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Comparative actions of cibenzoline and disopyramide on I_{Kr} and I_{Ks} currents in rat sino-atrial nodal cells

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Abstract

Modulation by class Ia antiarrhythmic drugs, cibenzoline and disopyramide, of the pacemaking activity and the underlying ionic currents in rat sino-atrial nodal cells was investigated using current-clamp and whole-cell patch-clamp techniques. Both drugs depressed the spontaneous activity and often caused sinus arrest. The negative chronotropic effect was significant at 10 μM cibenzoline and 30 μM disopyramide. The L-type Ca^{2+} current (I_{Ca}) and the hyperpolarization-activated inward current decreased by 69.7 ± 3.2% and by 45.8 ± 3.0% at 30 μM cibenzoline and by 51.2 ± 3.3% and by 48.3 ± 2.7% at 100 μM disopyramide, respectively. The delayed rectifier K^+ current, which is composed of rapidly and slowly activated currents (I_{Kr} and I_{Ks}), also decreased. The IC_{50} values of I_{Kr} for cibenzoline and disopyramide were 8.8 ± 1.1 and 25.1 ± 2.3 μM, respectively. In the presence of 5 μM E-4031 (1-[2-(6-methyl-2-pyridyl)ethyl]-4-(4-methylsulfonylaminobenzoyl) piperidine), the IC_{50} values of I_{Ks} for cibenzoline and disopyramide were 12.3 ± 1.8 and 81.1 ± 2.3 μM, respectively. The I_{Ks} was completely blocked by 30 μM 293B (*trans*-6-cyano-4-(*N*-ethylsulphonyl-*N*-methtamino)-3-hydroxy-2,2-dimethyl-chromane). These results indicate that the ionic currents are more sensitive to cibenzoline than disopyramide in rat sino-atrial nodal cells, and that I_{Ca} and I_{Kr} make major contributions to pacemaking activity.

Keywords: Cibenzoline; Disopyramide; Ionic current; I_{Kr}; I_{Ks}; Rat sino-atrial nodal cell

1. Introduction

Among antiarrhythmic drugs, cibenzoline and disopyramide clinically possess potent antiarrhythmic actions. Both drugs are Class Ia according to classification of Vaughan Williams (1992) and possess slow or intermediate kinetics (Campbell, 1989; Dangman and Miura, 1986). The drugs are not only Na^+ channel inhibitors, but also Ca^{2+} and K^+ channel inhibitors, i.e., multichannel inhibitors (Kodama et al., 1999; Satoh et al., 1987).

In sino-atrial nodal cells, it has been shown that spontaneous beating is caused by interactions with many ionic currents such as L-type Ca^{2+} (I_{Ca}), delayed rectifier K^+ currents (I_{Krec}), and hyperpolarization-activated inward (I_f) currents (Noble, 1984). Recently, the I_{Krec} has been classified on the basis of at least two components: an early activated (I_{Kr}) and a slowly activated (I_{Ks}) current (Sanguinetti and Jurkiewicz, 1990). For the first time, we have recently demonstrated the underlying ionic currents in rat spontaneously beating sino-atrial nodal cells, in which I_{Kr} is one of the major pacemaker currents, as are as I_{Ca} and I_f currents (Shinagawa et al., 2000). The spontaneous activity was blocked by E-4031 (a I_{Kr} inhibitor) but was not modulated by 293B (a I_{Ks} inhibitor).

Many electropharmacological effects of both drugs have already been reported (Sato et al., 1994; Wu et al., 1994; Hiraoka et al., 1989; Nakajima et al., 1998). In rabbit SA nodal preparations, cibenzoline also modulates pacemaker activity (Kotake et al., 1987). In sino-atrial nodal pacemaker cells, however, nothing is known about the effects of cibenzoline and disopyramide, which have different ionic channel kinetics. The aim of the present experiments was to examine the modulation of the chronotropic effect of both drugs in spontaneously beating rat sino-atrial nodal

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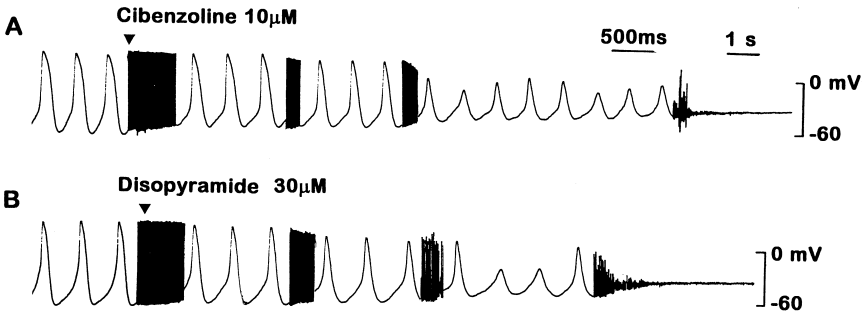

