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The effects of neuroleptics on the GABA-induced Cl^- current in rat dorsal root ganglion neurons: differences between some neuroleptics

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1 Several neuroleptics inhibited the 3 μM γ-aminobutyric acid induced-chloride current (GABA-current) on dissociated rat dorsal root ganglion neurons in whole-cell patch-clamp investigations.

2 The IC_{50} for clozapine, zotepine, olanzapine, risperidone and chlorpromazine were 6.95, 18.26, 20.30, 106.01 and 114.56 μM, respectively. The values for the inhibitory effects of neuroleptics on the GABA (3 μM)-current, which were calculated by the fitting Hill's equations where the concentrations represent the mean therapeutic blood concentrations, were ranked clozapine > zotepine > chlorpromazine > olanzapine > risperidone. These inhibitory effects, weighted with the therapeutic concentrations of neuroleptics, were correlated with the clinical incidences of seizure during treatment with neuroleptics.

3 Clozapine reduced the picrotoxin-inhibiton, and may compete with a ligand of the t-butylbicyclophosphorothionate (TBPS) binding site.

4 Haloperidol and quetiapine did not affect the peak amplitude of the GABA (3 μM)-current. However, haloperidol reduced the clozapine-inhibition, and may antagonize ligand binding to TBPS binding site.

5 Neuroleptics including haloperidol and quetiapine enhanced the desensitization of the GABA (3 μM)-current. However, haloperidol and quetiapine at 100 μM inhibited the desensitization at the beginning of application.

6 Blonanserin (AD-5423) at 30 and 50 μM potentiated the GABA (3 μM)-current to 170.1 ± 6.9 and $192.0 \pm 10.6\%$ of the control current, respectively. Blonanserin shifted GABA concentration-response curve leftward. Blonanserin only partly negatively interacted with diazepam. The blonanserin-potentiation was not reversed by flumazenil. Blonanserin is not a benzodiazepine receptor agonist.

7 The various effects of neuroleptics on the GABA-current may be related to the clinical effects including modifying the seizure threshold.

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Keywords: GABA-induced Cl^- current; dorsal root ganglion; whole-cell patch-clamp; serotonin-dopamine antagonist; neuroleptics; antipsychotics; clozapine; picrotoxin; blonanserin; seizure

Abbreviations: ANOVA, analysis of variance; DMEM, Dulbecco's modified Eagle's medium; DMSO, dimethylsulphoxide; DRG, dorsal root ganglion; Mg-ATP, adenosine 5′-triphosphate magnesium salt; SDA, serotonin-dopamine antagonist; TBPS, t-butylbicyclophosphorothionate

Introduction

Neuroleptics have been commonly used as an antipsychotic agent, even though they are known to have such adverse effects as oversedation, neuroleptic malignant syndrome, extrapyramidal symptoms, tardive dyskinesia, cardiovascular effects, anticholinergic symptoms and seizure (Casey, 1997; Marks & Luchins, 1991). Most neuroleptics lower the seizure threshold (Arana, 2000). Among serotonin-dopamine antagonists (SDA), which are atypical antipsychotics, the different liability to the seizure is noticed in the psychiatric medicational therapy (Casey, 1997). The clinical incidences of seizure during treatment with neuroleptics were 2.89% (41 of 1418 patients) (Devinsky *et al*., 1991) for clozapine, 2.34% (28 of 1196 patients, who were accumulated from several studies) (Manmaru, 1985) for zotepine, 0.88% (22 of 2500 patients) for olanzapine (Beasley *et al*., 1997), 0.75% (18 of 2387 patients) for quetiapine (Physicians' Desk Reference, 2000b) and 0.35% (9 of 2607 patients) for risperidone (Physicians' Desk Reference, 2000a) in previous studies. Among the conventional neuroleptics, which are typical antipsychotics, the clinical incidence of seizure during treatment with chlorpromazine was 1.25% (10 of 800 patients) (Lomas *et al*., 1955). Haloperidol rarely induces clinical seizure (Casey, 1997).

Neurotransmitters such as γ-aminobutyric acid (GABA), acetylcholine (ACh), glutamate, norepinephrine, dopamine

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and 5-hydroxytriptophan (5-HT) are related to the seizure threshold (Marks & Luchins, 1991). All conventional neuroleptics and SDA antagonize the D_2 receptor. SDA such as clozapine, zotepine (Manmaru, 1985), olanzapine, risperidone, quetiapine and blonanserin (Noda *et al*., 1993; Oka *et al*., 1993) also antagonize the 5-HT_2 receptor (Casey, 1997). Neuroleptics also bind to the muscarinic ACh, adrenergic and histamine receptor. (Casey, 1997; Marks & Luchins, 1991). Furthermore, several neuroleptics are reported to affect the voltage-gated ion channels such as the sodium, potassium, calcium channels (Ogata *et al*., 1989) and the ligand-gated ion channels such as the neuronal nicotinic ACh receptor, N-metyl-D-aspartate (NMDA) receptor (Yang & Zorumski, 1989), 5-HT_3 receptor (Squires & Saederup, 1999). However, the relationship between these numerous factors to neuroleptic-induced seizures has not yet been clarified.

Several neuroleptics including clozapine inhibit the GABA response on $GABA_A$ receptor-chloride (Cl^-) channel complex in previous ^{35}S-t-butylbicyclophosphorothionate ([^{35}S]-TBPS) binding studies (Korpi *et al*., 1995; Squires & Saederup, 1997; 1998). Clozapine was also reported to inhibit the GABA-induced Cl^- current in a previous $^{36}Cl^-$ flux-measured study (Korpi *et al*., 1995). Several phenothiazines including chlorpromazine were reported to inhibit the GABA-induced Cl^- current in previous patch-clamp studies (Yang & Zorumski, 1989; Zorumski & Yang, 1988). However, haloperidol, which is a butylophenon, only slightly affected the GABA-induced Cl^- current (Zorumski & Yang, 1988).

In this study, we investigated the effects of each neuroleptic agent on the GABA-induced Cl^- current evoked through the $GABA_A$ receptor-Cl^- channel complex in the dissociated rat dorsal root ganglion (DRG) neurons using the whole-cell patch-clamp technique. We also examined the relationship between the potency of each neuroleptic agent as an inhibitory agent of the GABA-induced Cl^- current and the clinical incidence of seizure.

Methods

Cell preparation

The DRGs were dissected from Wistar rats aged 1–9 days under diethylether anaesthesia and incubated at 37°C for 20 min in Ca^{2+}- and Mg^{2+}-free saline, containing 0.5% collagenase (Wako, Osaka, Japan). The DRG neurons were mechanically dissociated with fire-polished Pasteur pipettes in Dulbecco's modified Eagle's medium (DMEM, Nissui, Tokyo, Japan) supplemented with 10% (v v^{-1}) foetal bovine serum (GIBCO, Gland Island, NY, U.S.A.). The dissociated neurons were plated on a coverslip coated with poly-L-lysine (Sigma, St. Louis, MO, U.S.A.) and cultured at 37°C in DMEM supplemented with the serum, 50 u ml^{-1} penicillin and 50 μg ml^{-1} streptomycin (Sigma) in a humidified incubator containing 5% CO_2 in air. The neuron was then used for the patch-clamp experiment after 4 h to 5 days in culture.

