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Inhibition of G Protein-Activated Inwardly Rectifying K^+ Channels by Various Antidepressant Drugs

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G protein-activated inwardly rectifying K^+ channels (GIRK, also known as Kir3) are activated by various G protein-coupled receptors. GIRK channels play an important role in the inhibitory regulation of neuronal excitability in most brain regions and the heart rate. Modulation of GIRK channel activity may affect many brain functions. Here, we report the inhibitory effects of various antidepressants: imipramine, desipramine, amitriptyline, nortriptyline, clomipramine, maprotiline, and citalopram, on GIRK channels. In *Xenopus* oocytes injected with mRNAs for GIRK1/GIRK2, GIRK2 or GIRK1/GIRK4 subunits, the various antidepressants tested, except fluvoxamine, zimelidine, and bupropion, reversibly reduced inward currents through the basal GIRK activity at micromolar concentrations. The inhibitions were concentration-dependent with various degrees of potency and effectiveness, but voltage- and time-independent. In contrast, Kir1.1 and Kir2.1 channels in other Kir channel subfamilies were insensitive to all of the drugs. Furthermore, GIRK current responses activated by the cloned A_1 adenosine receptor were similarly inhibited by the tricyclic antidepressant desipramine. The inhibitory effects of desipramine were not observed when desipramine was applied intracellularly, and were not affected by extracellular pH, which changed the proportion of the uncharged to protonated desipramine, suggesting its action from the extracellular side. The GIRK currents induced by ethanol were also attenuated in the presence of desipramine. Our results suggest that inhibition of GIRK channels by the tricyclic antidepressants and maprotiline may contribute to some of the therapeutic effects and adverse side effects, especially seizures and atrial arrhythmias in overdose, observed in clinical practice.
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INTRODUCTION

Imipramine and other similar compounds: desipramine, amitriptyline, nortriptyline, and clomipramine, were the first successful antidepressants and have been widely used for the treatment of depression and other psychiatric disorders, such as panic and obsessive–compulsive disorders, bulimia nervosa, and chronic pain disorder (Baldessarini, 2001). Owing to their chemical structures with a three-ring molecular core, they are referred to as the tricyclic antidepressants. Later, a series of newer antidepressants have been developed: tetracyclic antidepressants, for example, maprotiline; atypical antidepressants, for example, bupropion; and selective serotonin reuptake inhibitors (SSRIs) such as fluoxetine, fluvoxamine, zimelidine, and citalopram (Baldessarini, 2001). Inhibition of norepinephrine and/or serotonin (5-hydroxytryptamine (5-HT)) transporters by antidepressants in the brain is generally thought to have important implications in their therapeutic effects (Baldessarini, 2001). In contrast, the interaction of antidepressants with muscarinic, α_1 adrenergic, and H_1 histamine receptors is involved in some of the adverse side effects (Baldessarini, 2001). It has also been shown that antidepressants inhibit the functions of several other receptors and ion channels, such as 5-HT_{2C} (Ni and Miledi, 1997) and 5-HT_3 receptors (Fan, 1994), nicotinic acetylcholine receptors (García-Colunga *et al*, 1997; Maggi *et al*, 1998), *N*-methyl-D-aspartate (NMDA) receptor channels (Sernagor *et al*., 1989), $P2X_2$ receptors (Nakazawa *et al*, 1999), voltage-gated Ca^{2+}, Na^+, and K^+ channels (Ogata *et al*, 1989; Mathie *et al*, 1998; Pancrazio *et al*, 1998; Teschemacher *et al*, 1999; Yeung *et al*, 1999; Deák *et al*, 2000; Choi *et al*, 2001; Cuellar-Quintero *et al*, 2001), Ca^{2+}-activated K^+ channels (Kamatchi and Ticku, 1991; Lee *et al*, 1997; Dreixler *et al*, 2000; Terstappen *et al*, 2001), and Cl^- channels (Maertens *et al*, 1999, 2002). The effects might also be involved in the molecular and cellular mechanisms underlying some of the therapeutic effects and side effects of various antidepressants.

G protein-activated inwardly rectifying K^+ (GIRK) channels (also known as Kir3 channels) are members of a family of inward-rectifier K^+ (Kir) channels that include

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seven subfamilies (Doupnik *et al*, 1995; Reimann and Ashcroft, 1999). Four GIRK channel subunits have been identified in mammals (Kubo *et al*, 1993b; Lesage *et al*, 1995; Wickman *et al*, 1997). Neuronal GIRK channels are predominantly heteromultimers composed of GIRK1 and GIRK2 subunits in most brain regions (Kobayashi *et al*, 1995; Lesage *et al*, 1995; Karschin *et al*, 1996; Liao *et al*, 1996) or homomultimers composed of GIRK2 subunits in the substantia nigra (Inanobe *et al*, 1999), whereas atrial GIRK channels are heteromultimers composed of GIRK1 and GIRK4 subunits (Krapivinsky *et al*, 1995). Various G protein-coupled receptors, such as M_2 muscarinic, α_2 adrenergic, D_2 dopaminergic, 5-HT_{1A}, opioid, nociceptin/orphanin FQ and A_1 adenosine receptors, activate GIRK channels (North, 1989; Ikeda *et al*, 1995, 1996, 1997) through direct action of G protein $\beta\gamma$ subunits (Reuveny *et al*, 1994). In addition, ethanol activates GIRK channels independent of G protein-coupled signaling pathways (Kobayashi *et al*, 1999; Lewohl *et al*, 1999). Activation of GIRK channels causes membrane hyperpolarization, and thus the channels play an important role in the inhibitory regulation of neuronal excitability and heart rate (North, 1989; Signorini *et al*, 1997; Wickman *et al*, 1998). Therefore, modulators of GIRK channel activity may affect many brain and cardiac functions. Using the *Xenopus* oocyte expression system, we demonstrated that various antipsychotic drugs including thioridazine and clozapine and an SSRI antidepressant drug, fluoxetine, inhibited GIRK channels (Kobayashi *et al*, 1998, 2000, 2003). Imipramine, a tricyclic antidepressant of the dibenzazepines, is chemically similar to the antipsychotic drugs thioridazine, a phenothiazine, and clozapine, a dibenzodiazepine. Therefore, we hypothesized that tricyclic antidepressants might interact with GIRK channels. In the present study, we examined the effects of various antidepressants including tricyclic antidepressants on brain-type GIRK1/2 and GIRK2 channels and cardiac-type GIRK1/4 channels by using the *Xenopus* oocyte expression assay.

