ELSEVIER

European Journal of Pharmacology 344 (1998) 287–297

ejp

Effects of pirmenol on action potentials and membrane currents in single atrial myocytes

Toshiaki Nakajima *, Kuniaki Iwasawa, Hisanori Hazama, Masao Omata

Second Department of Internal Medicine, Faculty of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113, Japan

Received 25 November 1997; accepted 9 December 1997

Abstract

Electrophysiological effects of pirmenol hydrochloride (pirmenol) were investigated in single atrial myocytes obtained from rabbit and guinea-pig hearts by using a whole-cell clamp technique. Under current clamp conditions, pirmenol (2–30 μM) prolonged action potential duration in a concentration-dependent manner without affecting resting membrane potential in rabbit atrial myocytes. However, in the presence of 4-aminopyridine (4 mM), pirmenol (10 μM) failed to prolong the action potential duration further. Pirmenol also suppressed acetylcholine-induced hyperpolarization and action potential duration shortening, resulting in a significant prolongation of the action potential duration in the presence of acetylcholine. Under voltage clamp conditions, pirmenol (1–1000 μM) inhibited transient outward current (I_{to}) in a concentration-dependent manner. The concentration for half-maximal inhibition (IC_{50}) of pirmenol on I_{to} was about 18 μM. Pirmenol did not show the use and frequency dependent inhibition of I_{to}. The voltage dependence of the steady-state inactivation of I_{to} and the recovery from inactivation were not significantly affected by pirmenol. Pirmenol accelerated the inactivation of I_{to} and blocked I_{to} as an exponential function of time, consistent with a time-dependent open channel blockade. Pirmenol (30 μM) did not affect the inwardly rectifying K^+ current significantly, but it decreased the voltage-dependent L-type Ca^{2+} current by about 20%. In guinea-pig atrial myocytes, both acetylcholine and adenosine induced a specific K^+ current activated by GTP-binding proteins. Pirmenol suppressed both the acetylcholine- and adenosine-induced K^+ current effectively. The IC_{50} of pirmenol for acetylcholine- and adenosine-induced current was about 1 and 8 μM, respectively. The present results suggest that pirmenol prolongs the action potential duration by primarily inhibiting the transient outward current in atrial myocytes. In addition, since pirmenol inhibits acetylcholine- and adenosine-induced K^+ current, pirmenol may effectively prolong the action potential duration in atrial myocytes under various physiological conditions as in the whole heart or ischemia. © 1998 Elsevier Science B.V.

Keywords: Pirmenol hydrochloride; Outward current, transient; Myocyte, single, atrial; K^+ current, muscarinic; Acetylcholine; Adenosine; Anticholinergic action; Antiarrhythmic drug, class Ia; Disopyramide; Cibenzoline

1. Introduction

Pirmenol hydrochloride (pirmenol), classified as a class Ia or Ic antiarrhythmic agent in the Vaughan Williams classification (Steffe et al., 1980; Vaughan Williams, 1984; Dukes et al., 1986; Kaplan et al., 1987), has been shown to suppress both supraventricular and ventricular arrhythmia (Hammill et al., 1982; Lee et al., 1983; Toivonen et al., 1987a,b). The major mechanism responsible for antiarrhythmic action of pirmenol is its ability to block the Na^+ channel (Nakaya et al., 1988), resulting in termination of re-entry or suppression of ectopic automaticity. In addition, pirmenol has been reported to lengthen the action potential duration in sinoatrial node cells (Kotake et al., 1988) and in ventricular myocytes (Sawanobori et al., 1990), possibly by inhibiting delayed outward current (I_K), and it also inhibits transient inward current (Sawanobori et al., 1990), which may account for the antiarrhythmic action of pirmenol. However, the effects of pirmenol on electrophysiological activities of atrial myocytes have not been investigated in detail. Therefore, the effects of pirmenol on action potentials and membrane currents were investigated in atrial myocytes obtained from rabbit heart, since the action potential configuration and basic ionic currents in human atrial myocytes are quite similar to those of rabbit atrial myocytes, in which the transient outward current (I_{to}) appears to have a prominent role in the regulation of the action potential duration (Giles and Imaizumi, 1988; Shibata et al., 1989; Fermini et al., 1992).

* Corresponding author. Tel.: +81-3-38155411; fax: +81-3-38140021.

In addition, class I drugs, such as disopyramide, are known to interact with muscarinic receptors (Endou et al., 1992) thus producing anticholinergic effects such as urine retention and constipation. In the whole heart, the actions of Ia drugs increase heart rate and accelerate atrioventricular conduction. However, the underlying mechanisms of anticholinergic action in hearts are different among the antiarrhythmic drugs as reported previously in both class Ia and class III drugs (Kurachi et al., 1987b; Nakajima et al., 1989; Wu et al., 1994; Mori et al., 1995). The anticholinergic effects of disopyramide can be accounted for the blockade of the muscarinic receptor (Nakajima et al., 1989), while quinidine or cibenzoline display anticholinergic effects by inhibiting the muscarinic K^+ channel ($I_{K.ACh}$) itself and/or GTP-binding proteins (Kurachi et al., 1987b; Wu et al., 1994). Since pirmenol has chemical structures close to disopyramide (Reder et al., 1980; Endou et al., 1986) and has been reported to have anticholinergic effects (Endou et al., 1992), the underlying mechanisms of the anticholinergic effects of pirmenol in atrial myocytes were also investigated in the present study and compared to those of other class Ia drugs as previously described by using guinea-pig atrial cells (Kurachi et al., 1987b; Nakajima et al., 1989; Wu et al., 1994).

Thus, the main goal of this study was to determine the electrophysiological effects of pirmenol on single atrial myocytes and to compare the effects of class Ia antiarrhythmic agents on I_{to} and $I_{K.ACh}$, which play important roles in regulating atrial electrical activity.

2. Materials and methods

2.1. Cell preparation

Single atrial myocytes from rabbit and guinea-pig hearts were obtained by an enzymatic dissociation procedure as described elsewhere (Kaibara et al., 1991; Nakajima et al., 1992; Yamashita et al., 1995a). Briefly, animals were anesthetized with sodium pentobarbital, their hearts rapidly removed and retrogradely Langendorff-perfused at 35–37°C with an oxygenated Tyrode solution. The hearts were then perfused with Ca^{2+}-free Tyrode solution for approximately 10 min and subsequently with the same solution containing collagenase (0.04% w/v Type 1, Sigma Chemical, St. Louis, MO) for 17–20 min. The digested hearts were stored in high K^+/low Cl^- solution at 4°C for later experimentation. The atria were then removed and cells were obtained by gentle mechanical agitation. This procedure consistently yielded an acceptable number of quiescent and relaxed atrial cells.

2.2. Solution and drugs

The composition of the normal Tyrode solution was (in mM): NaCl, 136.5; KCl, 5.4; $CaCl_2$, 1.8; $MgCl_2$, 0.53; glucose, 5.5 and 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid (HEPES)–NaOH buffer, 5; pH 7.4. The Ca^{2+}-free Tyrode solution was the same as the normal Tyrode solution except that $CaCl_2$ was omitted. To record K^+ currents, the pipette was filled with a solution containing the following elements (in mM): K-aspartate, 130; KCl, 20; KH_2PO_4, 1; disodium adenosine 5′-triphosphate (Na_2ATP), 3; GTP (guanosine-5′-triphosphate, sodium salt, Sigma), 0.1; $MgCl_2$, 1; EGTA, 5 and HEPES–KOH buffer, 5; pH 7.3. To block the Na^+ current (I_{Na}) and the Ca^{2+} current (I_{Ca}), tetrodotoxin (5 μM) and $CdCl_2$ (0.3 mM) were added to the bath solution. To block K^+ currents, 5 mM $BaCl_2$ was added to the bath solution. The patch pipette contained the Cs^+-internal solution (in mM): CsCl, 140; EGTA, 5; $MgCl_2$, 1; Na_2ATP, 3; GTP, 0.1 and HEPES–CsOH buffer, 5; pH 7.3. In several experiments, nonhydrolyzable GTP analogue (GTPγS, tetralithium salt, Boehringer Mannheim, Germany, 0.1 mM) was added to the pipette solution instead of GTP. The high K^+/low Cl^- solution contained (in mM): K-glutamate, 50; KCl, 40; KH_2PO_4, 20; taurine, 20; oxalic acid, 10; $MgCl_2$, 3; EGTA, 0.5 and HEPES–KOH buffer, 10 (pH 7.4). Acetylcholine chloride, atropine sulfate, 4-aminopyridine, disopyramide and adenosine were purchased from Sigma (St. Louis, MO). Pirmenol hydrochloride was provided by Warner-Lambert Pharmaceuticals, Japan. Cibenzoline was obtained from Fujisawa Pharmaceuticals, Japan. All experiments were performed at 33–35°C.

