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Antiarrhythmic and bradycardic drugs inhibit currents of cloned K^+ channels, $K_{V1.2}$ and $K_{V1.4}$

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Abstract

We investigated the effects of the antiarrhythmic drugs, quinidine, disopyramide, flecainide, clofilium, verapamil, and the bradycardic drug, bertosamil, on the currents of the cloned K^+ channels, $K_{V1.2}$ ($I_{K(V1.2)}$) and $K_{V1.4}$ ($I_{K(V1.4)}$), using the *Xenopus* oocyte expression system. Both $I_{K(V1.2)}$ and $I_{K(V1.4)}$ were inhibited in a concentration-dependent manner by quinidine (10 μM to 1 mM), flecainide (10 μM to 1 mM), clofilium (10–300 μM), verapamil (10 μM to 1 mM) and bertosamil (10 μM to 1 mM) but not by disopyramide (10 μM to 1 mM). The inhibitory effects of clofilium, verapamil and bertosamil on $I_{K(V1.2)}$ were time-dependent. The decay time course of $I_{K(V1.4)}$ was accelerated by clofilium, verapamil and bertosamil, but decelerated by quinidine and flecainide. These results indicate that $I_{K(V1.2)}$ and $I_{K(V1.4)}$ are targets for the four antiarrhythmic drugs and the bradycardic drug.

Keywords: K^+ channel, cloned; $K_{V1.2}$; $K_{V1.4}$; Antiarrhythmic drug; Bradycardic drug

1. Introduction

We have cloned the two cDNAs coding $K_{V1.2}$ and $K_{V1.4}$, and expressed them in *Xenopus* oocytes (Ishii et al., 1992; Okada et al., 1992). The current flowing through $K_{V1.2}$ ($I_{K(V1.2)}$) is very similar to a rapidly activating delayed rectifier current in rat atrium (Boyle and Nerbonne, 1991) and the current of $K_{V1.4}$ ($I_{K(V1.4)}$) closely resembles a 4-aminopyridine-sensitive component (I_{to1}) (Yamagishi et al., 1993) of the transient outward current (I_{to}) prominent in rat heart (Josephson et al., 1984) and human atrial cells (Escande et al., 1987; Shibata et al., 1989). These native K^+ currents are thought to contribute to repolarization of the action potential in cardiac cells where they exist. Thus, it was interesting to see whether $I_{K(V1.2)}$ and $I_{K(V1.4)}$ would be affected by class III antiarrhythmic drugs whose main action is to interfere with repolarization of the action potential. In a previous study we investigated the effects of the relatively pure class III antiarrhythmic drugs, *d*-sotalol, E-4031 and MS-551, on $I_{K(V1.2)}$ and $I_{K(V1.4)}$ using the *Xenopus* oocyte expression system (Yamagishi et al., 1993). Unexpectedly, however, neither $I_{K(V1.2)}$ nor $I_{K(V1.4)}$ was affected by *d*-sotalol, E-4031 and MS-551. Therefore, we designed the present study to see whether $K_{V1.2}$ and $K_{V1.4}$ would be targets for the class I antiarrhythmic drugs, quinidine, disopyramide, flecainide, the class III, clofilium, and the class IV, verapamil, and the bradycardic drug, bertosamil.

2. Materials and methods

2.1. Expression of $K_{V1.2}$ and $K_{V1.4}$

The plasmid (pBluescript) containing $K_{V1.2}$ or $K_{V1.4}$ was cut with appropriate restriction enzymes (Ishii et al., 1992; Okada et al., 1992), and capped run-off cRNA specific for them was synthesized in vitro with T7 RNA polymerase. Transcribed RNA was dissolved in water at a final concentration of 0.2 μg/μl for oocyte injection.

Xenopus laevis oocytes were treated for 2 h with collagenase (2 mg/ml) in modified Barth's medium and then were defolliculated manually with fine forceps. The oocytes were injected with $K_{V1.2}$ or $K_{V1.4}$ transcript (40–50 nl per oocyte) and incubated for 2–5

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days at 19° C in modified Barth's medium before electrophysiological assay. For electrophysiological assay, oocytes were continuously perfused at a flow rate of 0.5 ml/min with ND96 solution (96 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM Hepes, pH 7.4). After control data were obtained, bath perfusion was switched to drug-containing ND96 solution. The standard two-microelectrode voltage-clamp method was used for recording whole cell current with 3 M KCl-filled electrodes, and the current records were filtered at 1.0 kHz. All experiments were carried out at 20–23° C.

2.2. Drugs

Drugs and chemicals were obtained from the following sources: quinidine sulfate dihydrate (Wako, Osaka, Japan), disopyramide phosphate (Chugai, Tokyo, Japan), flecainide acetate (Eisai, Tokyo, Japan), clofilium phosphate (Eli Lilly, Indianapolis, USA), *d*-, *l*-, and *dl*-verapamil hydrochloride (Eisai, Tokyo, Japan) (*dl*-verapamil is simply denoted verapamil hereafter), bertosamil (Solvay Pharma Deutschland, Hannover, Germany), Hepes (*N*-2-hydroxyethyl-piperazine-*N*'-2-ethanesulphonic acid, Dojin, Kumamoto, Japan). All drugs were dissolved in ND96 solution just before using, and the pH of the solution was adjusted to 7.4.