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* A_1 adenosine (XA1) receptor, were obtained by using the polymerase chain reaction method as described previously (Kobayashi *et al*, 1995, 2000, 2002). In addition, cDNAs for rat Kir1.1 in pSPORT and mouse Kir2.1 in pcDNA1 were provided by Dr Steven C Hebert and Dr Lily Y Jan, respectively. These plasmids were linearized by digestion with an appropriate enzyme as described previously (Ho *et al*, 1993; Kubo *et al*, 1993a; Kobayashi *et al*, 2000), and the specific mRNAs were synthesized *in vitro* by using the mMESSAGE mMACHINE^{TM} *In vitro* Transcription Kit (Ambion, Austin, TX, USA).

Electrophysiological Analyses

Adult female *Xenopus laevis* frogs were purchased from Copacetic (Soma, Aomori, Japan) and maintained in the laboratory until used. 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 that were humanely killed after the final collection. Oocytes (Stages V and VI) were isolated manually from the ovary and maintained in Barth's solution (Kobayashi *et al*, 2002). *Xenopus laevis* oocytes were injected with mRNA(s) for GIRK1/GIRK2 or GIRK1/GIRK4 combinations (each $\sim$0.4 ng), GIRK2 ($\sim$5 ng), Kir1.1 ($\sim$5 ng) or Kir2.1 ($\sim$0.5 ng) and/or XA1 ($\sim$10 ng). The oocytes were incubated at 19°C in Barth's solution, and defolliculated following treatment with 0.8 mg ml^{-1} collagenase as described previously (Kobayashi *et al*, 2002). Whole-cell currents of the oocytes were recorded from 2 to 10 days after the injection with a conventional two-electrode voltage clamp (Kobayashi *et al*, 1999; Ikeda *et al*, 2003). The membrane potential was held at $-$70 mV, unless otherwise specified. Microelectrodes 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 in mM: KCl 96, NaCl 2, $MgCl_2$ 1, $CaCl_2$ 1.5, and HEPES 5, pH 7.4 with KOH) or a K^+-free high-sodium (ND98) solution (composition in mM: NaCl 98, $MgCl_2$ 1, $CaCl_2$ 1.5, and HEPES 5, pH 7.4 with NaOH) at a flow rate of 2.5 ml min^{-1}. For examining the effect of intracellular desipramine, 23 nl of 30 mM desipramine or 30 mM lidocaine *N*-ethyl bromide (QX-314) dissolved in distilled water was injected into an oocyte by using a Nanoliter injector (World Precision Instruments, Sarasota, FL, USA) as described previously (Kobayashi *et al*, 2003), and the oocyte currents were continuously recorded for approximately 30–40 min. Owing to a volume of $\sim$1 µl in the oocyte, the intracellular concentration of desipramine was presumed as $\sim$674 µM. In the hK solution, the K^+ equilibrium potential (E_K) was close to 0 mV, and inward K^+ current flow through Kir channels was observed at negative holding potentials. Data were fitted to a standard logistic equation by using KaleidaGraph (Synergy Software, Reading, PA, USA) for analysis of concentration–response relationships. The EC_{50} value, which is the concentration of a drug that produces 50% of the maximal current response for that drug, the IC_{25} and IC_{50} values, which are the concentrations 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 Analysis of Results

The values obtained are expressed as the mean $\pm$ SEM, and n is the number of oocytes tested. Statistical analysis of differences between groups was carried out by using paired *t*-test, Student's *t*-test, one-way ANOVA or two-way factorial ANOVA, followed by Bonferroni/Dunn *post hoc* test. A probability of 0.05 was taken as the level of statistical significance.

Compounds

All the antidepressants tested, except fluvoxamine (Tocris Cookson, Bristol, UK), and adenosine were purchased from Research Biochemicals Inc (Natick, MA, USA). Maprotiline,

fluvoxamine, and citalopram were dissolved in dimethyl sulfoxide (DMSO). Other drugs were dissolved in distilled water. The stock solutions of all of the compounds were stored at −30°C until used. Ethanol was purchased from Wako Pure Chemical Industries (Osaka, Japan). Each compound was added to the perfusion solution in appropriate amounts immediately before the experiments.

RESULTS

Inhibition of GIRK Channels by Antidepressants

To investigate whether various antidepressants interact with brain-type GIRK1/2 and cardiac-type GIRK1/4 channels, we conducted *Xenopus* oocyte expression assays. In oocytes co-injected with GIRK1 and GIRK2 mRNAs, basal GIRK currents, which are known to depend on free G protein $\beta\gamma$ subunits present in the oocytes because of the inherent activity of G proteins (Dascal, 1997), were observed under the conditions of a hK solution containing 96 mM K^+ and negative membrane potentials (Kobayashi *et al*, 2003; Figure 1a). Application of 300 μM imipramine, desipramine, amitriptyline or nortriptyline, tricyclic antidepressants, immediately and reversibly caused a reduction of the inward currents through the expressed GIRK channels in the hK solution (Figure 1a). The current responses were abolished in the presence of 3 mM Ba^{2+}, which blocks the Kir channel family including GIRK channels ($n=3$; data not shown). None of the antidepressants produced a significant response in the K^+-free ND98 solution containing 98 mM Na^+ instead of the hK solution ($n=3$; data not shown), suggesting that the antidepressant-sensitive current components show K^+ selectivity. In uninjected oocytes, the antidepressants tested, even at the highest concentrations used, and 3 mM Ba^{2+} caused no significant response (Figure 1c; $n=4$ and 10, respectively), suggesting no effect of the antidepressants and Ba^{2+} on intrinsic oocyte channels. These results suggest that the tricyclic antidepressants inhibit GIRK1/2 channels. In addition, since the magnitudes of inhibition of basal GIRK currents by 3 mM Ba^{2+} were almost equal to those by 5 mM Ba^{2+} in oocytes expressing GIRK channels, the 3 mM Ba^{2+}-sensitive current components (1048.6 ± 60.2 nA, $n=66$) were considered to correspond to the magnitudes of GIRK1/2 currents. Maprotiline, which is a tetracyclic antidepressant related structurally to tricyclic antidepressants, also inhibited basal GIRK currents by 44.8 ± 4.9% at 100 μM ($n=8$), whereas citalopram, an SSRI, and bupropion had a small or no effect on the currents (21.7 ± 2.9% inhibition and 4.7 ± 0.6% inhibition of the 3 mM Ba^{2+}-sensitive current component at 100 μM, $n=6$ and 3, respectively). In addition, application of DMSO, the solvent vehicle, at the highest concentration (0.3%) used had no significant effect on the current responses in oocytes co-injected with GIRK1 and GIRK2 mRNAs ($n=4$; data not shown). Similarly, in oocytes co-injected with GIRK1 and GIRK4 mRNAs (Figures 1b), basal GIRK currents were observed under the same conditions, and the current components sensitive to 3 mM Ba^{2+} were 880.2 ± 71.3 nA ($n=64$). The tricyclic antidepressants tested and maprotiline inhibited basal GIRK1/4 currents (Figures 1b, 2b), suggesting that the antidepressants also inhibit GIRK1/4 channels. However, citalopram and bupropion had little or no effect on basal GIRK1/4 currents (6.5 ± 1.7% inhibition and 3.7 ± 3.2% inhibition of the 3 mM Ba^{2+}-sensitive current component at 100 μM, $n=3$ and 8, respectively).