2.3. Recording technique and data analysis

Membrane currents and potentials were recorded with patch electrodes in an whole-cell clamp configuration (Hamill et al., 1981; Kurachi et al., 1986) using a patch-clamp amplifier (EPC-7, List Electronics, Darmstadt, Germany). Heat-polished patch pipettes, filled with the artificial internal solution (see composition in Section 2.2), had a tip-resistance of 3–6 MΩ. The membrane currents and potential were monitored using a high-gain storage oscilloscope (COS 5020-ST, Kikussui Electronics, Tokyo, Japan). At the start of each experiment, the series resistance was compensated. The data were stored on video tapes using a PCM converter system (RP-880, NF electronic circuit design, Tokyo). Later, the data were reproduced, low-passed filtered at 2 kHz (-3 dB) with a Bessel filter (FV-665, NF, 48 dB/octave slope attenuation), sampled at 5 kHz and analyzed off-line on a computer using p-Clamp software (Axon Instruments, CA). Statistical results are expressed as means $\pm$ S.D. Student's *t*-tests were performed and a value of $P < 0.05$ was considered significant.

The steady-state inactivation parameters of transient outward current (I_{to}) at various membrane potentials were estimated with the use of a double-pulse protocol. Conditioning voltage pulses (0.5 s in duration) for various membrane potentials between -70 and $+10$ mV were

applied from a holding potential of −80 mV. At 10 ms after the end of each conditioning pulse, a test pulse of +20 mV (0.5 s in duration) was applied to evoke I_{to}. The ratio of the amplitude of I_{to} with and without the conditioning pulse was plotted for the membrane potential of each conditioning pulse (inactivation curve). The interval between sets of double pulses was 30 s.

3. Results

3.1. Effects of pirmenol on membrane potentials of rabbit atrial myocytes

Fig. 1 and Table 1 show the effects of pirmenol on the action potential of atrial myocytes isolated from rabbit hearts. Pirmenol (2–30 μM) prolonged the action potential duration (APD) in a concentration-dependent manner (Fig. 1A). The mean % prolongation of action potential duration (APD_{25}, APD_{50} and APD_{90}) induced by pirmenol (10, 30 μM) was 100, 78 and 36% at 10 μM pirmenol and 143, 114 and 54% at 30 μM. Thus, it was much larger in APD_{25} and APD_{50} than APD_{90}. On the other hand, the resting membrane potential was not significantly altered by pirmenol (10–30 μM, Fig. 1A and Table 1). 4-aminopyridine, a blocker of transient outward current, markedly increased action potential duration without altering resting membrane potential (Fig. 1B). In the presence of 4-aminopyridine (4 mM), pirmenol (10 μM) failed to prolong APD further and pirmenol (30 μM, $n = 3$) rather shortened it as shown in Fig. 1B. These results suggest that the ionic basis of pirmenol-induced action potential prolongation may be mainly due to the inhibition of 4-aminopyridine sensitive K^+ current (I_{to}) in rabbit atrial myocytes. In addition, the effect of pirmenol on acetylcholine-induced action potential changes in rabbit atrial myocytes is presented in Fig. 1C and Table 1. The application of acetylcholine (1 μM) markedly reduced APD and hyperpolarized resting membrane potentials. Pirmenol (30 μM) completely suppressed the hyperpolarizing action of acetylcholine with a definite prolongation of action potential duration in rabbit atrial myocytes (Table 1).

3.2. Ionic mechanisms of pirmenol-induced action potential prolongation in rabbit atrial myocytes

To clarify the ionic mechanism of pirmenol effects on action potentials, we first examined the effects of pirmenol on transient outward current (I_{to}) as shown in Fig. 2. The cell was held at −80 mV and command voltage pulses to various membrane potentials were applied at 0.1 Hz. Pirmenol (30 μM) inhibited the peak amplitude of I_{to} (Fig. 2A). The current–voltage relationships measured at the peak are shown in Fig. 2Ab. Pirmenol suppressed I_{to} at all command voltage steps greater than −30 mV. Fig. 2B and C show the concentration-dependent effects of pirmenol on I_{to}. The amplitude of I_{to} was measured as the difference between the peak current and the current level at the end of the voltage pulses. The cell was held at −80 mV and the command voltage pulses to +20 mV were applied at 0.1 Hz (Fig. 2B). Pirmenol (3–100 μM) reduced the amplitude of I_{to} in a concentration-dependent manner. The concentration–response relationships of pirmenol on I_{to} are shown in Fig. 2C. Pirmenol (1–1000 μM) suppressed I_{to} in a concentration-dependent fashion. The concentration for half-maximal inhibition (IC_{50}) of pirmenol on I_{to} was about 18 μM. The inhibitory effects of pirmenol on I_{to} were fully reversible (data not shown). These results indicate that the inhibitory effects of pirmenol on I_{to} may account for the prolongation of action potential duration in rabbit atrial myocytes.

Since pirmenol has chemical structures close to disopyramide (Reder et al., 1980; Endou et al., 1986), we compared the effects of class Ia antiarrhythmic drugs, such as

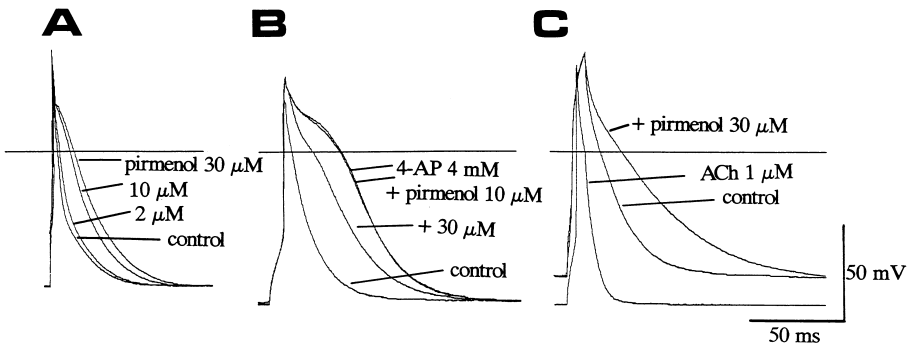

Fig. 1. Effects of pirmenol on action potentials of atrial myocytes obtained from rabbit hearts. (A) Effects of pirmenol on action potentials. Action potentials were elicited at a stimulation rate of 0.1 Hz and recorded in controls and after application of pirmenol (2, 10, 30 μM). (B) Effects of pirmenol on action potentials in the presence of 4-aminopyridine (4 mM). Pirmenol (10 and 30 μM) was applied in the presence of 4-aminopyridine. (C) Effects of pirmenol on acetylcholine-induced action potential changes. The action potentials were recorded in controls, after application of acetylcholine (1 μM) and additional application of pirmenol (30 μM). The zero potential levels are indicated by lines in each figure.