2.3. Data analysis

Experimental values are given as means ±S.E.M. The concentration-response curves for changes in peak $I_{K(V1.2)}$ and $I_{K(V1.4)}$ produced by drugs were computer-fitted to the following equation:

$$E = 1 - 1/\left[1 + (K/A)^{p}\right]$$

where E is the effect normalized by the control, A is the drug concentration, K is the IC_{50} value of the drug and p is the slope parameter. Peak currents (I) were converted into peak conductances (G) using the formula:

$$G = I/(V_m - V_{rev})$$

where V_m is the voltage of the depolarizing pulse, V_{rev}

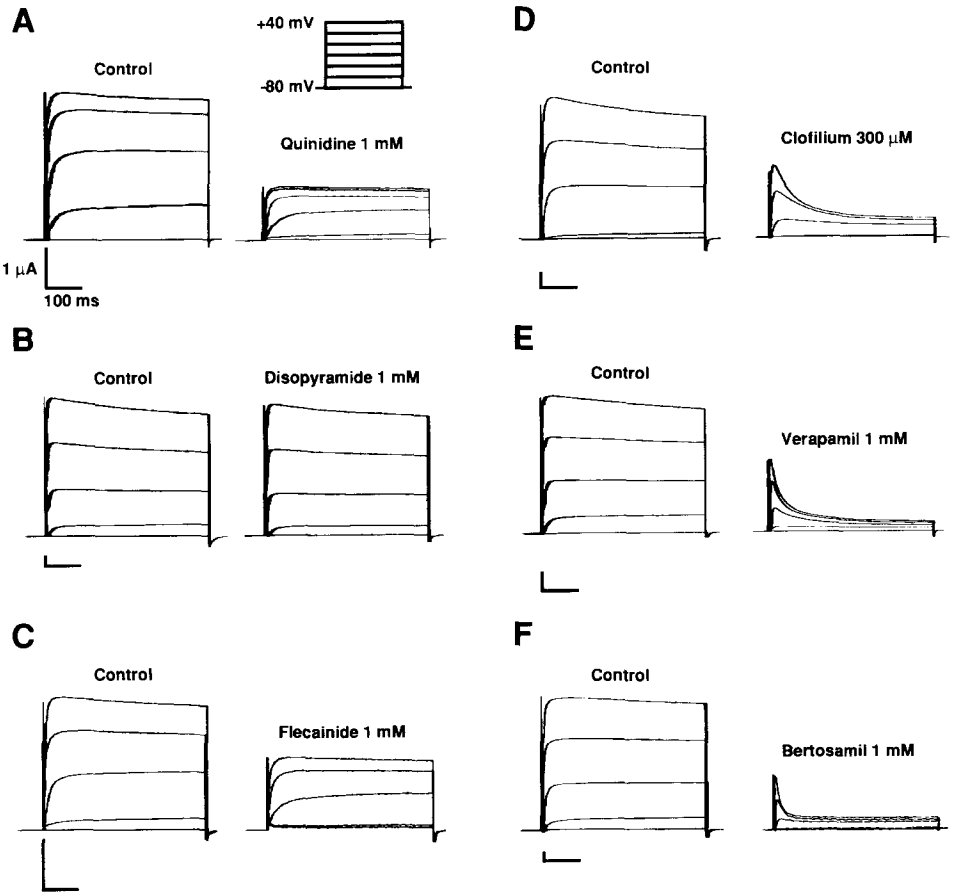

Fig. 1. Typical traces of $I_{K(V1.2)}$ in the control and with quinidine (1 mM), disopyramide (1 mM), flecainide (1 mM), clofilium (300 μM), verapamil (1 mM) and bertosamil (1 mM). The currents were measured during 400-ms test pulses to potentials between −80 and +40 mV from a holding potential of −80 mV at 30-s intervals.

is the reversal potential of currents. The steady state activation curves were fitted to Boltzmann distributions:

$$G/G_{max} = 1/[1 + \exp(-(V_m - V_a)/a_n)]$$

where G_{max} is the maximal conductance, V_a is the voltage for half-activation, a_n is the slope factor. The steady state inactivation curves were fitted to Boltzmann distributions (Perozo et al., 1992):

$$I/I_{max} = (1-\alpha)/[1 + \exp((V_p - V_h)/a_h)] + \alpha$$

where I_{max} is the maximal peak current, V_p is the prepulse voltage, V_h is the voltage for half-inactivation, a_h is the slope factor, α is the non-inactivating component of the curves.

The statistical significance of the results was evaluated using a one-way analysis of variance followed by the Dunnet multi-comparison test. A P value smaller than 0.05 was considered to be significant.