Solutions

The ionic composition of the normal external solution was (in mM): NaCl 140, KCl 5, $CaCl_2$ 2, $MgCl_2$ 2, HEPES 5, D-glucose 10. The composition of the patch pipette (internal) solution was (in mM): KCl 140, $MgCl_2$ 1, HEPES 5, EGTA 5, adenosine 5′-triphosphate magnesium salt (Mg-ATP) 5. The pH of both solutions were adjusted to 7.3 with NaOH (Kurata *et al*., 1993).

Electrical measurements

The ionic membrane current was measured using the whole-cell patch-clamp technique (Hamill *et al*., 1981). The membrane potential was held at −80 mV, except in the experiment investigating the current-voltage relationship. The patch pipette was made of a glass capillary, pulled on the vertical two stage puller and fire-polished on a microforge. The resistances of the patch pipettes filled with the internal solution ranged from 3 to 8 MΩ to the reference electrode of an Ag-AgCl wire in 3 M KCl-agar in the normal external solution. The current was measured with the patch-clamp amplifier (Axopatch 200B, Axon Instruments, Foster City, CA, U.S.A.), low-pass filtered at 5 kHz and carried out with the on-line system developed by Yoshii, M. and Ogata, N., using a personal computer (Motomura *et al*., 1995). The current was traced with a pen recorder. All experiments were performed at room temperature ranged from 22 to 26°C.

Drugs

GABA (Wako) was dissolved in distilled water. Bicuculline (Sigma), picrotoxin (Sigma), clozapine (Sigma), haloperidol, chlorpromazine, zotepine, olanzapine, risperidone, quetiapine, blonanserin (AD-5423: 2-(4-ethyl-1-piperazinyl)-4-(fluorophenyl)-5,6,7,8,9,10-hexahydrocycloocta[b]pyridine), diazepam and flumazenil were dissolved in dimethylsulphoxide (DMSO, Sigma). The dissolved drugs were diluted with the normal external solution before use. The final concentration of DMSO was less than 0.1% (v v^{-1}), at which concentration DMSO did not affect the GABA-induced Cl^- current. Neuroleptics and benzodiazepine receptor ligands were kindly donated by the Yoshitomi Pharmaceutical Corporation (Tokyo, Japan) for haloperidol and chlorpromazine, the Fujisawa Pharmaceutical Corporation (Tokyo, Japan) for Zotepine, Eli Lilly and Company (Indianapolis, IN, U.S.A.) for olanzapine, the Janssen-Kyowa Corporation (Tokyo, Japan) for risperidone, the Zeneca Pharmaceuticals (Cambridge, MA, U.S.A.) for quetiapine, the Dainippon Pharmaceutical Corporation (Osaka, Japan) for blonanserin and the Yamanouchi Pharmaceutical Corporation (Tokyo, Japan) for diazepam and flumazenil.

Drug applications

The drug solution was rapidly applied using a variant U-tube (Tatebayashi & Ogata, 1992) made of glass as near as 20–40 μm to a neuron. GABA was applied for 7 s, except in the experiment of the continuous GABA application. The other drugs were all diluted with GABA solution and co-applied with GABA. The interval time between applications was more than 2–3 min until the full recovery of the control current. The normal external solution was perfused at from 3 to 5 ml min^{-1} in a bath.

Data analysis

The fitting curve for a GABA concentration-response curve was obtained by Hill's equation as follows: $I = ImaxC^{nH}/$

($C^{nH} + EC_{50}{}^{nH}$), where I is the peak amplitude of the GABA-induced Cl^- current, Imax is the peak amplitude of the GABA-induced Cl^- current at the maximal GABA concentration of 300 μM, C is the GABA concentration, EC_{50} is the half maximal concentration and n_H is the Hill coefficient. The fitting curve for inhibition data of the neuroleptics and picrotoxin were obtained by Hill's equation as follows: $I = 1\text{-}C^{nH}/(C^{nH} + IC_{50}{}^{nH})$, where I is the peak amplitude of the 3 μM GABA-induced Cl^- current, C is the concentration of the neuroleptics or picrotoxin, IC_{50} is the concentration for half inhibition and n_H is the Hill coefficient. The fitting curves were carried out by non-linear regression using the least-squares method. The statistical significance of difference was assessed by a two-way repeated-measures analysis of variance (ANOVA) followed by the *post-hoc* Dunnett's test. A P value of <0.05 was considered to be statistically significant.

Results

The inhibitory effect on the 3 μM GABA-induced Cl^- current

The inward current was induced by GABA (Figure 1Aa). The GABA-induced current was confirmed a GABA concentration-dependent multiplication (Figure 4), a reduction by picrotoxin, a blockade by bicuculline and a flumazenil-sensitive potentiation by diazepam (data not shown). The reversal potential of the 3 μM GABA-induced current was -2.99 ± 1.14 mV (mean $\pm$ s.e.mean, $n = 6$), close to -1.9 mV, which is the theoretical Cl^- equilibrium potential with Nernst's equation. The 3 μM GABA-induced Cl^- current was used as a control response, since it was not largely desensitized and smoothly recovered after the washout of GABA within 2 min. The 3 μM GABA-induced Cl^- current was completely blocked by 100 μM bicuculline ($n = 5$, data not shown). The peak amplitude of the 3 μM GABA-induced Cl^- current was $7.98 \pm 0.94\%$ ($n = 5$) of the maximal response to 300 μM GABA (Figure 4).

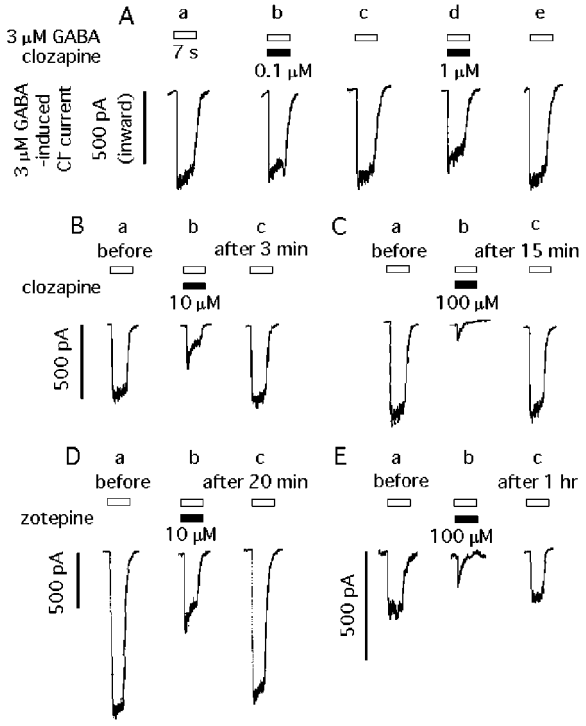

Figure 1 The 3 μM GABA-induced Cl^- current and the inhibition by clozapine and zotepine. (A) The inward current was obtained during the application of 3 μM GABA for 7 s (a). Clozapine at 0.1 μM (b) and 1 μM (d) inhibited the current reversibly (c,e). (B,C) Clozapine at 10 μM (B) and 100 μM (C) inhibited the current (b) reversibly (c). (D,E) Zotepine at 10 μM (D) and 100 μM (E) inhibited the current (b) irreversibly (c).