Table 1
Effects of pirmenol on action potential of the rabbit heart

	Control	Pirmenol, 10 μM	Pirmenol, 30 μM
(A) Effects of pirmenol on action potential of the rabbit heart			
APD_{25} (ms)	7 ± 3	$14 \pm 4^*$	$17 \pm 5^*$
APD_{50} (ms)	14 ± 5	$25 \pm 5^*$	$30 \pm 6^*$
APD_{90} (ms)	50 ± 9	$68 \pm 7^*$	$77 \pm 12^*$
Resting membrane potential (mV)	-69 ± 6	-67 ± 8	-68 ± 7
	Control	ACh, 1 μM	ACh, 1 μM + pirmenol, 30 μM
(B) Effects of pirmenol on acetylcholine (ACh)-induced action potential duration and resting membrane potential changes			
APD_{25} (ms)	6 ± 2	$3 \pm 1^*$	$13 \pm 3^*$
APD_{50} (ms)	14 ± 3	$5 \pm 2^*$	$28 \pm 4^*$
APD_{90} (ms)	48 ± 5	$14 \pm 3^*$	$75 \pm 6^*$
Resting membrane potential (m/V)	-70 ± 6	$-82 \pm 3^*$	-69 ± 5

Stimulatory frequency was 0.1 Hz.
(A) The data were obtained from 7 different cells.
(B) The data were obtained from 4 different cells.
* $p < 0.05$ v.s. control value.

disopyramide, on I_{to}. The typical experiments are shown in Fig. 2D. Disopyramide (30 μM, Fig. 2Da) and cibenzoline (30 μM, Fig. 2Db) also reduced the amplitude of I_{to}. The potency for inhibiting I_{to} at 30 μM is pirmenol $\gg$ disopyramide, and pirmenol $>$ cibenzoline. The IC_{50} value is 23 μM for cibenzoline and approximately 100 μM for disopyramide as shown in Fig. 2C.

To investigate the inhibitory action of pirmenol on I_{to}, the effects of pirmenol on voltage dependence of steady-state inactivation of I_{to} were studied. Fig. 3Aa and b show the steady-state inactivation curve of I_{to}. A double-pulse protocol was used (see Section 2). A 0.5 s conditioning pulse for various membrane potentials preceded the test pulse (0.5 s in duration) to +20 mV from a holding potential of −80 mV. The peak amplitude of I_{to} (I) at each test pulse was normalized to the maximum amplitude of I_{to} (I_{max}) elicited without a conditioning step. The normalized values were plotted against the conditioning voltages (Fig. 3Ab) and fitted to the Boltzmann function to the data points, using a least-square method:

$$I/I_{max} = 1/\{1 + \exp[(V - a)/b]\}$$

where V is the membrane potential of the conditioning pulse, a is the half-inactivation potential and b is a slope factor. The potential of half inactivation (a) was -40 ± 3 mV ($n = 4$) and the slope factor was 7.8 ± 0.9 ($n = 4$) in control and -41 ± 4 mV ($n = 4$) and 7.7 ± 0.8 ($n = 4$) in the presence of pirmenol (30 μM). Thus, pirmenol produced no significant shift in the steady-state inactivation curve, indicating a lack of drug effect on the inactivation gating of I_{to}.

Fig. 3B shows the use dependence of the effect of pirmenol on I_{to}. The command voltage steps from −80 to +20 mV (320 ms in duration) were applied at 0.1 Hz. The amplitude of I_{to} remained stable in the control solution (Fig. 3Ba). The depolarizing pulses were stopped and pirmenol (30 μM) were applied to the cells. Under application of pirmenol, the amplitude of I_{to} evoked by the first voltage step after a 60 s pause was already suppressed by $55 \pm 14\%$ (mean ± S.D., $n = 4$, Fig. 3Bb). During the repetitive pulse stimuli, the amplitude of I_{to} almost remained constant (Fig. 3Bb). These results suggest that pirmenol inhibition of I_{to} was not use-dependent. We also found no frequency dependence of the drug effect at stimulation rates of 1, 0.33 and 0.1 Hz. The cells were held at −80 mV and the command voltage pulses to +20 mV (320 ms in duration) were applied at various stimulation rates. The percent reduction of the peak I_{to} by 30 μM pirmenol was 56 ± 9, 54 ± 8 and $53 \pm 12\%$ of the control value at stimulation rates of 0.1, 0.33 and 1 Hz, respectively (P = N.S, $n = 5$ in each case).

Along with inhibiting the amplitude of I_{to}, pirmenol accelerated the inactivation time course of I_{to} as shown in Fig. 2. Fig. 4A and B show the detailed analysis of the inactivation of I_{to}. The cells were held at −80 mV and the command voltage pulses to +20 mV were applied. The points indicate experimental data and solid lines are fitted curves. In the control, the inactivation time course of I_{to} could be best fitted by double exponentials, i.e. the fast and slow components ($I_{to.f}$ and $I_{to.s}$) as described in the earlier papers (Yamashita et al., 1995a,b). The time constants of $I_{to.f}$ and $I_{to.s}$ (τ_1 and τ_2) at +20 mV were 15.9 ± 0.93 ms and 74.8 ± 19.8 ms ($n = 5$), respectively. After exposure to 10–100 μM pirmenol, the time constant for $I_{to.s}$ was not significantly different from that recorded under control conditions. On the other hand, $I_{to.f}$ in the presence of pirmenol was significantly faster than control ($P < 0.05$ versus control for each) in a concentration-dependent manner (Fig. 4A). Thus, the inhibitory effect of pirmenol on I_{to} was attributable mainly to the drug action on $I_{to.f}$. A possible explanation for its ability to accelerate inactivation of I_{to} is time-dependent open-channel blockade, resulting in more rapid removal of channels from the open state by drug binding than by spontaneous inactiva-