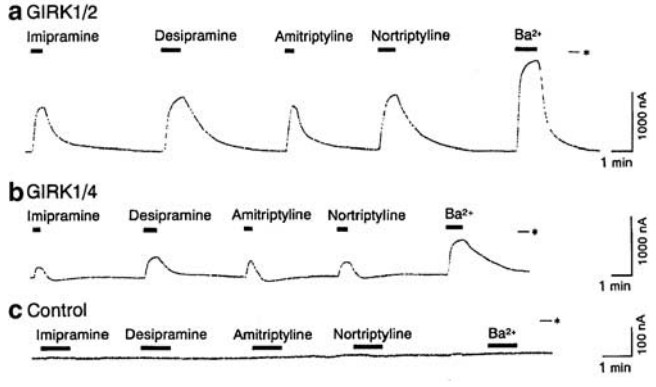

Figure 1 Inhibition by tricyclic antidepressants of brain-type GIRK1/2 channels and cardiac-type GIRK1/4 channels expressed in *Xenopus* oocytes. (a) In an oocyte co-injected with GIRK1 and GIRK2 mRNAs, current responses to imipramine, desipramine, amitriptyline, nortriptyline, and 3 mM Ba^{2+} are shown. The concentration of each antidepressant tested was 300 μM. (b) In an oocyte co-injected with GIRK1 and GIRK4 mRNAs, current responses to 100 μM of the same antidepressants as in (a) and 3 mM Ba^{2+} are shown. (c) In an uninjected oocyte, no significant current responses to 300 μM of the same antidepressants as in (a) and 3 mM Ba^{2+} are shown. Current responses were measured at a membrane potential of −70 mV in a high-potassium solution containing 96 mM K^+. Asterisks show the zero current level. Bars show the duration of application.

We next investigated the concentration–response relationship of the inhibitory effects of the various antidepressants on GIRK channels expressed in *Xenopus* oocytes, compared with the current components sensitive to 3 mM Ba^{2+}, which fully blocked basal GIRK currents (Kobayashi *et al*, 2002). Figure 2 shows that inhibition of GIRK1/2 and GIRK1/4 channels by the antidepressants is concentration-dependent with distinctive potency and effectiveness at micromolar concentrations. All the antidepressants inhibited both types of GIRK channels to a limited extent even at high concentrations. Table 1 shows the EC_{50} and n_H values obtained from the concentration–response relationships for the antidepressants tested and the percentage inhibition of the GIRK currents by the drugs at the highest concentrations tested. The rank order of the inhibition of GIRK1/2 and GIRK1/4 channels by 100 μM of these drugs was as follows: desipramine > clomipramine ⩾ imipramine > nortriptyline = amitriptyline ⩾ maprotiline ⩾ citalopram ⩾ bupropion for GIRK1/2 channels and desipramine > clomipramine ⩾ amitriptyline > maprotiline ⩾ nortriptyline > imipramine ⩾ citalopram = bupropion for GIRK1/4 channels. To further compare the effects of the antidepressants on GIRK channels, we also calculated the drug concentrations required to inhibit the GIRK currents by 25 or 50% (Table 1). Among these antidepressants, desipramine, a tricyclic antidepressant, was the most effective inhibitor of GIRK1/2 and GIRK1/4 channels. The effects of imipramine and clomipramine on GIRK1/2 channels were similar, and those of amitriptyline, nortriptyline, and maprotiline were similar on GIRK1/2 channels and GIRK1/4 channels. The effects of desipramine, amitriptyline, and maprotiline

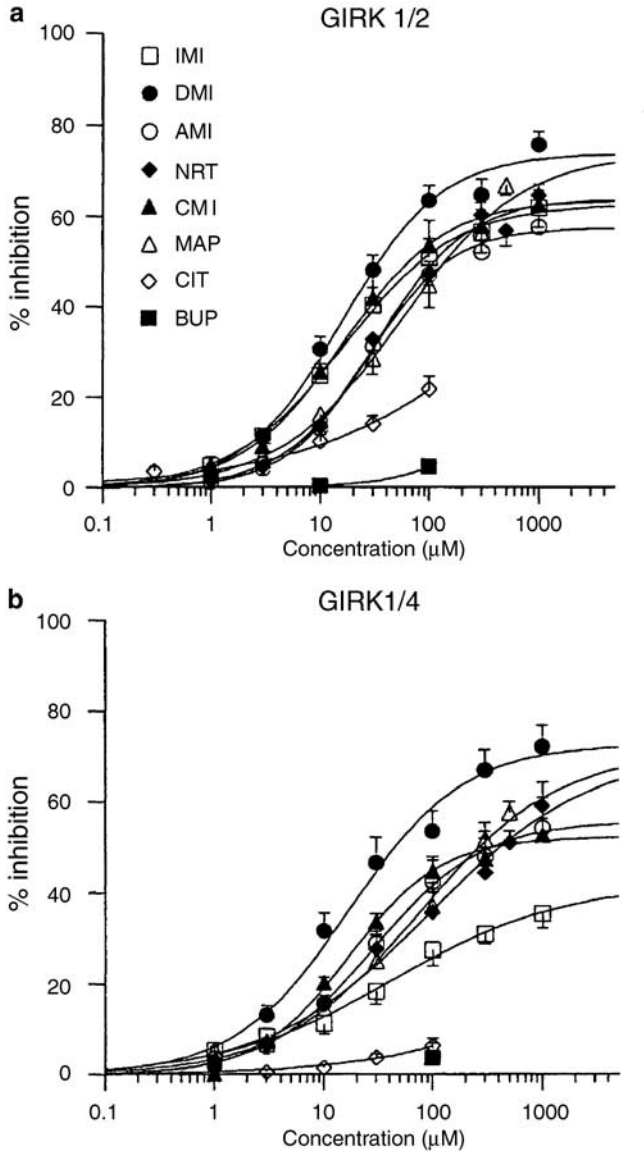

Figure 2 Concentration–response relationships for various antidepressants: imipramine (IMI), desipramine (DMI), amitriptyline (AMI), nortriptyline (NRT), clomipramine (CMI), maprotiline (MAP), citalopram (CIT), and bupropion (BUP), with regard to their effects on GIRK1/2 channels (a) and GIRK1/4 channels (b). The magnitudes of inhibition of GIRK current by antidepressants were compared with the 3 mM Ba^{2+}-sensitive current components, which were 1048.6 ± 60.2 nA ($n = 66$) in oocytes expressing GIRK1/2 channels and 880.2 ± 71.3 nA ($n = 64$) in oocytes expressing GIRK1/4 channels. Current responses were measured at a membrane potential of −70 mV in a high-potassium solution containing 96 mM K^+. Each point and error bar represents the mean and SEM of the percentage responses obtained from 3–14 oocytes. Data points were fitted by using a logistic equation.

