

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

Muneyoshi OKADA<sup>1)</sup>, Shinya WATANABE<sup>1)</sup>, Takashi MATADA<sup>1)</sup>, Yoko ASAO<sup>1)</sup>, Ramu HAMATANI<sup>1)</sup>, Hideyuki YAMAWAKI<sup>1)\*</sup> and Yukio HARA<sup>1)</sup>

<sup>1)</sup>Laboratory of Veterinary Pharmacology, Kitasato University, Towada, Aomori 034-8628, Japan

(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 (GTP $\gamma$ S). To elucidate mechanism for anticholinergic action, IC<sub>50</sub> ratio, the ratio of IC<sub>50</sub> for GTP $\gamma$ S-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<sub>50</sub> values were as follows; chlorpromazine 0.53  $\mu$ M, clozapine 0.06  $\mu$ M, fluphenazine 2.69  $\mu$ M, haloperidol 2.66  $\mu$ M, sulpiride 42.3  $\mu$ M, thioridazine 0.07  $\mu$ M, amitriptyline 0.03  $\mu$ M, imipramine 0.22  $\mu$ M and maprotiline 1.81  $\mu$ M. The drugs, except for sulpiride, inhibited GTP $\gamma$ S-activated  $I_{K,ACh}$  with following IC<sub>50</sub> values; chlorpromazine 1.71  $\mu$ M, clozapine 14.9  $\mu$ M, fluphenazine 3.55  $\mu$ M, haloperidol 2.73  $\mu$ M, thioridazine 1.90  $\mu$ M, amitriptyline 7.55  $\mu$ M, imipramine 7.09  $\mu$ M and maprotiline 5.93  $\mu$ M. The IC<sub>50</sub> ratio for fluphenazine and haloperidol was close to unity. The IC<sub>50</sub> 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.

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

doi: 10.1292/jvms.12-0511; *J. Vet. Med. Sci.* 75(6): 743–747, 2013

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 psychotropic 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 gua-

nosine 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, antianxiety drugs, 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.

\*CORRESPONDENCE TO: Yamawaki, H., Laboratory of Veterinary Pharmacology, Kitasato University, 23-35-1 Higashi, Towada, Aomori 034-8628, Japan.  
e-mail: yamawaki@vmas.kitasato-u.ac.jp

## 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  $I_{K,ACh}$  as an outward current at a holding potential of  $-40$  mV.  $I_{K,ACh}$  was induced by a superfusion of  $1 \mu\text{M}$  carbachol (CCh) or by an intracellular application of  $100 \mu\text{M}$  guanosine 5'-[ $\gamma$ -thio] triphosphate (GTP  $\gamma\text{S}$ ), a nonhydrolyzable guanosine 5'-triphosphate (GTP) analogue. The normal N-[2-hydroxyethyl] piperazine-N'-[2-ethanesulfonic 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,  $\text{CaCl}_2$  1.8,  $\text{MgCl}_2$  0.5,  $\text{NaH}_2\text{PO}_4$  0.33, glucose 5.5 and HEPES 5.0. The composition of the pipette solution was (mM): K-aspartate 110, KCl 20,  $\text{MgCl}_2$  1.0, adenosine-5'-triphosphate (ATP)- $\text{K}_2$  5.0, phosphocreatinine- $\text{K}_2$  5.0, ethylene glycol-bis (2-aminoethylether)-N,N,N',N'-tetraacetic acid (EGTA) 10 and HEPES 5.0 (pH 7.4, free  $\text{Ca}^{2+}$  concentration, pCa 8). GTP  $100 \mu\text{M}$  or GTP $\gamma\text{S}$   $100 \mu\text{M}$  was also added to the pipette solution.

Chlorpromazine and haloperidol were purchased from Wako Pure Chemical Industries (Osaka, Japan). CCh, GTP $\gamma\text{S}$ , Clozapine, fluphenazine, sulpiride, 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 is less than 1%, and this concentration of DMSO did not affect  $I_{K,ACh}$  recording.

