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Effects of clozapine on the δ- and κ-opioid receptors and the G-protein-activated K^+ (GIRK) channel expressed in *Xenopus* oocytes

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1 To investigate the effects of clozapine, an atypical antipsychotic, on the cloned μ-, δ- and κ-opioid receptors and G-protein-activated inwardly rectifying K^+ (GIRK) channel, we performed the *Xenopus* oocyte functional assay with each of the three opioid receptor mRNAs and/or the GIRK1 mRNA.

2 In the oocytes co-injected with either the δ- or κ-opioid receptor mRNA and the GIRK1 mRNA, application of clozapine induced inward currents which were attenuated by naloxone, an opioid-receptor antagonist, and blocked by Ba^{2+}, which blocks the GIRK channel. Since the opioid receptors functionally couple to the GIRK channel, these results indicate that clozapine activates the δ- and κ-opioid receptors and that the inward-current responses are mediated by the GIRK channel. The action of clozapine at the δ-opioid receptor was more potent and efficacious than that at the κ-opioid receptor. In the oocytes co-injected with the μ-opioid receptor and GIRK1 mRNAs, application of clozapine (100 μM) did not induce an inward current, suggesting that clozapine could not activate the μ-opioid receptor.

3 Application of clozapine caused a reduction of the basal inward current in the oocytes injected with the GIRK1 mRNA alone, but caused no current response in the uninjected oocytes. These results indicate that clozapine blocks the GIRK channel.

4 To test the antagonism of clozapine for the μ- and κ-opioid receptors, we applied clozapine together with each selective opioid agonist to the oocytes co-injected with either the μ- or κ-opioid receptor mRNA and the GIRK1 mRNA. Each of the peak currents induced by each selective opioid agonist together with clozapine was almost equal to the responses to a selective opioid agonist alone. These results indicate that clozapine has no significant antagonist effect on the μ- and κ-opioid receptors.

5 We conclude that clozapine acts as a δ- and κ-agonist and as a GIRK channel blocker. Our results suggest that the efficacy and side effects of clozapine under clinical conditions may be partly due to activation of the δ-opioid receptor and blockade of the GIRK channel.

Keywords: Clozapine; atypical antipsychotics; opioid receptor; G-protein-activated inwardly rectifying K^+ (GIRK) channel; *Xenopus* oocyte

Introduction

Clozapine, 8-chloro-11-(4-methyl-1-piperazinyl)-5H-dibenzo [b,e][1,4]diazepine, has been classified as an atypical antipsychotic agent because, in contrast to typical antipsychotics, it does not produce significant extrapyramidal side effects, such as dystonia, akathisia and dyskinesias (Baldessarini & Frankenburg, 1991). The clinical advantages of clozapine over typical antipsychotics are that it is effective in the treatment of neuroleptic-resistant schizophrenia and against negative schizophrenic symptoms, such as emotional withdrawal and motor retardation (Kane *et al*., 1988). Clozapine has high affinity for the D_4 dopamine, 5-hydroxytryptamine$_{2A}$ (5-HT_{2A}), 5-HT_{2C}, 5-HT_6, 5-HT_7, M_1 muscarinic receptors and α_1 adrenoceptors (Meltzer, 1994), as well as the ability to modulate the release of dopamine, 5-HT and acetylcholine (Moghaddam & Bunney, 1990; Yamamoto *et al*., 1994; Parada *et al*., 1997). Furthermore, previous studies showed that clozapine inhibited the binding of [^{3}H]-Met^5-enkephalin to synaptosome fractions prepared from rat brain with an IC_{50} value of 28.5 μM (Somoza *et al*., 1981) and chronic treatment with clozapine altered the density of opioid receptors (Giardino *et al*., 1991; Zhang *et al*., 1995), and the level of dynorphin (Nylander & Terenius, 1986) and proenkephalin mRNA (Angulo *et al*., 1990; Zhang *et al*., 1995) in certain areas of the brain, suggesting the interaction of clozapine with the opioid system. However, the molecular mechanisms underlying the effect of clozapine on the opioid system remain unknown.

