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Inhibition by various antipsychotic drugs of the G-protein-activated inwardly rectifying K^+ (GIRK) channels expressed in *Xenopus* oocytes

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1 To investigate the effects of various chemical classes of antipsychotic drugs: haloperidol, thioridazine, pimozide and clozapine, on the G-protein-activated inwardly rectifying K^+ (GIRK) channels, we carried out *Xenopus* oocyte functional assays with GIRK1 and GIRK2 mRNAs or GIRK1 and GIRK4 mRNAs.

2 In oocytes co-injected with GIRK1 and GIRK2 mRNAs, application of each of the various antipsychotic drugs immediately caused a reduction of inward currents through the basally active GIRK channels. These responses were not observed in the presence of 3 mM Ba^{2+}, which blocks the GIRK channels. In addition, in uninjected oocytes, none of the drugs tested produced any significant current response. These results indicate that all the antipsychotic drugs tested inhibited the brain-type GIRK1/2 heteromultimeric channels. Furthermore, similar results were obtained in oocytes co-injected with GIRK1 and GIRK4 mRNAs, indicating that the antipsychotic drugs also inhibited the cardiac-type GIRK1/4 heteromultimeric channels.

3 All the drugs tested inhibited, in a concentration-dependent manner, both types of GIRK channels with varying degrees of potency and effectiveness at micromolar concentrations. Only pimozide caused slight inhibition of these channels at nanomolar concentrations.

4 We conclude that the various antipsychotic drugs acted as inhibitors at the brain-type and cardiac-type GIRK channels. Our results suggest that inhibition of both types of GIRK channels by these drugs underlies some of the side effects, in particular seizures and sinus tachycardia, observed in clinical practice.

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Keywords: Antipsychotic drug; haloperidol; thioridazine; pimozide; clozapine; G-protein-activated inwardly rectifying K^+ (GIRK) channel: *Xenopus* oocyte

Abbreviations: CNS, central nervous system; DMSO, dimethyl sulphoxide; E_K, K^+ equilibrium potential; G$\beta\gamma$, G protein $\beta\gamma$-subunits; GIRK, G-protein-activated inwardly rectifying K^+ channel; hK, high-potassium; 5HT, 5-hydroxytryptamine; ND98, K^+-free high-sodium; n_H, Hill coefficient; NMDA, *N*-methyl-D-aspartate; PCR, polymerase chain reaction; XIR, endogenous *Xenopus* oocyte GIRK-related polypeptides

Introduction

Antipsychotic drugs are widely used in the treatment of psychiatric disorders, especially schizophrenia (Baldessarini, 1996). They belong to diverse chemical classes including phenothiazines, butyrophenones, diphenylbutylpiperidines and dibenzodiazepines, such as thioridazine, haloperidol, pimozide and clozapine, respectively (Reynolds, 1992; Baldessarini, 1996). The antipsychotic drugs have been shown to interact with dopaminergic, serotonergic, adrenergic, histaminergic receptors and several other types of receptors (Reynolds, 1992; Seeman & Van Tol, 1994). They also modulate the functions of voltage-gated Ca^{2+} and K^+ channels (Gould *et al*., 1983; Ogata *et al*., 1989) and *N*-methyl-D-aspartate (NMDA) receptor channels (Ilyin *et al*., 1996). Although blockade of dopaminergic receptors by antipsychotic drugs is thought to have important implications in the therapeutic actions and extrapyramidal side effects of antipsychotic drugs (Reynolds, 1992; Seeman & Van Tol, 1994), the cellular and molecular mechanisms underlying the clinical efficacy and side effects of various antipsychotic drugs remain poorly understood.