on GIRK1/2 channels were similar to those on GIRK1/4 channels, respectively, although inhibition of GIRK1/2 channels by maprotiline at high concentrations was more effective than that of GIRK1/4 channels. In addition, inhibition of GIRK1/2 channels by imipramine, nortriptyline, clomipramine, and citalopram was more effective than that of GIRK1/4 channels, respectively (Figure 2 and Table 1).

Although GIRK channels in the brain are predominantly GIRK1/2 heteromultimers (Liao *et al*, 1996), GIRK channels in the substantia nigra and ventral tegmental area are GIRK2 homomultimers (Karschin *et al*, 1996; Inanobe *et al*, 1999). To further address the functional relationship between various antidepressants and neuronal GIRK channels, we investigated the effects of the antidepressants on the homomeric channels (Figure 3). The inhibitory effects of the tricyclic antidepressants used and maprotiline on GIRK2 channels in oocytes expressing GIRK2 channels were less effective than those on GIRK1/2 channels ($p < 0.01$, significant interaction between the channel effect and the effect of the antidepressants, two-way factorial ANOVA; and $p < 0.01$, significant differences between the effects of each antidepressant on GIRK1/2 and GIRK2 channels, Bonferroni/Dunn *post hoc* test). For SSRIs, the inhibitory effect of citalopram on GIRK2 channels was similar to that on GIRK1/2 channels ($23.9 \pm 6.9\%$ inhibition of the 3 mM Ba^{2+}-sensitive current component at 100 μM, $n = 4$), whereas fluvoxamine and zimelidine had little effect on GIRK2 channels ($5.3 \pm 2.7\%$ inhibition and $2.9 \pm 0.3\%$ inhibition of the 3 mM Ba^{2+}-sensitive current component at 100 μM, $n = 3$ and 3, respectively), as were the cases for GIRK heteromeric channels (Kobayashi *et al*, 2003). In addition, bupropion had little effect on GIRK2 channels ($3.9 \pm 1.8\%$ inhibition of the 3 mM Ba^{2+}-sensitive current component at 100 μM, $n = 4$).

Furthermore, we examined whether the various antidepressants could interact with Kir1.1, an ATP-regulated inwardly rectifying K^+ channel, and Kir2.1, a constitutively active inward rectifier K^+ channel, in other Kir channel subfamilies (Figure 3). In oocytes expressing Kir1.1 or Kir2.1 channels, application of each antidepressant at 100 μM had no significant effect on the inward currents through the channels in the hK solution ($n \geqslant 3$ for Kir1.1 and $n = 3$ for Kir2.1).

Characteristics of Antidepressant Inhibition of GIRK Channels

As the various tricyclic antidepressants tested acted as inhibitors at GIRK channels in the present study, we further investigated the effect of the tricyclic antidepressants, particularly desipramine, in more detail. The instantaneous GIRK1/2 currents elicited by the voltage step to −100 mV from a holding potential of 0 mV diminished in the presence of 100 μM desipramine (Figure 4a). The percentage inhibition of the steady-state GIRK current at the end of the voltage step by desipramine was not significantly different from that of the instantaneous current (paired *t*-test, $p > 0.05$; $n = 4$ at −80, −100, and −120 mV, respectively). These results suggest that the channels were inhibited by desipramine primarily at the holding potential of 0 mV in a time-independent manner. Furthermore, similar results were obtained for imipramine, amitriptyline, nortriptyline, and clomipramine (data not shown).

Like 3 mM Ba^{2+}-sensitive currents corresponding to basal GIRK currents, desipramine-sensitive currents in oocytes expressing GIRK channels increased with negative membrane potentials, and the current–voltage relationships showed strong inward rectification (Figure 4b), indicating a characteristic of GIRK currents. Furthermore, similar

Table 1 Inhibitory Effects of Various Antidepressant Drugs on GIRK Channels

Compound	Imipramine	Desipramine	Amitriptyline	Nortriptyline	Clomipramine	Maprotiline	Citalopram	Bupropion
GIRK1/2								
EC_{50}	22.3±2.8	24.2±3.8	30.8±2.2	50.5±12.8	18.0±6.7	47.1±10.1	ND	ND
IC_{25}	18.4±3.8	11.7±1.9	24.4±3.5	23.6±3.3	17.6±8.7	36.6±10.6	ND	ND
IC_{50}	47.0±10.5	36.4±7.6	110.8±12.7	132.3±32.7	39.8±15.6	113.0±14.3	ND	ND
% max	62.0±4.0	75.9±2.9	57.7±3.8	64.8±7.2	62.8±4.5	67.0±2.0	21.7±2.9	4.7±0.6
(μM; *n*)	(1000; 14)	(1000; 10)	(1000; 8)	(1000; 8)	(1000; 9)	(500; 8)	(100; 6)	(100; 3)
n_H	0.83±0.07	0.89±0.05	1.10±0.04	1.06±0.10	1.10±0.12	0.70±0.02	ND	ND
GIRK1/4								
EC_{50}	30.4±9.9	26.3±6.3	33.4±12.0	84.1±3.9	24.0±3.8	70.8±16.6	ND	ND
IC_{25}	84.8±32.0	9.4±1.6	37.3±14.7	46.8±4.4	18.8±2.5	46.2±12.1	ND	ND
IC_{50}	ND	53.9±17.1	274.3±16.5	392.9±122.7	253.5±69.6	290.5±93.3	ND	ND
% max	35.5±3.2	72.2±4.8	54.3±6.3	59.2±5.3	52.8±3.6	57.6±2.4	6.5±1.7	3.7±3.2
(μM; *n*)	(1000; 8)	(1000; 9)	(1000; 10)	(1000; 10)	(1000; 8)	(500; 8)	(100; 3)	(100; 8)
n_H	0.98±0.13	0.88±0.09	1.07±0.09	0.89±0.09	1.02±0.08	0.83±0.07	ND	ND

The mean±SEM of the EC_{50} values and the drug concentrations required to reduce basal GIRK currents by 50 and 25% (IC_{50} and IC_{25}) are shown in μM. The values of % max indicate the mean±SEM% inhibition of basal GIRK currents by the drugs at the highest concentrations tested. The highest concentrations tested (μM) and the number of oocytes tested (*n*) are indicated in parantheses. The n_H values indicate the mean±SEM of the Hill coefficients. ND means that the value was not determined because of a low effectiveness of the drug.