The opioid receptor is implicated in many brain functions involved in emotion, euphoria, analgesia, opioid tolerance and dependence, and learning and memory (Pasternak *et al*., 1980; Koob *et al*., 1992; Nestler *et al*., 1993; Morris & Johnston, 1995). Activation of the opioid receptors modulates adenylyl cyclase, phospholipase C, an inwardly rectifying K^+ channel and a Ca^{2+} channel, each via G proteins, and ultimately results in the inhibition of neuronal firing and neurotransmitter release (Loh & Smith, 1990; Childers, 1993; North, 1993). Since activation of the G-protein-activated inwardly rectifying K^+ (GIRK) channel induces membrane hyperpolarization, the GIRK channel is thought to play an important role in decreasing the excitability of neuronal cells and slowing the heartbeat (North, 1989; Brown & Birnbaumer, 1990). The cDNAs for the μ-, δ- and κ-opioid receptors as well as the GIRK channel subunits have been cloned (Dascal *et al*., 1993; Kubo *et al*., 1993; Lesage *et al*., 1994; 1995; Minami & Satoh,

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1995; Knapp *et al*., 1995; Kobayashi *et al*., 1995; Krapivinsky *et al*., 1995; Hedin *et al*., 1996; Spauschus *et al*., 1996), and each opioid receptor was shown to couple functionally to the GIRK1 channel co-expressed in *Xenopus* oocytes (Chen & Yu, 1994; Henry *et al*., 1995; Ikeda *et al*., 1995; 1996; Kovoor *et al*., 1995; Ma *et al*., 1995). In addition, the GIRK channel expressed in *Xenopus* oocytes injected with the GIRK1 mRNA has been shown to form a heteromultimer of GIRK1 and XIR (GIRK5) which is an endogenous *Xenopus* GIRK-related polypeptide (Hedin *et al*., 1996). Using the coupling of the opioid receptors with the GIRK channel in a *Xenopus* oocyte expression system, we recently characterized the functional properties of ligands of various chemical and pharmacological classes for each opioid receptor (Kobayashi *et al*., 1996). In the present study, we examined the effects of clozapine on the opioid receptors and the GIRK channel by using the same assay system, and demonstrated that clozapine acts as an agonist at the δ- and κ-opioid receptors and also as a GIRK channel blocker.

Methods

Specific mRNA preparation

Plasmids containing the entire coding sequences for the mouse μ-, δ- and κ-opioid receptors and for the mouse GIRK1 channel were obtained by use of the polymerase chain reaction (PCR) method as described previously, and designated pSPORμ, pSPORδ, pSPORκ and pSPGIRK1, respectively (Ikeda *et al*., 1995; 1996; Kobayashi *et al*., 1995; 1996). pSPORμ, pSPORδ and pSPGIRK1 were linearized by digestion with EcoRI and pSPORκ by digestion with SacI. The specific mRNAs were synthesized *in vitro* from the linearized plasmids with SP6 RNA polymerase in the presence of cap dinucleotide ^{7}mGpppG (Ambion mMESSAGE mMACHINE^{TM} In Vitro Transcription Kit).