3. Results

3.1. Effects of antiarrhythmic and bradycardic drugs on $I_{K(V1.2)}$ and $I_{K(V1.4)}$

On large depolarization, $I_{K(V1.2)}$ showed a very slow inactivation and $I_{K(V1.4)}$ showed a rapid inactivation (Figs. 1 and 2). Thus, we investigated the rates of recovery from inactivation for $I_{K(V1.2)}$ and $I_{K(V1.4)}$ in the following way. A control 400-ms depolarizing pulse from a holding potential of −80 mV to +20 mV was given, and a second identical depolarization followed each control pulse after an increasing interval at −80 mV. The recovery time constants were 2.4 ± 0.4 s for $I_{K(V1.2)}$ ($n = 5$) and 5.6 ± 1.0 s for $I_{K(V1.4)}$ ($n = 5$). Full recovery was obtained after 5 s for $I_{K(V1.2)}$ ($n = 5$) and 30 s for $I_{K(V1.4)}$ ($n = 5$) from the control depolarization. Therefore, $I_{K(V1.2)}$ and $I_{K(V1.4)}$ were measured at 30-s intervals during 400-ms test pulses to potentials between −80 and +40 mV from a holding potential of −80 mV.

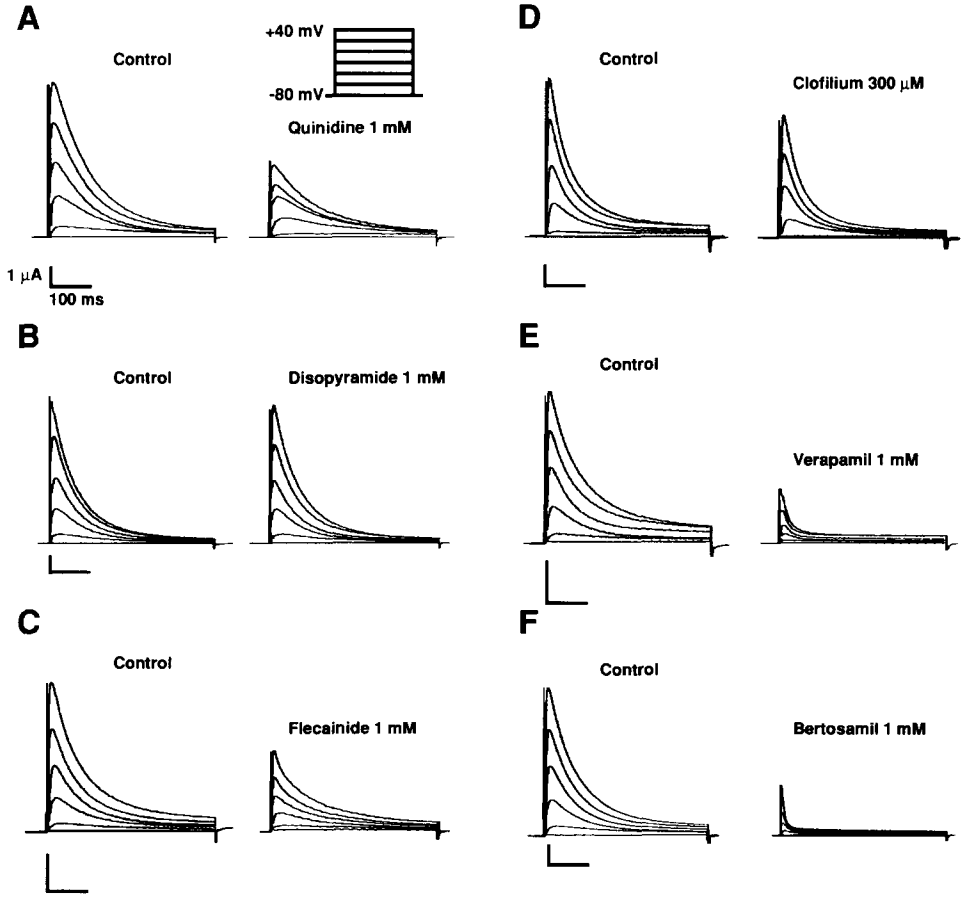

Fig. 2. Typical traces of $I_{K(V1.4)}$ in the control and with quinidine (1 mM), disopyramide (1 mM), flecainide (1 mM), clofilium (300 μM), verapamil (1 mM) and bertosamil (1 mM). The currents were measured during 400-ms test pulses to potentials between −80 and +40 mV from a holding potential of −80 mV at 30-s intervals.

Table 1
IC_{50} values of quinidine, disopyramide, flecainide, clofilium, verapamil and bertosamil for $I_{K(V1.2)}$ and $I_{K(V1.4)}$

	$I_{K(V1.2)}$		$I_{K(V1.4)}$	
	n	IC_{50} (μM)	n	IC_{50} (μM)
Quinidine	5	147.9	4	186.2
Disopyramide	3	> 1000	3	> 1000
Flecainide	5	1023.3	7	758.6
Clofilium	5	173.8	6	1071.5
Verapamil	5	251.2	5	707.9
Bertosamil	7	354.8	7	323.6

The concentration-response curves of peak $I_{K(V1.2)}$ and $I_{K(V1.4)}$ for each drug were analysed by computer-fitting to the logistic equation described in Methods.