**Data analysis:** In the recordings of  $I_{K,ACh}$  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  $\pm$  standard error of mean (SEM). The concentrations required to produce 50% of the maximal inhibitory effect ( $\text{IC}_{50}$ ) 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  $\text{IC}_{50}$  values for inhibition of the GTP $\gamma\text{S}$ -activated  $I_{K,ACh}$  to the carbachol-induced  $I_{K,ACh}$  was calculated using the following equation [6, 26]:

$$\text{IC}_{50} \text{ Ratio} = [\text{IC}_{50} \text{ for GTP}\gamma\text{S-activated current}] / [\text{IC}_{50} \text{ for carbachol-induced current}].$$

## RESULTS

**Influences of 6 antipsychotics on CCh-induced and GTP $\gamma\text{S}$ -activated  $I_{K,ACh}$  in single guinea-pig atrial myocytes:** Antipsychotics, including chlorpromazine, clozapine, fluphenazine, haloperidol, sulpiride and thioridazine, inhibited CCh-induced  $I_{K,ACh}$  in a concentration-dependent manner (Fig. 1). These drugs, except for sulpiride, inhibited GTP $\gamma\text{S}$ -activated  $I_{K,ACh}$  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 converged from  $\sim 150$  to  $\sim 400$  pA in each stimulant (CCh and GTP $\gamma\text{S}$ ) [26] and that there was no difference between the CCh-induced  $I_{K,ACh}$  and the GTP $\gamma\text{S}$ -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 GTP $\gamma\text{S}$ -activated  $I_{K,ACh}$  than CCh-induced  $I_{K,ACh}$ . The  $\text{IC}_{50}$  values are shown in Table 1. Of note, maximal percent inhibition of CCh-induced  $I_{K,ACh}$  by sulpiride  $300 \mu\text{M}$ , the highest concentration tested, was  $70.7 \pm 6.7\%$  ( $n=6$ ). Sulpiride  $300 \mu\text{M}$  had almost no inhibitory effect on GTP $\gamma\text{S}$ -activated  $I_{K,ACh}$  ( $5.7 \pm 4.0\%$ ,  $n=6$ ).

**Influences of 3 antidepressants on CCh-induced and GTP $\gamma\text{S}$ -activated  $I_{K,ACh}$  in single guinea-pig atrial myocytes:** Antidepressants, including amitriptyline, imipramine and maprotiline, inhibited both CCh-induced  $I_{K,ACh}$  and GTP $\gamma\text{S}$ -activated  $I_{K,ACh}$  in a concentration-dependent manner (Fig. 2). The  $\text{IC}_{50}$  values are shown in Table 1. Higher concentrations were necessary for inhibition of GTP $\gamma\text{S}$ -activated  $I_{K,ACh}$  than CCh-induced  $I_{K,ACh}$  in each drug.

**$\text{IC}_{50}$  ratio for inhibitory effects of psychotropic drugs in single guinea-pig atrial myocytes:** To elucidate mechanisms for the anticholinergic effects of antipsychotics, the  $\text{IC}_{50}$  ratio was calculated. The  $\text{IC}_{50}$  ratio for fluphenazine (1.32) and haloperidol (1.03) was close to unity (Table 1). The  $\text{IC}_{50}$  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 GTP $\gamma\text{S}$ -activated  $I_{K,ACh}$ , the  $\text{IC}_{50}$  ratio was not determined. The  $\text{IC}_{50}$  ratio for antidepressants was also calculated. The  $\text{IC}_{50}$  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  $I_{K,ACh}$  by different mechanisms. To elucidate mechanisms for the anticholinergic effects of psychotropic drugs, the  $\text{IC}_{50}$  ratio was calculated.  $\text{IC}_{50}$  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  $\text{M}_2$  receptor. Because sulpiride showed concentration-dependent inhibitory effect on CCh-induced  $I_{K,ACh}$  but it did not inhibit GTP $\gamma\text{S}$ -activated