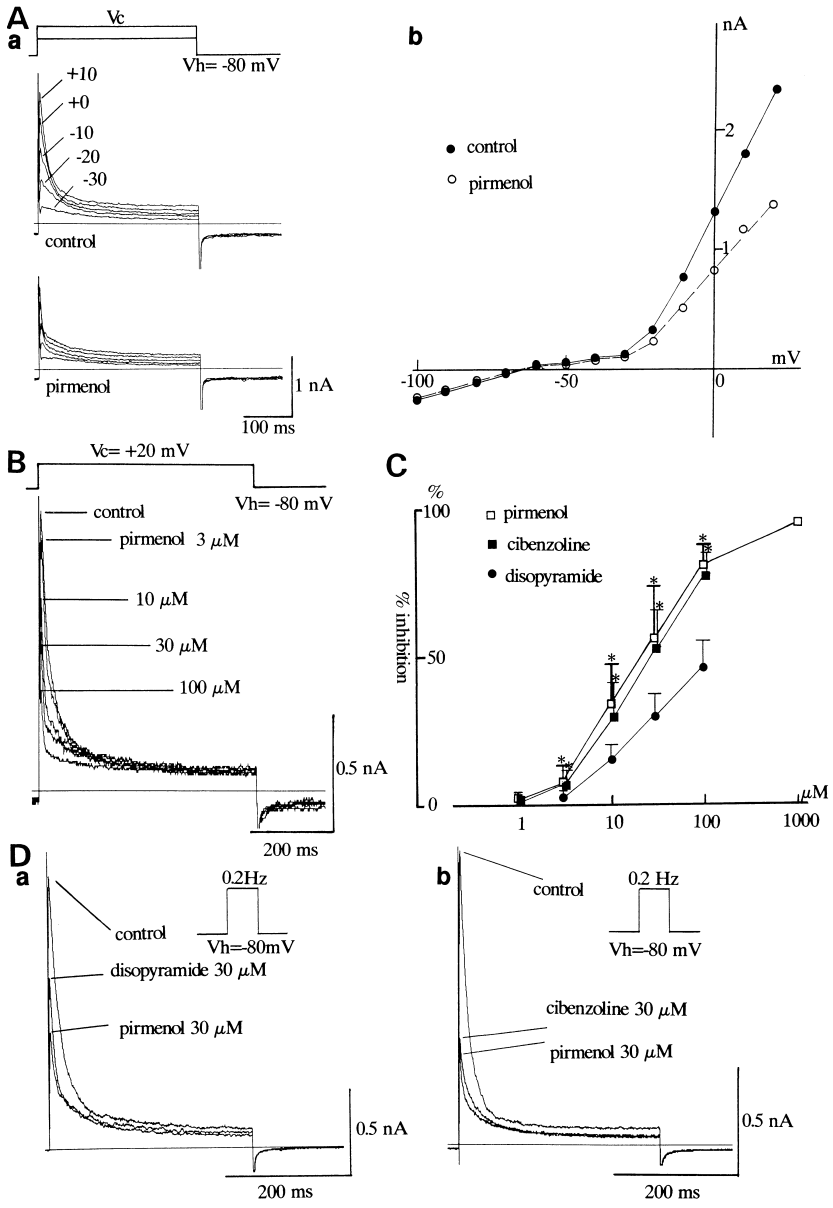

Fig. 2. Effects of pirmenol on transient outward current (I_{to}) of rabbit atrial myocytes. The cell was held at −80 mV and command voltage pulses for various membrane potentials were applied at 0.1 Hz. The cell was bathed in normal Tyrode solution containing tetrodotoxin (5 μM) and Cd^{2+} (0.3 mM). The patch pipette contained 5 mM EGTA to block Ca^{2+}-dependent currents. The original current traces are indicated in the controls (upper part of Aa) and in the presence of pirmenol (30 μM) (lower part of Aa). The zero current level is indicated by lines. In (A)b, the current–voltage relationships of the peak outward current are shown in controls (closed circles) and in the presence of pirmenol (open circles). (B and C) Effects of pirmenol, disopyramide and cibenzoline on I_{to}. The cells were held at −80 mV and the command voltage pulses to +20 mV were applied at 0.1 Hz. Current traces are shown for controls and in the presence of pirmenol (3, 10, 30 and 100 μM) in (B). Concentration–response curves of I_{to} inhibition by pirmenol (open square), cibenzoline (closed square) and disopyramide (closed circle) are shown in (C). Symbols and bars (obtained from 5–7 different cells) represent mean ± S.D. * Significant difference between pirmenol or cibenzoline and disopyramide in percentage of inhibition of I_{to}. The half-maximal inhibitory concentration was estimated as 18 μM for pirmenol, 23 μM for cibenzoline and approximately 100 μM for disopyramide. (D) The comparative potency of pirmenol, cibenzoline and disopyramide to inhibit I_{to}. Disopyramide and subsequently pirmenol (30 μM) were applied in (D)a. Cibenzoline and subsequently disopyramide (30 μM) were applied in (D)b. The cells were held at −80 mV and command voltage steps to +20 mV were applied at 0.1 Hz.

tion (Dukes et al., 1990). To explain this possibility, we plotted the degree of the drug-induced I_{to} blockade as a function of time after the onset of a depolarizing pulse (Fig. 4C). The fraction of current blocked by 30 μM pirmenol ($I_{to.control} - I_{to.pir}/I_{to.control}$) increased as a function of time after the depolarizing pulse with a single exponential curve, reaching a steady-state value. The time constants for the onset of block were 6.1 ± 2.0 ms at 30 μM pirmenol. These values were the same order as the inactivation time constants for $I_{to.f}$ of the same concentration as shown in Fig. 4B. The exponential curve fits crossed the vertical axis near to the origin, suggesting that

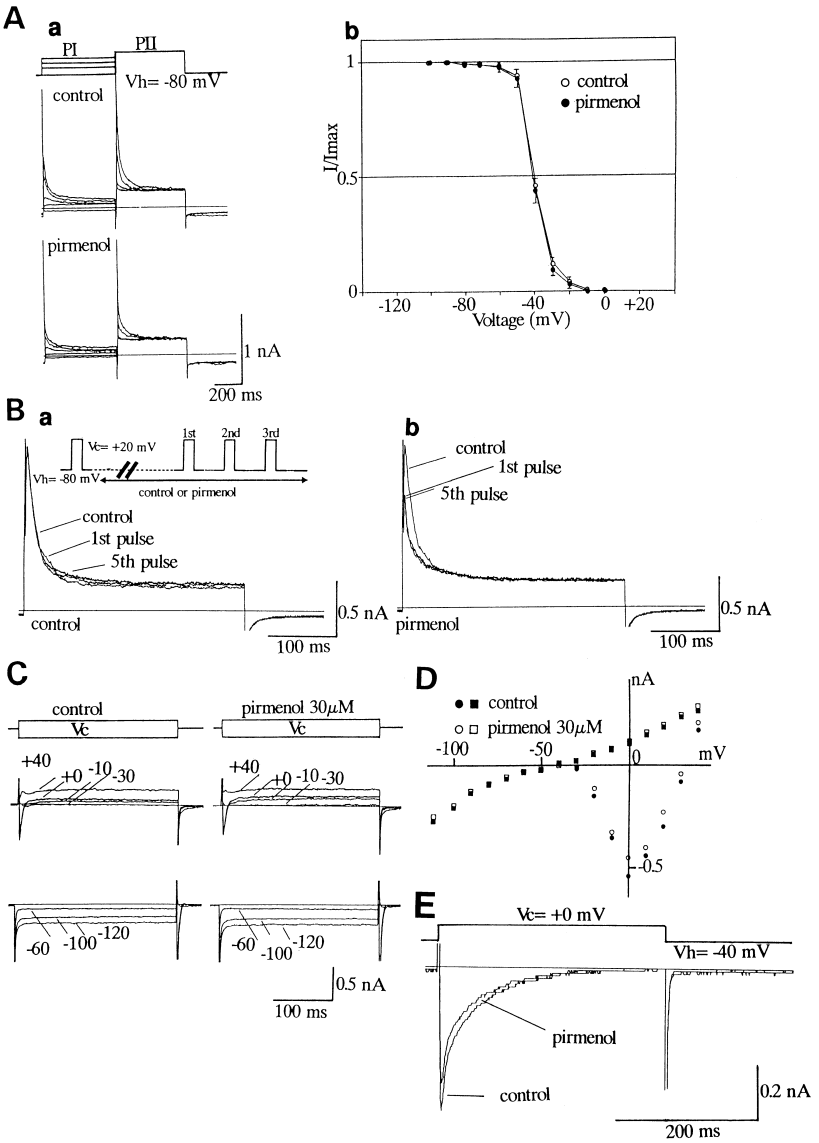

Fig. 3. Inhibitory effects of pirmenol on transient outward current (I_{to}) and other membrane currents in rabbit atrial myocytes. (A) Effects of pirmenol on steady-state inactivation curve of I_{to} with and without pirmenol (30 μM). Using a double-pulse protocol, the steady-state inactivation parameter of I_{to} was obtained in the controls and in the presence of pirmenol (30 μM). The original current traces in controls and in the presence of pirmenol (30 μM) are presented in (A)a. In (A)b, the relative amplitude of I_{to} in response to the test pulse was determined at each membrane potential of the conditioning pulses. Open and closed circles represent I/I_{max} in controls and in the presence of pirmenol (30 μM). (B) Use-dependent effects of pirmenol on I_{to}. The cells were held at -80 mV and command voltage pulses to $+20$ mV (300 ms in duration) were applied at 0.1 Hz. The control current traces are indicated in (B)a and (B)b. After the voltage steps were stopped, the bath solution without (Ba) or with pirmenol (Bb) was applied. After approximately 60 s in the control or pirmenol (30 μM)-containing solution, repeated depolarizing pulses to $+20$ mV at 0.1 Hz were applied again. Currents produced by the first and 5th command voltage pulse in the control (Ba) and after application of pirmenol (Bb) are shown. Note that there was no significant change in current amplitude in control conditions (Ba), but in the presence of pirmenol, the amplitude of 1st pulse decreased and significantly failed to decrease with successive pulses. (C, D and E) Effects of pirmenol on other membrane currents besides I_{to} in rabbit atrial myocytes. In (C), the cell was held at -40 mV and command voltage pulses for various membrane potentials were applied. The original current traces in the control and in the presence of pirmenol (30 μM) are illustrated. The current–voltage relationships measured at the peak and at the steady-state are shown in the lower part. Note that pirmenol (30 μM) did not inhibit the inwardly rectifying K^+ current significantly, but reduced the voltage-dependent L-type Ca^{2+} current. In (E), typical current recordings, where the cell was held at -40 mV and command voltage step to $+0$ mV was applied are shown in the control and in the presence of pirmenol (30 μM). The bath solution contained 5 μM tetrodotoxin to block the Na^+ current and the patch pipette contained Cs^+-internal solution.

there is no drug-induced blockade at the onset of depolarizing pulses and that the channel opening may be a necessary step in the blockade. Similarly, both cibenzoline and disopyramide accelerated the inactivation time course for I_{to} (Fig. 2D).