G-protein-activated inwardly rectifying K^+ (GIRK) channels are gated directly by G protein $\beta\gamma$-subunits (G$\beta\gamma$) (Reuveny *et al*., 1994). They are activated by various G-protein-coupled receptors, such as the M_2 muscarinic, D_2 dopaminergic, α_2 adrenergic, 5-hydroxytryptamine$_{1A}$ (5-HT$_{1A}$), μ-, δ- and κ-opioid and nociceptin/orphanin FQ receptors (North, 1989; Ikeda *et al*., 1995; 1996; 1997). Activation of the channels plays an important role in the inhibitory regulation of the excitability of neuronal cells and the heart rate (North, 1989; Brown & Birnbaumer, 1990; Signorini *et al*., 1997; Wickman *et al*., 1998). The cDNAs for the four GIRK channel subunits have been cloned from mammalian tissues (Doupnik *et al*., 1995; Kobayashi *et al*., 1995; Lesage *et al*., 1995; Wickman *et al*., 1997). Recent studies have indicated that neuronal GIRK channels are heteromultimers composed of GIRK1 and GIRK2 subunits (Kobayashi *et al*., 1995; Lesage *et al*., 1995; Karschin *et al*., 1996; Liao *et al*., 1996) and atrial GIRK channels are heteromultimers composed of GIRK1 and GIRK4 subunits (Krapivinsky *et al*., 1995). Using the *Xenopus*

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oocyte expression system, we recently demonstrated that clozapine, an atypical antipsychotic, inhibits GIRK channels (Kobayashi *et al*., 1998) composed of GIRK1 subunits and endogenous *Xenopus* oocyte GIRK-related polypeptides (XIR) (Hedin *et al*., 1996). In the present study, we examined the effects of various chemical classes of antipsychotic drugs on the brain-type GIRK1/2 and cardiac-type GIRK1/4 heteromultimeric channels using the *Xenopus* oocyte expression system. We demonstrated that a variety of antipsychotic drugs: thioridazine, haloperidol, pimozide and clozapine, acted as inhibitors at both the brain-type and cardiac-type GIRK heteromultimeric channels.

Methods

Preparation of specific mRNAs

Plasmid containing the entire coding sequence for the mouse GIRK1 subunit was obtained using the polymerase chain reaction (PCR) method as described previously, and designated as pSPGIRK1 (Kobayashi *et al*., 1995). Based on the cDNA sequences for the mouse GIRK2 (Patil *et al*., 1995) and GIRK4 subunits (Wickman *et al*., 1997), pairs of oligonucleotide primers corresponding to the regions containing either a translational initiation codon or a stop codon were synthesized. Primers for GIRK2 were 5′-GCAAGCTTATGACAATGGCCAAGTTAAC-3′ and 5′-GCTCTAGAATCACCCATTCCTCTCCGTC-3′, and those for GIRK4 were 5′-GCGATATCATGGCCGGTGATTCTAGGAA-3′ and 5′-GCTCTAGATTCACATTGAGCCCCT-3′. PCR was performed using C57BL/6NJcl mouse brain cDNA and *Pfu* DNA polymerase (Stratagene) as described previously (Ikeda *et al*., 1995). The PCR products containing the entire coding sequences for GIRK2 and GIRK4 were inserted into the plasmid pSP35T to yield the plasmids pSPGIRK2 and pSPGIRK4, respectively. The nucleotide sequence of the inserted region of pSPGIRK2 was identical with the reported coding sequence for GIRK2 (Patil *et al*., 1995), and that of pSPGIRK4 revealed four nucleotide differences from the reported coding sequence for 129 Sv/J mouse GIRK4 (Wickman *et al*., 1997), which resulted in four amino acid substitutions: C514G, Pro172Ala; T642G, Asn214Lys; C887A, Pro296Gln; C1052T, Ser351Phe. Since the amino acids at these positions are identical with those of another reported sequence for the channel from an unidentified strain mouse (Lesage *et al*., 1995), these differences may be derived from polymorphisms or differences between strains. The nucleotide sequence of the GIRK4 cDNA appears in the DDBJ, EMBL and GenBank databases under the accession number AB019560. pSPGIRK1, pSPGIRK2 and pSPGIRK4 were linearized by digestion with *Eco*RI, *Sac*I and *Not*I, respectively. The specific mRNAs were synthesized *in vitro* from the linearized plasmids using the mMESSAGE mMACHINE™ *In Vitro* Transcription Kit (Ambion).