Several neuroleptics reduced the 3 μM GABA-induced Cl^- current in a concentration-dependent manner. The IC_{50} for clozapine, zotepine, olanzapine, risperidone and chlorpromazine were 6.95, 18.26, 20.30, 106.01 and 114.56 μM (Table 1), and the Hill coefficients were 0.51, 0.50, 0.76, 0.74 and 0.59, respectively (Figure 2). The recoveries from the inhibitory effects of olanzapine and risperidone (data not shown), chlorpromazine (data not shown) and clozapine (Figure 1C) at the maximal concentration of 100 μM were complete after a washout lasting 3, 5 and 15 min, respectively. However, the recovery for zotepine was more prolonged and remained incomplete even after 1 h from an application of 100 μM zotepine (Figure 1D,E). Haloperidol and quetiapine did not affect the peak amplitude of the 3 μM GABA-induced Cl^- current (Figure 2).

Picrotoxin also inhibited the 3 μM GABA-induced Cl^- current in a concentration-dependent manner (data not shown). The IC_{50} for picrotoxin was 9.44 μM. The Hill coefficient was 0.61. The current did not completely recover after the washout of picrotoxin.

The clinical incidence of seizure and the inhibitory effect on the 3 μM GABA-induced Cl^- current

It is possible to calculate the value for the inhibitory effect of neuroleptics at any concentration on the 3 μM GABA-induced Cl^- current using Hill's equation, to which the inhibitory data of each neuroleptic agent was fitted (Figure 2). The inhibition of the 3 μM GABA-induced Cl^- current at the therapeutic concentration (Figure 1) was calculated by Hill's equation (Figure 2) where the concentration represents the mean value of the therapeutic concentration of neuroleptics reported in the literature (Table 1). The inhibition by clozapine at 922.8 nM (Cirimele *et al*., 2000), zotepine at 180.9 nM (Otani *et al*., 1990), chlorpromazine at 365.1 nM (Chetty *et al*., 1999), olanzapine at 69.5 nM (Olesen & Linnet, 1999) and risperidone at 34.1 nM (Nagasaki *et al*., 1999) were thus calculated on 26.29, 9.05, 3.27, 1.81 and 0.27% of the control 3 μM GABA-induced Cl^- current, respectively (Table 1).

The clinical incidence of seizure during the treatment with neuroleptics in the literatures (Table 1) represented a positive correlation relationship with the calculated value for the inhibition of the 3 μM GABA-induced Cl^- current by neuroleptics at the mean value of the therapeutic serum or plasma concentration (Table 1, Figure 3). A correlation curve was carried out by linear regression using the least-squares method and $y = 0.089x + 0.81$, where x is the inhibition by neuroleptics at the therapeutic concentration (%) (Table 1) and y is the clinical incidence of seizure (%) (Table 1). The correlation coefficient was 0.82 (Figure 3).

Table 1 The clinical incidence of seizure and the inhibitory effect of neuroleptics on the 3 μM GABA-induced Cl⁻current

Neuroleptics	*IC_{50}* (μM)	*Mean of therapeutic serum or plasma concentration* (nM)	*Inhibition of 3 μM GABA induced Cl^- current at therapeutic concentration* (%)	*Clinical incidence of seizure* (%)
clozapine	6.95	922.8[a]	26.29	2.89[f]
zotepine	18.26	180.9[b]	9.05	2.34[g]
chlorpromazine	114.56	365.1[c]	3.27	1.25[h]
olanzapine	20.30	69.5[d]	1.81	0.88[i]
risperidone	106.01	34.1[e]	0.27	0.35[j]
quetiapine	—	—	0	0.75[k]
haloperidol	—	—	0	rare[l]
picrotoxin	9.44			

[a]Cirimele *et al*., 2000, [b]Otani *et al*., 1990, [c]Chetty *et al*., 1999, [d]Olesen & Linnet, 1999, [e]Nagasaki *et al*., 1999, [f]Devinsky *et al*., 1991, [g]Manmaru, 1985, [h]Lomas *et al*., 1955, [i]Beasley *et al*., 1997, [j]Physicians' Desk Reference, 2000a, [k]Physicians' Desk Reference, 2000b, [l]Casey, 1997. The IC_{50} is the concentration for half inhibition of the 3 μM GABA-induced Cl^- current on the fitted Hill's equation for the concentrations-responce relationship. The inhibition of 3 μM GABA-induced Cl^- current at therapeutic concentration represents the value for the inhibitory effect of each neuroleptics on the 3 μM GABA-induced Cl^- current, calculated by Hill's equation (Figure 2) where the concentration represents the mean therapeutic serum or plasma concentration.

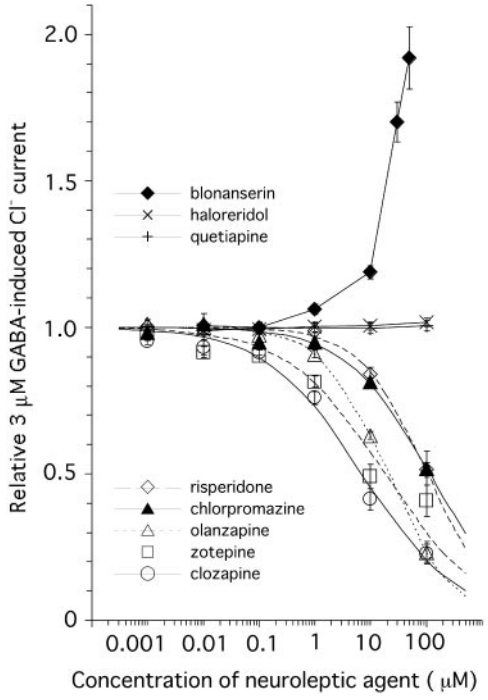

Figure 2 The concentration of neuroleptics and the effects on the 3 μM GABA-induced Cl^- current. The peak amplitude of the 3 μM GABA-induced Cl^- current in the presence of neuroleptics, relative to the control 3 μM GABA-induced Cl^- current, was plotted against each concentration of neuroleptics. The inhibitory data of clozapine, zotepine, olanzapine, risperidone and chlorpromazine was fitted to Hill's equation. Haloperidol and quetiapine did not affect the peak amplitude of the current. Blonanserin potentiated the current. Each point represents the mean ± s.e.mean ($n \geq 4$).

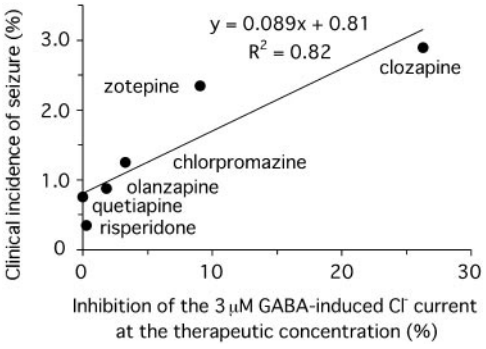

Figure 3 The clinical incidence of seizure and the inhibitory effect of neuroleptics on the 3 μM GABA-induced Cl^- current. The ordinate represents the clinical incidence of seizure during treatment with neuroleptics. The abscissa represents the calculated value for the percentage inhibition of the 3 μM GABA-induced Cl^- current induced by neuroleptics at the therapeutic concentration (Table 1). The obtained correlation curve was $y = 0.089x + 0.81$. The correlation coefficient was 0.82.