We also examined the effects of pirmenol on the time course of recovery of I_{to} from inactivation (reactivation). The reactivation time course was studied by using a double-pulse protocol in which a conditioning pulse (PI) was followed by an identical test pulse (PII) from -80 to $+20$

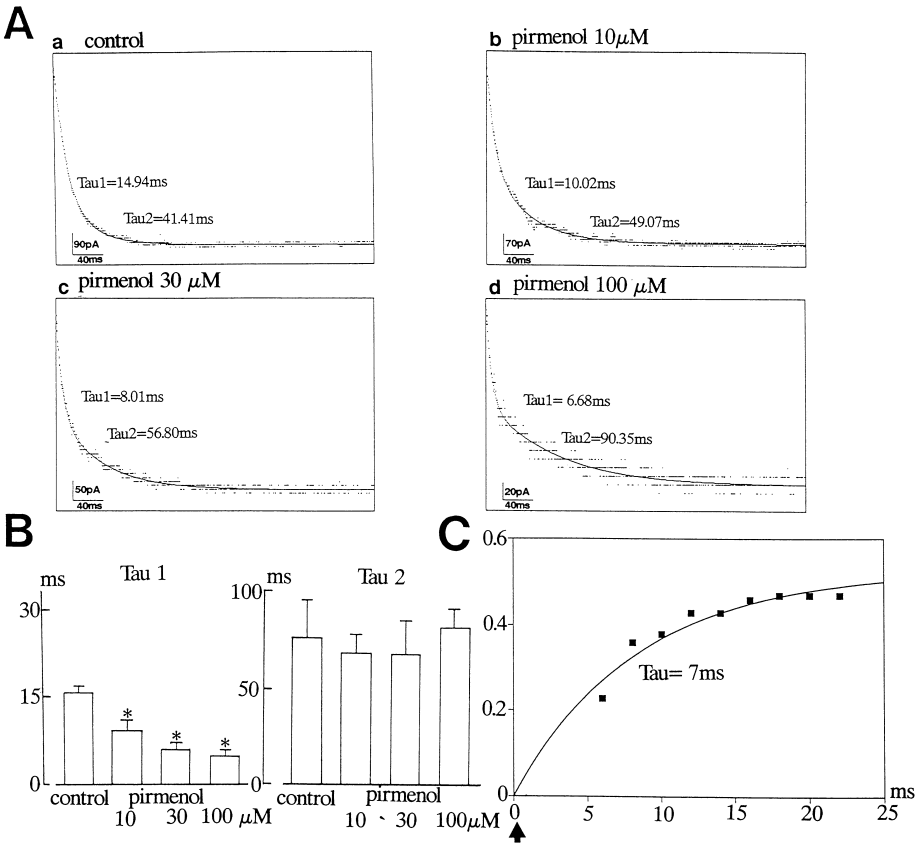

Fig. 4. Effects of pirmenol on the time course of inactivation of I_{to}. Representative current recordings from one cell after depolarizing to +20 mV from a holding potential of −80 mV are indicated in (A)a–d. The points are the inactivation portion of the current data and the solid lines are least squares curve fits obtained with the curve fit program Clampfit. The data (a–d) are indicated in control (a) and in the presence of pirmenol (10, 30 and 100 μM, b–d). In each case, the inactivation course was well-fitted by a biexponential process, with a fast time constant (τ_1) and a slow time constant (τ_2). In (B), the effects of pirmenol (30 μM) on the inactivation time course of I_{to} are shown. The data were obtained from 5 different cells. Note that pirmenol accelerated the time course of inactivation of I_{to}, resulting in the decrease of τ_1. τ_2 was not significantly different from control value ($^*P < 0.05$ versus control). (C) Time-dependent block of I_{to} by pirmenol. The cell was held at −80 mV and command voltage pulse to +20 mV was applied at 0.1 Hz. The fraction of current blocked by pirmenol (30 μM) ($I_{to.control} - I_{to.pir}/I_{to.control}$) shown on the *Y*-axis is plotted against a function of time. The arrow indicates the onset of the depolarizing pulses. $I_{to.control}$ and $I_{to.pir}$ denote the current amplitude in the control and in the presence of pirmenol (30 μM), respectively. The actual data points are shown by closed squares. The data are fitted by a single exponential function and the fitted curve $\{A(1 - e^{-t/\tau}) + B\}$ is shown as a line. In this cell, the value of A, τ and B was 0.53, 7 ms and 0, respectively.

mV for 500 ms, with a various interpulse interval. Recovery from inactivation in the absence and presence of the drug was simply fitted by a single exponential, with time constants of 0.76 ± 0.11 s and 0.78 ± 0.13 s ($n = 4$, P = N.S.), respectively. This suggests that the recovery of I_{to} from inactivation is very slow and significantly unaltered by pirmenol.

Fig. 3C and D show the effects of pirmenol on other membrane currents besides I_{to} in rabbit atrial myocytes. The cell was held to −40 mV and command voltage pulses for various membrane potentials were applied in controls and in the presence of pirmenol (30 μM, Fig. 3C). The current–voltage relationships measured at the initial peak and at the steady state are illustrated in Fig. 3D. Pirmenol (30 μM) decreased the amplitude of the voltage-dependent L-type Ca^{2+} current (I_{Ca}) from −550 to −460 pA at +0 mV in this case and pirmenol also reduced I_{Ca} at each command pulse. Thus, pirmenol (30 μM) inhibited I_{Ca} by 21 ± 5% ($n = 4$). Similar results were obtained under the conditions where CsCl was included in the patch pipettes to block K^+ currents and 5 μM tetrodotoxin was added to the bathing solution to block Na^+ current (I_{Na}) (Fig. 3E). Pirmenol did not affect the inwardly-rectifying K^+ current (I_{K1}) significantly. This result is consistent with the lack of an effect of pirmenol on resting membrane potential as shown in Fig. 1A and B.

3.3. Effects of pirmenol on muscarinic K^+ current in atrial myocytes

Fig. 1C shows that pirmenol suppressed the acetylcholine effect in rabbit atrial myocytes. Therefore, to investigate its molecular mechanism, the effect of pirmenol on muscarinic K^+ currents was investigated and compared among the class Ia antiarrythmic agents in atrial myocytes obtained from the guinea-pig hearts as described previ-

ously in guinea-pig cells (Kurachi et al., 1986; Mori et al., 1995; Nakajima et al., 1989; Wu et al., 1994). When ACh (1 μM) was added to the perfusion medium in GTP-loaded cells (Fig. 5Aa), there was a significant increase of a specific K^+ current, i.e. the membrane current at a holding potential of -53 mV was markedly increased in the outward direction. Under this sustained clamp condition, immediately after the accelerated increase in K^+ current, the acetylcholine-induced current was gradually desensitized and then reached to a steady state level as shown previously (Kurachi et al., 1987a). Atropine (1 μM) blocked the acetylcholine-induced current completely, indicating that the effect of acetylcholine is mediated by its specific binding to muscarinic receptors. Pirmenol (0.2–100 μM) also inhibited the acetylcholine-induced current in a concentration-dependent manner, and pirmenol at concentrations more than 10 μM completely abolished it (Fig. 5Aa). On the other hand, when the recording pipette was filled with GTPγS, the holding current was gradually increased even in the absence of acetylcholine after rupture of the membrane (Fig. 5Ab). Subsequent addition of acetylcholine markedly increased the holding current in the outward direction. Unlike with GTP-loaded cells, this K^+ current elicited by intracellular application of GTPγS was not blocked by atropine (Fig. 5Ab), indicating that the K^+ channel was functionally uncoupled from muscarinic receptors and irreversibly activated by GTPγS as shown in the earlier studies (Kurachi et al., 1986; Nakaya et al., 1988; Nakajima et al., 1989). Pirmenol (1–100 μM) also inhibited the GTPγS-induced K^+ current in a concentration-dependent manner (Fig. 5Ab) and 100 μM pirmenol completely abolished the current. The concentration–response relationships of pirmenol and other class Ia antiarrhythmic drugs (disopyramide and cibenzoline) for the