Expression in Xenopus *oocytes and electrophysiological analyses*

Xenopus laevis oocytes were injected with the GIRK1 mRNA along with either GIRK2 or GIRK4 mRNA (~0.6 ng of each mRNA per oocyte). The oocytes were incubated at 19°C in Barth's solution, and defolliculated following treatment with 1 mg ml^{-1} collagenase as described (Kobayashi *et al*., 1998). Whole-cell currents of the oocytes were recorded from 2 to 5 days after the injection with a conventional two-electrode voltage clamp (Ikeda *et al*., 1997). The membrane potential was held at −70 mV, unless otherwise specified. Microelectrodes were filled with 3 M KCl. The oocytes were superfused with a high-potassium (hK) solution (composition in mM): KCl 96, NaCl 2, $MgCl_2$ 1 and $CaCl_2$ 1.5. In this bath solution, the K^+ equilibrium potential (E_K) is close to 0 mV and enables K^+ inward current flow through inward-rectifier K^+ channels at negative holding potentials. Drug effect was expressed as a percentage reduction of inward current through the basally active GIRK channels. Data were fitted to a standard logistic equation using SigmaPlot (Jandel Scientific) in analysis of concentration-response relationships. The EC_{50} value, which is the concentration of an antipsychotic drug that produce 50% of the maximal current response for that drug, and the Hill coefficient (n_H) were obtained from the concentration-response relationships. The values obtained are expressed as mean ± s.e.mean, and n is the number of oocytes tested.

Compounds

Haloperidol (a butyrophenone), thioridazine hydrochloride (a phenothiazine) and pimozide (a diphenylbutylpiperidine) were purchased from Research Biochemicals Inc. Clozapine (a dibenzodiazepine) was purchased from Sigma Chemical Co. Thioridazine was dissolved in distilled water. Other compounds were dissolved in dimethyl sulphoxide (DMSO). The stock solutions of all the compounds were stored at −20°C until use. Each compound was added to the perfusion solution in appropriate amounts immediately before the experiments. Haloperidol, pimozide and clozapine were tested at concentrations up to 300, 30 and 300 μM, respectively, due to the relatively low solubility of these drugs in aqueous media.

Results

Inhibition of the brain-type and cardiac-type GIRK channels by antipsychotics

To investigate whether various chemical classes of antipsychotic drugs interact with the brain-type GIRK1/2 and cardiac-type GIRK1/4 channels, we used the *Xenopus* oocyte expression system. In oocytes co-injected with GIRK1 and GIRK2 mRNAs, application of 100 μM haloperidol, 100 μM clozapine, 30 μM pimozide or 100 μM thioridazine immediately and reversibly caused a reduction of the inward currents through basally active channels in a hK solution containing 96 mM K^+ (Figure 1a). These responses were not observed in the presence of 3 mM Ba^{2+}, which blocks the IRK channel family including GIRK channels ($n=3$; data not shown). The difference between the 3 mM Ba^{2+}-sensitive current components and the current level at the resting membrane potential was 112.5 ± 15.7 nA in oocytes co-injected with GIRK1 and GIRK2 mRNAs ($n=13$) and 84.3 ± 9.0 nA in uninjected oocytes of the same batch ($n=15$), respectively (Figure 1), and there was no significant difference between two groups ($P>0.1$, Student t-test), indicating that the current components insensitive to 3 mM Ba^{2+} were composed of intrinsic currents independent of the GIRK currents. To confirm that the 3 mM Ba^{2+}-sensitive current components correspond to blockade of the basally active GIRK channels, we analysed the Ba^{2+}-sensitive current components. The current-voltage relationships of the 3 mM Ba^{2+}-sensitive currents in oocytes expressing GIRK channels showed strong inward rectification (Figure 2), indicating a characteristic of GIRK channels. Ba^{2+} at