Fig. 1. Drug effects on a spontaneously beating rat sino-atrial nodal cell. (A) Modulation of the spontaneous action potentials by application of 10 μM cibenzoline. (B) Application of 30 μM disopyramide on the spontaneous action potentials. Different cells were used in A and B.

cells. This approach was chosen because of difficulty in recording pacemaker action potentials by inserting conventional microelectrodes into rat sino-atrial nodal tissue. Furthermore, the effects of cibenzoline (which inhibits I_{Kr} and I_{Ks}) and disopyramide (which inhibits I_{Kr}, but not I_{Ks}) on the underlying ionic currents, especially I_{Kr} and I_{Ks}, were examined.

2. Methods

2.1. Cell preparation

Wister rats, weighing 200–300 g, were anaesthetized with sodium pentobarbital (30 mg/kg, i.p.). Rat sino-atrial nodal cells are not isolated successfully with enzyme solution using the Langendorff apparatus. Therefore, a new dissociation technique has recently been developed by Noma's group, as previously described (Shinagawa et al., 2000). Under artificial respiration, the chest cavity was opened. An injection needle, connected to a perfusion line, was inserted through the right atrial wall, and Tyrode solution was directly infused into the atrial cavity at a rate of ~10 ml/min with a hydrostatic pressure of ~70 cm H_2O. To avoid mixing of the perfusate with venous return, and also to expand the atrial cavity by using the perfusion pressure, the superior and inferior venae were ligated. Then, the inferior vena cava was cut distal to the ligature to allow drainage of perfusate, which passed through the pulmonary and then the systemic circulation. Within several minutes, the drained perfusate became largely blood-free. The spontaneous heart beat was stopped by switching the perfusate from normal Tyrode to a nominally Ca^{2+}-free Tyrode solution. Then, Ca^{2+}-free solution containing 0.4 mg/ml trypsin (Wako, Osaka, Japan) was applied for 5–6 min to remove the endocardial endothelium. Ca^{2+}-free solution containing 0.85 mg/ml collagenase (Wako) was perfused for approximately 5 min. Then, the heart was dissected out into fresh collagenase solution. The right atrium was opened by cutting along the atrial septum and also by cutting the ventral wall of the superior vena cava. The atrial tissue including the sino-atrial node was dissected out and was gently shaken in the collagenase plus elastase (0.1 mg/ml) solution (Boehringer Mannheim,

Table 1
Modulation of the spontaneous action potential in rat sino-atrial nodal cells

	n	APA (mV)	MDP (mV)	APD_{50} (ms)	V_{max} (V/s)	CL (ms)
Control	14	76.7 ± 2.2	−60.2 ± 2.5	57.4 ± 3.2	11.4 ± 1.1	289 ± 21
Cibenzoline						
1 μM	10	−3.8 ± 1.1	−2.8 ± 0.7	+2.1 ± 1.0	−1.8 ± 0.3	+6.3 ± 1.1
3 μM	13	−7.3 ± 1.7	−7.6 ± 1.3	+4.8 ± 1.2	−8.1 ± 2.2	+12.5 ± 2.3
10 μM	12	−15.8 ± 2.4[a]	−13.3 ± 1.9	+11.7 ± 2.1	−27.6 ± 2.7[b]	+48.3 ± 3.6[b]
30 μM	11	−34.7 ± 2.5[b]	−28.8 ± 3.1[b]	+24.5 ± 2.5[a]	−27.6 ± 3.3[b]	+48.3 ± 3.6[c]
Control	15	77.3 ± 3.6	−61.4 ± 3.4	57.8 ± 3.3	11.2 ± 2.1	296 ± 23
Disopyramide						
3 μM	13	−1.2 ± 0.7	−1.4 ± 1.7	+3.3 ± 1.0	−0.9 ± 0.3	+4.3 ± 1.1
10 μM	15	−4.8 ± 1.3	−6.8 ± 1.7	+9.9 ± 2.1	−3.9 ± 1.5	+12.6 ± 1.8
30 μM	9	−20.2 ± 2.6[a]	−12.2 ± 2.4	+13.5 ± 2.5	−15.8 ± 2.6[a]	+24.3 ± 2.3[b]
100 μM	7	−38.3 ± 3.7[b]	−21.0 ± 3.3[a]	+29.1 ± 3.2[b]	−29.3 ± 3.3[b]	+63.3 ± 3.9[c]

Values (%) are presented as means ± S.E.M. APA: action potential amplitude. MDP: maximum diastolic potential. APD_{50}: 50% repolarization of action potential duration. V_{max}: Maximum rate of depolarization. CL: cycle length.