Expression in Xenopus *oocytes and electrophysiological analyses*

Xenopus laevis oocytes were injected with each opioid-receptor mRNA (~10 ng per oocyte) and/or the GIRK1 mRNA (~12 ng per oocyte). The oocytes were incubated at 19°C in Barth's solution (composition in mM: NaCl 88, KCl 1, $Ca(NO_3)_2$ 0.33, $CaCl_2$ 0.41, $MgSO_4$ 0.82, $NaHCO_3$ 2.4, Tris-HCl (pH 7.4) 7.5, and gentamicin sulphate 0.1 mg ml^{-1}). Oocytes were defolliculated by manual dissection after 1 mg ml^{-1} collagenase (Wako) treatment for 1 h. Whole-cell currents of the oocytes were recorded from 4 to 10 days after injection with a conventional two-micropipette voltage clamp (Sakimura *et al*., 1992). The oocytes were superfused with a high-potassium solution (composition in mM: KCl 96, NaCl 2, $MgCl_2$ 1 and $CaCl_2$ 1.5). In this solution, the K^+ equilibrium potential (E_K) is close to 0 mV and enables K^+ inward current flow through inwardly rectifying K^+ channels at negative holding potentials. In this study, the membrane potential was held at −70 mV. Data were fitted to a standard logistic equation by use of SigmaPlot (Jandel Scientific) to compute the EC_{50} value for analysis of concentration-response relationships. The values obtained are expressed as mean ± s.e.mean and n is the number of oocytes tested. Statistical analysis of differences between groups was carried out with Student's t test. A probability of 0.05 was taken as the level of statistical significance.

Compounds

Clozapine, an atypical antipsychotic agent, [D-Ala^2, N-Me-Phe^4, Gly^5-ol]enkephalin (DAMGO), a selective μ-opioid-receptor agonist, [D-Pen^{2,5}]enkephalin (DPDPE), a selective δ-opioid-receptor agonist, *trans*-(±)-3,4-dichloro-N-methyl-N-(2-[1-pyrrolidinyl]cyclohexyl)benzeneacetamide (U50488H), a selective κ-opioid-receptor agonist and naloxone, an opioid-receptor antagonist, were purchased from Sigma Chemical Co. Clozapine was dissolved in dimethyl sulphoxide (DMSO). Other compounds were dissolved in distilled water. The stock solutions of all compounds were stored at −20°C until use. They were added to the high-potassium solution in appropriate amounts immediately before the experiments.

Results

Effects of clozapine on the opioid receptors

Taking advantage of the functional coupling of the opioid receptors to the GIRK channel, we investigated the effects of clozapine on the cloned μ-, δ- and κ-opioid receptors co-expressed with the GIRK channel in *Xenopus* oocytes. In the oocytes co-injected with either the δ- or κ-opioid receptor mRNA and the GIRK1 mRNA, application of clozapine induced inward currents (Figure 1a, b). In these oocytes, the current induced by clozapine at high concentrations reached its peak amplitude immediately after the switch from application of clozapine to perfusion with a high-potassium solution as shown in Figure 1b. All of the current responses induced by clozapine were reduced by 10 μM naloxone, an opioid-receptor antagonist (Figure 1c, d; middle). Application of naloxone (10 μM) to the oocytes injected with the GIRK1 mRNA caused no current response (n = 3; data not shown). In addition, application of DMSO, a solvent vehicle, at the highest concentration (0.1%) in this experiment had no effect on the current responses in the oocytes injected with each opioid receptor mRNA and/or the GIRK1 mRNA (n = 3; data not shown). These results suggest that clozapine directly activated the δ- and κ-opioid receptors. The responses to clozapine were rapidly blocked by 300 μM Ba^{2+}, which blocks a family of inward-rectifier K^+ channels including the GIRK channel (Kovoor *et al*., 1995) (Figure 1c, d; right). Application of clozapine induced no inward-current response in the oocytes injected with each of the opioid receptor mRNAs alone (n = 3; data not shown). These results suggest that the current responses were mediated by the GIRK channel expressed. In the oocytes co-injected with the μ-opioid receptor mRNA and the GIRK1 mRNA, application of clozapine, even at 100 μM, did not induce an inward current but caused an upward shift of membrane current traces. However, application of DAMGO 200 nM, to these oocytes, induced inward currents (212.5 ± 49.4 nA, range 150–310 nA; n = 3) (data not shown), suggesting that clozapine does not activate the μ-opioid receptor but activates the GIRK channel via the δ- or κ-opioid receptors expressed in oocytes.