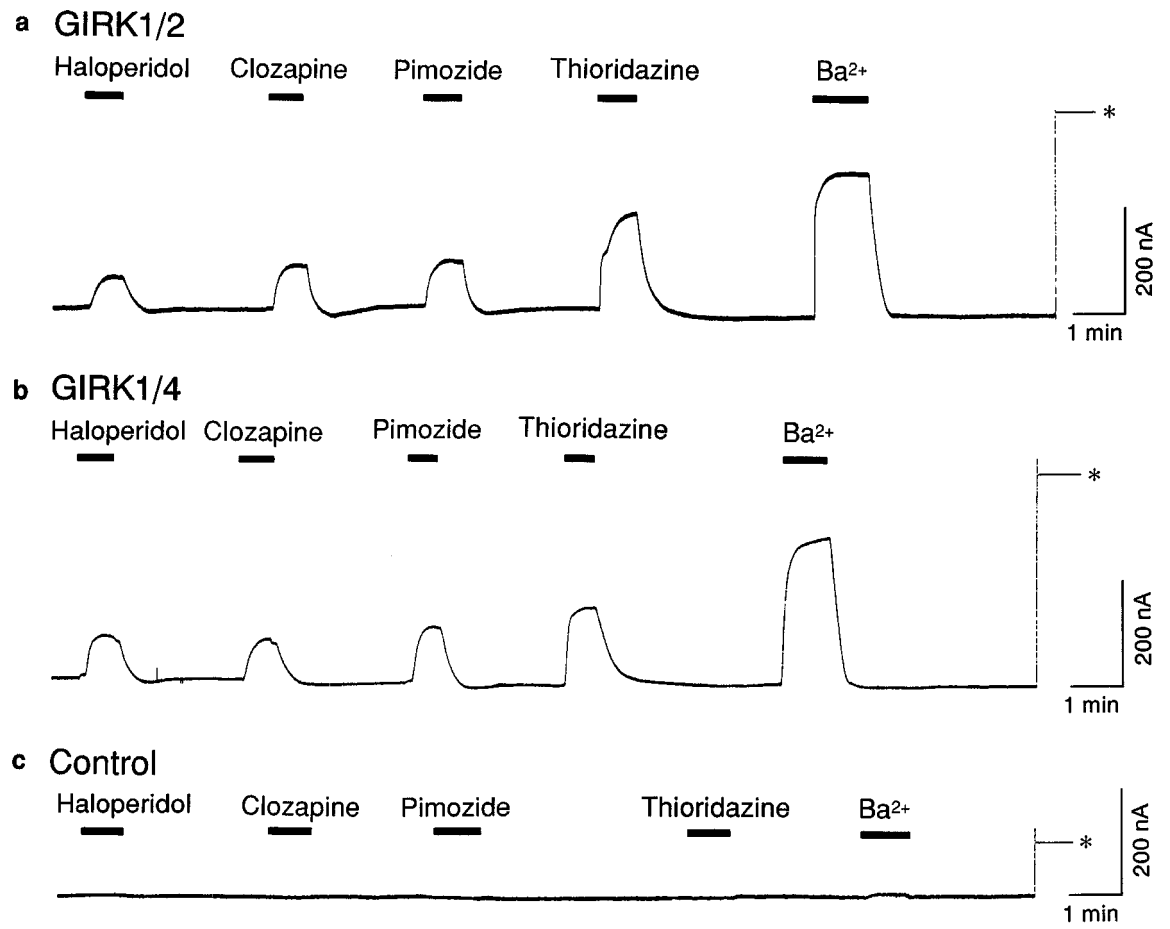

Figure 1 Inhibition by various antipsychotic drugs of the brain-type GIRK1/2 channels and the cardiac-type GIRK1/4 channels expressed in *Xenopus* oocytes. (a) In an oocyte co-injected with GIRK1 mRNA and GIRK2 mRNA, current responses to 100 μM haloperidol, 100 μM clozapine, 30 μM pimozide, 100 μM thioridazine and 3 mM Ba^{2+} are shown. (b) Same as in (a), except that GIRK4 mRNA instead of GIRK2 mRNA was used. (c) In an uninjected oocyte, no significant current response to 300 μM haloperidol, 300 μM clozapine, 30 μM pimozide, 300 μM thioridazine and 3 mM Ba^{2+} is shown. Current responses were measured at a membrane potential of -70 mV in a high-potassium solution containing 96 mM K^+. In this external solution, the K^+ equilibrium potential (E_K) is close to 0 mV and enables K^+ inward current flow through GIRK channels at the holding potential. Asterisks show the current level at the resting membrane potential. Bars show the duration of application.

