.5 ± 4.9</td>
<td>20.8 ± 2.7</td>
</tr>
<tr>
<td>IC(_{25})</td>
<td>2.70 ± 0.48</td>
<td>13.8 ± 2.2</td>
<td>14.1 ± 2.3</td>
</tr>
<tr>
<td>IC(_{50})</td>
<td>13.8 ± 2.9</td>
<td>58.0 ± 13.3</td>
<td>203 ± 54</td>
</tr>
<tr>
<td>% max</td>
<td>83.0 ± 2.5</td>
<td>64.0 ± 6.3</td>
<td>55.8 ± 3.2</td>
</tr>
<tr>
<td>n(_H)</td>
<td>(500; 9)</td>
<td>(300; 9)</td>
<td>(300; 9)</td>
</tr>
<tr>
<td></td>
<td>0.82 ± 0.06</td>
<td>1.04 ± 0.05</td>
<td>0.86 ± 0.04</td>
</tr>
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</table>

The mean ± S.E.M. of the EC\(_{50}\) values and the concentrations required to reduce basal GIRK currents by 25% and 50% (IC\(_{25}\) and IC\(_{50}\), respectively) are shown in µM. The values of % max indicate the mean ± S.E.M.% inhibition of basal GIRK currents by paroxetine at the highest concentrations tested. The highest concentrations tested (µM) and the number of oocytes tested (n) are indicated in parentheses. The n\(_H\) values indicate the mean ± S.E.M. of Hill coefficients.

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**Table 2. Effects of milnacipran and trazodone on GIRK channels**

<table>
<thead>
<tr>
<th>Channels</th>
<th>Milnacipran (10 µM)</th>
<th>Trazodone (10 µM)</th>
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<tr>
<td>GIRK1/2</td>
<td>5.3 ± 2.9</td>
<td>13.9 ± 0.3</td>
</tr>
<tr>
<td>GIRK2</td>
<td>0.0 ± 0.0</td>
<td>6.3 ± 4.2</td>
</tr>
<tr>
<td>GIRK1/4</td>
<td>1.1 ± 1.1</td>
<td>10.5 ± 1.4</td>
</tr>
</tbody>
</table>

The values indicate the mean ± S.E.M.% inhibition of basal GIRK currents by the two antidepressants. The drug concentrations tested are indicated in parentheses.
paroxetine-sensitive inward currents in oocytes expressing GIRK1/2+ channels. Current responses were normalized to the 3 mM Ba2+ solution containing 96 mM K+. The arrow indicates the zero current level. B: Current-voltage relationships of 3 mM Ba2+-sensitive inward currents and 10 µM paroxetine-sensitive inward currents in oocytes expressing GIRK1/2 channels. Current responses were normalized to the 3 mM Ba2+-sensitive current component measured at a membrane potential of −100 mV (1932.0 ± 557.5 nA, n = 5). C: Percentage inhibition of GIRK1/2 channels by paroxetine over the voltage range of −120 to −40 mV. All values are the mean and S.E.M.

Kir 2.1 channels were insensitive to 100 µM of milnacipran (0.9 ± 0.9% and 0.2 ± 0.2% inhibition, n = 4, respectively) and trazodone (3.4 ± 3.0% and 1.0 ± 0.7% inhibition, n = 4, respectively).

Characteristics of paroxetine inhibition of GIRK channels

Next we investigated the inhibitory effects of paroxetine on GIRK channels in more detail. The instantaneous GIRK1/2 currents elicited by the voltage step to −100 mV from a holding potential of 0 mV were diminished in the presence of 10 µM paroxetine (Fig. 3A). The percentage inhibition of the steady-state GIRK current at the end of the voltage step by paroxetine was not significantly different from that of the instantaneous current (paired t-test, P > 0.05; n = 5 at −40, −60, −80, −100, and −120 mV). These results suggest that the channels were inhibited by paroxetine primarily at the holding potential of 0 mV and in a time-independent manner during each voltage pulse. Similarly, inhibition by paroxetine of the GIRK1/4 currents elicited by each voltage pulse was observed primarily at the instantaneous phase of the voltage pulse (n = 4, data not shown).