The peak amplitudes of the inward current responses induced by clozapine were concentration-dependent, and were compared with the full response induced by a selective opioid agonist, at 1 μM (Figure 2). The maximum current responses induced by the selective δ- and κ-opioid agonists, DPDPE and U50488H, were 52.6 ± 5.9 nA (range 40.0–68.8 nA; n = 5) and 166.0 ± 41.8 nA (range 63.8–307.5 nA; n = 5), respectively. The EC_{50} values and Hill coefficients (n_H) for clozapine, obtained from the concentration-response relationships, were

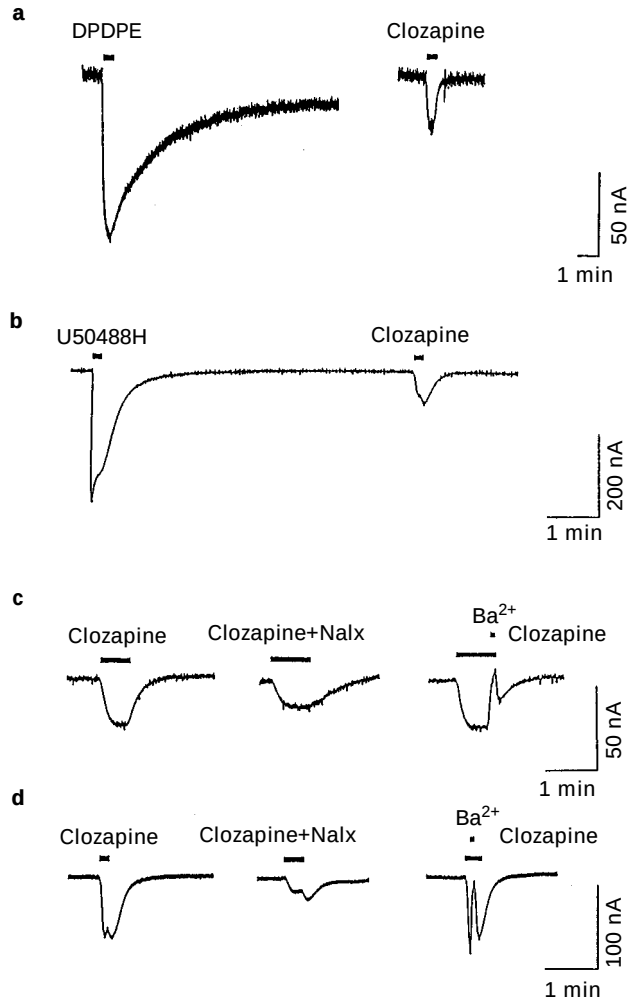

Figure 1 Activation of the δ- and κ-opioid receptors by clozapine and inhibition of clozapine-induced currents by naloxone and Ba^{2+}. (a) Current responses in the oocyte co-injected with the δ-opioid receptor mRNA and GIRK1 mRNA to 400 nM DPDPE and 10 μM clozapine. (b) Current responses in the oocyte co-injected with the κ-opioid receptor mRNA and GIRK1 mRNA to 100 nM U50488H and 100 μM clozapine. (c) Current responses in the oocyte co-injected with the δ-opioid receptor mRNA and GIRK1 mRNA to 10 μM clozapine, 10 μM clozapine plus 10 μM naloxone (Nalx) and 10 μM clozapine plus 300 μM Ba^{2+}. (d) Current responses in the oocyte co-injected with the κ-opioid receptor mRNA and GIRK1 mRNA to 100 μM clozapine, 100 μM clozapine plus 10 μM Nalx and 100 μM clozapine plus 300 μM Ba^{2+}. Current responses were measured at a −70 mV membrane potential in a high-potassium solution. Bars above the traces show the duration of application. Inward current is downward.