3 mM produced no significant response in a K^+-free high-sodium (ND98) solution (composition in mM): NaCl 98, $MgCl_2$ 1 and $CaCl_2$ 1.5, instead of the hK solution ($n=3$; data not shown), suggesting that the Ba^{2+}-sensitive current components show K^+ selectively. In uninjected oocytes, 3 mM Ba^{2+} caused no significant response (2.9 ± 0.5 nA at a membrane potential of -70 mV, $n=19$; Figure 1c). Taken together, the 3 mM Ba^{2+}-sensitive current components were considered to represent the magnitudes of currents through the expressed GIRK channels which were basally active. Moreover, application of each of the antipsychotic drugs tested to oocytes co-injected with GIRK1 and GIRK2 mRNAs produced no significant response in ND98 solution ($n=4$; data not shown). In uninjected oocytes, none of the antipsychotic drugs, even at the highest concentrations used, induced any significant current response (Figure 1c; $n=5$). In addition, application of DMSO, the solvent vehicle, at the highest concentration (0.3%) used had no effect on the current responses in oocytes co-injected with GIRK1 and GIRK2 mRNAs ($n=7$; data not shown). These results suggest that all the four drugs tested inhibited the GIRK1/2 channels. Furthermore, similar results were obtained in oocytes co-injected with GIRK1 and GIRK4 mRNAs (Figure 1b), suggesting that all the drugs tested also inhibited the GIRK1/4 channels.

It is known that GIRK currents recorded in oocytes co-injected with mRNAs for GIRK1/GIRK2 or GIRK1/GIRK4 combinations are very significantly larger than those in oocytes injected with the same amount of a single GIRK mRNA (Duprat *et al.*, 1995). In the present study therefore, we injected a small amount of GIRK mRNAs to minimize the expression of GIRK homomeric channels and the effects of XIR, which does not exists in the brain and heart, and to investigate the effects of various antipsychotic drugs on the GIRK1/2 or GIRK1/4 heteromultimeric channels. The 3 mM Ba^{2+}-sensitive current components were 365.4 ± 32.9 nA ($n=26$) in oocytes co-injected with GIRK1 and GIRK2 mRNAs and 316.6 ± 67.0 nA ($n=21$) in oocytes co-injected with GIRK1 and GIRK4 mRNAs, respectively. In contrast those in the same donor oocytes injected with the same small amount of each of GIRK1, GIRK2 or GIRK4 mRNA alone were 13.8 ± 2.6 nA ($n=5$), 16.0 ± 4.5 nA ($n=6$) and 10.2 ± 0.6 nA ($n=5$), respectively, indicating that inward currents through the expressed GIRK channels were much smaller than those in the oocytes co-injected with mRNAs for GIRKs combinations. Moreover, all of the antipsychotic drugs tested induced little or no current response (data not shown; $n=5$), suggesting that the distinct effects of these drugs on the GIRK/XIR channels and the injected-GIRK channels were not detected under the injection of the small amount of a single

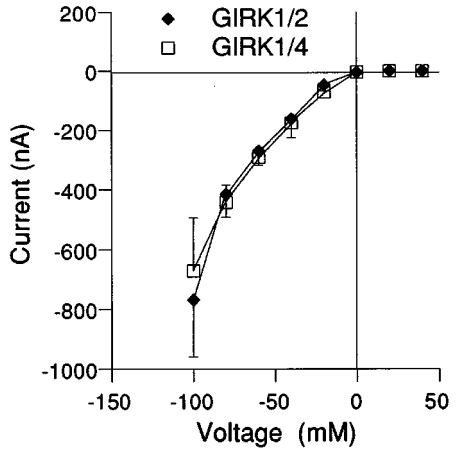

Figure 2 Current-voltage relationships of 3 mM Ba^{2+}-sensitive inward-currents in oocytes expressing GIRK1/2 channels or GIRK1/4 channels. Current responses were measured in a high-potassium solution containing 96 mM K^+. Each point and error bar represents the mean and s.e.mean of the responses obtained from five oocytes.