The mechanism of the inhibitory effect of clozapine

Regarding the GABA concentration-response curve, the EC_{50} for GABA and the Hill coefficient were 16.51 μM and 1.29 (Figure 4). Clozapine (Figure 4) and picrotoxin (data not shown) reduced the GABA-induced Cl^- current at each concentration of GABA and shifted the GABA concentration-response curve downward. In the presence of 1 μM clozapine and 1 μM picrotoxin, the EC_{50} for GABA were 16.17 and 15.84 μM and the Hill coefficients were 1.40 and 1.16, respectively. The maximal responses to 300 μM GABA in the presence of clozapine and picrotoxin, relative to the peak amplitude of the normal 300 μM GABA-induced Cl^- current, were 83.37 ± 5.04 ($n = 7$) and 84.25 ± 3.80% ($n = 10$), respectively.

The 3 μM GABA-induced Cl^- current was bicuculline-sensitive in the presence of clozapine at each concentration less than 10 μM (Figure 5). However, in the presence of 100 μM clozapine, the 3 μM GABA-induced Cl^- current was only slightly reduced by 0.1 μM bicuculline. Clozapine reduced the inhibitory effect of 1 μM picrotoxin on the 3 μM GABA-induced Cl^- current in a clozapine concentration-dependent manner (Figure 5).

The effects of haloperidol and quetiapine on the clozapine-induced inhibition of the 3 μM GABA-induced Cl^- current

Haloperidol decreased the inhibitory effects of 0.1 and 1 μM clozapine on the 3 μM GABA-induced Cl^- current in a haloperidol concentration-dependent manner (Figure 6A).

Haloperidol at 10 μM ($n=5$) and 100 μM ($n=5$) almost completely blocked the inhibitory effects of 0.1 μM clozapine. Haloperidol at 100 μM reduced the inhibitory effects of 1 μM clozapine to 37.62±9.38% ($n=4$) of that in the absence of haloperidol. However, quetiapine did not influence the inhibitory effects of 0.1 and 1 μM clozapine (Figure 6B).

The modulations of the desensitization of the 3 μM GABA-induced Cl⁻ current

The 3 μM GABA-induced Cl⁻ current gradually decreased in the continuous application (Figure 7). The decrease in the amplitude of the 3 μM GABA-induced Cl⁻ current at the end of the application for 120 s was 27.82±2.60% of the peak amplitude ($n=6$).

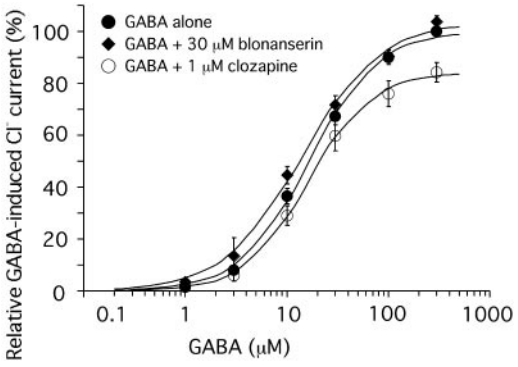

Figure 4 The modulations of the GABA concentration-response relationship by clozapine and blonanserin. The peak amplitude of the GABA-induced Cl⁻ current in the absence and presence of clozapine or blonanserin, relative to the normal 300 μM GABA-induced Cl⁻ current, was plotted against each concentration of GABA. The fitting curve was obtained by Hill's equation. Clozapine at 1 μM and blonanserin at 30 μM shifted the GABA concentration-response curve downward and leftward, respectively. Each point represents the mean±s.e.mean ($n\geqslant 4$).

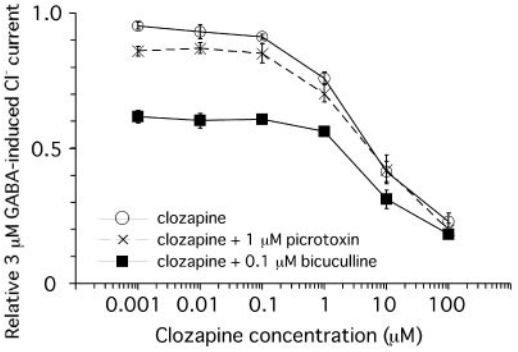

Figure 5 The effects of picrotoxin and bicuculline on the clozapine-induced inhibition of the 3 μM GABA-induced Cl⁻ current. The peak amplitudes of the 3 μM GABA-induced Cl⁻ current in the presence of the three series of clozapine (same as Figure 2), clozapine and either picrotoxin or bicuculline, relative to the control 3 μM GABA-induced Cl⁻ current, were plotted against each concentration of clozapine. Clozapine decreased the inhibitory effect of 1 μM picrotoxin. The current in the presence of clozapine at each concentration of less than 10 μM was bicuculline-sensitive. The current was only slightly reduced by 0.1 μM bicuculline in the presence of 100 μM clozapine. Each point represents the mean± s.e.mean ($n\geqslant 4$).

The desensitization was enhanced by picrotoxin and neuroleptics (Figure 1). The amplitudes at the end of the application for 7 s in the presence of picrotoxin, clozapine, zotepine, chlorpromazine, olanzapine and risperidone at 10 μM were 21.41±6.60 ($n=4$), 43.54±4.07 ($n=5$), 42.27±6.15 ($n=5$), 61.78±11.09 ($n=4$), 73.18±2.49 ($n=4$) and 80.07±4.35% ($n=4$) of the peak amplitude of each current, respectively. That for picrotoxin and each neuroleptic agent at 100 μM were 10.89±3.39, 11.51±6.51, 6.20±3.57, 29.80±7.23, 13.92±2.85 and 74.72±4.78%, respectively ($n=4$). Haloperidol and quetiapine also slightly enhanced the desensitization in a concentration-dependent manner (Figure 7).

The desensitization of the 3 μM GABA-induced Cl⁻ current in the presence of haloperidol or quetiapine at 100 μM was smaller than that even in the absence of haloperidol nor quetiapine at the beginning of the application for 10 s. Then the desensitization of the 3 μM GABA-induced Cl⁻ current in the presence of haloperidol or quetiapine at 100 μM remarkably increased more than 10 s later (Figure 7).

After the washout of haloperidol or quetiapine, the peak amplitude of the 3 μM GABA-induced Cl⁻ current recovered completely for several minutes (data not shown). DMSO at 0.1% (v v^{-1}) did not affect the desensitization of the 3 μM GABA-induced Cl⁻ current (Figure 7B).