[a] $P < 0.05$, with respect to control values.

[b] $P < 0.01$, with respect to control values.

[c] $P < 0.001$, with respect to control values.

Table 2
Incidence of sinus arrest in rat sino-atrial nodal cells

	1	3	10	30	100	300 μM
Cibenzoline	0/10	0/13	2/12	3/11	7/7	–
Disopyramide	–	0/13	0/15	1/9	3/7	5/6

Values are presented as a number of occurrence/experimental number.

Germany). The enzyme treatment lasted for 20–25 min, depending on the extent of tissue digestion seen under a dissection microscope. Finally, the digested tissue was put in the modified KB (Kraftbrühe) solution, and trimmed by scissors into a small sino-atrial node fragment of ~1 mm in width and 3–4 mm in length. The major bundle of atrial cells running through the crista terminalis was discarded. The small sino-atrial node tissue was placed in a 35-mm plastic Petri dish with a fresh KB solution and SA node cells were dissociated by gentle puffing with KB solution to the tissue. The dissociated cells were stored in the same solution at 4°C for experimentation.

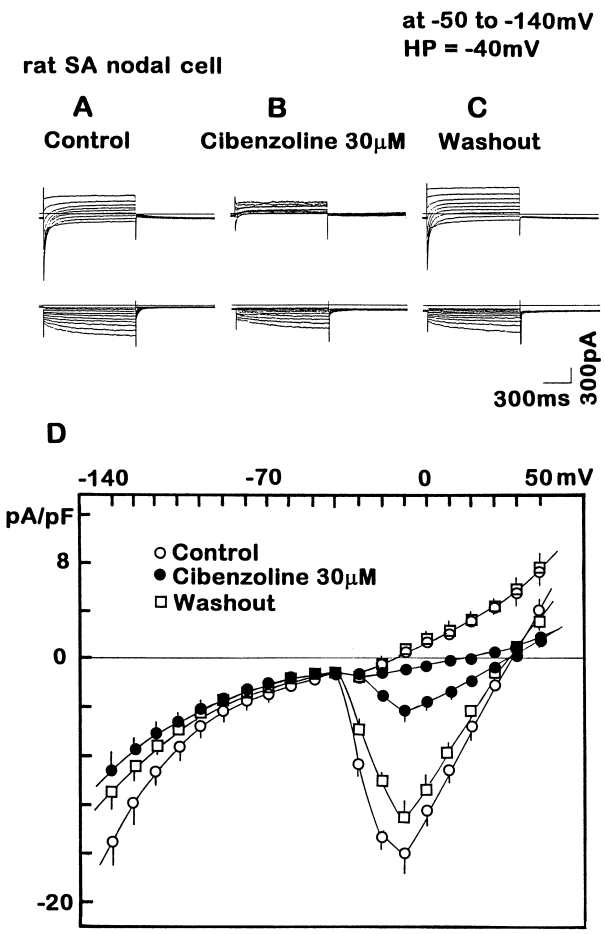

Fig. 2. Modulation by cibenzoline of the ion currents in rat sino-atrial nodal cells. Test pulses for 1 s were applied from −30 to 50 mV and from −50 to −140 mV. (A) Control. (B) Cibenzoline (30 μM). (C) Washout (10 min later). Horizontal line indicates zero current level. (D) Current–voltage relation for the ion currents in rat SA nodal cell. Cibenzoline (30 μM) was administered and the ionic current was recorded 3 min later. The values are presented as means ± S.E.M.