GIRK mRNA. We previously observed the small amplitude of reduction of GIRK1/XIR currents by clozapine in a different batch of oocytes injected with ~20 fold greater amount of mRNA encoding GIRK1 subunit (Kobayashi *et al*., 1998), which can form functional GIRK channels by associating with XIR (Hedin *et al*., 1996). In addition, functional GIRK current expression in injection with GIRK1 mRNA alone depends on the oocyte batch (Duprat *et al*., 1995). Taken together, the negligible responses to clozapine in oocytes injected with GIRK1 mRNA in this study may be involved in the amount of GIRK1 mRNA injected and XIR in the different batch of oocytes. Therefore, the present observations in oocytes injected with mRNAs for GIRKs combinations indicate that all of the four antipsychotic drugs tested predominantly inhibited the brain-type GIRK1/2 and the cardiac-type GIRK1/4 heteromultimeric channels.

Concentration-dependent inhibition of the GIRK channels by antipsychotics

We further investigated the concentration-response relationships of the inhibitory effects of the various antipsychotic drugs on the GIRK heteromultimeric channels expressed in *Xenopus* oocytes. Because the magnitudes of reduction of basally active GIRK currents by 3 mM Ba^{2+} were almost equal to those by 5 mM Ba^{2+}, the effect of 3 mM Ba^{2+} may cause full blockade of GIRK channels. We, therefore, compared the magnitudes of reduction of the GIRK currents by the antipsychotic drugs with the 3 mM Ba^{2+}-sensitive current components. Figure 3 shows that inhibition of the GIRK1/2 and GIRK1/4 channels by all of the antipsychotic drugs tested is concentration-dependent with distinctive potency and effectiveness at micromolar concentrations, and only pimozide caused slight inhibition of these channels at nanomolar concentrations. Thioridazine could almost fully block the basally active GIRK currents, while the other drugs: haloperidol, clozapine and pimozide, inhibited the GIRK currents to a limited extent. The rank order of the maximal inhibition of both types of GIRK channels by these drugs was as follows: thioridazine > clozapine > pimozide > haloperidol. Table 1 shows the EC_{50} and Hill coefficient (n_H) values obtained from the concentration-response relationships for the antipsychotic drugs tested and the percentage inhibition of the

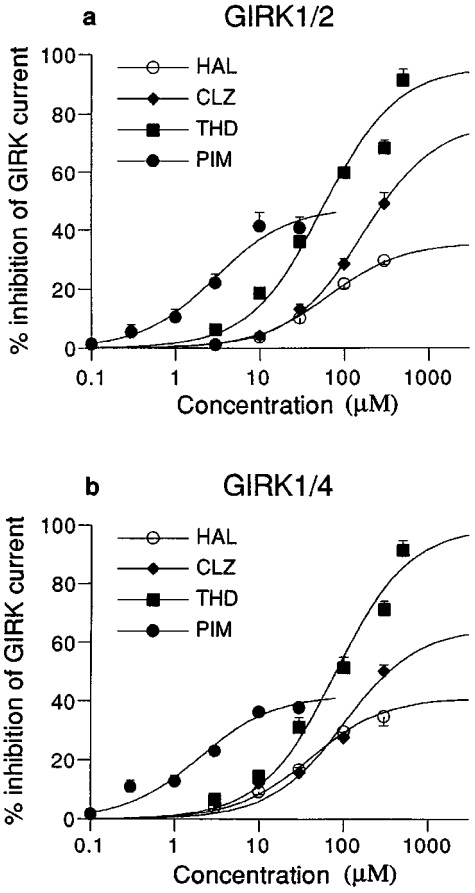

Figure 3 Concentration-response relationships for various antipsychotic drugs, haloperidol (HAL), clozapine (CLZ), thioridazine (THD) and pimozide (PIM), in regard to their effects on the GIRK1/2 channels (a) and the GIRK1/4 channels (b). The magnitudes of inhibition of GIRK current by various antipsychotics were compared with the 3 mM Ba^{2+}-sensitive current components which were 365.4±32.9 nA (n=26) in oocytes expressing GIRK1/2 channels and 316.6±67.0 nA (n=21) in oocytes expressing GIRK1/4 channels, respectively. 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 s.e.mean of the percentage responses obtained from four to eight oocytes. Data points were fitted using a logistic equation.

GIRK currents by the drugs at the highest concentrations tested. In addition, to compare the effects of the antipsychotic drugs on the GIRK channels, the drug concentrations required to inhibit the GIRK currents by 20% are shown in Table 1. Each of the four antipsychotic drugs inhibited both types of GIRK channels in a similar manner.