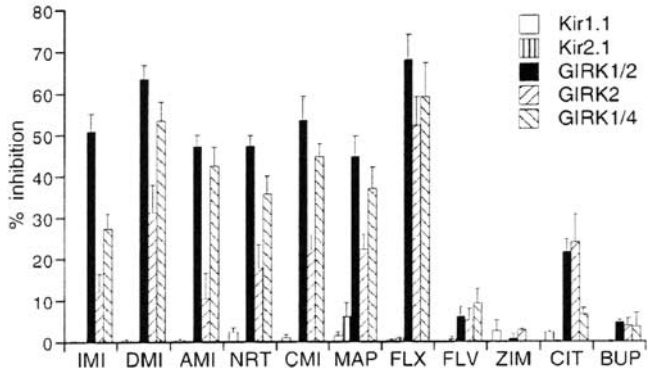

Figure 3 Comparison of the effects of various antidepressants: imipramine (IMI), desipramine (DMI), amitriptyline (AMI), nortriptyline (NRT), clomipramine (CMI), maprotiline (MAP), fluoxetine (FLX), fluvoxamine (FLV), zimelidine (ZIM), citalopram (CIT), and bupropion (BUP), on GIRK, Kir1.1 and Kir2.1 channels. The concentration of each antidepressant tested was 100 μM. The magnitudes of inhibition of Kir currents by the various antidepressants were compared with the 3 mM Ba^{2+}-sensitive current components in oocytes expressing Kir1.1, Kir2.1, GIRK1/2 channels, GIRK2 channels or GIRK1/4 channels (949.3±234.1 nA, $n=6$; 1452.2±274.7 nA, $n=26$; 1168.2±80.4 nA, $n=84$; 493.6±30.2 nA, $n=40$; 961.4±90.6 nA, $n=70$, respectively). Data on FLX are cited from our previous report (Kobayashi *et al*, 2003). The bars represent the means and SEM of the percentage responses obtained from 3–20 oocytes. Current responses were measured at a membrane potential of −70 mV in a high-potassium solution.

results were obtained for imipramine, amitriptyline, nortriptyline, clomipramine, and maprotiline (data not shown).

The percentage inhibition of GIRK currents by 100 μM desipramine was measured at membrane potentials between −100 and −20 mV. For GIRK1/2 and GIRK1/4 channels, the percentage inhibition showed no significant difference across the voltages ($p>0.05$, one-way ANOVA; Figure 4c). These results suggest that inhibition of GIRK channels by desipramine was voltage-independent. Furthermore, similar results were obtained for imipramine, amitriptyline, nortriptyline, clomipramine, and maprotiline (data not shown).

At physiological pH or below, desipramine exists mainly in a protonated form, and the proportion of the uncharged form increases with an increase in pH, because of a pK_a value of 10.2 (Green, 1967). We examined whether changes in pH affect the inhibition by desipramine of GIRK channels expressed in oocytes prepared from the same donor. For GIRK1/2 channels, no effect of pH on the inhibition was observed in the concentration–response relationships for desipramine ($p>0.05$ at each concentration, $n=5$, Student's *t*-test, Figure 5). For GIRK1/4 channels, similar results were obtained at 30 and 100 μM desipramine ($p>0.05$, $n=4$, Student's *t*-test). These results suggest that the inhibition may be mediated by both forms of desipramine with almost the same effectiveness. It also appears unlikely that the inhibition by desipramine is caused by hydrophobic interactions with GIRK channels within the membrane bilayer.

Moreover, we examined the effects of desipramine on GIRK channels activated by G protein-coupled receptors. In oocytes co-expressing GIRK1/2 channels and XA1 receptors (Kobayashi *et al*, 2002), application of 10 nM adenosine induced inward GIRK currents (Figure 6a). The effects of desipramine were evaluated by measuring the amplitude of the adenosine-induced current response during application of desipramine at different concentrations. The current responses to 10 nM adenosine were reversibly inhibited by desipramine with an IC_{50} value of 38.6±8.7 μM and an n_H value of 0.76±0.06 ($n=6$, Figure 6a, b). The percentage inhibition by desipramine was similar to that of basally active GIRK1/2 channels ($p>0.1$ at each concentration,