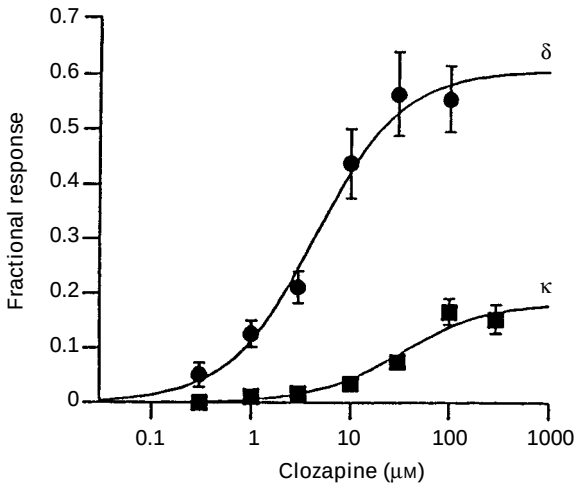

Figure 2 Concentration-response relationships for clozapine in the oocytes co-injected with the δ-opioid receptor mRNA and GIRK1 mRNA (δ) and in the oocytes co-injected with the κ-opioid receptor mRNA and GIRK1 mRNA (κ). The fractional responses are the ratios of clozapine-induced responses to the control response to either a δ- or κ-selective opioid receptor agonist, DPDPE or U50488H, respectively. Each point and vertical line represents the mean and s.e. mean of the fractional responses obtained from 4 to 5 oocytes. Data points were fitted by use of a logistic equation.

4.56 ± 0.46 μM and 1.18 ± 0.20 in the oocytes co-injected with the δ-opioid receptor and GIRK1 mRNAs, and 30.2 ± 1.9 μM and 1.69 ± 0.22 in the oocytes co-injected with the κ-opioid receptor and GIRK1 mRNAs, respectively. The action of clozapine at the δ-opioid receptor was more potent and efficacious than that at the κ-opioid receptor (Figure 2).

To determine whether clozapine has an antagonist effect on the opioid receptors, we applied clozapine together with a selective opioid agonist to the oocytes co-injected with either the μ- or κ-opioid receptor mRNA and the GIRK1 mRNA. The selective opioid agonists were used at the concentrations of 10 fold the respective EC_{50} values, since each current response at that concentration was near the full response (Ikeda *et al*., 1996). The control current responses to DAMGO (180 nM) and U50488H (150 nM) were 355.9 ± 95.0 nA ($n=5$) and 72.9 ± 3.8 nA ($n=3$), respectively. In the oocytes co-injected with the μ-opioid receptor mRNA and the GIRK1 mRNA, the current induced by DAMGO together with clozapine (30 μM) reached its peak amplitude immediately after the switch from co-application to perfusion with a high-potassium solution (Figure 3a). In the case of application of DAMGO alone, an additional inward current after the solution switch was not observed (Figure 3a). In the case of co-application of DAMGO and clozapine, although the inward current responses during co-application of DAMGO and clozapine were smaller than those induced by DAMGO alone (Figure 3a), the amplitudes of the peak currents after the solution switch were $97.8 \pm 1.6\%$ of the control current response to DAMGO ($n=5$). The maximum amplitudes of current responses in each case were not significantly different. These results suggest that the activation of the μ-opioid receptor by DAMGO is not significantly affected by clozapine at 30 μM. In addition, the results obtained during co-application of DAMGO and clozapine suggest that the GIRK channel may be affected by clozapine. Next, since the application of clozapine, even at 10 μM, produced only a small current response in the oocytes co-injected with the κ-opioid receptor mRNA and the GIRK1 mRNA (Figure 2), we investigated the possible antagonism of the κ-opioid receptor by 10 μM clozapine. The percentage reduction of the control current response to U50488H by clozapine (10 μM) was only $1.1 \pm 1.6\%$ ($n=3$) (Figure 3b). Hence, the activation of the κ-opioid receptor by U50488H was not significantly affected by clozapine. This indicates that clozapine, at 10 μM, has no obvious antagonist effect on the κ-opioid receptor.