2.2. Whole-cell voltage- and current-clamp experiments

Whole-cell voltage-clamp recordings were performed using an Axopatch patch-clamp amplifier (Axon Instruments, Burlingame, CA, USA) and standard techniques. Patch pipettes from borosilicate glass capillaries were fabricated using a two-stage puller; they had a resistance of 5–7 MΩ. The series resistance was less than 10 mV, and no compensation was used. The liquid junction potential between the pipette solution and the external solution (less than 10 mV) was corrected for all membrane potential recordings. For the action potential parameters, the action potential amplitude was measured as the difference between the peak of the action potential and the maximum diastolic potential, the action potential duration as the duration at 50% repolarization, and the cycle length as the interval between each peak action potential peak. The I_{Ca} was measured as the difference between the peak current and the zero current, and the I_f was the difference between the current at the end of a 1-s test pulse and the zero current. The I_{Kr} tail current was as measured the differ-

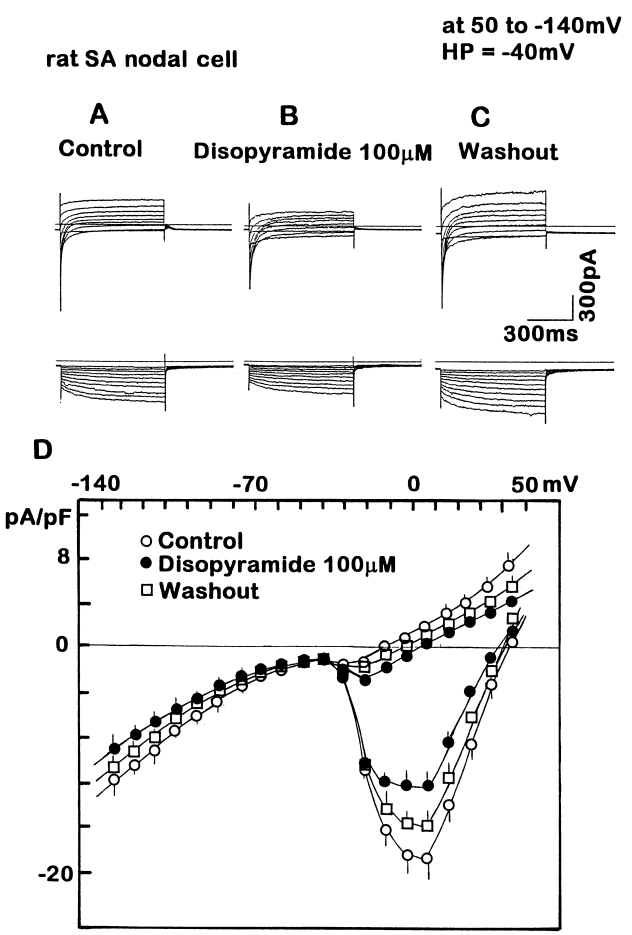

Fig. 3. Changes in the ion currents during exposure to disopyramide and washout. (A) Control. (B) Disopyramide (100 μM). (C) Washout (10 min later). Horizontal line indicates zero current level. (D) Voltage dependence of the ion currents in the absence and presence of disopyramide. Symbols used are control (○), in 100 μM (●) and washout (□). The values are presented as means ± S.E.M.

ence between the peak current using depolarizing test pulses of 200-ms duration and the zero current. The tail current of I_{Ks} was similarly measured by application of a test pulse of 1–2 s duration in the presence of 5 μM E-4031.

Experiments were carried out at 36°C. The data were stored and analyzed on an IBM-AT microcomputer, using the PCLAMP analysis program (Axon Instruments). Current traces were filtered using a cut-off frequency of 2 kHz for plotting. All the values are given as means ± S.E.M. The differences between the mean values were analyzed by analysis of variance (ANOVA) and Student's *t*-test for paired data, and a *P*-value less than 0.05 was considered significant.

2.3. Experimental solutions

The composition of the modified Tyrode solution was (in mM): NaCl 137, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 1.0, NaH_2PO_4 0.3, glucose 5.0, and HEPES [*N*-(2-hydroxyethyl)piperazine-*N*′-2-ethansulfonic acid] (Wako) 5.0. The pH was adjusted to 7.4 with NaOH. The pipette solution (intracellular) contained (in mM): K-aspartate 110, KCl 20, $MgCl_2$ 2, EGTA 5, Mg-ATP 5, creatine phosphate 5, and HEPES 5 (pH 7.2).

The drugs used are cibenzoline (Fujisawa Pharmaceutical, Osaka), and disopyramide (Sigma, St. Louis, MO, USA), which were dissolved to the desired concentrations directly in the bath solution. Also, E-4031, 1-[2-(6-methyl-2-pyridyl)ethyl]-4-(4-methylsulfonylaminobenzoyl)piperidine, (Eizai Pharmaceutical, Tokyo, Japan) and 293B, *trans*-6-cyano-4-(*N*-ethylsulphonyl-*N*-methtamino)-3-hydroxy-2,2-dimethyl-chromane (Hoechst Pharmaceutical, Germany) were used.