Like 3 mM Ba2+-sensitive currents corresponding to basal GIRK currents, paroxetine-sensitive currents in oocytes expressing GIRK1/2 channels increased with negative membrane potentials, and the current-voltage relationships showed strong inward rectification (n = 5, Fig. 3B), indicating a characteristic of GIRK currents. Furthermore, similar results were obtained in oocytes expressing GIRK1/4 channels (n = 4, data not shown).

The percentage inhibition of GIRK currents by 10 µM paroxetine was measured at membrane potentials between −120 and −40 mV. For GIRK1/2 channels, the percentage inhibition showed no significant difference across the voltages (P > 0.05, n = 5, one-way ANOVA; Fig. 3C). Furthermore, similar results were obtained for GIRK1/4 channels (n = 4; data not shown). These results suggest that inhibition of GIRK channels by paroxetine was voltage-independent.

At physiological pH or below, paroxetine exists mainly in a protonated form, approximately 99.7% at pH 7.4; and the proportion of the uncharged form increases with an increase in pH because of a pK value of 9.9 (5). We examined whether changes in pH would affect the inhibition by paroxetine of GIRK channels expressed in oocytes. However, no significant effect of pH on the inhibition was observed in the concentration-response relationships for paroxetine at extracellular pH 7.4 and 9.9 in oocytes expressing GIRK1/2 channels (P > 0.05, two-way factorial ANOVA; P > 0.05 at each concentration, Tukey-Kramer post hoc test, Fig. 4). The results suggest that a marked increase in the proportion of the uncharged form may not affect the inhibitory effects on GIRK channels. Furthermore, if the charged form was the only form that caused inhibition of GIRK channels, the percentage inhibition plotted as a function of the charged concentration as derived from the data points at the two pHs would be shown as the open symbols in Fig. 4. The two curves for these calculated data are not significantly different. Taken together, these results suggest that the inhibition of paroxetine may be mediated primarily by the protonated form, although there is a possibility that the uncharged form may slightly affect it. Also, it seems unlikely that the inhibition by paroxetine was caused primarily by its action on membrane lipids.

Moreover, we examined the effects of paroxetine on GIRK channels activated by a G-protein-coupled receptor. In oocytes co-expressing GIRK1/2 channels and A1Rs (32), application of 10 nM adenosine induced inward GIRK currents, and application of paroxetine alone inhibited basal GIRK currents consistently at
the concentrations tested (Fig. 5A). The effects of paroxetine were evaluated by measuring the amplitude of the adenosine-induced current response during application of paroxetine at different concentrations. The current responses to 10 nM adenosine (587.2 ± 58.3 nA) were reversibly inhibited by paroxetine with an IC$_{50}$ value of 5.0 ± 1.2 µM and an n$_H$ value of 0.77 ± 0.07 (n = 6, Fig. 5: A and B). The percentage inhibition by paroxetine was not significantly different from that of basal GIRK1/2 currents in oocytes injected with mRNAs for GIRK1 and GIRK2 subunits (P > 0.05 at each concentration, Tukey-Kramer post hoc test), suggesting that the effects of paroxetine on basally active and receptor-activated GIRK channels were similar. In addition, the adenosine-induced GIRK currents were not significantly affected by intracellularly applied paroxetine (97.5 ± 8.2% of untreated control current, paired t-test, P > 0.1, n = 4, Fig. 5C), whereas the GIRK currents were significantly inhibited by intracellularly applied lidocaine N-ethyl bromide (QX-314), as reported previously (8, 38). Moreover, in oocytes expressed with only GIRK channels, the injection of paroxetine had no significant effect on the amplitude of the basal currents (94.3 ± 5.6% of control current, paired t-test, P > 0.1, n = 6). These results indicate that intracellular paroxetine could not inhibit GIRK channels and G-proteins mediated by A1R activation. Also, it appears unlikely that the inhibition by paroxetine was caused by its action from the intracellular side. Furthermore, since the protonated form could not readily permeate the cell membrane, the main component of extracellularly applied paroxetine at physiological pH may exist in the extracellular side. Taken together, our data suggest that extracellular paroxetine inhibited the GIRK channels activated by the A1R.