Quinidine (10 μM to 1 mM), flecainide (10 μM to 1 mM), clofilium (10–300 μM), verapamil (10 μM to 1 mM) and bertosamil (10 μM to 1 mM), inhibited $I_{K(V1.2)}$ and $I_{K(V1.4)}$ but disopyramide (10 μM to 1 mM) failed to inhibit them. Figs. 1 and 2 show the typical traces of $I_{K(V1.2)}$ and $I_{K(V1.4)}$, respectively. The inhibition of $I_{K(V1.2)}$ caused by clofilium, verapamil and bertosamil progressed during 400-ms depolarizing pulses. Fig. 3 shows the concentration-response curves for inhibition of peak $I_{K(V1.2)}$ and $I_{K(V1.4)}$ for each drug at a test potential of +20 mV. The IC_{50} values for these drugs to inhibit peak $I_{K(V1.2)}$ or $I_{K(V1.4)}$ are shown in Table 1. Clofilium was less effective on $I_{K(V1.4)}$ than on $I_{K(V1.2)}$, although the effects of the other drugs on $I_{K(V1.2)}$ and $I_{K(V1.4)}$ were similar. We also investigated the effects of *d*- or *l*-verapamil on $I_{K(V1.2)}$ and $I_{K(V1.4)}$. Both isomers of verapamil inhibited $I_{K(V1.2)}$ and $I_{K(V1.4)}$ but there was no difference in the IC_{50} values between them (n = 3, data not shown). After 30-min washout of each drug $I_{K(V1.2)}$ and $I_{K(V1.4)}$ recovered partially (data not shown).

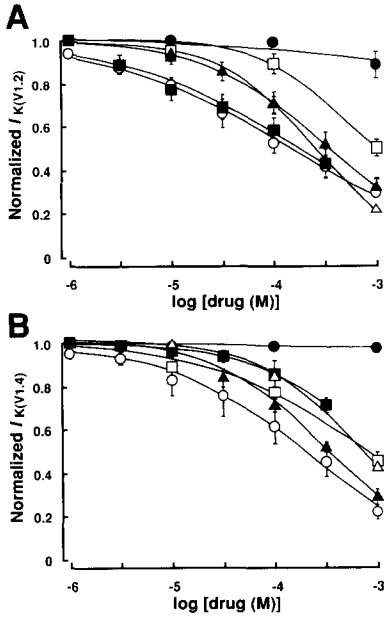

Fig. 3. Concentration-response curves of peaks of $I_{K(V1.2)}$ (A) and $I_{K(V1.4)}$ (B) at a test potential of +20 mV for quinidine (1 μM to 1 mM, ○), disopyramide (1 μM to 1 mM, ●), flecainide (1 μM to 1 mM, □), clofilium (1–300 μM, ■), verapamil (1 μM to 1 mM, △) and bertosamil (1 μM to 1 mM, ▲). Data are expressed as means ± S.E.M. (vertical bars) (n = 4–7). The curves were fitted by the logistic equation described in Methods.

Table 2
Characteristics of steady state activation and inactivation curves of $I_{K(V1.2)}$ and $I_{K(V1.4)}$ in the absence and presence of quinidine, flecainide, clofilium, verapamil and bertosamil

	Activation				Inactivation			
	n	G_{max} (μS)	V_a (mV)	a_n (mV)	n	V_h (mV)	a_h (mV)	α
$I_{K(V1.2)}$								
Control	31	55.0 ± 6.4	−8.6 ± 1.1	9.6 ± 0.4		ND		
Quinidine (1 mM)	7	23.2 ± 7.9 [a]	−30.4 ± 3.1 [a]	6.5 ± 1.4 [a]		ND		
Flecainide (1 mM)	5	22.7 ± 7.7 [a]	−22.7 ± 3.4 [a]	7.2 ± 0.7 [a]		ND		
Clofilium (300 μM)	6	24.8 ± 6.8 [a]	0.6 ± 2.1 [a]	8.9 ± 0.6	6	−10.4 ± 2.0	4.5 ± 0.3	0.46 ± 0.07
Verapamil (1 mM)	6	24.2 ± 5.8 [a]	−3.9 ± 2.8 [a]	10.6 ± 0.8	5	−27.4 ± 3.0	4.2 ± 0.3	0.27 ± 0.05
Bertosamil (1 mM)	7	32.7 ± 10.3 [a]	−2.4 ± 2.8 [a]	11.1 ± 1.0	7	−21.8 ± 3.0	3.2 ± 0.6	0.51 ± 0.06
$I_{K(V1.4)}$								
Control	28	25.6 ± 2.4	−14.6 ± 0.9	17.0 ± 0.5	27	−48.9 ± 1.1	4.5 ± 0.2	0.12 ± 0.01
Quinidine (1 mM)	4	10.9 ± 5.0 [a]	−14.3 ± 3.3	15.6 ± 0.7	4	−36.8 ± 2.8 [a]	6.5 ± 0.8	0.23 ± 0.04 [a]
Flecainide (1 mM)	6	14.3 ± 3.5 [a]	−16.7 ± 1.9	15.8 ± 1.1	5	−45.7 ± 2.5	4.4 ± 0.2	0.19 ± 0.03 [a]
Clofilium (300 μM)	6	21.1 ± 5.3	−5.3 ± 2.7 [a]	14.2 ± 0.3	5	−38.7 ± 1.9 [a]	3.9 ± 0.3	0.12 ± 0.05
Verapamil (1 mM)	5	7.6 ± 1.4 [a]	−8.7 ± 2.2 [a]	19.5 ± 1.9	6	−50.8 ± 1.1	5.0 ± 0.6	0.31 ± 0.03 [a]
Bertosamil (1 mM)	7	9.1 ± 1.5 [a]	−9.8 ± 2.6 [a]	16.9 ± 0.8	7	−49.3 ± 2.5	4.3 ± 0.1	0.21 ± 0.03 [a]