3. Results

3.1. Effects on spontaneous action potentials

Cibenzoline at 10 μM caused a significant negative chronotropic effect, as shown in Fig. 1A. The action potential amplitude and the maximum diastolic potential decreased. The effects on the action potential parameters are summarized in Table 1. The responses were concentration dependent and almost reached steady state approximately 3–5 min after application. In this cell, sinus arrest

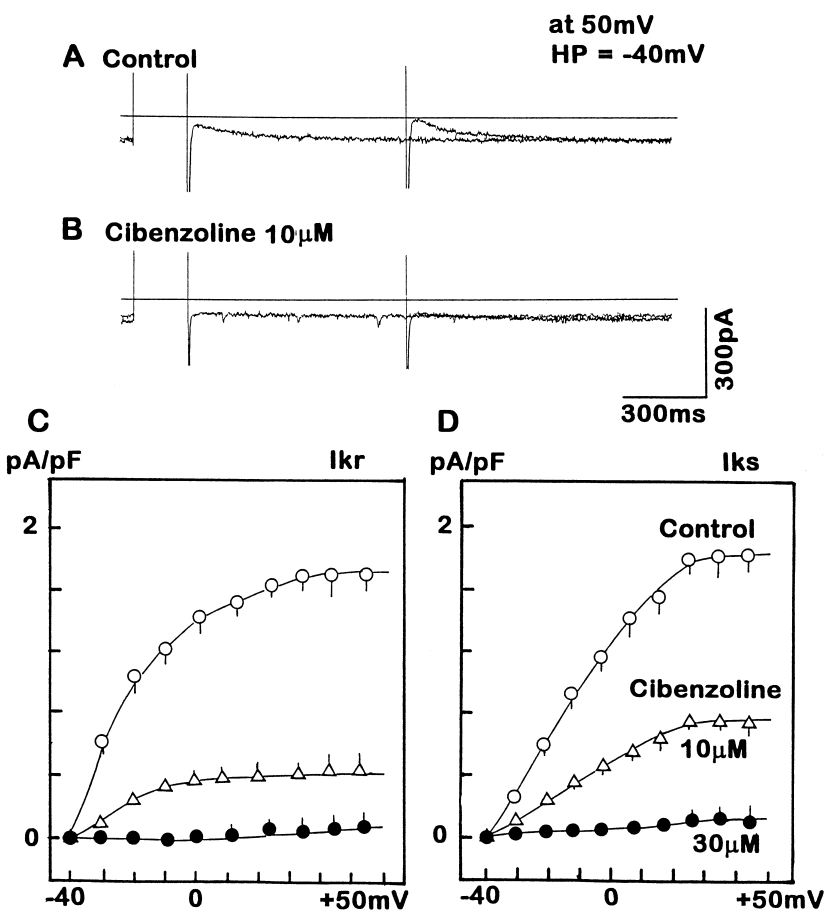

Fig. 4. Modulation of the outward tail current by cibenzoline in a spontaneously beating rabbit sino-atrial nodal cell. (A–B) Current traces in control and in the presence of 10 μM cibenzoline. The traces corresponding to 200 ms pulse duration and to 1-s pulse duration (50 mV test pulse from a holding potential of −40 mV) are superimposed. Horizontal line indicates zero current level. (C) Effects on I_{Kr}. (D) Effects on I_{Ks}. Symbols used are control (○), 10 μM cibenzoline (△) and 30 μM cibenzoline (●).

occurred approximately 5 min later. The resting potential was -38.6 ± 1.1 mV ($n = 7$). Cibenzoline elicited sinus arrest in 3 out of 11 cells at 30 μM and in 7 out of 7 cells at 100 μM. The incidence of sinus arrest is presented in Table 2. Disopyramide 30 μM significantly depressed the spontaneous action potentials and then elicited sinus arrest (Fig. 1B). The resting potential was -37.9 ± 1.2 mV ($n = 5$). Disopyramide similarly elicited sinus arrest, but the incidence was relatively lower. Both drugs induced concentration-dependent depression of spontaneous activity, accompanied by prolongation of action potential duration. At 10 to 15 min after washout of the drugs, the regular rhythm recovered to approximately 70–80% of the control level.