The potentiation of the 3 μM GABA-induced Cl⁻ current by blonanserin

Blonanserin potentiated the 3 μM GABA-induced Cl⁻ current in a concentration-dependent manner at higher than 1 μM (Figures 2, 8). The peak amplitudes of the 3 μM GABA-induced Cl⁻ current in the presence of 10, 30 and 50 μM blonanserin, relative to the peak amplitude of the control current in the absence of blonanserin, were 1.19±0.02 ($n=25$), 1.70±0.07 ($n=32$) and 1.92±0.11 ($n=26$), respec-

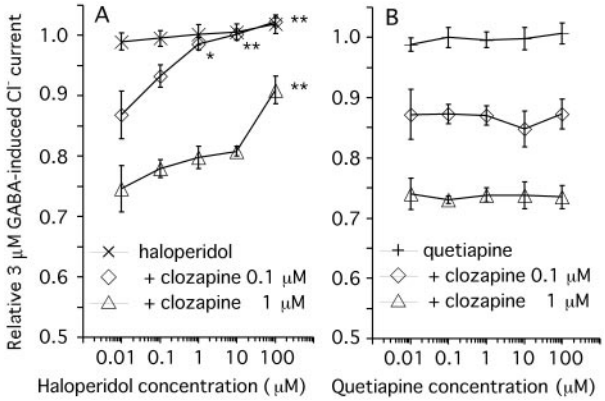

Figure 6 The effects of haloperidol and quetiapine on the clozapine-induced inhibition of the 3 μM GABA-induced Cl⁻ current. The peak amplitudes of the 3 μM GABA-induced Cl⁻ current in the presence of clozapine at each concentration of 0 (same as Figure 2), 0.1, 1 μM and either haloperidol (A) or quetiapine (B), relative to the control 3 μM GABA-induced Cl⁻ current, was plotted against each concentration of haloperidol or quetiapine. The clozapine-induced inhibition was reduced by haloperidol, but was not involved by quetiapine ($P>0.05$). The statistical significance of difference between the clozapine-induced inhibitions in the presence and absence of haloperidol was obtained (*$P<0.05$, **$P<0.01$). Each point represents the mean±s.e.mean ($n\geqslant 4$).

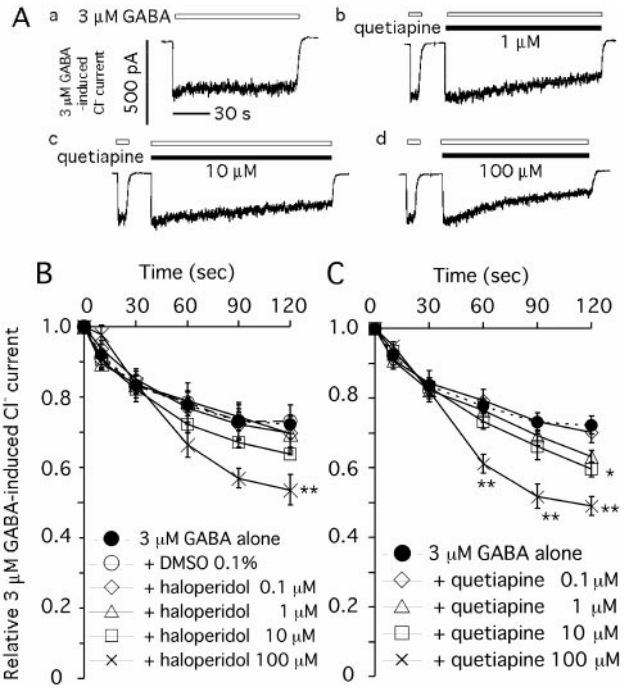

Figure 7 The modulations of the desensitization of the 3 μM GABA-induced Cl^- current by haloperidol and quetiapine. (A) The 3 μM GABA-induced Cl^- current decreased in the continuous application (a). The desensitization was enhanced by quetiapine at 1 μM (b), 10 μM (c), 100 μM (d). Quetiapine at 100 μM did not enhance at the beginning of the application for 10 s and thereafter, did remarkably enhance the desensitization of the current. (B,C) The amplitudes of the 3 μM GABA-induced Cl^- current in the absence and presence of either haloperidol, 0.1% DMSO (B) or quetiapine (C), relative to the peak amplitude of the current, were plotted against each time from the start of application. The statistical significance of difference between the amplitudes in the presence and absence of either haloperidol or quetiapine was obtained (*$P<0.05$, **$P<0.01$). The amplitude at 10 s in the presence of haloperidol or quetiapine at 100 μM was smaller than that for GABA alone. DMSO at 0.1% ($v v^{-1}$) did not affect the desensitization ($P>0.05$). Each point represents the mean$\pm$s.e.mean ($n\geqslant 4$).

tively. The potentiations by blonanserin widely ranged and the ranges of the peak amplitude of the 3 μM GABA-induced Cl^- current in the presence of 30 and 50 μM blonanserin, relative to the control current, were 1.07 to 2.71 and 1.14 to 3.43, respectively. The effect of blonanserin was not observed after the washout of blonanserin for several minutes (data not shown). Blonanserin at 50 μM produced no current in the absence of GABA ($n=4$, data not shown). The 3 μM GABA-induced Cl^- current, potentiated by blonanserin, exerted a bicuculline-sensitive manner in each concentration of blonanserin (Figure 9).

Blonanserin at 30 μM shifted the GABA concentration-response curve leftward (Figure 4). The EC_{50} for GABA in the presence of 30 μM blonanserin was 14.12 μM. The Hill coefficient was 1.12. The maximal response to 300 μM GABA in the presence of 30 μM blonanserin was 103.70$\pm$2.39% ($n=17$), relative to the peak amplitude of the normal 300 μM GABA-induced Cl^- current.

Diazepam at 1 μM potentiated the 3 μM GABA-induced Cl^- current almost maximally (data not shown). The additive potentiation of the 3 μM GABA-induced Cl^- current by 1 μM diazepam in the presence of blonanserin decreased at concentrations of blonanserin higher than 10 μM (Figure 9). Flumazenil at 10 μM slightly potentiated the 3 μM GABA-induced Cl^- current (Figure 9). The potentiation by 1 μM diazepam was almost completely reversed by 10 μM flumazenil in the presence of 0.01 or 50 μM blonanserin. Flumazenil at 10 μM did not reversed the effect of blonanserin at each concentration (Figure 9).

Discussion

The diverse effects of neuroleptics on the GABA-induced Cl^- current evoked through the $GABA_A$ receptor-Cl^- channel complex were indicated in this study. The effects were (1) an inhibition of the current and (2) an enhancement of desensitization. Furthermore, the other effects of (3) a reduction in the inhibitory effect of clozapine, (4) an inhibition of desensitization and (5) a potentiation of the current were also indicated.

The inhibition of the GABA-induced Cl^- current

Neuroleptics is related to clinical seizure. The clozapine-induced seizure was prevented by the co-administration of some anticonvulsants clinically (Lane *et al*., 1999; Usiskin *et*

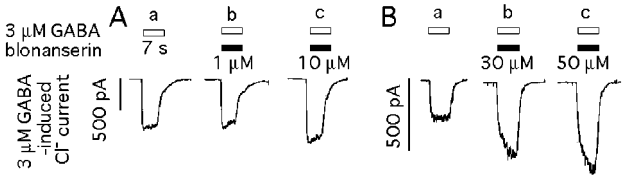

Figure 8 The potentiation of the 3 μM GABA-induced Cl^- current by blonanserin. (A) The 3 μM GABA-induced Cl^- current was little potentiated by 1 μM blonanserin (b) and was potentiated by 10 μM blonanserin (c). (B) Blonanserin at 30 μM (b) and 50 μM (c) gradually potentiated the current.