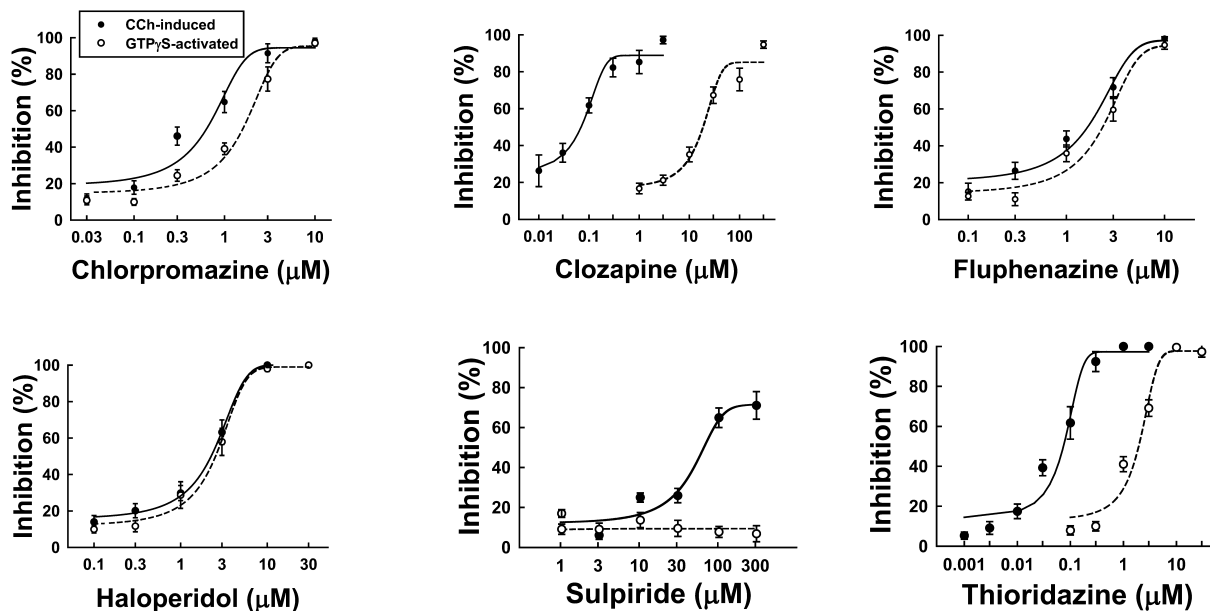


Fig. 1. Concentration-response curves for inhibitory effects of six antipsychotics on carbachol (CCh)-induced (closed circle) and guanosine 5'-[ $\gamma$ -thio] triphosphate (GTP $\gamma$ S)-activated (open circle)  $I_{K,ACh}$  in guinea-pig single atrial myocytes. CCh (1  $\mu$ M) and GTP $\gamma$ S (100  $\mu$ M) were applied extracellularly and intracellularly, respectively. Results are expressed as means  $\pm$  SEM of four to ten myocytes. Each antipsychotic, except for sulpiride, inhibited both current in a concentration-dependent manner. Almost no inhibitory effect of sulpiride on the GTP $\gamma$ S-activated current was observed.

$I_{K,ACh}$ , 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 GIRK channels which are gated directly by GTP-binding protein  $\beta\gamma$ -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  $I_{K,ACh}$  with a threshold of  $\sim 30$  nM in rat atrial cardiomyocyte [1]. In the present experiments, it was found that antipsychotics and antidepressants inhibited  $I_{K,ACh}$  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 GTP $\gamma$ S-activated  $I_{K,ACh}$  to CCh-induced  $I_{K,ACh}$ , has been proposed [6, 26]. CCh induces  $I_{K,ACh}$  through binding to muscarinic  $M_2$  receptor and subsequent activation of G $\beta\gamma$ -potassium channel interaction [14, 18], whereas intracellular loading of GTP $\gamma$ S can directly activate the interaction and thus evokes antagonist-resistant activation of  $I_{K,ACh}$  [5]. Thus, the muscarinic potassium channel opening through stimulation of G $\beta\gamma$ -potassium channel interaction is a common pathway for induction of  $I_{K,ACh}$ . 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  $I_{K,ACh}$  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  $I_{K,ACh}$  as follows: 1) direct inhibition of K.ACh channel, 2) inhibition of G $\beta\gamma$ -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 GTP $\gamma$ S-activated  $I_{K,ACh}$ .