Data were fitted to Boltzmann distributions, $G/G_{max} = 1/[1 + \exp\{-(V_m - V_a)/a_n\}]$, to obtain the voltage for half-activation (V_a) and the slope factor (a_n) of the curves. Data were fitted to Boltzmann distributions, $I/I_{max} = (1 - \alpha)/\{1 + \exp((V_p - V_h)/a_h\} + \alpha$, to obtain the voltage for half-inactivation (V_h), the slope factor (a_h) and the non-inactivating component (α) of the curves. All values are given as the means ± S.E.M.
[a] $P < 0.05$ compared with control. ND, not determined.

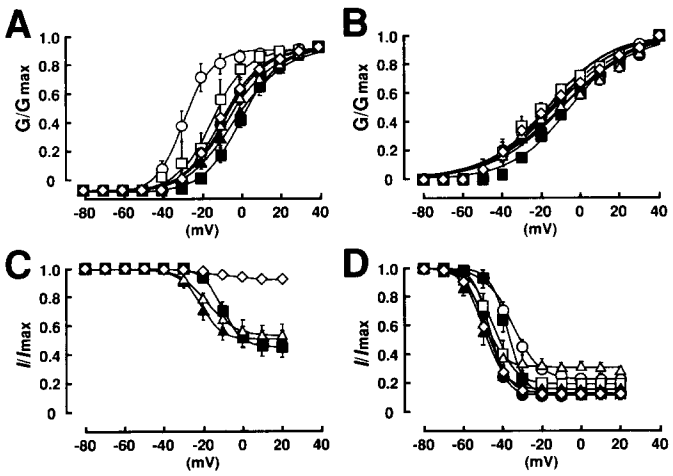

Fig. 4. Steady state activation curves of $I_{K(V1.2)}$ (A) and $I_{K(V1.4)}$ (B) and steady state inactivation curves of $I_{K(V1.2)}$ (C) and $I_{K(V1.4)}$ (D) in the absence (control, ◇, $n = 31$ for $I_{K(V1.2)}$ and $n = 28$ for $I_{K(V1.4)}$) and presence of quinidine (1 mM, ○), disopyramide (1 mM, ●), flecainide (1 mM, □), clofilium (300 μM, ■), verapamil (1 mM, △) and bertosamil (1 mM, ▲) ($n = 4$–7). Data are expressed as means ± S.E.M. (vertical bars). Each curve was obtained by fitting the data to the Boltzmann distributions given in Methods.

3.2. Effects of antiarrhythmic and bradycardic drugs on steady state activation and inactivation of $I_{K(V1.2)}$ and $I_{K(V1.4)}$

Fig. 4 shows the steady state activation and inactivation curves for $I_{K(V1.2)}$ and $I_{K(V1.4)}$ measured in the absence (control) and presence of quinidine, flecainide, clofilium, verapamil and bertosamil. The effects of the drugs were evaluated at 10 min after their application. Therefore, we measured the steady state activation and inactivation curves in control solution at 0 and 10 min to see if the time-dependent shift occurred. None of the parameters of steady state activation and inactivation curves of $I_{K(V1.2)}$ and $I_{K(V1.4)}$ differred significantly between 0 and 10 min (data not shown, $n = 5$–6). Table 2 shows the characteristics of the curves. The steady state activation curves for $I_{K(V1.2)}$ in the control had V_a of -8.6 ± 1.1 mV and a_n of 9.6 ± 0.4 mV ($n = 31$). The steady state activation curves for $I_{K(V1.2)}$ were shifted in the negative direction by quinidine and flecainide and in the positive direction by clofilium, verapamil and bertosamil. Quinidine and flecainide decreased a_n but clofilium, verapamil and bertosamil did not change a_n. As $I_{K(V1.2)}$ in the control showed a very slow inactivation, the values of steady state inactivation characteristics, V_h and a_h, were not determined. However, V_h and a_h were obtained in the case of clofilium, verapamil and bertosamil since they accelerated the inactivation of $I_{K(V1.2)}$.