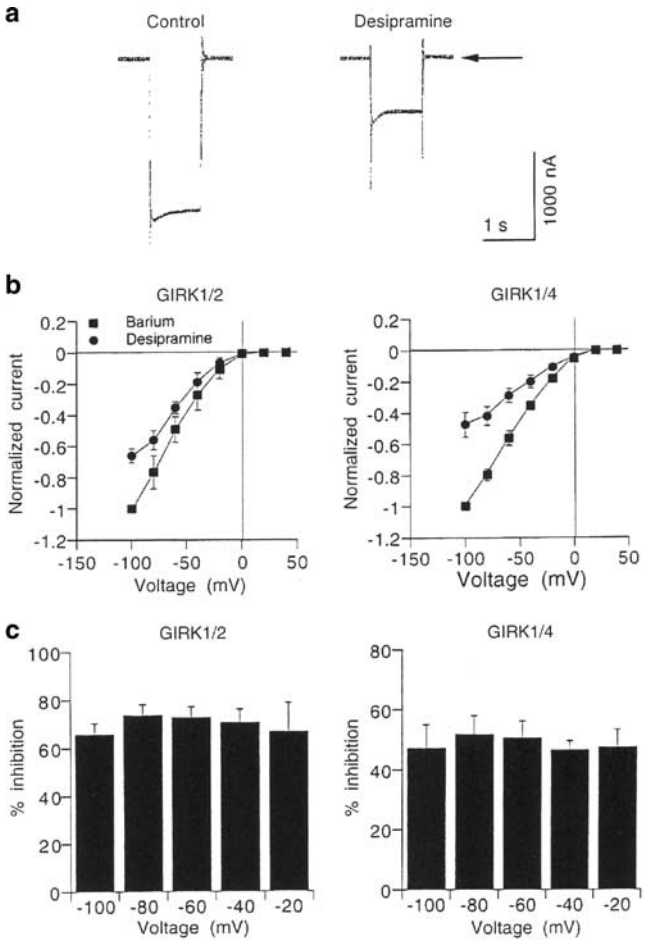

Figure 4 Characteristics of the inhibitory effects of desipramine on GIRK currents. (a) Representative GIRK1/2 currents elicited by a voltage step to −100 mV for 1 s from a holding potential of 0 mV in the absence and presence of 100 μM desipramine. Current responses were recorded in a high-potassium solution containing 96 mM K^+. Arrow indicates the zero current level. (b) Current–voltage relationships of 3 mM Ba^{2+}-sensitive inward currents and 100 μM desipramine-sensitive inward currents in oocytes expressing GIRK1/2 channels or GIRK1/4 channels. Current responses were normalized to the 3 mM Ba^{2+}-sensitive current component measured at a membrane potential of −100 mV. The Ba^{2+}-sensitive current components were 1019.4 ± 162.4 nA ($n = 4$) in oocytes expressing GIRK1/2 channels and 615.0 ± 103.2 nA ($n = 4$) in oocytes expressing GIRK1/4 channels. (c) Percentage inhibition of GIRK channels by desipramine over the voltage range of −100 to −20 mV. There was no significant interaction between the desipramine effect and the membrane potential effect ($p > 0.05$ for GIRK1/2, $n = 4$ for each group, and $p > 0.05$ for GIRK1/4, $n = 4$ for each group; one-way ANOVA). All values are the mean and SEM.

Student *t*-test), suggesting the interaction of desipramine with GIRK channels. In addition, the adenosine-induced GIRK currents were not significantly affected by intracellularly applied desipramine (90.8 ± 10.6% of pretreated control current, paired *t*-test, $p > 0.1$, $n = 5$, Figure 6c), whereas the GIRK currents were significantly inhibited by intracellularly applied QX-314 as reported previously (Zhou *et al*, 2001; Kobayashi *et al*, 2003). The results, therefore, suggest that extracellular desipramine can directly inhibit GIRK channels activated by the XA1 receptors.

GIRK channels are also activated by ethanol independent of G protein signaling pathways (Kobayashi *et al*, 1999). We

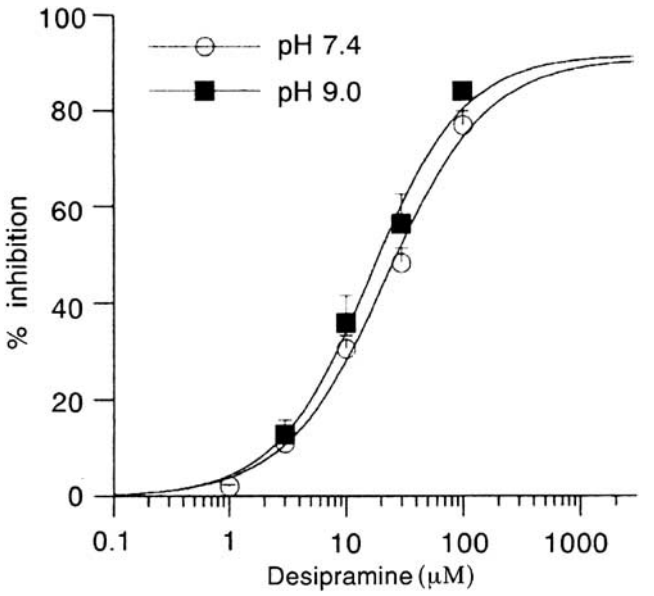

Figure 5 Concentration–response relationships for inhibition of GIRK channels by desipramine at different pH values. The magnitudes of inhibition by desipramine of GIRK current in oocytes expressing GIRK1/2 channels were compared with the 3 mM Ba^{2+}-sensitive current components, which were 865.3 ± 61.0 nA at pH 7.4 ($n = 5$) and 927.4 ± 72.6 nA at pH 9.0 ($n = 5$). Current responses were measured at a membrane potential of −70 mV in a high-potassium solution. Each point and error bar represents the mean and SEM of the percentage responses obtained. Data points were fitted by using a logistic equation.

next examined the effect of desipramine on ethanol-induced GIRK currents. In oocytes expressing GIRK1/2 channels, the GIRK currents induced by ethanol were attenuated in the presence of desipramine, with an IC_{50} value of 34.6 ± 13.4 μM and an n_H value of 1.10 ± 0.08, in a reversible manner (62.3 ± 4.9% inhibition at 100 μM, $n = 5$; Figure 7). The results, therefore, suggest that desipramine can inhibit GIRK channels activated by ethanol.

DISCUSSION

We demonstrated that all of the tricyclic antidepressants tested, namely, desipramine, imipramine, amitriptyline, nortriptyline, and clomipramine, inhibited brain-type GIRK1/2 and GIRK2 channels and cardiac-type GIRK1/4 channels at micromolar concentrations in a distinctive manner. Furthermore, maprotiline, which also possesses a similar tricyclic structure, inhibited GIRK channels. These results indicate that tricyclic moiety of these antidepressants may be involved in GIRK channel inhibition. Among SSRIs, fluoxetine significantly inhibited GIRK channels even at low micromolar levels, whereas fluvoxamine and zimelidine had little effect on the channels (Kobayashi *et al*, 2003). In the present study, citalopram weakly inhibited brain-type GIRK1/2 and GIRK2 channels, but not cardiac-type GIRK1/4 channels. These distinctive effects on GIRK channels may be due to the diverse chemical structures of SSRIs. In addition, both types of GIRK channels were insensitive to bupropion, an atypical antidepressant, suggesting that the drugs with the chemical