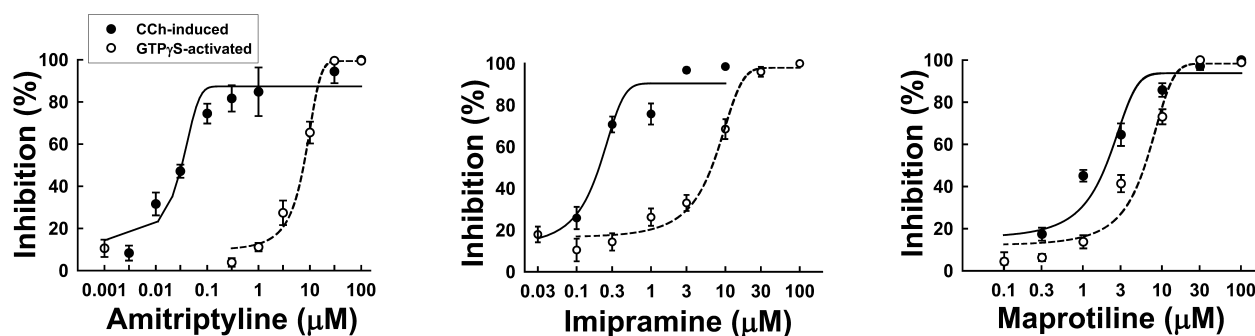


Fig. 2. Concentration-response curves for inhibitory effects of three antidepressants on CCh-induced (closed circle) and GTP $\gamma$ S-activated (open circle)  $I_{K,ACh}$  in guinea-pig single atrial myocytes. Results are expressed as means  $\pm$  SEM of four to ten myocytes. Each antidepressant inhibited both current in a concentration-dependent manner.

The  $IC_{50}$  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  $I_{K,ACh}$  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  $\mu$ M for chlorpromazine, 0.92 to 1.84  $\mu$ M for clozapine, 0.46 to 9 nM for fluphenazine, 0.013 to 0.045  $\mu$ M for haloperidol, 0.15 to 1.76  $\mu$ M for sulpiride and 0.27 to 5.4  $\mu$ 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  $I_{K,ACh}$ . On the other hand, higher concentration is necessary for fluphenazine, haloperidol and sulpiride to inhibit CCh-induced  $I_{K,ACh}$ . Chlorpromazine and thioridazine can inhibit GTP $\gamma$ S-activated  $I_{K,ACh}$  at close to clinical concentration, while higher concentration than clinical settings is necessary for fluphenazine and haloperidol to inhibit GTP $\gamma$ S-activated  $I_{K,ACh}$ . The therapeutic plasma concentrations of various antidepressants in human range approximately from 0.18 to 1.1  $\mu$ M for amitriptyline, 0.17 to 1.25  $\mu$ M for imipramine and 0.36 to 2.2  $\mu$ M for maprotiline [29]. Therefore, the present studies suggest that  $I_{K,ACh}$  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  $I_{K,ACh}$  through acting on the common pathway.

In summary, it is concluded that the psychotropic drugs studied, six antipsychotics and three antidepressants, suppress  $I_{K,ACh}$  in a concentration-dependent manner. Chlorpromazine, clozapine, thioridazine, amitriptyline, imipramine, maprotiline and sulpiride 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  $I_{K,ACh}$  by different mechanisms.