GIRK channels are also activated by ethanol independently of G-protein signaling pathways (25). So we next examined the effect of paroxetine on GIRK channel activation by ethanol. In oocytes expressing GIRK1/2 channels, the GIRK currents induced by 100 mM ethanol (312.2 ± 26.4 nA) were reversibly attenuated in the presence of paroxetine, with an IC$_{50}$ value of 16.1 ± 3.8 µM and an n$_H$ value of 0.76 ± 0.11 (n = 7, Fig. 6: A and B). In addition, since the carboxyl terminal domains of GIRK channels are crucial for the ethanol sensitivity of the channel (26, 38), we examined whether intracellular paroxetine would affect ethanol activation of the GIRK channels. However, the ethanol-induced GIRK currents were not significantly affected by intra-
cellularly applied paroxetine (94.8 ± 9.3% of untreated control current, paired t-test, P > 0.1, n = 6; Fig. 6C). These results, therefore, suggest that extracellular paroxetine inhibited the GIRK channels activated by ethanol.

**Discussion**

We have demonstrated that paroxetine, a commonly used SSRI antidepressant, inhibited GIRK1/2 heteromeric channels, which exist as a major component in the brain, at submicromolar concentrations or more, and GIRK2 homomeric channels and cardiac-type GIRK1/4 heteromeric channels at micromolar concentrations, although paroxetine is structurally different from the SSRI fluoxetine and TCAs, which inhibit GIRK channels as previously shown (8, 9). The inhibition of GIRK channels by paroxetine was concentration-dependent, but voltage-independent and time-independent during each voltage pulse. The GIRK currents were not completely blocked by paroxetine even at high concentrations. Our results also suggest that paroxetine may act at the channels from the extracellular side of the cell membrane. On the other hand, blockade by extracellular Ba²⁺ and Cs⁺, typical of Kir channel blockers that occlude the pore of the open channel, shows a concentration-dependence, a strong voltage-dependence, and a time-dependence with a comparatively small effect on the instantaneous current but a marked inhibition on the steady-state current at the end of voltage pulses (12). These observations suggest that paroxetine may allosterically cause a conformational change in GIRK channels rather than blockade of the open channel. Such an action mechanism may account for the incomplete blockade of GIRK channels by paroxetine. Interestingly, the action mechanisms of paroxetine for GIRK channels are also similar to those of fluoxetine and some TCAs (8, 9). Moreover, the effects of paroxetine on the brain-type GIRK channels were more potent than those of fluoxetine and the TCAs. In addition, paroxetine similarly inhibited GIRK currents induced by basally free G-protein βγ subunits present in oocytes, by G-proteins mediated by A1R activation, or by ethanol. Further studies using single channel experiments may be useful for understanding the mechanism of the action of paroxetine and the antidepressants on GIRK channels.

Moreover, the potency and effectiveness of inhibition by paroxetine of GIRK1/2 channels were higher than those of GIRK1/4 and GIRK2 channels. Additionally, Kir1.1 and Kir2.1 channels in other Kir channel subfamilies were insensitive to paroxetine. Further studies using GIRK/Kir1.1 and GIRK/Kir2.1 chimeric channels and mutant GIRK channels may clarify the critical sites mediating the effects of paroxetine on GIRK channels. In addition, fluoxetine and TCAs, at micromolar concentrations, inhibited GIRK channels (8, 9). Among SSRIs, fluvoxamine and zimelidine had little effect on the channels; and citalopram slightly inhibited GIRK1/2 and GIRK2 channels, but not GIRK1/4 channels (9). Bupropion, an atypical antidepressant, had little effect on GIRK channels (9). In the present study, two different classes of antidepressants: milnacipran and trazodone, slightly inhibited these types of GIRK channels. The above antidepressants exhibit diverse chemical structures (1). The present results suggest that GIRK channels may also interact with agents with the structural moiety of paroxetine, a phenylpiperidine derivative, but not agents with the structure similar to those of milnacipran and trazodone. Studies on the relationship between the structures of paroxetine and drugs interacting with GIRK channels and the structures of their binding sites on the channels may provide the basis for designing novel GIRK modulators.