3.2. Effects on ionic currents

Whole-cell voltage-clamp experiments were performed using isolated single rat SA nodal cells (Figs. 2 and 3). The L-type Ca^{2+} current (I_{Ca}) and the hyperpolarization-activated inward current (I_f) were inhibited by approximately $69.7 \pm 3.2\%$ ($n = 7$, $P < 0.001$) and $45.8 \pm 3.0\%$ ($n = 7$, $P < 0.01$) at 30 μM cibenzoline (Fig. 2D), and by $51.2 \pm 3.3\%$ ($n = 6$, $P < 0.01$) and $48.3 \pm 2.7\%$ ($n = 6$, $P < 0.01$) at 100 μM disopyramide, respectively (Fig. 3D). The responses were almost reversible and recovered to 70–80% of control levels after a 15- to 20-min washout (Figs. 2C–D and 3C–D).

The delayed rectifier K^+ current, which is composed of rapidly and slowly activated currents (I_{Kr} and I_{Ks}), also decreased. Cibenzoline 10 μM blocked the tail current of I_{Kr} and simultaneously that of I_{Ks} (Fig. 4A and B). The inhibition of the tail current of I_{Kr} is summarized in Fig. 4C. Cibenzoline inhibited the I_{Kr} tail current by $60.2 \pm 3.7\%$ ($n = 6$, $P < 0.01$) at 10 μM and by $93.8 \pm 2.6\%$ ($n = 6$, $P < 0.001$) at 30 μM. In the presence of 5 μM E-4031, cibenzoline inhibited the I_{Ks} tail current by $24.3 \pm 3.4\%$ ($n = 6$, $P < 0.05$) at 10 μM and by $85.6 \pm 2.3\%$ ($n = 6$, $P < 0.001$) at 30 μM. The I_{Ks} tail current was completely blocked by 30 μM 293B. Disopyramide at 30

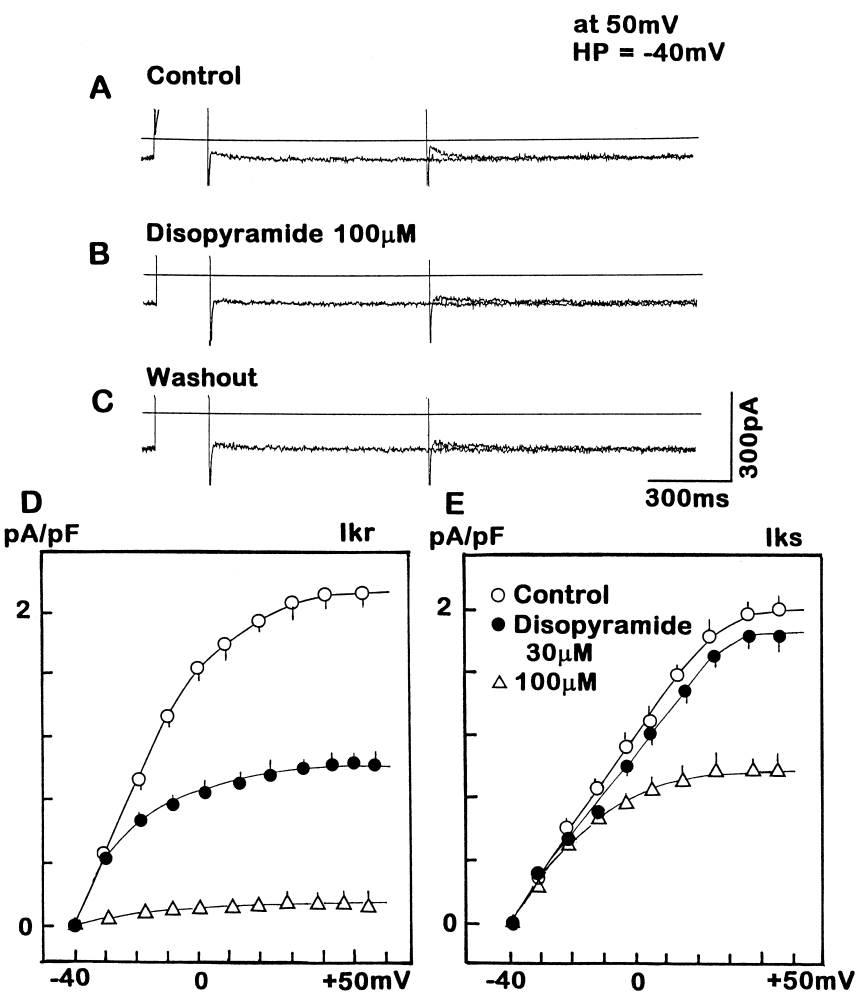

Fig. 5. Modulation of the outward tail current by disopyramide in a spontaneously beating rabbit sino-atrial nodal cell. (A–C) Current traces in control, in 100 μM disopyramide and in washout. The traces corresponding to 200-ms pulse duration and to 1-s pulse durations (50 mV test pulse from a holding potential of −40 mV) are superimposed. Horizontal line indicates zero current level. (D) Effects on I_{Kr}. (E) Effects on I_{Ks}. Symbols used are control (○), 30 μM of (●) and 100 μM of disopyramide (△).