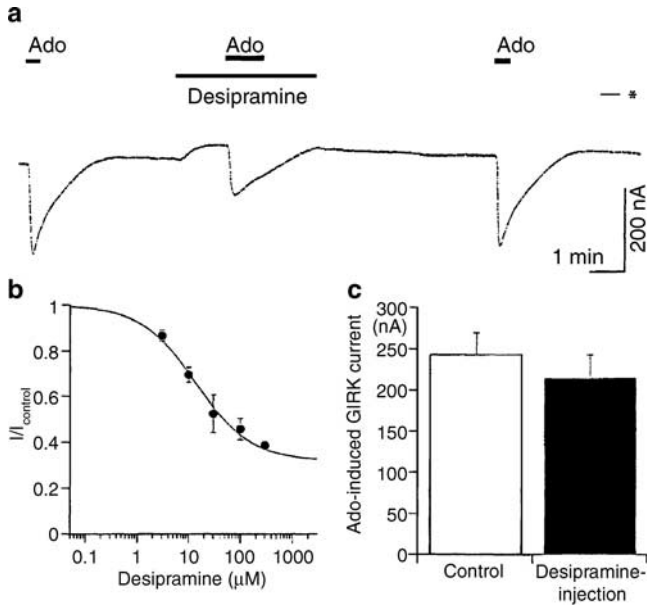

Figure 6 Inhibitory effect of desipramine on GIRK channels activated by a G protein-coupled receptor. (a) In an oocyte co-injected with mRNAs for GIRK1 and GIRK2 channels and XA1 receptor, current responses to 10 nM adenosine (Ado), 10 nM Ado in the presence of 30 μM desipramine and 10 nM Ado are shown. Bars show the duration of application. Asterisk indicates the zero current level. (b) Concentration-dependent inhibition by desipramine of Ado-induced GIRK currents. $I_{control}$ is the amplitude of GIRK currents induced by 10 nM Ado (213.8 ± 30.3 nA, $n = 6$) and I is the current amplitude in the presence of desipramine. Data points were fitted by using a logistic equation. (c) Lack of effect of intracellular desipramine on Ado-induced GIRK currents. There was no significant difference in Ado-induced GIRK currents between the groups before and after intracellular injection of desipramine ($p > 0.05$, $n = 5$, paired t-test). Current responses were measured at a membrane potential of −70 mV in a high-potassium solution. All values are the mean and SEM.

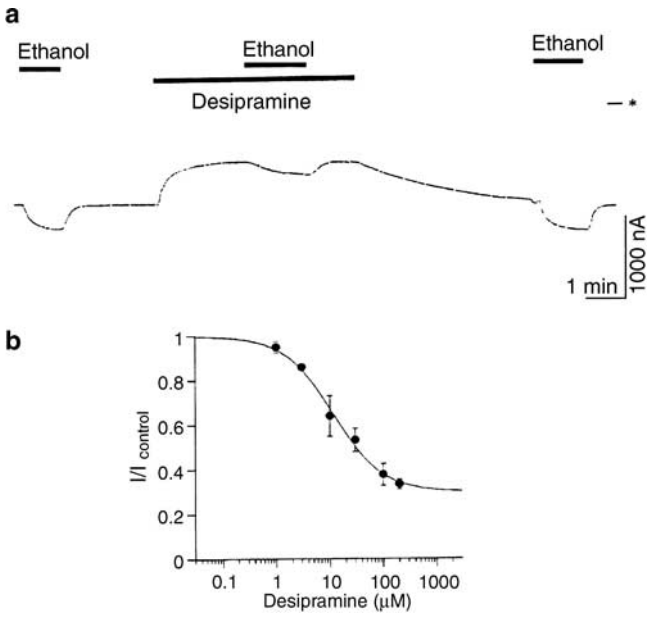

Figure 7 Inhibitory effect of desipramine on the ethanol-induced GIRK currents in *Xenopus* oocytes expressing GIRK1/2 channels. (a) In an oocyte co-injected with GIRK1 and GIRK2 mRNAs, current responses to 100 mM ethanol, 100 mM ethanol in the presence of 30 μM desipramine, and 100 mM ethanol are shown. Asterisk indicates the zero current level. Bars show the duration of application. (b) Concentration-dependent inhibition by desipramine of ethanol-induced GIRK currents. $I_{control}$ is the amplitude of GIRK currents induced by 100 mM ethanol (309.0 ± 24.3 nA, $n = 5$) and I is the current amplitude in the presence of desipramine. Current responses were measured at a membrane potential of −70 mV in a high-potassium solution. Each point and error bar represents the mean and SEM of the relative responses. Data points were fitted by using a logistic equation.

structure similar to bupropion may have no significant effect on GIRK channels.

The inhibition of GIRK channels by the tricyclic and tetracyclic antidepressants tested was concentration-dependent but voltage- and time-independent. The GIRK currents were not completely blocked by the antidepressants even at high concentrations. Our results also suggest that desipramine, a tricyclic antidepressant, acts at the channels from the extracellular side of the cell membrane. On the other hand, blockade by extracellular Ba^{2+} 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 (Lesage *et al*, 1995). These observations suggest that the antidepressants may probably cause a conformational change in the channels, but not act as open channel blockers of Kir channels like Ba^{2+} and Cs^{+}. The difference in action mechanism between the antidepressants and Ba^{2+} may be involved in the incomplete blockade of GIRK channels by the antidepressants. In addition, the inhibitory effects of the tricyclic and tetracyclic antidepressants tested on GIRK2 homomeric channels were weaker than those on GIRK1/2 heteromeric channels. Moreover, Kir1.1 and Kir2.1 channels among members of the Kir channel family were insensitive to all of the antidepressants. 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 the antidepressants on GIRK channels.

The therapeutic plasma concentrations of various antidepressants range approximately from 0.7 to 1.1 μM for imipramine, 0.47 to 1.1 μM for desipramine, 0.36 to 0.9 μM for amitriptyline, 0.23 to 0.57 μM for nortriptyline, 0.48 to 1.6 μM for clomipramine, 0.7 to 1.4 μM for maprotiline, and 0.23 to 0.46 μM for citalopram (Baldessarini, 2001). The plasma concentrations after their overdoses become significantly higher than those in the therapeutic use (Prouty and Anderson, 1990; Barbey and Roose, 1998; Rosenstein *et al*, 1993). As most antidepressants have highly lipophilic properties, they accumulate in the brain (Prouty and Anderson, 1990): namely, the brain-to-plasma ratios are approximately 13−20:1 for imipramine (Besret *et al*, 1996), 15−25:1 for desipramine (Baumann *et al*, 1983), 10−35:1 for amitriptyline (Glotzbach and Preskorn, 1982; Baumann *et al*, 1984; Miyake *et al*, 1990), 8−15:1 for nortriptyline (Baumann *et al*, 1984; Miyake *et al*, 1990), 10−48:1 for clomipramine (Friedman and Cooper, 1983; Eschalier *et al*, 1988), 44−59:1 for maprotiline (Miyake *et al*, 1991), and 4−8:1 for citalopram (Kugelberg *et al*, 2001). In addition, the mean brain concentrations of the

tricyclic antidepressants in postmortem humans were approximately 20 times higher than the corresponding blood levels (Prouty and Anderson, 1990). Therefore, the present findings suggest that GIRK channels in the brain may be inhibited by the various tricyclic antidepressants and maprotiline at clinically relevant brain concentrations.