Table 1. Inhibitory effects of six antipsychotics and three antidepressants on carbachol (CCh)-induced and guanosine 5'-[ $\gamma$ -thio] triphosphate (GTP $\gamma$ S)-activated  $I_{K,ACh}$  in guinea-pig single atrial myocytes

Drugs	IC <sub>50</sub> value (μM)		IC <sub>50</sub> ratio
	Carbachol-induced I <sub>K,ACh</sub>	GTPγS-activated I <sub>K,ACh</sub>	
Antipsychotics			
Chlorpromazine	0.53 ± 0.19	1.71 ± 0.45	3.23
Clozapine	0.06 ± 0.01	14.9 ± 3.5	248.3
Fluphenazine	2.69 ± 0.98	3.55 ± 3.77	1.32
Haloperidol	2.66 ± 0.32	2.73 ± 0.41	1.03
Sulpiride	42.3 ± 13.0	> 300 <sup>a)</sup>	ND
Thioridazine	0.07 ± 0.01	1.90 ± 0.37	27.1
Antidepressants			
Amitriptyline	0.03 ± 0.01	7.55 ± 0.74	251.7
Imipramine	0.22 ± 0.13	7.09 ± 1.44	32.7
Maprotiline	1.81 ± 0.46	5.93 ± 1.18	3.28

$IC_{50}$  values were determined by a mathematical curve fitting of concentration-response curves described in Figs. 1 and 2 ( $n=4-10$ ).  $IC_{50}$  ratio was calculated by a following equation;  $IC_{50}$  ratio = [ $IC_{50}$  for GTP $\gamma$ S-activated current] / [ $IC_{50}$  for CCh-induced current]. a) Sulpiride 300  $\mu$ M had almost no effect on GTP $\gamma$ S-activated  $I_{K,ACh}$ . ND: Not determined.

## REFERENCES

1. Abi-Gerges, N., Eschenhagen, T., Hove-Hadsen, L., Fischmeister, R. and Mery, P.F. 1997. Methylene blue is a muscarinic antagonist in cardiac myocytes. *Mol. Pharmacol.* **52**: 482-490. [Medline]
2. Alvarez, P. A. and Pahissa, J. 2010. QT alterations in psychopharmacology: proven candidates and suspects. *Curr. Drug Saf.* **5**: 97-104. [Medline] [CrossRef]
3. Baldessarini, R. J. 2006. Drug therapy of depression and anxiety disorders. pp. 429-459. In: Goodman & Gilman's The Pharmacological Basis of Therapeutics, 11th ed. (Brunton, L., Lazo, J., Parker, K., Buxton, I. and Blumenthal, D. eds.), McGraw-Hill, New York.