The steady state activation curves for $I_{K(V1.4)}$ in the control had V_a of -14.6 ± 0.9 mV and a_n of 17.0 ± 0.5 mV ($n = 28$). The steady state activation curves for $I_{K(V1.4)}$ were shifted in the positive direction by clofilium, verapamil and bertosamil. Quinidine and flecainide did not change V_a significantly. None of the

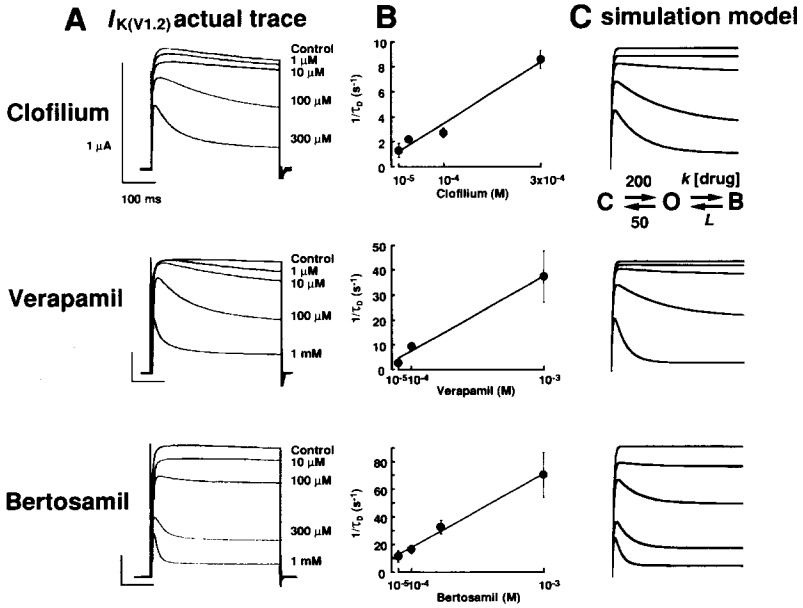

Fig. 5. (A) The currents were measured at +20 mV in the absence and presence of clofilium, verapamil or bertosamil. (B) The reciprocal of the drug-induced time constant ($1/\tau_D$) of $I_{K(V1.2)}$ at a test potential of +20 mV was plotted against concentrations of each drug according to the equation shown in the text. Data are expressed as means ± S.E.M. (vertical bars) ($n = 5$). (C) The computer simulation of $I_{K(V1.2)}$ before and after application of clofilium, verapamil or bertosamil was performed according to the three-state model shown in this figure. The time constant of activation of $I_{K(V1.2)}$ was 3.7 ± 0.4 ms ($n = 6$) at +20 mV in the control. This simulation was performed assuming this value was not changed in the presence of clofilium, verapamil or bertosamil.

drugs changed a_n. The steady state inactivation curves for $I_{K(V1.4)}$ in the control had V_h of -48.9 ± 1.1 mV and a_h of 4.5 ± 0.2 mV ($n = 27$). The steady state inactivation curves for $I_{K(V1.4)}$ were shifted in the positive direction by quinidine and clofilium. Flecainide, verapamil and bertosamil did not affect V_h. None of the five drugs changed a_h.

3.3. Effects of antiarrhythmic and bradycardic drugs on decay time course of $I_{K(V1.2)}$ and $I_{K(V1.4)}$

Time-dependent blockade occurred in $I_{K(V1.2)}$ in the presence of clofilium, verapamil or bertosamil in a concentration-dependent manner (Fig. 5). These results suggest that the block of $K_{V1.2}$ produced by these drugs can be described in terms of an open channel blocking scheme similar to that employed by previous workers (Snyders et al., 1992; Rampe et al., 1993). The current decay of $I_{K(V1.2)}$ in the presence of clofilium (300 μM), verapamil (1 mM) or bertosamil (1 mM) was fitted to a single exponential function (Table 3). Following the methods proposed by Rampe et al. (1993), we obtained a drug-induced time constant (τ_D) as an approximation of the drug-channel interaction kinetics which was calculated according to the equation:

$$1/\tau_D = k[X] + L$$

where [X] is the concentration of drugs and k and L are apparent association and dissociation rates for the drugs, respectively. Here we assume that the drugs do not affect the gating of $K_{V1.2}$. The plots of $1/\tau_D$ for $I_{K(V1.2)}$ against the concentration of clofilium, verapamil and bertosamil at a test potential of +20 mV are shown in Fig. 5. An apparent association rate, k, an apparent dissociation rate, L, and the apparent K_d value, L/k, were obtained from the slope and intercept of the least square fit to the data. The k, L, K_d values of clofilium, verapamil and bertosamil for $I_{K(V1.2)}$ are shown in Table 4. Fig. 5 shows $I_{K(V1.2)}$ before and after application of clofilium, verapamil and bertosamil each with the corresponding computer-derived model. Here we assumed that 80% of channels were open at +20 mV and the drugs did not affect the rate constants for opening and closing. The slower inactivation present in the control currents was ignored.

Table 3
The time constants of inactivation for $I_{K(V1.2)}$ and $I_{K(V1.4)}$ in the absence and presence of quinidine, flecainide, clofilium, verapamil and bertosamil

	Time constant (ms)			
	n	$I_{K(V1.2)}$	n	$I_{K(V1.4)}$
Control		ND	22	71.7±3.6
Quinidine (1 mM)		ND	4	150.6±16.1 [a]
Flecainide (1 mM)		ND	4	103.2±1.6 [a]
Clofilium (300 μM)	4	113.4±18.5	4	46.4±10.9 [a]
Verapamil (1 mM)	5	31.2± 5.1	5	27.3±4.3 [a]
Bertosamil (1 mM)	5	29.5±11.0	5	10.3±1.6 [a]

The current decay of $I_{K(V1.2)}$ in the presence of clofilium, verapamil or bertosamil was fitted to a single exponential function. Inactivation of $I_{K(V1.4)}$ in the absence and presence of drugs was also fitted to a single exponential function. [a] $P < 0.05$, compared with control. ND, not determined.