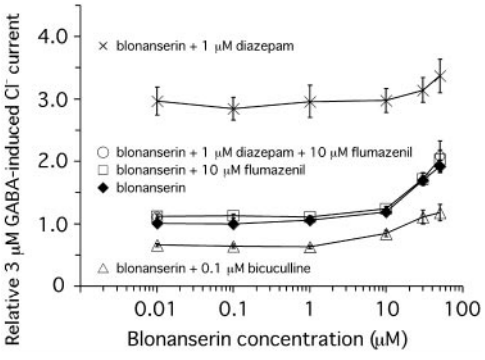

Figure 9 The modulation of the 3 μM GABA-induced Cl^- current potentiated by blonanserin. The peak amplitudes of the 3 μM GABA-induced Cl^- current in the presence of the five series of blonanserin (same as Figure 2), blonanserin and either bicuculline, diazepam, flumazenil or both diazepam and flumazenil, relative to the control 3 μM GABA-induced Cl^- current, were plotted against each concentration of blonanserin. The potentiation was not sensitive to 10 μM flumazenil but partly interacted with 1 μM diazepam. The potentiation by 1 μM diazepam was completely reversed by 10 μM flumazenil even in the presence of blonanserin at 0.01 or 50 μM. Each point represents the mean$\pm$s.e.mean ($n\geqslant 4$).

al., 2000). The clinical incidence of seizure depends on both dose (Devinsky *et al*., 1991; Manmaru, 1985) and individuality (Table 1) of neuroleptics.

The inhibitory effect of neuroleptics on the GABA-induced Cl^- current (Figures 2 & 4) was similar to picrotoxin (data not shown), which is a proconvulsant agent. The clinical incidence of seizure was correlated with the inhibitory effect on the GABA-induced Cl^- current weighted with the therapeutic concentration of each neuroleptic agent (Table 1, Figure 3). The individuality of neuroleptics for the inhibitory effect on the GABA-induced Cl^- current is thus suggested to involve the agent-dependent manner of the neuroleptics-induced seizure.

The clinical incidences of seizure were reported to be 1.0, 2.7 and 4.4% during the treatment with clozapine at doses of less than 300 mg day^{-1}, 300 to 600 mg day^{-1} and more than 600 mg day^{-1}, respectively (Devinsky *et al*., 1991). The plasma concentration of clozapine when a seizure occurred was elevated to 4.02 μM much higher than the previous level ranged from 0.45 to 2.42 μM. The clozapine concentrations were 6.71 and 6.32 μM at twice of the occurrence of seizure in the other case (Simpson & Cooper, 1978). The neuroleptic-induced seizure may be concentration-dependent.

The Hill coefficients as a slope factor for olanzapine and risperidone were higher than the other neuroleptics (Figure 2). Olanzapine and risperidone only slightly exerted the inhibitory effect at lower than 1 μM (Figure 2). The therapeutic serum or plasma concentrations were 25–150 nM for olanzapine (Olesen & Linnet, 1999) and less than 108 nM for risperidone (Nagasaki *et al*., 1999). In contrast olanzapine at higher than 1 μM exerted a marked inhibitory effect (Figure 2). Olanzapine occasionally induced clinical seizure (Lee *et al*., 1999) and one fatal case has also been reported (Wyderski *et al*., 1999). Risperidone is reported to induce clinical seizures at overdose levels (Acri & Henretig, 1998). The concentration-dependent manner for the inhibitory effect on the GABA-induced Cl^- current may be related to clinical seizure at elevated concentration levels of neuroleptics.

It must be considered that the effect of neuroleptics on the GABA-induced Cl^- current influence the seizure threshold because of a dependent manner on individualities and concentrations of neuroleptics in the present investigation. On the other hand, the combined effects of neuroleptics on the many other ion channels must be also investigated hereafter.

Picrotoxin binds to the TBPS binding site on the $GABA_A$ receptor-Cl^- channel complex (Höld *et al*., 2000; Ikeda *et al*., 1999). Clozapine antagonized the inhibitory effect of picrotoxin on the GABA-induced Cl^- current (Figure 5) and inhibited [^{35}S]-TBPS binding in the absence of GABA in a [^{35}S]-TBPS binding study (Korpi *et al*., 1995). These results therefore suggest that clozapine affects the TBPS binding site and competes with picrotoxin and [^{35}S]-TBPS to bind to the TBPS binding site.

The recovery from the inhibition of the GABA-induced Cl^- current varied for each ligand of the TBPS binding site (Höld *et al*., 2000; Ikeda *et al*., 1999, 2001) including neuroleptics (Figure 1; Yang & Zorumski, 1989; Zorumski & Yang, 1988) in patch-clamp studies. The recovering times (data not shown) were not merely proportional to the degrees of inhibition of the GABA-induced Cl^- current. However, the mechanism regarding the differences in reversibility remains to be elucidated.

Clozapine at higher than 10 μM reduced the bindings of [^{3}H]-muscimol and [^{3}H]-SR 95531, which are $GABA_A$ receptor agonist and antagonist, in a binding study (Korpi *et al*., 1995). Bicuculline exerted only a slight effect in the presence of 100 μM clozapine (Figure 5) probably because of the interfering effect of clozapine on the binding of both GABA and bicuculline on $GABA_A$ receptor. However, it is not clear whether this effect of clozapine is mediated by the TBPS binding site.

The reduction in the inhibitory effect of clozapine

Haloperidol reduced the inhibitory effects of clozapine (Figure 6A) and decreased [^{35}S]-TBPS binding in a [^{35}S]-TBPS binding study (Korpi *et al*., 1995). Haloperidol may thus be suggested to inhibit clozapine and [^{35}S]-TBPS binding to the TBPS binding site. Haloperidol may be an antagonist of the TBPS binding site. This finding also indicates that clozapine is a ligand of the TBPS binding site same as [^{35}S]-TBPS.

The enhancement and inhibition of desensitization

Every non-competitive antagonists of the GABA-induced Cl^- current (Ikeda *et al*., 1999; 2001) including neuroleptics (Figure 1; Yang & Zorumski, 1989) enhance the desensitization of the current. However, haloperidol and quetiapine also enhanced the desensitization (Figure 7). Haloperidol exerted the antagonism against clozapine instead of the inhibitory effect (Figure 6). While quetiapine exerts neither effect (Figure 6). The enhancement of the desensitization was thus differentiated from the inhibition of the current and the antagonism against clozapine. Furthermore, haloperidol and quetiapine at 100 μM either facilitate the current or interfere with the desensitization of the current (Figure 7). The mechanism of all these effects was not unified. The mechanisms and the affecting sites of every effects of neuroleptics on the $GABA_A$ receptor-Cl^- channel complex are not clear.

The potentiation of the GABA-induced Cl^- current

The current in the presence of blonanserin exerted the blonanserin concentration-dependent augmentation and was bicuculline-sensitive (Figures 8 & 9). Blonanserin alone produced no current (data not shown). Therefore, the increase in the current represented the potentiation of the GABA-induced Cl^- current by blonanserin.