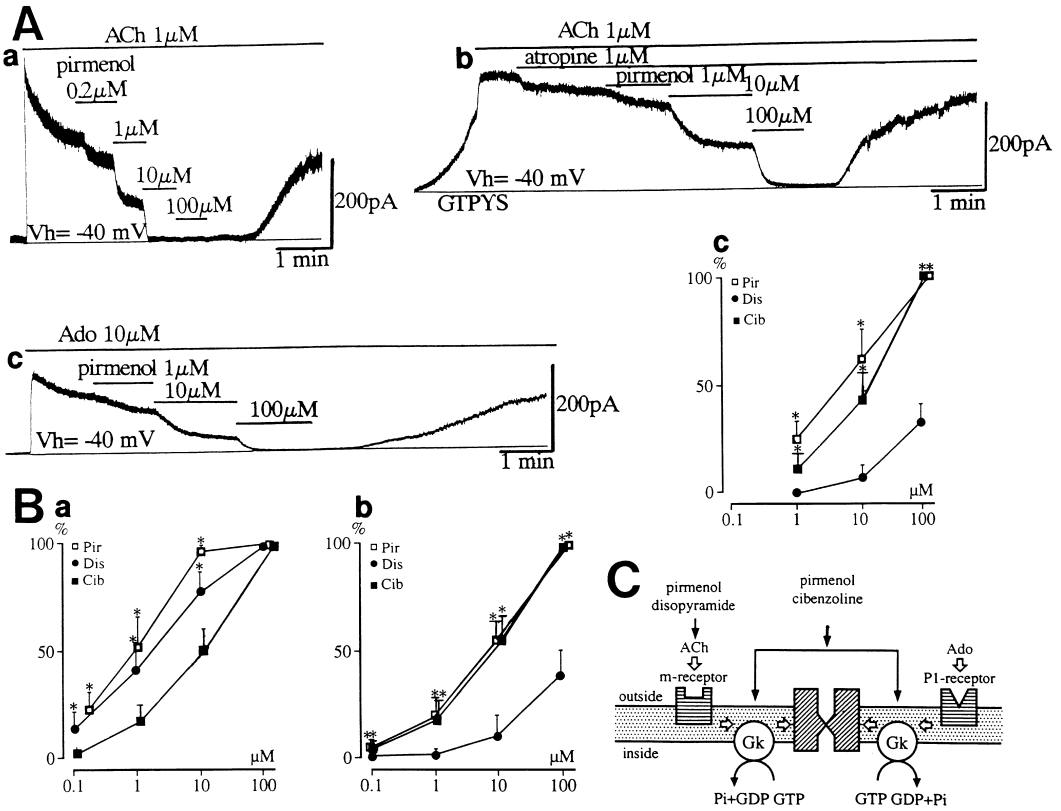

Fig. 5. Effects of pirmenol and other class Ia antiarrhythmic agents, on acetylcholine (ACh)-induced (a and b) and adenosine (Ado)-induced (c) K^+ current in single atrial myocytes obtained from guinea-pig hearts. The cells were loaded with GTP (100 μM), in (A)a and c, and GTPγS (100 μM) in (A)b. Protocols (drug sequences) are shown in the upper part of each original trace. The cells were held at -53 mV. The zero current level is shown by the line. Immediately after the rupture of the membrane, the holding current was continuously recorded. In GTPγS-loaded cells (b), the holding current was progressively increased even in the absence of ACh. (B) Concentration–response relationships for percent inhibition of acetylcholine (ACh)- or adenosine (Ado)-induced K^+ current caused by class I antiarrhythmic drugs. Concentration–response curves of pirmenol (Pir), disopyramide (Dis) and cibenzoline (Cib) on ACh-(a and b) or Ado-(c) induced current in GTP-loaded and GTPγS-loaded cells are shown. In GTP-loaded cells, the difference between the steady-state current in superfusion medium containing ACh (1 μM) or Ado (10 μM) and the current level without ACh or Ado was taken as 100%. In GTPγS-loaded cells, the difference between the current level with the application of atropine (1 μM) and the initial current level just after the rupture of the patch membrane was taken as 100%. Symbols and bars (obtained from four different cells) represent mean $\pm$ S.D. * Significant difference between Pir or Dis and Cib in (B)a. * Significant difference between Pir or Cib and Dis in (B)b and c. (D) Different inhibitory mechanisms of class Ia antiarrhythmic agents on the ACh-induced K^+ current. Note that pirmenol and disopyramide suppress the ACh-induced K^+ current by inhibiting muscarinic M_2 receptors, while pirmenol also inhibits the K^+ channel itself or the GTP-binding proteins coupled to the channel, then inhibiting the Ado-induced K^+ current in a similar way as cibenzoline does. G_k, GTP-binding proteins coupled to the channels; ACh, acetylcholine and Ado, adenosine.

percent inhibition of acetylcholine-induced K^+ current in GTP-loaded cells and in GTPγS-loaded cells are compared in Fig. 5Ba and Bb, respectively. The concentration required for half-maximal inhibition (IC_{50}) of pirmenol was 1 μM (Fig. 5Ba) and 8 μM (Fig. 5Bb), respectively.

In guinea-pig atrial myocytes, not only acetylcholine but also adenosine elicited the K^+ current activated by GTP-binding proteins (Kurachi et al., 1986). The activation of the K^+ currents by adenosine (10 μM) was not blocked by atropine (1 μM), but completely inhibited by theophylline (500 μM), a purinoceptor blocker, suggesting that activation of the K^+ channels by acetylcholine and adenosine is mediated by different membrane receptors. Therefore, to examine whether pirmenol also suppresses K^+ current induced by adenosine, 10 μM adenosine was added to the superfusion medium.

Immediately after addition of adenosine, the holding current rapidly increased in an outward direction and gradually decreased to a steady-state level (Fig. 5Ac). Pirmenol (1–100 μM) inhibited the adenosine-induced current in a concentration-dependent manner and 100 μM pirmenol completely suppressed it (Fig. 5A). Fig. 5Bc shows the concentration–response curve of pirmenol and other class Ia drugs on the adenosine-induced current in GTP-loaded cells. The IC_{50} value of pirmenol was about 8 μM. Thus, pirmenol suppressed both the adenosine-induced and GTPγS-induced current in a similar manner, proposing that pirmenol inhibits adenosine-induced K^+ current, primarily by inhibiting the K^+ channel itself and/or GTP-binding proteins coupled to the channel, but not the purinoceptor as cibenzoline.

4. Discussion

The major findings of the present study are as follows: (1) pirmenol lengthened the action potential duration in rabbit atrial myocytes without altering the resting membrane potential in a concentration-dependent manner, primarily by inhibiting transient outward current (I_{to}). (2) Pirmenol also suppressed the acetylcholine-induced action potential shortening in atrial myocytes resulting in definite prolongation of the action potential duration in the presence of acetylcholine.

4.1. Ionic mechanisms of pirmenol-induced action potential prolongation in single rabbit atrial myocytes

The present results indicate that pirmenol prolonged the action potential duration in a concentration-dependent manner in rabbit atrial myocytes by inhibiting transient outward current (I_{to}). The inhibitory effect of I_{to} was obtained at therapeutic concentrations (1–5 μM) (Hashimoto et al., 1988) and IC_{50} value was about 18 μM. Since in guinea-pig papillary muscle, pirmenol inhibits $\dot{V}_{max}$ in a concentration-dependent manner with IC_{50} more than 30 μM (Nakaya et al., 1988), pirmenol appears to have potent inhibitory effects on I_{to}. In the presence of 4-aminopyridine, where I_{to} was completely suppressed, pirmenol (10 μM) failed to increase action potential duration (Fig. 1B), suggesting that action potential prolongation caused by pirmenol is mainly due to the inhibition of I_{to} and moreover pirmenol at doses of 30 μM affected the other membrane currents, resulting in shortening of the action potential duration in the presence of 4-aminopyridine. The pirmenol-induced shortening of the action potential duration may be explained by several ionic mechanisms such as a block of tetrodotoxin-sensitive 'window' Na^+ current (Attwell et al., 1979; Coraboeuf et al., 1979) or a block of Ca^{2+} current. In the present study, pirmenol (30 μM) decreased the voltage-dependent L-type Ca^{2+} current, which may account for the pirmenol-induced action potential shortening. The Ca^{2+}-antagonistic effect of pirmenol has already been reported in rabbit ventricular myocytes and in multicellular sinus node preparations (Kotake et al., 1988; Sawanobori et al., 1990), which was also observed for other class Ia antiarrhythmic drugs such as quinidine (Hiraoka et al., 1986), disopyramide (Coraboeuf et al., 1988) and cibenzoline (Holck and Osterrieder, 1986). However, since pirmenol (10 μM) in the presence of 4-aminopyridine did not affect the action potential duration significantly, this effect may be relatively small compared to that on transient outward current at clinical concentrations. In single rabbit ventricular myocytes and sinus node preparations (Kotake et al., 1988; Sawanobori et al., 1990), it has been reported that pirmenol also decreases the time-dependent outward K^+ current (I_K), which prolongs the final repolarization in these cells. In rabbit atrial myocytes of the present study, pirmenol failed to affect other outward currents besides I_{to}. Thus, the contribution of I_K on the pirmenol-induced action potential prolongation may be relatively small since I_K is small in atrial cells isolated from rabbit hearts as previously reported (Giles and Imaizumi, 1988). The IC_{50} value of pirmenol on I_{to} was approximately 18 μM, which is relatively higher than that of quinidine (~7 μM) (Imaizumi and Giles, 1987) and tedisamil (~6 μM) (Dukes et al., 1990), but similar to that of flecainide (~17 μM) (Yamashita et al., 1995b). Pirmenol has, also, a more potent inhibitory effect on I_{to}, compared to the other class Ia drugs (cibenzoline and disopyramide), suggesting that it has a potent inhibitory action on I_{to}.