Discussion

To determine the effects of various antipsychotic drugs on the brain-type and cardiac-type GIRK channels, we used the *Xenopus* oocyte system in which GIRK1/2 or GIRK1/4 channels were expressed. We demonstrated that all of the four antipsychotic drugs tested, namely, thioridazine, haloperidol, pimozide and clozapine, inhibited both the brain-type GIRK1/2 and cardiac-type GIRK1/4 channels in a distinctive manner. The brain-type GIRK channels are widely present in the brain (Kobayashi *et al*., 1995; Karschin *et al*., 1996; Liao *et al*., 1996), while the cardiac-type GIRK channels are abundantly present in the atrium of the heart (Krapivinsky *et al*., 1995). Therefore, inhibition of these GIRK channels by the various

Table 1 Inhibitory effects of various antipsychotic drugs on the GIRK heteromultimeric channels

	GIRK 1/2				*GIRK* 1/4			
Compound	EC_{50}	*20% inhibition*	*% inhibition* (μM; n)	n_H	EC_{50}	*20% inhibition*	*% inhibition* (μM; n)	n_H
Haloperidol	75.5±14.4	95.4±15.5	29.7±1.2 (300; 8)	0.97±0.05	40.9±7.8	43.5±8.9	34.7±3.0 (300; 6)	0.92±0.05
Thioridazine	57.6±4.5	11.8±1.2	91.6±3.6 (500; 6)	1.10±0.07	84.3±8.4	21.3±2.3	91.7±3.4 (500; 6)	0.80±0.07
Pimozide	2.96±0.59	2.45±0.56	40.7±3.6 (30; 4)	1.06±0.12	2.24±0.32	1.89±0.36	37.7±1.2 (30; 4)	0.87±0.10
Clozapine	178.9±28.9	60.3±5.9	48.9±4.0 (300; 7)	0.93±0.04	106.6±14.7	45.2±7.0	50.2±2.2 (300; 5)	0.65±0.04

The mean±s.e.mean of the EC_{50} values and the drug concentrations required to reduce basal GIRK currents by 20% (20% inhibition) are shown in μM. The values of % inhibition indicate the mean±s.e.mean % 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 parentheses. The n_H values indicate the mean±s.e.mean of the Hill coefficients.

antipsychotic drugs may explain some of the actions of these drugs in the central nervous system (CNS) and the heart.

Antipsychotic drugs have not only therapeutic efficacy but also many side effects, including sedation, extrapyramidal effects, seizures, sialorrhea, orthostatic hypotension, tachycardia and agranulocytosis (Baldessarini, 1996). Among these side effects, the occurrence of seizures is a serious CNS side effect. The risk of seizures during clozapine treatment has been reported to be the highest among various antipsychotics and also to be dose-dependent (Marks & Luchins, 1991). In addition, the risk with thioridazine has been reported to be almost the same as with other antipsychotics, with the exception of clozapine (Buckley *et al*., 1995). The plasma concentrations of clozapine in two patients who experienced seizures and in its therapeutic use were reported to be approximately 4–6.7 μM (Marks & Luchins, 1991) and 1 μM (Perry *et al*., 1991), respectively. During thioridazine treatment, the peak plasma concentrations were reported to be approximately 2–4 μM (Vanderheeren & Muusze, 1977; Tune *et al*., 1981). The brain concentrations were approximately 24 times higher in the case of clozapine (Baldessarini *et al*., 1993) and approximately two (Sunderland & Cohen, 1987) or ten times higher (Kilts *et al*., 1984) in the case of thioridazine, than the corresponding blood drug levels. The present study showed that clozapine and thioridazine inhibited, in a concentration-dependent manner, the brain-type GIRK1/2 channels by 28.5±1.9% at 100 μM ($n=7$) and 18.7±1.3% at 10 μM ($n=6$), respectively (Figure 3a), suggesting that these drugs can inhibit the GIRK channels at the predicted brain concentrations. Activation of the GIRK channels causes membrane hyperpolarization (North, 1989), and GIRK2-deficient mice show spontaneous seizures (Signorini *et al*., 1997). Thus, GIRK channels are thought to play an important role in inhibiting the excitability of neuronal cells. Therefore, these antipsychotics-related seizures may be induced by the inhibition of the brain-type GIRK channels. In addition, some inhibition of the GIRK channels by thioridazine or clozapine at clinically relevant concentrations in the CNS may be partly related to some of other CNS side effects and some effects in use of the therapeutic dosages. In contrast, seizures during pimozide and haloperidol treatment are low incidence (Pinder *et al*., 1976; Marks & Luchins, 1991). The plasma concentrations during pimozide therapy and high-dose haloperidol therapy were reported to be less than 0.1 μM (Pinder *et al*., 1976; McCreadie *et al*., 1979) and 0.7 μM (Zarifian *et al*., 1982), and the brain concentrations were approximately two times higher (Janssen & Allewijn, 1968) and 20 times higher (Sunderland & Cohen, 1987) than the corresponding blood levels, respectively. The present study suggests that pimozide and haloperidol may have little or no inhibitory effect on the GIRK1/2 channels at the predicted brain concentrations in clinical practice (Figure 3a). Therefore, the low incidence of seizures in their therapeutic use may be attributable to the little effects of these drugs on the GIRK channels. Under toxicity conditions of these drugs, however, seizures were observed in animal studies (Janssen *et al*., 1968). Since the brain levels of the drugs under toxicity conditions may reach considerably high concentrations, inhibition of the brain GIRK channels by the drugs at such concentrations may be partly related to the incidence of seizures.