The plasma concentrations during treatment with paroxetine at the therapeutic doses of 20 to 50 mg daily
range from approximately 0.1 to 0.3 \( \mu \text{M} \) (1). However, paroxetine is extensively distributed into most tissues, and only 1% of the drug in the body resides in the systemic circulation (39). Indeed, the brain concentrations in patients with major depression who were taking 20 mg per day were shown to be approximately 2 to 14 \( \mu \text{M} \) by a fluorine magnetic resonance spectroscopy study (40). Therefore, the present findings suggest that neuronal GIRK channels, especially GIRK1/2 channels, may be inhibited by paroxetine at clinically relevant brain concentrations. Inhibition of the channels leads to a depolarization of the membrane potential, resulting in an increase in cell excitability (41). Therefore, paroxetine might affect some of brain functions via the inhibition of GIRK channels.

Interestingly, GIRK2 knockout mice show reduced anxiety and an increase in motor activity (42). Paroxetine has anxiolytic properties (43, 44) and enhances locomotor activity in animals (45). Indeed, paroxetine is effective in the treatment of depression and anxiety disorders (2). The therapeutic effects of paroxetine are generally thought to be primarily due to inhibition of the reuptake of serotonin in the brain (1). In the treatment of depression, comparative studies of SSRIs have shown no significant difference in efficacy between individual agents (2). However, previous studies have also shown that switching to a second SSRI including paroxetine may be a useful alternative in some depressed patients who failed to respond to the initial SSRI treatment (46, 47). Inhibition of GIRK channels by paroxetine might contribute partly to additive effects for improving anxiety and decreased activity in some neuropsychiatric disorders including depression. Additionally, the differences in efficacy of GIRK inhibition among paroxetine and other SSRIs including fluoxetine might affect their therapeutic and adverse effects.

GIRK2 knockout mice show spontaneous seizures and are more susceptible to seizures induced by pentylenetetrazol than wild-type mice (27). The resting membrane potentials of neurons in GIRK knockout mice are depolarized compared with those in wild-type mice (48, 49). The inhibition of GIRK channels leads to a depolarization of the membrane potential (41). The overall incidence of seizures during treatment with paroxetine for depression is 0.1% (2). However, the incidence of seizures in paroxetine overdose is boosted to 2% (50). High dosages of paroxetine are associated with convulsions in animals (51). The blood levels in postmortem cases involving toxicity from paroxetine overdoses are approximately 4 to 40 times higher than the upper limit of the therapeutic levels (52, 53), thereby leading to the high brain concentrations (40). Therefore, inhibition of neuronal GIRK channels by paroxetine after overdose might contribute to an increased susceptibility to seizures by causing an increase in neuronal excitability.

In the heart, acetylcholine opens atrial GIRK channels via activation of the M2 muscarinic cholinergic receptor and thereby causes slowing of the heart rate (54). Paroxetine overdoses are associated with the incidence of sinus tachycardia (50). The binding affinity of paroxetine for the receptor exhibits high nanomolar concentrations (3). The heart blood concentrations in two fatal cases after paroxetine overdoses were reported to be approximately 11 and 12 \( \mu \text{M} \) (52), although the corresponding heart tissue concentrations were not determined. In the present study, micromolar concentrations of paroxetine inhibited cardiac-type GIRK1/4 channels, which are abundantly present in the atrium (19). GIRK1 or GIRK4 knockout mice show mild tachycardia (29). Taken together, sinus tachycardia after paroxetine overdoses might be related to not only antagonism of the M2 muscarinic cholinergic receptor but also partial inhibition of atrial GIRK channels.

Paroxetine affects ethanol-related behaviors in animals (55, 56), and was shown to be effective for psychiatric patients with comorbid alcoholism (57). Interestingly, GIRK2 knockout mice show reduced ethanol-induced conditioned taste aversion and conditioned place preference (58); and they are less sensitive to some of the acute effects of ethanol, including anxiolysis, habituated locomotor stimulation and acute handling-induced convulsions, than wild-type mice (42). In the present study, paroxetine inhibited GIRK1/2 currents induced by ethanol, as also do the antidepressants fluoxetine and desipramine (8, 9). Therefore, paroxetine might suppress some of the GIRK-related ethanol effects. Further studies using GIRK knockout mice and mutant mice with GIRK channels insensitive to these antidepressants may be useful for understanding the GIRK-mediated effects of the drugs.

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