Inhibitory Effects of Psychotropic Drugs on the Acetylcholine Receptor-Operated Potassium Current (I_{K,ACH}) in Guinea-Pig Atrial Myocytes

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(Received 19 November 2012/Accepted 10 January 2013/Published online in J-STAGE 24 January 2013)

ABSTRACT. Influences of psychotropic drugs, six antipsychotics and three antidepressants, on acetylcholine receptor-operated potassium current (I_{K,ACH}) were examined by a whole-cell patch clamp method in freshly isolated guinea-pig atrial myocyte. I_{K,ACH} was induced by a superfusion of carbachol (CCh) or by an intracellular application of guanosine 5’-thio triphosphate (GTPγS). To elucidate mechanism for anticholinergic action, IC_{50} ratio, the ratio of IC_{50} for GTPγS-activated I_{K,ACH} to CCh-induced I_{K,ACH}, was calculated. Antipsychotics and antidepressants inhibited CCh-induced I_{K,ACH} in a concentration-dependent manner. The IC_{50} values were as follows; chlorpromazine 0.53 µM, clozapine 0.06 µM, fluphenazine 2.69 µM, haloperidol 2.66 µM, sulpiride 42.3 µM, thioridazine 0.07 µM, amitriptyline 0.03 µM, imipramine 0.22 µM and maprotiline 1.81 µM. The drugs, except for sulpiride, inhibited GTPγS-activated I_{K,ACH} with following IC_{50} values; chlorpromazine 1.71 µM, clozapine 14.9 µM, fluphenazine 3.55 µM, haloperidol 2.73 µM, thioridazine 1.90 µM, amitriptyline 7.55 µM, imipramine 7.09 µM and maprotiline 5.93 µM. The IC_{50} ratio for fluphenazine and haloperidol was close to unity. The IC_{50} ratio for chlorpromazine, clozapine, thioridazine, amitriptyline, imipramine and maprotiline was much higher than unity. The present findings suggest that the psychotropics studied suppress I_{K,ACH}. Chlorpromazine, clozapine, thioridazine, amitriptyline, imipramine, maprotiline and sulpiride are preferentially acting on muscarinic receptor. Fluphenazine and haloperidol may act on G protein and/or potassium channel.

KEYWORDS: acetylcholine receptor-operated potassium current, antidepressants, antipsychotics, atrial myocyte, patch clamp method.


Psychotropic drugs are diverse classes of chemicals that alter mental functions. Some of psychotropic drugs are applied to psychiatric disturbances, such as schizophrenic syndrome, depression, mania and anxiety in human clinical field[3, 4]. In veterinary clinical settings, these drugs are utilized for treating animal behavioral disorders [10]. Although these drugs have a high therapeutic index and are generally safe agents, cardiovascular side effects by direct actions and/or indirect actions through central nervous system and autonomic reflexes were reported. Several antipsychotics and antidepressants inhibited cardiac repolarization and prolonged QTc, resulting in an increased risk of malignant arrhythmia, such as torsades de pointes and sudden death, in psychiatric patients taking these drugs [23–25, 30, 32, 34]. Psychotropic drugs demonstrating QT prolongation can block voltage-gated potassium channel, a human ether-a-go-go related-gene (HERG) channel, thereby decreasing a delayed rectifier potassium current [2, 15, 20, 21, 27, 31]. Thus, influences of psychototropic drugs on the voltage-gated potassium channel were well examined. On the other hand, influences of psychotropic drugs on ligand-gated potassium channel were not extensively examined.