In cardiac myocytes, pirmenol causes a shift of the curve relating membrane potential and $\dot{V}_{max}$ along the voltage axis in a hyperpolarizing direction (Nakaya et al., 1988), suggesting that the block of Na^+ current appears to be voltage-dependent. On the other hand, pirmenol did not show a significant voltage dependence of I_{to} block. The block of I_{to} appears to be intimately linked to channel opening. The onset of block is a first-order function of time after the onset of a depolarizing pulse and the fitted curves cross near the origin, suggesting that channel open-

ing is essential for the blocking reaction. Our findings regarding I_{to} block by pirmenol are consistent with phenomena noted in previous studies of the I_{to} blocking action of tedisamil (Dukes et al., 1990) and flecainide (Yamashita et al., 1995b) and may be interpreted as indicative of open-channel block. In addition, the lack of significant change in I_{to} reactivation kinetics by pirmenol suggests rapid unblocking on return to the holding potential, consistent with the lack of use and frequency dependence of pirmenol block. Since the I_{to} channel is highly present in atrial myocytes, Purkinje fibers and ventricular myocytes of some species including human atrial cells (Shibata et al., 1989; Fermini et al., 1992), I_{to} appears to have a prominent role in the regulation of the action potential duration in these cells. The action potential prolongation has long been recognized as a potent antiarrhythmic mechanism (Vaughan Williams, 1984), which suggests that the effect of pirmenol on I_{to} may be responsible for the observed antiarrhythmic effects of the drug (Toivonen et al., 1987a,b).

4.2. Inhibitory effects of pirmenol on muscarinic K^+ current in atrial myocytes

Class Ia antiarrythmic drugs, such as disopyramide, are known to interact with muscarinic receptors (Endou et al., 1992), thus producing anticholinergic effects such as urine retention, constipation, increase of heart rate and acceleration of atrioventricular conduction in whole hearts. Pirmenol bears a chemical resemblance to disopyramide (Reder et al., 1980; Endou et al., 1986). Also, according to results from earlier studies of inhibition of the specific binding of [^{3}H]*N*-methylscopolamine, pirmenol has been shown to be as potent as disopyramide in competing for [^{3}H]*N*-methylscopolamine binding sites in heart preparations (Endou et al., 1992), suggesting that pirmenol has the same anticholinergic actions as disopyramide. In atrial myocytes of guinea-pig, muscarinic M_2 receptor or adenosine purinoceptor is linked to a specific K^+ channel activated by GTP-binding proteins (Kurachi et al., 1986) as presented schematically in Fig. 5C. Pirmenol inhibited both acetylcholine- and adenosine-induced K^+ current in a concentration-dependent manner, however, the IC_{50} values were about 1 and 8 μM, respectively. The IC_{50} value of pirmenol to block the acetylcholine-induced K^+ current was quite similar to that of disopyramide as shown in Fig. 5Ba, but much lower than that of quinidine or cibenzoline (in the present study; Kurachi et al., 1987b; Nakajima et al., 1989; Wu et al., 1994). These results suggest that pirmenol has a potent blocking effect on muscarinic M_2 receptors as disopyramide. GTPγS, a non-hydrolyzable GTP analogue, can activate GTP-binding proteins irreversibly, resulting in persistent activation of K^+ channels (Kurachi et al., 1986; Nakajima et al., 1989; Clark et al., 1990). The fact that the GTPγS-induced current was not inhibited by atropine indicates that the K^+ channels were not only elicited but also were uncoupled from the mediation of muscarinic receptors. Pirmenol effectively inhibited the K^+ current activated by GTPγS, suggesting that pirmenol may also block the K^+ channel itself and/or the functions of GTP-binding proteins coupled to the channel as indicated in Fig. 5C. The inhibition of adenosine-induced current by pirmenol may also be best explained by these blocking effects. The IC_{50} value of pirmenol for both adenosine-induced and GTPγS-induced current (Fig. 5Bb and Bc) was about 8 μM in both cases. The IC_{50} value of pirmenol was quite similar to that of quinidine or cibenzoline as reported previously (Kurachi et al., 1987b; Nakajima et al., 1989; Wu et al., 1994; also in the present study). From these observations, it is likely that pirmenol inhibits the acetylcholine-induced K^+ current mainly by inhibiting the muscarinic M_2 receptors as disopyramide, while in addition, pirmenol inhibits the adenosine-induced K^+ current by the direct action of the K^+ channel itself or GTP-binding proteins as cibenzoline and quinidine, as schematically described in Fig. 5C.

4.3. Clinical implications

The present results indicate that pirmenol effectively prolonged action potential duration by inhibiting I_{to} in atrial myocyte and antagonized acetylcholine-induced action potential shortening. The duration of action potential and effective refractory period of atrium are important factors for the vulnerability to atrial arrhythmias such as atrial fibrillation (Moe, 1962; Allessie et al., 1977; Rensma et al., 1988). In fact, intravenous infusion of metacholine substantially reduces atrial effective refractory period and potentiates the inducibility of atrial tachyarrhythmia (Elvan et al., 1995). Since pirmenol prolonged action potential duration and antagonized acetylcholine-induced action potential shortening in atrium, pirmenol may prevent atrial arrhythmia. In addition, endogenous adenosine produced and released from myocardial cells during hypoxic conditions (Meghji et al., 1988; Belardinelli et al., 1989) may contribute largely to action potential shortening in the atrium. Based on the present results, pirmenol inhibited adenosine-induced K^+ current in atrial myocytes, which may exert an antiarrythmic action during myocardial ischemia in atrial tissues.

Acknowledgements

This work was partly supported by grants from the Ministry of Education, Science and Culture of Japan.

References

Allessie, M.A., Bonke, F.I.M., Schopman, F.J.G., 1977. Circus movement in rabbit atrial muscle as a mechanism of tachycardia. III. The 'leading circle' concept: A new model of circus movement in cardiac tissue without the involvement of an anatomical obstacle. Circ. Res. 41, 9.

Attwell, D., Cohen, J., Eisner, D., Ohba, M., Ojeda, C., 1979. The steady-state tetrodotoxin-sensitive ('window') current in cardiac Purkinje fibers. Pflüg. Arch. 379, 137.

Belardinelli, L., Linden, J., Berne, R.M., 1989. The cardiac effects of adenosine. Prog. Cardiovasc. Dis. 32, 73.

Clark, R.B., Nakajima, T., Giles, W., Kanai, K., Momose, Y., Szabo, G., 1990. Two distinct types of inwardly rectifying K channels in bull-frog atrial myocytes. J. Physiol. 424, 229.

Coraboeuf, E., Deroubainx, E., Coulombe, A., 1979. Effect of tetrodotoxin on action potentials of the conducting system in dog heart. Am. J. Physiol. 236, H561.

Coraboeuf, E., Deroubaix, E., Escande, D., Coulombe, A., 1988. Comparative effects of three class I antiarrhythmic drugs on plateau and pacemaker currents of sheep cardiac Purkinje fibers. Cardiovasc. Res. 22, 375.

Dukes, I.D., Vaughan Williams, E.M., Dennis, P.D., 1986. Electrophysiological and cardiovascular effects of pirmenol, a new class I antiarrhythmic drug. J. Cardiovasc. Pharmacol. 8, 227.