Blockade of the GIRK channel by clozapine

In the oocytes co-injected with the μ-opioid receptor mRNA and the GIRK1 mRNA, application of clozapine, at 100 μM, caused an upward shift of membrane current traces as described above. Since a high-potassium solution in oocytes expressing the GIRK channel produces a basal inward current at a holding potential of −70 mV (Kovoor *et al*., 1995), we assumed that clozapine at high concentrations attenuated the

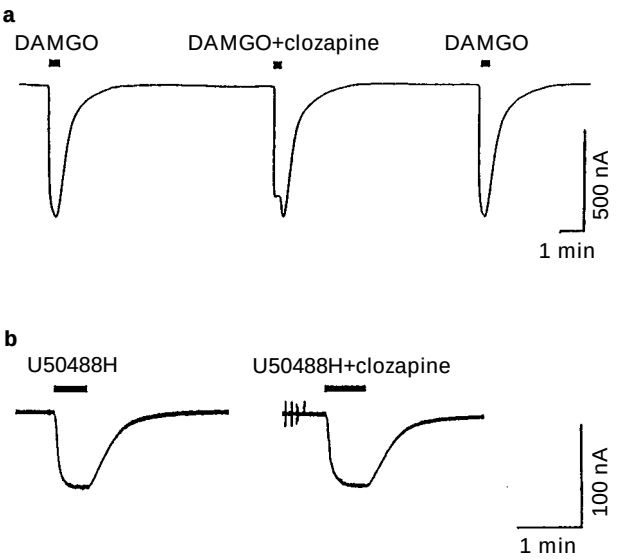

Figure 3 Effects of clozapine on the current responses to a selective opioid-receptor agonist in *Xenopus* oocytes co-injected with opioid receptor mRNA and GIRK1 mRNA. (a) Current responses to 180 nM DAMGO, 180 nM DAMGO plus 30 μM clozapine, and 180 nM DAMGO in the oocyte co-injected with the μ-opioid receptor mRNA and GIRK1 mRNA. (b) Current responses to 150 nM U50488H and 150 nM U50488H plus 10 μM clozapine in the oocyte co-injected with the κ-opioid receptor mRNA and GIRK1 mRNA. Current responses were measured at a membrane potential of -70 mV in a high-potassium solution. Bars above the traces show the duration of application. Inward current is downward.

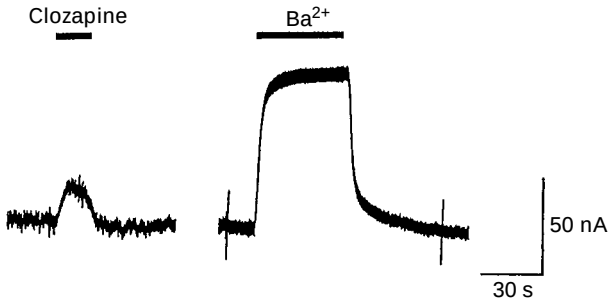

Figure 4 Blockade by clozapine of the GIRK channel in *Xenopus* oocytes injected with the GIRK1 mRNA alone. Current responses to 100 μM clozapine and 300 μM Ba^{2+} in one oocyte. Current responses were measured at a membrane potential of -70 mV in a high-potassium solution. Bars show the duration of application.