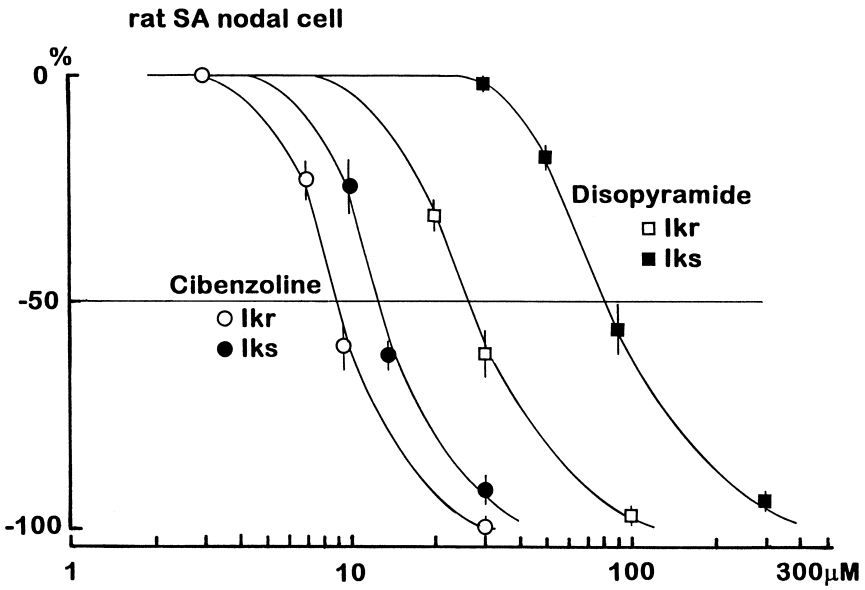

Fig. 6. Dose–response curves for the effects on the I_{Kr} and I_{Ks} in rat sino-atrial nodal cells. Cibenzoline and disopyramide caused a concentration-dependent inihibition. Symbols are the I_{Kr} (○) and I_{Ks} (●) in the presence of cibenzoline, and the I_{Kr} (□) and I_{Ks} (■) in the presence of disopyramide.

and 100 μM inhibited the I_{Kr} tail current by 65.0 ± 3.3% ($n = 6$, $P < 0.01$) and by 97.0 ± 2.1% ($n = 6$, $P < 0.001$), respectively (Fig. 5). In the presence of 5 μM E-4031, disopyramide at 30 and 100 μM inhibited the tail current of I_{Ks} by 1.7 ± 0.5% ($n = 6$, $P > 0.05$) and by 61.6 ± 3.5% ($n = 6$, $P < 0.01$), respectively. After a 15-min washout of both drugs, the responses recovered to almost 60% to 80% of control values.

As shown in Fig. 6, the concentration–response curve for I_{Kr} and I_{Ks} currents was fitted with a sigmoidal curve. The IC_{50} values for I_{Kr} of cibenzoline and disopyramide were 8.8 ± 1.1 and 25.1 ± 2.3 μM, respectively. In the presence of 5 μM E-4031, the IC_{50} values for I_{Ks} of cibenzoline and disopyramide were 12.3 ± 1.8 and 81.1 ± 2.3 μM, respectively.

4. Discussion

Cibenzoline (sensitive to I_{Kr} and I_{Ks}) and disopyramide (sensitive to just I_{Kr}) as class Ia antiarrhythmic drugs, possess characteristic of slow and intermediate kinetics for I_{Na} channels, respectively, and are multichannel inhibitors. In rat sino-atrial nodal cells, the inhibitory potency was $I_{Kr} > I_{Ks} > I_{Ca} > I_f$. The inhibitory potency reported previously was as follows: $I_{Na} > I_{Ca} > I_{Krec}$ (I_{Kr} and I_{Ks}) $> I_f$ (Koumi et al., 1992; Sato et al., 1994; Nakajima et al., 1998; Hiraoka et al., 1989; Wang et al., 1996; Virág et al., 1998). The order is almost consistent, but the small discrepancy might result from the differences in species and tissues.