In the heart, acetylcholine released from the stimulated vagus nerve opens atrial GIRK channels *via* activation of the M_2 muscarinic acetylcholine receptors, and ultimately causes slowing of the heart rate (Brown & Birnbaumer, 1990). In isolated atria, acetylcholine reduced the rate of spontaneous beating, while thioridazine at concentrations above 5 μM attenuated the effect of acetylcholine in a non-competitive manner (Haffener & Landmark, 1972). Although thioridazine has anticholinergic properties (Miller & Hiley, 1974), the non-competitive effect of thioridazine may not be entirely attributed to blockade of acetylcholine receptors. In the present study, micromolar concentrations of thioridazine inhibited the cardiac-type GIRK1/4 channels (Figure 3b). Therefore, the effect of thioridazine may be, in part, due to inhibition of the cardiac GIRK channels by thioridazine. In clinical practice, thioridazine and clozapine cause sinus tachycardia (Gerlach *et al*., 1989; Buckley *et al*., 1995). In contrast, adverse cardiac effects of haloperidol are uncommon (Ayd, 1978). Also, pimozide causes no significant change in heart rate, although it occasionally causes electrocardiographic abnormalities, including prolongation of the QTc interval and T wave abnormalities (Fulop *et al*., 1987). The present study showed that these drugs at micromolar concentrations caused inhibition of the cardiac-type GIRK1/4 channels in a distinctive manner. The clinically relevant plasma concentrations of thioridazine and clozapine are in the micromolar range, while those of haloperidol and pimozide are in the nanomolar range, although the corresponding heart concentrations of these drugs have not been determined. Since atrial GIRK channels play a critical role in slowing of the heart rate (Brown & Birnbaumer, 1990; Wickman *et al*., 1998), tachycardia during the treatment with thioridazine or clozapine may be related to inhibition of the cardiac-type GIRK1/4 channels.

Further studies using the *Xenopus* oocyte expression system may be useful for the development of novel antipsychotic

drugs with low seizurogenic properties and fewer adverse cardiac effects. In addition, since the amino acid sequences of the pore portion of the members of the IRK channel family, including the GIRK channels, exhibit high homology (Doupnik *et al*., 1995), further studies with other members of the IRK channel family may clarify the interactions of various antipsychotic drugs with other IRK channels.

In conclusion, we demonstrated that all the various chemical classes of antipsychotic drugs tested inhibited both the brain-type GIRK1/2 channels and cardiac-type GIRK1/4 channels. Our results suggest that inhibition of these GIRK channels by these drugs underlies some of the side effects, in particular seizures and sinus tachycardia, observed in clinical practice.

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