Interestingly, GIRK2-deficient mice showed reduced anxiety and an increase in motor activity (Blednov *et al*, 2001). Although it is generally thought that the therapeutic effects of antidepressants are primarily due to inhibition of the reuptake of norepinephrine and/or serotonin in the brain, inhibition of neuronal GIRK channels by the antidepressants may contribute to additive therapeutic effects for depression and some psychiatric disorders. Also, antidepressant drugs have various side effects, including sedation, tremor, seizures, orthostatic hypotension, tachycardia, and weight gain (Baldessarini, 2001). Among these side effects, the incidence of seizures is a serious side effect. The risk of seizures for most antidepressants increases with the dose (Rosenstein *et al*, 1993; Barbey and Roose, 1998; Baldessarini, 2001). The mean plasma concentrations of tricyclic antidepressants in several patients who experienced seizures following overdoses were reported to be approximately eight times higher than those in the therapeutic doses (Boehnert and Lovejoy, 1985; Rosenstein *et al*, 1993). Therefore, the tricyclic antidepressants and maprotiline at the brain levels after the overdoses may potently inhibit neuronal GIRK channels. As opposed to these drugs, citalopram, which only slightly inhibits GIRK channels even at toxic concentrations, has a lower seizure risk (Barbey and Roose, 1998). The inhibition of GIRK channels leads to depolarize the membrane potential, resulting in an increase in neuronal excitability (Kuzhikandathil and Oxford, 2002). In addition, GIRK2-deficient mice show spontaneous seizures (Signorini *et al*, 1997). Therefore, potent inhibition of neuronal GIRK channels by some antidepressants may contribute to the cause of the neuropsychiatric toxicity, particularly seizures.

Antidepressant drugs including tricyclic antidepressants and SSRIs show analgesic activity (Messing *et al*, 1975; Lin *et al*, 1980; Schreiber *et al*, 1996; Korzeniewska-Rybicka and Plaznik, 2000; Galeotti *et al*, 2001). Studies using GIRK2-deficient mice and *weaver* mutant mice, which have mutant GIRK2 channels insensitive to G proteins and ethanol, have shown that the analgesic effects induced by opioids or ethanol are remarkably reduced in these mice, suggesting that the GIRK channel activation induces analgesia (Kobayashi *et al*, 1999; Ikeda *et al*, 2000, 2002; Blednov *et al*, 2003; Mitrovic *et al*, 2003). However, our studies have demonstrated that various tricyclic antidepressants and fluoxetine (Kobayashi *et al*, 2003) inhibit GIRK channels. Therefore, the analgesic effects of these antidepressants may not be mediated by GIRK channels. On the other hand, it has been shown that the analgesic effects of various antidepressant drugs may be caused by interaction with other several targets: voltage-gated Na^+ channels (Pancrazio *et al*, 1998) and voltage-gated, ATP-sensitive and Ca^{2+}-activated K^+ channels (Galeotti *et al*, 2001), by the action of 5-HT (Messing *et al*, 1975; Lin *et al*, 1980; Tura and Tura, 1990) and by opioid mechanisms (Reichenberg *et al*, 1985).

In the heart, acetylcholine released from the stimulated vagus nerve opens atrial GIRK channels via activation of the M_2 muscarinic acetylcholine receptor, and ultimately causes slowing of the heart rate (Brown and Birnbaumer, 1990). Sinus tachycardia during tricyclic antidepressant treatment is frequently observed (Baldessarini, 2001). Various tricyclic antidepressants exhibit nanomolar affinity for the receptor (Stanton *et al*, 1993). The present study demonstrated that micromolar concentrations of various tricyclic antidepressants and maprotiline inhibited cardiac-type GIRK1/4 channels. The heart concentrations of the tricyclic antidepressants are approximately 20–200 times higher than the corresponding blood levels (Elonen *et al*, 1975; Jandhyala *et al*, 1977). No exact heart-to-plasma ratio for maprotiline has been reported. However, because maprotiline has a similar tricyclic structure, it may also highly accumulate in the heart. Therefore, atrial GIRK channels may also be inhibited by the antidepressants in clinical practice. Taken together, sinus tachycardia during the treatment with antidepressants may be related to not only antagonism of the M_2 muscarinic acetylcholine receptor, but also inhibition of atrial GIRK channels. Moreover, toxic plasma levels after overdoses of tricyclic antidepressants are associated with cardiac side effects including atrial premature contractions and supraventricular tachycardia (Frommer *et al*, 1987). Cardiac-type GIRK1/4 channels are abundantly present in the atrium of the heart (Krapivinsky *et al*, 1995). Therefore, greater inhibition of atrial GIRK channels by the antidepressants after overdoses may contribute to the incidence of these atrial arrhythmias in cardiac toxicity. In contrast, the cardiac side-effects of bupropion and citalopram in clinical practice are uncommon (Baldessarini, 2001). These drugs have weak antimuscarinic properties (Baldessarini, 2001) and no significant effect on GIRK1/4 channels (Figure 2b). Therefore, the minimal cardiotoxic effects of bupropion and citalopram may be partly attributable to their lack of any significant effect on atrial GIRK channels, together with their weak antimuscarinic effects.

Desipramine, a norepinephrine reuptake inhibitor, reduces ethanol consumption like SSRIs including fluoxetine (Murphy *et al*, 1985; Naranjo and Knoke, 2001), suggesting a relation between the antidepressants and ethanol effects. Recently, we demonstrated that fluoxetine inhibited ethanol-induced GIRK1/2 currents (Kobayashi *et al*, 2003). The present study also demonstrates that desipramine at micromolar concentrations can inhibit GIRK1/2 currents induced by ethanol. However, GIRK2-deficient mice displayed no significant change in ethanol consumption (Blednov *et al*, 2001). Further studies using mice treated with antisense oligodeoxynucleotides for the GIRK mRNAs, mice lacking other GIRK subunit(s) in addition to the GIRK2 subunit and mutant mice with GIRK channels insensitive to ethanol alone may help to advance the understanding of the antagonism by desipramine of ethanol-induced GIRK effects *in vivo* as well as the understanding of the functions of GIRK channels.

Lastly, our electrophysiological studies have clarified the functional actions of various psychotropic drugs on GIRK channels using *Xenopus* oocyte expression assays (Kobayashi *et al*, 1999, 2000, 2003). Recent studies using GIRK2-deficient mice suggested that GIRK activators may be useful for the treatment of epilepsy and pain (Signorini *et al*, 1997; Ikeda *et al*, 2002; Blednov *et al*, 2003). In addition, since

GIRK knockout mice show decreased cocaine self-administration (Morgan *et al*, 2003), selective GIRK channel inhibitors might be potential agents for the treatment of abusers of cocaine. Therefore, as GIRK channels are considered candidates for clinically relevant targets, it may be important to clarify the pharmacological and physiological effects of various agents interacting with GIRK channels.

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