The IC_{50} value for I_{Na} was 7.8 μM for cibenzoline and 32.0 μM for disopyramide. The ratio of the IC_{50} values of disopyramide and cibenzoline was approximately 4- to 5-fold. From previous reports, however, the ratio of sensitivity for different currents of both drugs is not always consistent (Koumi et al., 1992; Sato et al., 1994; Nakajima et al., 1998). The IC_{50} value for I_{Ca} is 14.4 μM for cibenzoline (Sato et al., 1994) and 11 μM for disopyramide (Hiraoka et al., 1989). The ratio of the IC_{50} values of disopyramide and cibenzoline was 0.76. In the present experiments, the values were 15 μM for cibenzoline and 100 μM for disopyramide. The ratio was 6.67. The effects of the drugs on the I_f current of isolated single sino-atrial nodal cells have not yet been shown. The IC_{50} values were 30 μM for cibenzoline and 100 μM for disopyramide. The ratio of disopyramide and cibenzoline was 3.33.

The prominent component of the delayed rectifier K^+ current is the rapid type (I_{kr}, HERG, human ether-a-go-go related gene, -type) in rabbit sino-atrial nodal cells (Ono and Ito, 1995; Verheijck et al., 1995) and the slow type (I_{ks}, KvLQT, voltage-gated potassium channel gene with a strong candidate for long QT syndrome, -type) in guinea pig sino-atrial nodal cells (Anumonwo et al., 1992; Guo et al., 1997). The IC_{50} values of I_{Kr} and I_{Ks} for cibenzoline and disopyramide are also quite different. The IC_{50} value for I_{Kr} is 30 μM for cibenzoline and 1.8 μM for disopyramide (Wang et al., 1996; Virág et al., 1998). In the present experiments, however, the ratio of the IC_{50} values of disopyramide and cibenzoline was approximately 2-fold for I_{Kr} and 4-fold for I_{Ks}. The data for each channel in different experiments are independent, and may not necessarily permit comparison of the effective concentrations. The results of this study indicate that cibenzoline possesses a much higher sensitivities to cardiac ionic currents than disopyramide, consistent with the sensitivity to I_{Na}. Furthermore, in our laboratory, the effective concentrations for ionic currents were lower in rat sino-atrial nodal cells than in guinea pig ventricular cardiomyocytes (unpublished data).

4.1. Pacemaker activity

Both cibenzoline and disopyramide inhibited all the currents (I_{Ca}, I_{Krec} and I_f). Pacemaker activity is regulated by the rate of the slow diastolic potential (phase 4 depolarization) of sino-atrial nodal action potentials. The heart rate varies considerably among different species. The pacemaker potential is not regulated by only one current, but by the modulation of all the currents (Satoh, 1991, 1993). In general, the negative chronotropic effect is mainly considered to be due to (1) I_{Ca} inhibition, (2) decrease in I_K conductance, and (3) I_f inhibition (Noble, 1984). Recently, Noma's group has shown that the pacemaker mechanism also involves the rapidly activated delayed K^+ current (I_{Kr}) and the sustained inward current (I_{st}) (Guo et al., 1995, 1997; Shinagawa et al., 2000). Therefore, the modulation of I_{Kr} is a chronotropic mechanism in rat sino-atrial nodal cells. The effective concentrations of both drugs for I_{Kr} were quite different. Since I_{Kr} is a major pacemaker current, the inhibition of I_{Kr} would cause a depressant effect on pacemaker activity similar to that on the other pacemaker currents (Verheijck et al., 1995).

Both drugs often caused sinus arrest (or dysrhythmias). Increasing the concentration increased the incidence of sinus arrest. Both drugs inhibited the I_{Ca} as well as the I_{Kr}, which can result in a potent negative chronotropic effect. Simultaneously, the drugs inhibited the I_f current. In rat sino-atrial nodal cells, however, the I_f current makes a minor contribution to pacemaker activity (Shinagawa et al., 2000), because regular spontaneous action potentials are generated in spite of the disappearance of the I_f current. The spontaneous action potentials in rat sino-atrial nodal cells were also less sensitive to CsCl (a selective I_f inhibitor). Therefore, the occurrence of sinus arrest is due to the potent inhibition of currents such as I_{Ca} and I_{Kr}.

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