The ligand-gated potassium channels are members of a family of inward-rectifier potassium channels and are guanosine 5’-triphosphate binding protein (G protein)-activated inwardly rectifying potassium (GIRK) channels [11]. Inhibition of monoamine transporters by antidepressants in the brain is generally thought to have important implications in their therapeutic effect. In contrast, the interaction of antidepressants with muscarinic, adrenergic and histaminergic receptors is involved in some of the adverse side effects [3, 4]. In this context, the interaction with ligand-gated potassium channel is another target for cardiac side effects of psychotropic drugs. Our group previously reported that benzodiazepines, anxiolytics, inhibited the acetylcholine receptor-operated potassium current (I_{K,ACH}) in relatively higher concentration than that of clinical concentration [26]. It was reported that a couple of antipsychotics and antidepressants inhibited GIRK channel current in an over-expression system [16, 17]. Moreover, chlorpromazine inhibited I_{K,ACH} in rat cardiac myocytes [1]. However, influences of most psychotropic drugs on native cardiac myocytes were not fully examined.

In the present study, influences of 6 antipsychotics, including chlorpromazine, clozapine, fluphenazine, haloperidol, sulpiride and thioridazine, and 3 antidepressants, including amitriptyline, imipramine and maprotiline, on I_{K,ACH} were examined by a whole-cell patch clamp method in freshly isolated guinea-pig atrial myocytes. And, mechanisms of the anticholinergic action of these drugs were explored. From this study, it is concluded that the psychotropic drugs studied had anticholinergic effects in atrial myocytes through suppressing I_{K,ACH} via different mechanisms.
MATERIALS AND METHODS

This study was performed in accordance with the “Guiding principles for the Care and Use of Laboratory Animals” approved by the Japanese Pharmacological Society and the Kitasato University. The methods for cell preparations and current recordings were the same as the previous ones [7–9]. Briefly, guinea-pig (male, 250–750 g body weight) hearts were harvested under sodium pentobarbital (50 mg/kg i.p.) anesthesia and set on a modified Langendorff apparatus for isolation of single atrial myocytes by an enzymatic digestion with collagenase. Whole-cell patch clamp method was used for recording of I_{ACh} as an outward current at a holding potential of −40 mV. I_{ACh} was induced by a superfusion of 1 µM carbachol (CCh) or by an intracellular application of 100 µM guanosine 5'-[γ-thio] triphosphate (GTPγS), a nonhydrolysable guanosine 5'-triphosphate (GTP) analogue. The normal N-[2-hydroxyethyl]piperazine-N'-[2-ethylene sulfonic acid (HEPES)-Tyrode solution (pH 7.4) and the standard pipette solution were used as superfusate and inner solution, respectively. The composition of HEPES-Tyrode solution was (mM): NaCl 143, KCl 5.4, CaCl2 1.8, MgCl2 0.5, NaH2PO4 0.33, glucose 5.5 and HEPES 5.0. The composition of the pipette solution was (mM): K-aspartate 110, KCl 20, MgCl2 1.0, adenosine-5'-triphosphate (ATP)-K2 5.0, phosphocreatinine-K2 5.0, ethylene glycol-bis (2-aminoethylether)-N,N,N',N'-tetraacetic acid (EGTA) 10 and HEPES 5.0 (pH 7.4, free Ca²⁺ concentration, pCa 8). GTP 100 µM or GTPγS 100 µM was also added to the pipette solution.

Chlorpromazine and haloperidol were purchased from Wako Pure Chemical Industries (Osaka, Japan). CCh, GTPγS, Clozapine, fluphenazine, sulphiride, thioridazine, amitriptyline, imipramine and maprotiline were obtained from Sigma-Aldrich (St. Louis, MO, U.S.A.). All psychotropic drugs were dissolved in dimethyl sulfoxide (DMSO) as a stock solution and added to the superfusate. The concentrations of psychotropic drugs applied were increased in a stepwise fashion every three min. The final concentration of DMSO was less than 1%, and this concentration of DMSO did not affect I_{ACh} recording.

Data analysis: In the recordings of I_{ACh} current, the activated current is followed by a continuous decline by desensitization [19]. Continuous current decline before psychotropic drug treatment was assumed as quasi steady state (QSS). We used QSS as a maximum current. All values are presented as mean ± standard error of mean (SEM). The concentrations required to produce 50% of the maximal inhibitory effect (IC50) were calculated from concentration-response curves using Math Curve Fitter (SigmaPlot, Jandel Scientific, CA, U.S.A.) to solve nonlinear equations. To elucidate mechanisms for the anticholinergic effect of psychotropic drugs, a ratio of IC50 values for inhibition of the GTPγS-activated I_{ACh} to the carbachol-induced I_{ACh} was calculated using the following equation [6, 26]:

IC50 Ratio = [IC50 for GTPγS-activated current]/[IC50 for carbachol-induced current].