Regarding the shift of the GABA concentration-response curve, blonanserin (Figure 4) was similar to diazepam (data not shown). However, the potentiation by blonanserin was not reversed by flumazenil (Figure 9) and blonanserin only slightly antagonized the binding of [^{3}H]-GABA on $GABA_A$ receptor and [^{3}H]-diazepam on benzodiazepine receptor in a binding study (Oka *et al*., 1993). These results suggest that blonanserin is not a ligand of benzodiazepine receptor. The potentiation of the GABA-induced Cl^- current mediated the blonanserin binding site was slightly interfered with the potentiation by diazepam (Figure 9). Regarding the potentiations mediated the blonanserin binding site, the approximate

3 fold differences were observed among neurons. These differences mediated the blonanserin binding site may possibly be related to the diverse subtypes of the $GABA_A$ receptor-Cl^- channel complex.

Blonanserin is a newer SDA and exerts an antipsychotic effect (Noda *et al*., 1993; Oka *et al*., 1993). Blonanserin exerts an anticonvulsant effect in mice at high doses in contrast with the other neuroleptics. The data showed that electroshock-induced convulsion was inhibited in two of six mice by the oral administration of high dose blonanserin at 100 mg kg^{-1}, and was not inhibited by blonanserin at a moderate dose of 30 mg kg^{-1}. The pentetrazole-induced convulsion was inhibited in one of six mice by blonanserin at 30 mg kg^{-1} and two of six mice by blonanserin at 100 mg kg^{-1} (Dainippon Pharmaceutical Corporation, private communication). These results thus suggest that the potentiation of the GABA-induced Cl^- current by blonanserin at high concentrations may involve the anticonvulsant effect of blonanserin at high doses. Not only an inhibitory effect but also a potentiating effect of neuroleptics on the GABA-induced Cl^- current is indicated to possibly be related to the modulation of the seizure threshold.

The modulations of the $GABA_A$ receptor-Cl^- channel complex and the effects of neuroleptics

The $GABA_A$ receptor-Cl^- channel complex is mainly composed by α, β, γ subunits. There are variations in each subunit. The combination of the various subunits induces diversity in the $GABA_A$ receptor-Cl^- channel complex. The inhibitory effect of clozapine also varied for several subtypes of the $GABA_A$ receptor-Cl^- channel complex in a previous [^{35}S]-TBPS binding study (Korpi *et al*., 1995). Clozapine antagonized the GABA response in most brain regions. However, the antagonism of the GABA response induced by clozapine exhibited diversity between brain regions, probably because of the different distribution of various $GABA_A$ receptor-Cl^- channel complex subtypes (Korpi *et al*., 1995).

The non-competitive inhibitions of the GABA-induced Cl^- current by various agents were investigated on the rat DRG neurons in previous patch-clamp studies (Höld *et al*., 2000; Ikeda *et al*., 1998; 1999; 2001). The $GABA_A$ receptor-Cl^- channel complex on the DRG neuron is composed of α1-3, β2-3 and γ2 subunits (Persohn *et al*., 1991; Serafini *et al*., 1998; Zhai *et al*., 1998). The $\alpha1\beta2\gamma2$ subtype is major in the most regions of the central nervous system (Korpi *et al*., 1995) and also exists on the rat DRG neuron (Zhai *et al*., 1998). However, it is possible that the effect on the rat DRG neuron may be different from that on other neurons in the central nervous system. The influence of neuroleptics on raising or lowering the seizure threshold must be also examined on some neurons of the central nervous system.

The $GABA_A$ receptor-Cl^- channel complex is widely distributed in the central nervous system and modulated by many factors such as endogenous modulators, exogenous modulators and second messengers. GABA and other neurotransmitters such as 5-HT and glutamate modulate each other (Zorumski & Isenberg, 1991). The clinical adverse effects of neuroleptics, which may be related to the GABA function, are not only seizure but also akathisia, restless leg syndrome, tardive dyskinesia and so on (Verghese *et al*., 1996). These adverse effects of neuroleptics are sometimes affected by clonazepam, which is a benzodiazepine receptor agonist. Furthermore, the inhibitory effect of clozapine on the GABA response is also suggested to be related to an antipsychotic effect (Farnbach-Pralong *et al*., 1998; Squires & Saederup, 1999). The effects of neuroleptics on the GABA-induced Cl^- current may involve various clinical effects of neuroleptics through the modulation of diverse functions mediated by the $GABA_A$ receptor-Cl^- channel complex in the central nervous system.

References

ACRI, A.A. & HENRETIG, F.M. (1998). Effects of risperidone in overdose. *Am. J. Emerg. Med.,* **16,** 498 – 501.

ARANA, G.W. (2000). An overview of side effects caused by typical antipsychotics. *J. Clin. Psychiatry,* **61**(suppl 8), 5 – 11.

BEASLEY, JR, C.M., TOLLEFSTON, G.D. & TRAN, P.V. (1997). Safety of olanzapine. *J. Clin. Psychiatry,* **58**(suppl 10), 13 – 17.

CASEY, D.E. (1997). The relationship of pharmacology to side effects. *J. Clin. Psychiatry,* **58**(suppl 10), 155 – 162.

CHETTY, M., GOUWS, E., MILLER, R. & MOODLEY, S.V. (1999). The use of a side effect as a qualitative indicator of plasma chlorpromazine levels. *Eur. Neuropsychopharmacol.,* **9,** 77 – 82.

CIRIMELE, V., KINTZ, P., GOSSELIN, O. & LUDES, B. (2000). Clozapine dose-concentration relationships in plasma, hair and sweat specimens of schizophrenic patients. *Forensic Sci. Int.,* **107,** 289 – 300.

DEVINSKY, O., HONIGFELD, G. & PATIN, J. (1991). Clozapine-related seizures. *Neurology,* **41,** 369 – 371.

FARNBACH-PRALONG, D., BRADBURY, R., COPOLOV, D. & DEAN, B. (1998). Clozapine and olanzapine treatment decreases rat cortical and limbic $GABA_A$ receptors. *Eur. J. Pharmacol.,* **349,** R7 – R8.

HAMILL, O.P., MARTY, A., NEHER, E., SAKMANN, B. & SIGWORTH, F.J. (1981). Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflügers Arch.,* **391,** 85 – 100.

HÖLD, K.M., SIRISOMA, N.S., IKEDA, T., NARAHASHI, T. & CASIDA, J.E. (2000). α-thujone (the active component of absinthe): γ-aminobutyric acid type A receptor modulation and metabolic detoxification. *Proc. Natl. Acad. Sci. U.S.A.,* **97,** 3826 – 3831.

IKEDA, T., NAGATA, K., SHONO, T. & NARAHASHI, T. (1998). Dieldrin and picrotoxinin modulation of GABA(A) receptor single channels. *Neuroreport,* **9,** 3189 – 3195.

IKEDA, T., OZOE, Y., OKUYAMA, E., NAGATA, K., HONDA, H., SHONO, T. & NARAHASHI, T. (1999). Anistin modulation of the γ-aminobutyric acid receptor-channel in rat dorsal root ganglion neurons. *Br. J. Pharmacol.,* **127,** 1567 – 1576.

IKEDA, T., ZHAO, X., NAGATA, K., KONO, Y., GHONO, T., YEH, J.Z. & NARAHASHI, T. (2001). Fipronil modulation of γ-aminobutyric $acid_A$ receptors in rat dorsal root ganglion neurons. *J. Pharmacol. Exp. Ther.,* **296,** 914 – 921.

KORPI, E.R., WONG, G. & LÜDDENS, H. (1995). Subtype specificity of γ-aminobutyric acid type A receptor antagonism by clozapine. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **352,** 365 – 373.