basal inward current by blockade of the GIRK channel. Hence, to clarify the effects of clozapine on the GIRK channel, we performed the *Xenopus* oocyte functional assay with the GIRK1 mRNA alone. Application of clozapine caused a reduction of the basal inward current in the oocytes injected with the GIRK1 mRNA (Figure 4), but caused no current response in the uninjected oocytes ($n=5$; data not shown). These results indicate that clozapine acts as a GIRK channel blocker, although the mechanisms underlying the blockade by clozapine are not completely understood. Ba^{2+}, a GIRK channel blocker, also caused a reduction of the basal inward current in the oocytes injected with the GIRK1 mRNA (72.7 ± 11.7 nA at 10 mM; $n=6$) (Figure 4), but caused no response in the uninjected oocytes ($n=3$; data not shown). Therefore, the Ba^{2+}-sensitive component was considered to be the basal activity of the GIRK channel. The magnitudes of the blockade by clozapine at various concentrations were compared with the full blockade by 10 mM Ba^{2+}. The percentage blockade of the GIRK channel by clozapine was $1.3\pm0.83\%$ at 10 μM ($n=6$), $14.3\pm3.0\%$ at 30 μM ($n=5$) and $15.5\pm0.86\%$ at 100 μM ($n=3$).

In the oocytes co-injected with the μ-opioid receptor mRNA and the GIRK1 mRNA, the maximum current responses during co-application of DAMGO and clozapine (30 μM) were reduced by $14.7\pm1.2\%$ ($n=5$) when compared with the control current responses to DAMGO (Figure 3a). Since clozapine blocked the GIRK channel as described above, this observation indicates that the DAMGO-induced GIRK currents during the co-application were attenuated by clozapine. The percentage reduction by clozapine was almost equal to the percentage blockade of the GIRK channel by clozapine at the same concentration. This indicates that the reduction of the current responses by clozapine was caused by the blockade of the GIRK channel by clozapine and support the finding that clozapine, even at 30 μM, may have no significant antagonist effect on the μ-opioid receptor. Furthermore, in the oocytes co-injected with either the δ- or κ-opioid receptor mRNA and the GIRK1 mRNA, the peak currents induced by clozapine at high concentrations were observed immediately after the switch from application of clozapine to washout with a high-potassium solution, suggesting that washout of clozapine abolished blockade by clozapine of inward current flow through the activated GIRK channel. These results also suggest that clozapine at high concentrations blocks the GIRK channel.

Discussion

To determine the interaction of clozapine with the μ-, δ- and κ-opioid receptors and the GIRK channel, we used the *Xenopus* oocyte system in which mRNAs for each of the opioid receptors and/or the GIRK channel were expressed. An earlier study showed that clozapine inhibited the binding of [^{3}H]-Met^5-enkephalin, an endogenous opioid agonist, to synaptosome fractions prepared from rat brain with an IC_{50} value of 28.5 μM (Somoza *et al.*, 1981). However, since Met^5-enkephalin exhibits high affinity for the μ- and δ-opioid receptors (Raynor *et al.*, 1994), the interaction of clozapine with each subtype of opioid receptors was not known. Here we demonstrated that clozapine acts as an agonist at the δ- and κ-opioid receptors but has no significant effect on the μ-opioid receptor. Somoza *et al.* (1981) showed that clozapine could block nearly all of the binding of Met^5-enkephalin, but there was a clozapine-resistant component. Since clozapine did not interact with the μ-opioid receptor in this study, the clozapine resistant component might be partly due to its effect on the μ-opioid receptor. Furthermore, we demonstrated that clozapine blocks the GIRK channel.

In man, plasma concentrations of clozapine associated with clinical responses are approximately 1 μM at about 12 h after the last dose (Perry *et al.*, 1991; Hasegawa *et al.*, 1993; Miller *et al.*, 1994). Brain and serum levels of clozapine correlate closely, and the brain concentration of clozapine was 24 times higher than the corresponding serum drug level (Baldessarini *et al.*, 1993). In addition, clinical pharmacokinetic studies with clozapine have revealed that the time to peak plasma concentration after oral doses and the elimination half-life from blood average about 2 and 14 h, respectively (Choc *et al.*, 1987; 1990; Cheng *et al.*, 1988; Baldessarini & Frankenburg, 1991). Although precise bioactive concentrations of clozapine in the brain have not been determined, clozapine in the brain under clinical conditions may mediate many brain functions