RESULTS

Influences of 6 antipsychotics on CCh-induced and GTPγS-activated I_{ACh} in single guinea-pig atrial myocytes: Antipsychotics, including chlorpromazine, clozapine, fluphenazine, haloperidol, sulpiride and thioridazine, inhibited CCh-induced I_{ACh} in a concentration-dependent manner (Fig. 1). These drugs, except for sulpiride, inhibited GTPγS-activated I_{ACh} in a concentration-dependent manner (Fig. 1). It should be noted that amplitudes of the currents varied depending on size of myocytes used. Our previous study demonstrated that the maximum amplitudes of currents were converted from −150 to −400 pA in each stimulant (CCh and GTPγS) [26] and that there was no difference between the CCh-induced I_{ACh} and the GTPγS-activated one. Fluphenazine and haloperidol possessed inhibitory effects on both currents in the same concentration ranges. In the case of chlorpromazine, clozapine and thioridazine, higher concentrations were necessary for inhibition of GTPγS-activated I_{ACh} than CCh-induced I_{ACh}. The IC50 values are shown in Table 1. Of note, maximal percent inhibition of CCh-induced I_{ACh} by sulpiride 300 µM, the highest concentration tested, was 70.7 ± 6.7% (n=6). Sulpiride 300 µM had almost no inhibitory effect on GTPγS-activated I_{ACh} (5.7 ± 4.0%, n=6).

Influences of 3 antidepressants on CCh-induced and GTPγS-activated I_{ACh} in single guinea-pig atrial myocytes: Antidepressants, including amitriptyline, imipramine and maprotiline, inhibited both CCh-induced I_{ACh} and GTPγS-activated I_{ACh} in a concentration-dependent manner (Fig. 2). The IC50 values are shown in Table 1. Higher concentrations were necessary for inhibition of GTPγS-activated I_{ACh} than CCh-induced I_{ACh} in each drug.

IC50 ratio for inhibitory effects of psychotropic drugs in single guinea-pig atrial myocytes: To elucidate mechanisms for the anticholinergic effects of antipsychotics, the IC50 ratio was calculated. The IC50 ratio for fluphenazine (1.32) and haloperidol (1.03) was close to unity (Table 1). The IC50 ratio for chlorpromazine (3.23), clozapine (248.3) and thioridazine (27.1), was much higher than unity (Table 1). Because sulpiride showed almost no inhibition for GTPγS-activated I_{ACh}, the IC50 ratio was not determined. The IC50 ratio for antidepressants was also calculated. The IC50 ratio for amitriptyline (251.7), imipramine (32.7) and maprotiline (3.28) was much higher than unity (Table 1).

DISCUSSION

The psychotropic drugs studied had anticholinergic effects in freshly isolated atrial myocytes through suppressing I_{ACh} by different mechanisms. To elucidate mechanisms for the anticholinergic effects of psychotropic drugs, the IC50 ratio was calculated. IC50 ratio for chlorpromazine, clozapine, thioridazine, amitriptyline, imipramine and maprotiline was much higher than unity. So, these drugs are assumed to be preferentially acting on muscarinic M1 receptor. Because sulpiride showed concentration-dependent inhibitory effect on CCh-induced I_{ACh}, but it did not inhibit GTPγS-activated
Inhibition of IK.ACh: it might act on M\(_2\) receptor. In contrast, the IC\(_{50}\) ratio for fluphenazine and haloperidol was close to unity. G protein and/or potassium channel are acting points for these antipsychotics for anticholinergic action in atrial myocytes.