4. Baldessarini, R. J. and Tarazi, F. I. 2006. Pharmacotherapy of psychosis and mania. pp. 461–500. In: Goodman & Gilman's The Pharmacological Basis of Therapeutics, 11th ed. (Brunton, L., Lazo, J., Parker, K., Buxton, I. and Blumenthal, D. eds.), McGraw-Hill, New York.
5. Breitwieser, G. E. and Szabo, G. 1985. Uncoupling of cardiac muscarinic and  $\beta$ -adrenergic receptors from ion channels by a guanine nucleotide analogue. *Nature* **317**: 538–540. [Medline] [CrossRef]
6. Hara, Y. and Kizaki, K. 2002. Antimalarial drugs inhibit the acetylcholine-receptor-operated potassium current in atrial myocytes. *Heart Lung Circ.* **11**: 112–116. [Medline] [CrossRef]
7. Hara, Y. and Nakaya, H. 1995. SD-3212, a new class I and IV antiarrhythmic drugs: a potent inhibitor of the muscarinic acetylcholine receptor-operated potassium current in atrial myocytes. *Br. J. Pharmacol.* **116**: 2750–2756. [Medline] [CrossRef]
8. Hara, Y., Kizaki, K., Temma, K., Chugun, A. and Kondo, H. 2004. Effects of anticancer chemotherapeutic drugs on the acetylcholine receptor-operated potassium current in guinea pig atrial myocytes. *Basic Clin. Pharmacol. Toxicol.* **95**: 234–240. [Medline] [CrossRef]
9. Hara, Y., Yamawaki, H., Shimada, M., Okada, K., Tanai, T., Ichikawa, D., Miyake, K. and Kizaki, K. 2007. Anticholinergic effects of artemisinin, an antimalarial drug, in isolated guinea pig heart preparations. *J. Vet. Med. Sci.* **69**: 697–702. [Medline] [CrossRef]
10. Hart, B. L. 1985. Behavioral indications for phenothiazine and benzodiazepine tranquilizers in dogs. *J. Am. Vet. Med. Assoc.* **186**: 1192–1194. [Medline]
11. Hibino, H., Inanobe, A., Furutani, K., Murakami, S., Finglay, I. and Kurachi, Y. 2010. Inwardly rectifying potassium channels: their structure, function, and physiological roles. *Physiol. Rev.* **90**: 291–366. [Medline] [CrossRef]
12. Inomata, N., Ohno, T., Ishihara, T. and Akaike, N. 1993. Antiarrhythmic agents act differently on the activation phase of the ACh-response in guinea-pig atrial myocytes. *Br. J. Pharmacol.* **108**: 111–115. [Medline] [CrossRef]
13. Ito, H., Takikawa, R., Kurachi, Y. and Sugimoto, T. 1989. Anticholinergic effect of verapamil on the muscarinic acetylcholine receptor-gated  $K^+$  channel in isolated guinea-pig atrial myocytes. *Naunyn Schmiedebergs Arch. Pharmacol.* **339**: 244–246. [Medline] [CrossRef]
14. Kaibara, M., Nakajima, T., Irisawa, H. and Giles, W. 1991. Regulation of spontaneous opening of muscarinic  $K^+$  channels in rabbit atrium. *J. Physiol.* **433**: 589–613. [Medline]
15. Kim, K. S. and Kim, E. L. 2005. The phenothiazine drugs inhibit hERG potassium channels. *Drug Chem. Toxicol.* **28**: 303–313. [Medline] [CrossRef]
16. Kobayashi, T., Ikeda, K. and Kumanishi, T. 2000. Inhibition by various antipsychotic drugs of the G-protein-activated inwardly rectifying  $K^+$  (GIRK) channels expressed in *Xenopus* oocytes. *Br. J. Pharmacol.* **129**: 1716–1722. [Medline] [CrossRef]
17. Kobayashi, T., Washiyama, K. and Ikeda, K. 2004. Inhibition of G protein-activated inwardly rectifying  $K^+$  channels by various antidepressant drugs. *Neuropsychopharmacology* **29**: 1841–1851. [Medline] [CrossRef]
18. Kurachi, Y. 1995. G protein regulation of muscarinic potassium channel. *Am. J. Physiol.* **269**: C821–C830. [Medline]
19. Kurachi, Y., Nakajima, T. and Sugimoto, T. 1987. Short-term desensitization of muscarinic  $K^+$  channel current in isolate atrial myocytes and possible role of GTP-binding proteins. *Pflügers Arch.* **410**: 227–233. [Medline] [CrossRef]