Table 4
The apparent association rates (k), dissociation rates (L) and K_d (L/k) values of clofilium, verapamil and bertosamil for $I_{K(V1.2)}$ and $I_{K(V1.4)}$

	k (M^{-1} s^{-1})	L (s^{-1})	K_d (μM)
$I_{K(V1.2)}$			
Clofilium	2.4×10^4	1.0	42
Verapamil	3.3×10^4	4.0	120
Bertosamil	5.9×10^4	11.5	190
$I_{K(V1.4)}$			
Clofilium	2.9×10^4	1.1	38
Verapamil	1.9×10^4	3.9	210
Bertosamil	6.9×10^4	4.7	680

The reciprocal of the drug-induced time constant ($1/\tau_D$) of $I_{K(V1.2)}$ at a test potential of +20 mV is plotted against concentrations of drugs, according to the equation, $1/\tau_D = k[X] + L$, where [X] is the concentration of drugs and k and L are the apparent association and dissociation rates. For $I_{K(V1.4)}$, the difference between $1/\tau_D$ and the reciprocal of control time constant ($1/\tau_{control}$) of $I_{K(V1.4)}$ at a test potential of +20 mV is plotted against concentrations of drugs, according to the equation, $1/\tau_D - 1/\tau_{control} = k[X] + L$.

The decay time course of $I_{K(V1.4)}$ in the control was fitted to a single exponential function with a decay time constant of 71.7 ± 3.6 ms (n = 22, Table 3). $I_{K(V1.4)}$ decayed more slowly in the presence of quinidine (1 mM) or flecainide (1 mM) than in their absence. On the contrary, $I_{K(V1.4)}$ decayed faster in the presence of clofilium (300 μM), verapamil (1 mM) or bertosamil (1 mM) than in the control. The k, L, K_d values of clofilium, verapamil and bertosamil for $I_{K(V1.4)}$ are also shown in Table 4.

4. Discussion

All the drugs used in the present study have been reported to inhibit voltage-dependent K^+ currents in native cardiac cells. Among class I antiarrhythmic drugs, quinidine (Furukawa et al., 1989) and disopyramide (Coraboeuf et al., 1988) block I_{to} and I_K, and flecainide blocks I_K (Follmer et al., 1992). Clofilium, a class III antiarrhythmic drug, blocks I_{to} and I_K (Arena and Kass, 1988; Castle, 1991). Bertosamil, a bradycardic drug, is a derivative of tedisamil which also has properties of a class III antiarrhythmic drug (Dukes et al., 1990), and inhibits I_{to} (Weis et al., 1990). Verapamil, a class IV antiarrhythmic drug, blocks voltage-dependent K^+ channels (Terada et al., 1987; Pancrazio et al., 1991), and has recently been shown to block a rapidly activating delayed rectifier K^+ channel cloned

from human heart (Rampe et al., 1993). In the present study all the drugs used except disopyramide inhibited both $I_{K(V1.2)}$ and $I_{K(V1.4)}$, although there was a distinct difference in mode of inhibition of $I_{K(V1.2)}$ between quinidine and flecainide on the one hand and clofilium, verapamil and bertosamil on the other. Taking into consideration the fact that $I_{K(V1.2)}$ is very similar to a rapidly activating delayed rectifier recently recorded from rat atrium (Boyle and Nerbonne, 1991), and that $I_{K(V1.4)}$ is similar to the transient outward current (I_{to1}) (Yamagishi et al., 1993), the present results are not unexpected. The question arises about what properties of these antiarrhythmic drugs are responsible for their blocking activities on $I_{K(V1.2)}$ and $I_{K(V1.4)}$. The antiarrhythmic potencies of class I antiarrhythmic drugs are thought to be associated with their lipid solubilities (Vaughan Williams, 1989). The rank order of octanol/water partition coefficients of class I antiarrhythmic drugs used in the present experiments is quinidine > disopyramide > flecainide. This rank order is not consistent with their potencies to block $I_{K(V1.2)}$ and $I_{K(V1.4)}$ (Table 1). Furthermore, discrepancies have been known between the lipid solubilities of disopyramide and its blocking effects on glibenclamide-sensitive K^+ currents induced by K^+ channel openers in *Xenopus* oocytes (Sakuta et al., 1992). Thus, the lipid solubilities of antiarrhythmic and bradycardic drugs do not appear likely to contribute to their blocking actions on $I_{K(V1.2)}$ and $I_{K(V1.4)}$. The reason for the virtual ineffectiveness of disopyramide on $I_{K(V1.2)}$ and $I_{K(V1.4)}$ is unknown. We previously showed that the class III antiarrhythmic drugs, *d*-sotalol, E-4031 and MS-551, had no effects on $I_{K(V1.2)}$ and $I_{K(V1.4)}$, using the *Xenopus* oocyte expression system (Yamagishi et al., 1993). One might suspect that these drugs may not have been able to reach their binding sites because of membrane properties of oocytes. However, Sakuta et al. (1993) reported that E-4031 and MS-551 blocked glibenclamide-sensitive K^+ currents in *Xenopus* oocytes, indicating that this is not the case.