Cardiac side effects of psychotropic drugs, such as prolongation of QT, cardiac arrhythmias and sudden death are main concerns for treatment of psychiatric patients. Some drugs were withdrawn from a market, because of these types of side effects [33]. Psychotropic drugs demonstrating QT prolongation are known to block a voltage-gated potassium channel, HERG channel, thereby decreasing a delayed rectifier potassium current [2, 15, 20, 21, 27, 31]. Thus, influences of psychotropic drugs on the voltage-gated potassium channel were well examined. However, influences of psychotropic drugs on a ligand-gated potassium channel were not extensively examined. The ligand-gated potassium channels are members of a family of inward-rectifier potassium channels and are GIRQ channels which are gated directly by GTP-binding protein βγ-subunit [11, 28]. It was already reported that some psychotropic drugs with higher concentration ranges inhibited a cardiac type of GIRK channel in an over-expression system of *Xenopus* oocytes and CHO cells [16, 17, 28]. Moreover, it was reported that chlorpromazine inhibited the acetylcholine-induced IK.ACh with a threshold of ~30 nM in rat atrial cardiomyocyte [1]. In the present experiments, it was found that antipsychotics and antidepressants inhibited IK.ACh in native cardiac myocytes within or close to clinical plasma concentrations.

Possible mechanisms of anticholinergic actions of drugs in the heart have been proposed as follows: some drugs may block the muscarinic receptor, and others inhibit the muscarinic potassium channel itself and/or GTP-binding proteins [6–9, 12, 13, 22]. To elucidate the mechanisms for the anticholinergic effects of psychotropic drugs, the IC\(_{50}\) ratio, a ratio of IC\(_{50}\) for GTPγS-activated IK.ACh to CCh-induced IK.ACh, has been proposed [6, 26]. CCh induces IK.ACh through binding to muscarinic M\(_2\) receptor and subsequent activation of Gβγ-potassium channel interaction [14, 18], whereas intracellular loading of GTPγS can directly activate the interaction and thus evokes antagonist-resistant activation of IK.ACh [5]. Thus, the muscarinic potassium channel opening through stimulation of Gβγ-potassium channel interaction is a common pathway for induction of IK.ACh. In the case of drugs acting on the common pathway, the IC\(_{50}\) ratio would be close to unity. On the other hand, if the inhibitory action for IK.ACh was caused through a blockade of the muscarinic receptor binding, the IC\(_{50}\) ratio would be higher than unity. The IC\(_{50}\) value and IC\(_{50}\) ratio obtained from the present study are listed in Table 1. The IC\(_{50}\) ratio for fluphenazine (1.32) and haloperidol (1.03) was close to unity. Both drugs would thus act on the common pathway, as was previously demonstrated in diazepam, a benzodiazepine derivative [26]. Several mechanisms are presumed for the fluphenazine and/or haloperidol to inhibit the IK.ACh as follows: 1) direct inhibition of K.ACh channel, 2) inhibition of Gβγ-potassium channel interaction. Further experiments to determine the mechanisms are necessary. In contrast, the IC\(_{50}\) ratio for chlorpromazine (3.23), clozapine (248.3) and thioridazine (27.1) was much higher than unity. In addition, sulpiride showed almost no inhibition for GTPγS-activated IK.ACh.
The IC\textsubscript{50} ratio of antidepressants, including amitriptyline (251.7), imipramine (32.7) and maprotiline (3.28) was much higher than unity. Therefore, the main mechanism of these psychotropic drugs for inhibiting I\textsubscript{K.ACh} in atrial myocytes was assumed to be a muscarinic receptor level. Because all psychotropic drugs studied are highly lipid-soluble, we assume that the lipid solubility of drugs is not always related to the acting points of the drugs.