20. Lee, S.Y., Kim, Y.J., Kim, K.T., Choe, H. and Jo, S.H. 2006. Blockade of HERG human  $K^+$  channels and  $I_{Kr}$  of guinea-pig cardiomyocytes by the antipsychotic drug clozapine. *Br. J. Pharmacol.* **148**: 499–509. [Medline] [CrossRef]
21. Milnes, J. T., Witchel, H. J., Leaney, J. L., Leishman, D. J. and Hancox, J. C. 2006. hERG  $K^+$  channel blockade by the antipsychotic drug thloridazine: an obligatory role for the S6 helix residue F656. *Biochem. Biophys. Res. Commun.* **351**: 273–280. [Medline] [CrossRef]
22. Mori, K., Hara, Y., Saito, T., Masuda, Y. and Nakaya, H. 1995. Anticholinergic effects of class III antiarrhythmic drugs in guinea pig atrial cells. Different molecular mechanisms. *Circulation* **91**: 2834–2843. [Medline] [CrossRef]
23. Moss, A. J. 1999. The QT interval and trossades de pointes. *Drug Saf.* **21**: 5–10. [Medline] [CrossRef]
24. Moss, A. J., Schwartz, P. J., Crampton, R. S., Tzivoni, D., Locati, E. H., MacCluer, J., Hall, W. J., Weitkamp, L., Vincent, G. M. and Garson, A. Jr. 1991. The long QT syndrome: Prospective longitudinal study of 328 families. *Circulation* **84**: 1136–1144. [Medline] [CrossRef]
25. Nishimoto, M., Hashimoto, H., Ozaki, T., Taguchi, T., Ohara, T. and Nakashima, M. 1994. Effects of imipramine and amitriptyline on intraventricular conduction, effective refractory period, incidence of ventricular arrhythmias induced by programmed stimulation, and on electrocardiogram after myocardial infarction in dog. *Arch. Int. Pharmacodyn. Ther.* **328**: 39–53. [Medline]
26. Okada, M., Mizuno, W., Nakarai, R., Matada, T., Yamawaki, H. and Hara, Y. 2012. Benzodiazepines inhibit the acetylcholine receptor-operated potassium current ( $I_{K,ACH}$ ) by different mechanisms in guinea-pig atrial myocytes. *J. Vet. Med. Sci.* **74**: 879–884. [Medline] [CrossRef]
27. Punke, M. A. and Friederich, P. 2007. Amitriptyline is a potent blocker of human Kv1.1 and Kv7.2/7.3 channels. *Anesth. Analg.* **104**: 1256–1264. [Medline] [CrossRef]
28. Reuveny, E., Slesinger, P. A., Inglese, J., Morales, J. M., Iniguez-Lluhi, J. A., Lefkowitz, R. J., Mourné, H. R., Jan, Y. N. and Jan, L. Y. 1994. Activation of the cloned muscarinic potassium channel by G protein  $\beta\gamma$  subunits. *Nature* **370**: 143–146. [Medline] [CrossRef]
29. Schulz, M. and Schmoldt, A. 2003. Therapeutic and toxic blood concentrations of more than 800 drugs and other xenobiotics. *Pharmazie* **58**: 447–474. [Medline]
30. Stollberger, C., Huber, J. O. and Fensterer, J. 2005. Antipsychotic drugs and QT prolongation. *Int. Clin. Psychopharmacol.* **20**: 243–251. [Medline] [CrossRef]
31. Teschemacher, A. G., Seward, E. P., Hancox, J. C. and Witchel, H. J. 1999. Inhibition of the current of heterologously expressed HERG potassium channels by imipramine and amitriptyline. *Br. J. Pharmacol.* **128**: 479–485. [Medline] [CrossRef]
32. van Noord, C., Straus, S. M. J. M., Sturkenboom, M. C. J. M., Hofman, A., Aarnoudse, A. J. L. H. J., Bagnardi, V., Kors, J. A., Newton-Cheh, C., Witteman, J. C. M. and Stricker, B. H. C. 2009. Psychotropic drugs associated with corrected QT interval prolongation. *J. Clin. Psychopharmacol.* **29**: 9–15. [Medline] [CrossRef]
33. WHO. 2005. Pharmaceuticals Newsletter [cited 2012 November 1]. Available from [http://www.who.int/medicines/publications/newsletter/en/news2005\\_1.pdf](http://www.who.int/medicines/publications/newsletter/en/news2005_1.pdf).
34. Zareba, W. and Lin, D. A. 2003. Antipsychotic drugs and QT interval prolongation. *Psychiatr. Q.* **74**: 291–306. [Medline] [CrossRef]