via activation of the δ-opioid receptor. Similar to morphine, which is clinically used as an analgesic, clozapine inhibits the nociceptive reaction in tests utilizing benzoquinone or electric stimulation as the noxious stimulus (Stille *et al*., 1971). Since δ-opioid-receptor agonists induce analgesia (Galligan *et al*., 1984; Porreca *et al*., 1984), the antinociceptive effect of clozapine may be partly mediated by the δ-opioid receptor. Moghaddam & Bunney (1990) showed that clozapine increased the extracellular concentration of dopamine in the nucleus accumbens. Since a selective δ-opioid-receptor agonist also increased the extracellular concentration of dopamine in the nucleus accumbens (Spanagel *et al*., 1990), the increase in dopamine concentration may be partly caused by activation of the δ-opioid receptor by clozapine. In addition, characterization of clozapine analogues by using our *Xenopus* oocyte assay system may lead to the development of a novel class of non-peptide selective δ-opioid receptor agonists.

The GIRK1–4 subunits have been cloned (Dascal *et al*., 1993; Kubo *et al*., 1993; Lesage *et al*., 1994; 1995; Kobayashi *et al*., 1995; Krapivinsky *et al*., 1995; Spauschus *et al*., 1996), and have been shown to form homo- or hetero-multimeric channels (Duprat *et al*., 1995; Kofuji *et al*., 1995; Krapivinsky *et al*., 1995; Velimirovic *et al*., 1996). In the *Xenopus* oocytes injected with the GIRK1 mRNA, the GIRK channel expressed has been shown to form a heteromultimer of GIRK1 and XIR (GIRK5), which is an endogenous *Xenopus* oocyte polypeptide homologous to rat CIR (GIRK4) (Hedin *et al*., 1996). The present study shows that clozapine blocked the GIRK1/GIRK5 channel. Since the amino acid sequences of the pore portion of the members of the inwardly rectifying K^+ channel family including the GIRK subunits exhibit high homology (Lesage *et al*., 1994), clozapine may block other inwardly rectifying K^+ channels. Further studies with the *Xenopus* oocyte expression system with members of the inwardly rectifying K^+ channel family may clarify the interaction of clozapine with the GIRK channels as well as with other inwardly rectifying K^+ channels.

Clozapine has many side effects, such as sedation, sialorrhea, tachycardia, seizures and agranulocytosis, resulting from its clinical use (Baldessarini and Frankenburg, 1991). However, the molecular mechanisms underlying the side effects of clozapine are largely unknown. Several studies, in which Northern blot analysis and *in situ* hybridization histochemistry were used, have revealed that GIRK1 mRNA is present both in the brain and heart, GIRK2 and GIRK3 mRNAs are present mainly in the brain and GIRK4 mRNA is present mainly in the heart (Karschin *et al*., 1994; 1996; Lesage *et al*., 1994; Kobayashi *et al*., 1995; Krapivinsky *et al*., 1995; Spauschus *et al*., 1996). Activation of the GIRK channel plays an important role in slowing the heartbeat and inhibiting neuronal excitability (North, 1989; Brown & Birnbaumer, 1990). Tachycardia and seizures, which are among the side effects of clozapine, may be partly due to the blockade of the GIRK channel by clozapine. Furthermore, since administration of δ-opioid receptor agonists into the hippocampus or the lateral ventricles has been shown to lead to convulsions (Cain *et al*., 1990), clozapine-induced seizures may be partly mediated by the δ-opioid receptor.

In conclusion, we demonstrated that clozapine, an atypical antipsychotic, activates the δ- and κ-opioid receptors, and blocks the GIRK channel. Our results suggest that the efficacy and side effects of clozapine under clinical conditions may be in part due to activation of the δ-opioid receptor and blockade of the GIRK channel.

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