KURATA, Y., MARSZALEC, W., HAMILTON, B.J., CARTER, D.B. & NARAHASHI, T. (1993). Alcohol modulation of cloned $GABA_A$ receptor-channel complex expressed in human kidney cell lines. *Brain Res.,* **631,** 143 – 146.

LANE, H.Y., SU, K.P. & CHANG, W.H. (1999). Seizures after discontinuation of low-dose lorazepam from originally seizure-free clozapine regimen: combined effects? *J. Clin. Psychiatry,* **60,** 408 – 409.

LEE, J.W., CRISMON, M.L. & DORSON, P.G. (1999). Seizure associated with olanzapine. *Ann. Pharmacother.,* **33,** 554 – 556.

LOMAS, J., BOARDMAN, R.H. & MARKOWE, M. (1955). Complications of chlorpromazine therapy in 800 mental-hospital patients. *Lancet,* **1,** 1144 – 1147.

MANMARU, S. (1985). Five cases of schizophrenia with convulsive seizures during zotepine administration (in Japanese). *Seishin Igaku (Clinical Psychiatry),* **27,** 59–70.

MARKS, R.C. & LUCHINS, D.J. (1991). Antipsychotic medications and seizure. *Psychiatric Medicine,* **9,** 37–52.

MOTOMURA, H., FUJIKAWA, S., TASHIRO, N., ITO, Y. & OGATA, N. (1995). Single-channel analysis of two types of Na^+ currents in rat dorsal root ganglia. *Pflügers Arch.,* **431,** 221–229.

NAGASAKI, T., OHKUBO, T., SUGAWARA, K., YASUI, N., FURUKORI, H. & KANEKO, S. (1999). Determination of risperidone and 9-hydroxyrisperidone in human plasma by high-performance liquid chromatography: application to therapeutic drug monitoring in Japanese patients with schizophrenia. *J. Pharm. Biomed. Anal.,* **19,** 595–601.

NODA, Y., KURUMIYA, S., MIURA, Y. & OKA, M. (1993). Comparative study of 2-(4-ethyl-1-piperazinyl)-4-(fluorophenyl)-5,6,7,8,9,10-hexahydrocycloocta[b]pyridine (AD-5423) and haloperidol for their pharmacological activities related to antipsychotic efficacy and/or adverse side-effects. *J. Pharmacol. Exp. Ther.,* **265,** 745–751.

OGATA, N., YOSHII, M. & NARAHASHI, T. (1989). Psychotropic drugs block voltage-gated ion channels in neuroblastoma cells. *Brain Res.,* **476,** 140–144.

OKA, M., NODA, Y., OCHI, Y., URUKAWA, K., UNE, T., KURUMIYA, S., HINO, K. & KARASAWA, T. (1993). Pharmacological profile of AD-5423, a novel antipsychotic with both potent dopamine-D_2 and serotonin-S_2 antagonist properties. *J. Pharmacol. Exp. Ther.,* **264,** 158–165.

OLESEN, O.V. & LINNET, K. (1999). Olanzapine serum concentrations in psychiatric patients given standard doses: the influence of comedication. *Ther. Drug Monit.,* **21,** 87–90.

OTANI, K., HIRANO, T., KONDO, T., KANEKO, S., FUKUSHIMA, Y., NODA, K. & TASHIRO, Y. (1990). Biperiden and piroheptine do not affect the serum level of zotepine, a new antipsychotic drug. *Br. J. Psychiatry,* **157,** 128–130.

PERSOHN, E., MALHERBE, P. & RICHARDS, J.G. (1991). In situ hybridization histochemistry reveals a diversity of $GABA_A$ receptor subunit mRNAs in neurons of the rat spinal cord and dorsal root ganglia. *Neuroscience,* **42,** 497–507.

PHYSICIANS' DESK REFERENCE. (2000a). Risperdal® (risperidone). In *Physicians' Desk Reference* 54th edn. pp. 1453–1457. Montvale, NJ: Medical Economics.

PHYSICIANS' DESK REFERENCE. (2000b). Seroquel® (quetiapine). In *Physicians' Desk Reference*, 54th edn. pp. 562–566. Montvale, NJ: Medical Economics.

SERAFINI, R., MARIC, D., MARIC, I., MA, W., FRITSCHY, J.M., ZHANG, L. & BARKER, J. (1998). Dominant $GABA_A$ receptor/Cl^- channel kinetics correlate with the relative expression of α_2, α_3, α_5 and β_3 subunits in embryonic rat neurones. *Eur. J. Neurosci.,* **10,** 334–349.

SIMPSON, G.M. & COOPER, T.A. (1978). Clozapine plasma levels and convulsions. *Am. J. Psychiatry,* **135,** 99–100.

SQUIRES, R.F. & SAEDERUP, E. (1997). Clozapine and some other antipsychotic drugs may preferentially block the same subset of $GABA_A$ receptors. *Neurochem. Res.,* **22,** 151–162.

SQUIRES, R.F. & SAEDERUP, E. (1998). Clozapine and several other antipsychotic/antidepressant drugs preferentially block the same'core'fraction of $GABA_A$ receptors. *Neurochem. Res.,* **23,** 1283–1290.

SQUIRES, R.F. & SAEDERUP, E. (1999). Clozapine's antipsychotic effects don't depend on blockade of 5-HT_3 receptors. *Neurochem. Res.,* **24,** 659–667.

TATEBAYASHI, H. & OGATA, N. (1992). Kinetic analysis of the $GABA_B$-mediated of the high-threshold Ca^{2+} current in cultured rat sensory neurones. *J. Physiol. (Lond.),* **447,** 391–407.

USISKIN, S.I., NICOLSON, R., LENANE, M. & RAPOPORT, J.L. (2000). Gabapentin prophylaxis of clozapine-induced seizures. *Am. J. Psychiatry,* **157,** 482–483.

VERGHESE, C., DELEON, J., NAIR, C. & SIMPSON, G.M. (1996). Clozapine withdrawal effects and receptor profiles of typical and atypical neuroleptics. *Biol. Psychiatry,* **39,** 135–138.

WYDERSKI, R.J., STARRETT, W.G. & ABOU-SAIF, A. (1999). Fatal status epilepticus associated with olanzapine therapy. *Ann. Pharmacother.,* **33,** 787–789.

YANG, J. & ZORUMSKI, C.F. (1989). Trifluoperazine block GABA-gated chloride currents in cultured chick spinal cord neurons. *J. Neurophysiol.,* **61,** 363–373.

ZHAI, J., STEWART, R.R., FREIDBERG, M.W. & LI, C. (1998). Phosphorylation of the GABA receptor γ2L subunit in rat sensory neurons may not be necessary for ethanol sensitivity. *Brain Res.,* **805,** 116–122.

ZORUMSKI, C.F. & ISENBERG, K.E. (1991). Insights into the structure and function of GABA-benzodiazepine receptors: ion channels and psychiatry. *Am. J. Psychiarty,* **148,** 162–173.

ZORUMSKI, C.F. & YANG, J. (1988). Non-competitive of GABA currents by phenothiazines in cultured chick spinal cord and rat hippocampal neurons. *Neurosci. Lett.,* **92,** 86–91.

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