Dukes, I.D., Cleemann, L., Morad, M., 1990. Tedisamil blocks the transient and delayed rectifier K^+ currents in mammalian cardiac and glial cells. J. Pharmacol. Exp. Ther. 254, 560.

Elvan, A., Pride, H.P., Eble, J.N., Zipes, D.P., 1995. Radiofrequency catheter ablation of the atria reduces inducibility and duration of atrial fibrillation in dogs. Circulation 91, 2235.

Endou, M., Hattori, Y., Gando, S., Kanno, M., 1992. Selectivity of class I antiarrhythmic agents, disopyramide, pirmenol, and pentisomide for peripheral muscarinic M_2 and M_3 receptors. J. Cardiovasc. Pharmacol. 19, 674.

Endou, K., Yamamoto, H., Sato, T., Nakata, F., 1986. Effects of CM 7857, a derivative of disopyramide, on electrophysiologic properties of canine Purkinje fibers and inotropic properties of canine ventricular muscle. J. Cardiovasc. Pharmacol. 8, 507.

Fermini, B., Wang, Z., Duan, D., Nattel, S., 1992. Differences in rate-dependence of transient outward current in rabbit and human atrium. Am. J. Physiol. 263, H1747.

Giles, W.R., Imaizumi, Y., 1988. Comparison of potassium currents in rabbit atrium and ventricular cells. J. Physiol. 405, 123.

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. Pflüg. Arch. 391, 85.

Hammill, S.C., Shand, D.G., Routledge, P.A., Hindman, M.C., Baker, J.T., Pritchett, E.C.L., 1982. Pirmenol, a new antiarrhythmic agent: Initial study of efficacy, safety and pharmacokinetics. Circulation 65, 369.

Hashimoto, K., Watanabe, K., Sugiyama, A., 1988. Antiarrhythmic plasma concentrations of pirmenol on canine ventricular arrhythmias. Jpn. J. Pharmacol. 48, 273.

Hiraoka, M., Sawada, K., Kawano, S., 1986. Effects of quinidine on plateau currents of guinea-pig ventricular myocytes. J. Mol. Cell. Cardiol. 18, 1097.

Holek, M., Osterrieder, W., 1986. Inhibition of the myocardial Ca^{2+} inward current by the class I antiarrhythmic agent, cibenzoline. Br. J. Pharmacol. 87, 705.

Imaizumi, Y., Giles, W.R., 1987. Quinidine-induced inhibition of transient outward current in cardiac muscle. Am. J. Physiol. 253, H704.

Kaibara, M., Nakajima, T., Irisawa, H., Giles, W., 1991. Regulation of spontaneous opening of muscarinic K^+ channels in rabbit atrium. J. Physiol. 433, 589.

Kaplan, H.R., Mertz, T.E., Steffe, T.J., 1987. Preclinical pharmacology of pirmenol. Am. J. Cardiol. 59, H2.

Kotake, H., Hirai, S., Kinugawa, T., Ito, T., Yamasaki, J., Hasegawa, T., Mashiba, H., 1988. Effects of pirmenol hydrochloride on the spontaneous action potentials and membrane current systems of rabbit sinoatrial node cells. J. Electrocardiol. 21, 355.

Kurachi, Y., Nakajima, T., Sugimoto, T., 1986. On the mechanism of activation of muscarinic K^+ channels by adenosine in isolated atrial cells: Involvement of GTP-binding proteins. Pflüg. Arch. 407, 264.

Kurachi, Y., Nakajima, T., Sugimoto, T., 1987a. Short-term desensitization of muscarinic K^+ channel current in isolated atrial myocytes and possible role of GTP-binding proteins. Pflüg. Arch. 410, 227.

Kurachi, Y., Nakajima, T., Sugimoto, T., 1987b. Quinidine inhibition of the muscarinic receptor-activated K^+ channel current in atrial cells of guinea-pig. Naunyn-Schmiedebergs Arch. Pharmacol. 335, 216.

Lee, T.G., Goldberg, A.D., Chang, T., Serkland, M.T., Yakatan, G.J., Johnson, E.L., Toole, J.G., Goldstein, S., 1983. Pharmacokinetics and efficacy of pirmenol hydrochloride in the treatment of ventricular dysrhythmia. J. Cardiovasc. Pharmacol. 5, 632.

Meghji, P., Middleton, K.M., Newby, A.C., 1988. Absolute rates of adenosine formation during ischemia in rat and pigeon hearts. Biochem. J. 249, 695.

Moe, G.K., 1962. On the multiple wavelet hypothesis of atrial fibrillation. Arch. Int. Pharmacodyn. Ther. 140, 183.

Mori, K., Hara, Y., Saito, T., Masuda, Y., Nakaya, H., 1995. Anticholinergic effects of class III antiarrhythmic drugs in guinea-pig atrial cells: Different molecular mechanisms. Circulation 91, 2834.

Nakajima, T., Kurachi, Y., Ito, H., Takikawa, R., Sugimoto, T., 1989. Anti-cholinergic effects of quinidine, disopyramide and procainamide in isolated atrial myocytes: Mediation by different molecular mechanisms. Circ. Res. 64, 297.

Nakajima, T., Sugimoto, T., Kurachi, Y., 1992. Effects of anions on the G protein-mediated activation of the muscarinic K^+ channel in the cardiac atrial cell membrane: Intracellular chloride inhibition of the GTPase activity of G_K. J. Gen. Physiol. 99, 665.

Nakaya, H., Tohse, N., Kanno, M., 1988. Frequency- and voltage-dependent depression of maximum upstroke velocity of action potentials by pirmenol in guinea pig ventricular muscles. Jpn. J. Pharmacol. 48, 423.

Reder, R.F., Danilo, P. Jr., Rosen, M.R., 1980. Effects of pirmenol HCl on electrophysiologic properties of cardiac Purkinje fibers. Eur. J. Pharmacol. 61, 321.

Rensma, P.L., Allessie, M.A., Lammers, W.J.E.P., Bonke, F.I.M., Schalig, M.J., 1988. The length of the excitation wave as an index for the susceptibility to reentrant atrial arrhythmia. Circ. Res. 62, 395.

Sawanobori, T., Adaniya, H., Yamashita, K., Kawano, S., Hayami, H., Kuga, K., Hiraoka, M., 1990. Electrophysiologic and antiarrhythmic actions of pirmenol on rabbit and guinea-pig cardiac preparations. J. Cardiovasc. Pharmacol. 16, 975.

Shibata, E.F., Drury, T., Refsum, H., Aldrete, V., Giles, W., 1989. Contribution of a transient outward current to repolarization in human atrium. Am. J. Physiol. 257, H1773.

Steffe, T.J., Mertz, T.E., Hastings, S.G., Potoczak, R.E., Kaplan, H.R., 1980. CI-845 (pirmenol hydrochloride): A new orally effective long-acting antiarrhythmic agent. J. Pharmacol. Exp. Ther. 214, 50.

Toivonen, L.K., Nieminen, M.S., Manninen, V., Frick, H., 1987a. Pirmenol in the termination of paroxysmal supraventricular tachycardia. Am. J. Cardiol. 59, H35.

Toivonen, L.K., Nieminen, M.S., Manninen, V., Frick, H., 1987b. Conversion of paroxysmal atrial fibrillation to sinus rhythm by intravenous pirmenol. Am. J. Cardiol. 59, H39.

Vaughan Williams, E.M., 1984. A classification of antiarrhythmic actions reassessed after a decade of new drugs. J. Clin. Pharmacol. 24, 129.

Wu, S.N., Nakajima, T., Yamashita, T., Hamada, E., Hazama, H., Iwasawa, K., Omata, M., Kurachi, Y., 1994. Molecular mechanism of cibenzoline-induced anticholinergic action in single atrial myocytes: Comparison with effect of disopyramide. J. Cardiovasc. Pharmacol. 23, 618.

Yamashita, T., Nakajima, T., Hazama, H., Hamada, E., Murakawa, Y., Sawada, K., Omata, M., 1995a. Regional differences in transient outward current density and inhomogeneities of repolarization in rabbit right atrium. Circulation 92, 3061.

Yamashita, T., Nakajima, T., Hamada, E., Hazama, H., Omata, M., Kurachi, Y., 1995b. Flecainide inhibits the transient outward current in atrial myocytes isolated from the rabbit heart. J. Pharmacol. Exp. Ther. 274, 315.