Clinical implication of the present study should be discussed. The therapeutic plasma concentrations of antipsychotics tested range approximately from 0.09 to 1.57 \textmu M for chlorpromazine, 0.92 to 1.84 \textmu M for clozapine, 0.46 to 9 nM for fluphenazine, 0.013 to 0.045 \textmu M for haloperidol, 0.15 to 1.76 \textmu M for sulphide and 0.27 to 5.4 \textmu M for thioridazine [29]. From the present results, it is indicated that therapeutic concentration of chlorpromazine, clozapine and thioridazine can produce anticholinergic actions on the heart via inhibiting CCh-induced I\textsubscript{K.ACh}. On the other hand, higher concentration is necessary for fluphenazine, haloperidol and sulphide to inhibit CCh-induced I\textsubscript{K.ACh}. Chlorpromazine and thioridazine can inhibit GTP\gamma S-activated I\textsubscript{K.ACh} at close to clinical concentration, while higher concentration than clinical settings is necessary for fluphenazine and haloperidol to inhibit GTP\gamma S-activated I\textsubscript{K.ACh}. The therapeutic plasma concentrations of various antidepressants in human range approximately from 0.18 to 1.1 \textmu M for amitriptyline, 0.17 to 1.25 \textmu M for imipramine and 0.36 to 2.2 \textmu M for maprotiline [29]. Therefore, the present studies suggest that I\textsubscript{K.ACh} may be inhibited by these antidepressants through blockade of muscarinic receptor at clinically relevant plasma concentrations. On the other hand, higher concentrations for these antidepressants are necessary to inhibit GTP\gamma S-activated I\textsubscript{K.ACh} through acting on the common pathway.

In summary, it is concluded that the psychotropic drugs studied, six antipsychotics and three antidepressants, suppress I\textsubscript{K.ACh} in a concentration-dependent manner. Chlorpromazine, clozapine, thioridazine, amitriptyline, imipramine, maprotiline and sulphide are presumed to be preferentially acting on muscarinic receptor. Fluphenazine and haloperidol may act on G protein and/or potassium channel. Thus, psychotropic drugs had the anticholinergic effects on atrial myocytes through inhibiting I\textsubscript{K.ACh} by different mechanisms.

<table>
<thead>
<tr>
<th>Drugs</th>
<th>IC\textsubscript{50} value (\textmu M)</th>
<th>IC\textsubscript{50} ratio</th>
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<td></td>
<td>Carbachol-induced I\textsubscript{K.ACh}</td>
<td>GTP\gamma S-activated I\textsubscript{K.ACh}</td>
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<td>Antipsychotics</td>
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<tr>
<td>Chlorpromazine</td>
<td>0.53 ± 0.19</td>
<td>1.71 ± 0.45</td>
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<tr>
<td>Clozapine</td>
<td>0.06 ± 0.01</td>
<td>14.9 ± 3.5</td>
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<tr>
<td>Fluphenazine</td>
<td>2.69 ± 0.98</td>
<td>3.55 ± 3.77</td>
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<tr>
<td>Haloperidol</td>
<td>2.66 ± 0.32</td>
<td>2.73 ± 0.41</td>
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<tr>
<td>Sulphide</td>
<td>42.3 ± 13.0</td>
<td>&gt; 300\textsuperscript{a}</td>
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<tr>
<td>Thioridazine</td>
<td>0.07 ± 0.01</td>
<td>1.90 ± 0.37</td>
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<tr>
<td>Antidepressants</td>
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<tr>
<td>Amitriptyline</td>
<td>0.03 ± 0.01</td>
<td>7.55 ± 0.74</td>
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<tr>
<td>Imipramine</td>
<td>0.22 ± 0.13</td>
<td>7.09 ± 1.44</td>
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<tr>
<td>Maprotiline</td>
<td>1.81 ± 0.46</td>
<td>5.93 ± 1.18</td>
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IC\textsubscript{50} values were determined by a mathematical curve fitting of concentration-response curves described in Figs. 1 and 2 (n=4–10). IC\textsubscript{50} ratio was calculated by a following equation; IC\textsubscript{50} ratio = [IC\textsubscript{50} for GTP\gamma S-activated current] / [IC\textsubscript{50} for CCh-induced current]. a) Sulphide 300 \textmu M had almost no effect on GTP\gamma S-activated I\textsubscript{K.ACh}. ND: Not determined.

REFERENCES