The major point of the present results is that high concentrations of drugs were required to inhibit $I_{K(V1.2)}$ and $I_{K(V1.4)}$. In native cardiac tissues or cells, quinidine blocked I_{to} with the IC_{50} value of 10 μM (Imaizumi and Giles, 1987) and I_K with the IC_{50} value of 2 μM (Furukawa et al., 1989), flecainide blocked I_K with the IC_{50} value of 2.1 μM (Follmer et al., 1992), clofilium blocked I_K with the IC_{50} value of 50 μM (Arena and Kass, 1988). Bertosamil at 10 μM nearly abolished I_{to} (Weis et al., 1990). These values are smaller by one or two orders of magnitude than those needed to block $I_{K(V1.2)}$ and $I_{K(V1.4)}$. Some other studies concerning the effects of antiarrhythmic drugs on cloned K^+ channels have shown that higher drug concentrations were needed to block the cloned K^+ channels. For example, quinidine blocked a cloned I_{to}-type cardiac K^+ channel (RHK1, $K_{V1.4}$) with the IC_{50} value of 1.69 mM at 0 mV (Yatani et al., 1993), and the currents of a cloned K^+ channel (MK-1, $K_{V1.1}$) expressed in CHO cells were inhibited by higher concentrations of quinidine (100 μM) and tedisamil (100 μM) (Robertson and Owen, 1993). Although the reason why the drugs studied were far less potent in $I_{K(V1.2)}$ and $I_{K(V1.4)}$ than in native K^+ channels is unknown, one possibility is the contribution of other subunits. Voltage-dependent Na^+ and Ca^{2+} channels consist of more than two subunits. It has been reported that, in both channels, coexpression of the main pore-forming subunits with the other subunits results in normalization of current kinetics (Isom et al., 1992; Singer et al., 1991). Recently two different β-subunits of voltage-gated K^+ channels, $K_v\beta_1$ and $K_v\beta_2$, were cloned from rat brain (Rettig et al., 1994). It has been shown that the $K_v\beta_1$ subunit may associate with the *Shaker*-related rat K^+ channel family changing their characteristics. Therefore, it is possible that cloned K^+ channels might lack some unidentified structure proteins, which are responsible for reduced sensitiveness to the antiarrhythmic drugs.

In the present study the time-dependent block of $I_{K(V1.2)}$ occurred in the presence of clofilium, verapamil, and bertosamil (Fig. 5, Table 3). These experimental data of $I_{K(V1.2)}$ were well-fitted by the open density function, $O(t)$ (Pancrazio et al., 1991), predicted by the binding scheme first employed by Armstrong (1969). The model of $I_{K(V1.2)}$ supports the idea that clofilium, verapamil and bertosamil block $I_{K(V1.2)}$ as open channel blockers. $I_{K(V1.4)}$ has an inactivation state, which is explained by a ball and chain model (Hoshi et al., 1990; Zagotta et al., 1990). Yatani et al. (1993) have reported that quinidine acts on $I_{K(V1.4)}$ as an open channel blocker, slowing the rate of current decay of $I_{K(V1.4)}$. Although it is not clear whether or not the drugs studied block $I_{K(V1.4)}$ as open channel blockers, the five drugs affected the inactivation kinetics of $I_{K(V1.4)}$. That is, $I_{K(V1.4)}$ decayed faster in the presence of clofilium, verapamil and bertosamil and more slowly in the presence of quinidine and flecainide than the control. The k, L, K_d values of clofilium, verapamil and bertosamil for $I_{K(V1.4)}$ are of similar magnitude to those values for $I_{K(V1.2)}$.

Rampe et al. (1993) have reported that S6 and the following carboxy-terminal region of the cloned K^+ channel, fHK, has significant homology to the verapamil binding site identified in Ca^{2+} channels (Striessnig et al., 1990). Since verapamil inhibited $I_{K(V1.2)}$ and $I_{K(V1.4)}$ to a great extent, we also searched $K_{V1.2}$ and $K_{V1.4}$ for the putative verapamil binding sequence. $K_{V1.2}$ and $K_{V1.4}$ had significant homology to the verapamil binding site of the Ca^{2+} channel at the same region as identified in fHK. The sequence identified as forming part of the verapamil binding site of the Ca^{2+} channel is compared with the 44-residue peptide con-

taining S6 and the following carboxy-terminal region of $K_{V1.2}$ and $K_{V1.4}$, which is thought to form part of the inner mouth of the K^+ channels pore (Choi et al., 1993; Lopez et al., 1994). The identified residues were identical to those of fHK. This site may be one of the binding sites of verapamil in $K_{V1.2}$ and $K_{V1.4}$.

In conclusion, $I_{K(V1.2)}$ and $I_{K(V1.4)}$ are targets for the antiarrhythmic drugs, quinidine, flecainide, clofilium, verapamil and the bradycardic drug, bertosamil. Further study will reveal the sites of action for these drugs in cloned K^+ channels.

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