Effects of Dofetilide on Membrane Currents in Sinoatrial Node Cells of Rabbit

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ABSTRACT—The effects of dofetilide (UK 68798) on membrane currents were examined in isolated sinoatrial node cells of rabbits by using patch clamping. At a concentration of 1 μM, dofetilide decreased the delayed rectifier K^+ current (I_K) (50.2±10.2%, mean±S.E.). The Ca^{2+} current was slightly decreased during the application of dofetilide. However, the decrease in the current may be attributed to the "run down" phenomenon. The drug did not affect the hyperpolarization-activated inward current. Therefore, dofetilide exhibited class III antiarrhythmic activity in rabbit sinoatrial node cells. Similarly, E-4031 (1-[2-(6-methyl-2-pyridyl)ethyl]-4-(4-methylsulfonylaminobenzoyl)pireridine) (1 μM), a standard class III agent, also showed specific inhibition of I_K. Furthermore, dofetilide depolarized the maximum diastolic potentials, reduced the slope of the pacemaker potential and then abolished spontaneously firing action potentials in the nodal cells. The results demonstrate that dofetilide may produce negative chronotropic effects as a result of its class III activity.

Keywords: Dofetilide, E-4031, Ion channel, Delayed rectifier K^+ current, Sinoatrial node cell

Class III antiarrhythmic agents are defined as those that prolong the duration of an action potential, without affecting its maximal velocity of upstroke (1), resulting in a prolongation of the effective refractory period. It is also reported that class III agents exhibit negative chronotropic activity in experimental situations (2-4), but the mechanism behind this effect has not been fully investigated.

Dofetilide (UK 68798) is a newly developed derivative of the class III antiarrhythmic agent *d*-sotalol (5), which has been reported to prolong the action potential duration and effective refractory period in canine ventricular muscle and Purkinje fibers, without affecting the maximum upstroke velocity (5). In voltage clamp studies, dofetilide has been shown to reduce the rapid component of the delayed rectifier K^+ current (I_{Kr}), without affecting the Ca^{2+} current and inward rectifier K^+ current in guinea pig ventricular myocytes (5, 6). Therefore, the class III action of dofetilide may be attributed to a highly selective blockade of I_{Kr}.

The I_K plays a pivotal role in the pacemaker mechanism of the sinoatrial node (7). The repolarization and pacemaker phases of an action potential are produced by activation and deactivation of I_K, respectively, and it is therefore likely that the negative chronotropic activity of dofetilide is attributable to blockade of this current. However, there are no published reports that assess the effect of dofetilide on the membrane currents of sinoatrial node cells. Consequently, the effects of dofetilide on the membrane current of isolated rabbit sinoatrial node cells was assessed and compared with effects produced by E-4031, another agent reputed to selectively block I_{Kr} (8).

MATERIALS AND METHODS

Isolation of single cells from the sinoatrial node region was carried out according to the method described by Hagiwara et al. (9). Heparinized (300 units/kg) New Zealand White rabbits (1.5-2.0 kg; Ichikawaya, Tokyo) were sacrificed by intravenous injection of pentobarbital (40 mg/kg). The heart was isolated and immersed in normal Tyrode solution (Table 1), and the sinoatrial node region was excised and transferred into the Ca^{2+}-free Tyrode solution. The specimen ceased spontaneous contraction within 20 min and was then incubated in 2.5 ml Ca^{2+}-free Tyrode solution containing 0.4% collagenase (500 U/mg; Yakuruto, Tokyo) for 140 min at 37℃. The

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Table 1. Composition of solutions used in this study (mM)

	Tyrode	KB	Pipette
NaCl	143	—	—
KCl	5.4	40	20
KOH	—	70	100
$CaCl_2$	1.8	—	—
$MgCl_2$	0.5	3	1
NaH_2PO_4	0.33	—	—
KH_2PO_4	—	20	—
l-Glutamate	—	50	—
Aspartate	—	—	100
Taurine	—	20	—
ATP-K_2	—	—	5
Creatine-Pi-K_2	—	—	5
Glucose	5.5	10	—
EGTA	—	1	10
HEPES	5	10	5
pH (titer)	7.4 (NaOH)	7.4 (KOH)	7.4 (KOH)

digested specimen was then transferred to Kraftbrühe (KB) solution (Table 1) and stored at 4℃ for later use. Only single rod-shaped cells, which revealed a relatively smooth surface and contracted spontaneously in a regular rhythm, were used in this study.

The whole-cell patch-clamp mode (10) was applied to the isolated sinoatrial cells using glass patch pipettes, with a tip diameter of 3–4 μm and a resistance between 4–10 MΩ, containing internal solution (Table 1). The Ag-AgCl electrode in the patch pipette was connected with a patch clamp amplifier (EPC-7; List, Darmstadt, Germany). Signals were displayed on a storage oscilloscope (Tektronix 5113 OP03; Beaverton, OR, USA) and were simultaneously fed to a data recording system consisting of a video cassette recorder (NV-F1; National, Osaka) and a PCM converter system (PCM-501ES; Sony, Tokyo). The current and voltage signals were filtered at 1 kHz, digitized by an AD converter (ADX-98; Canoopus Electronics, Kobe) at 2 kHz and stored in a personal computer

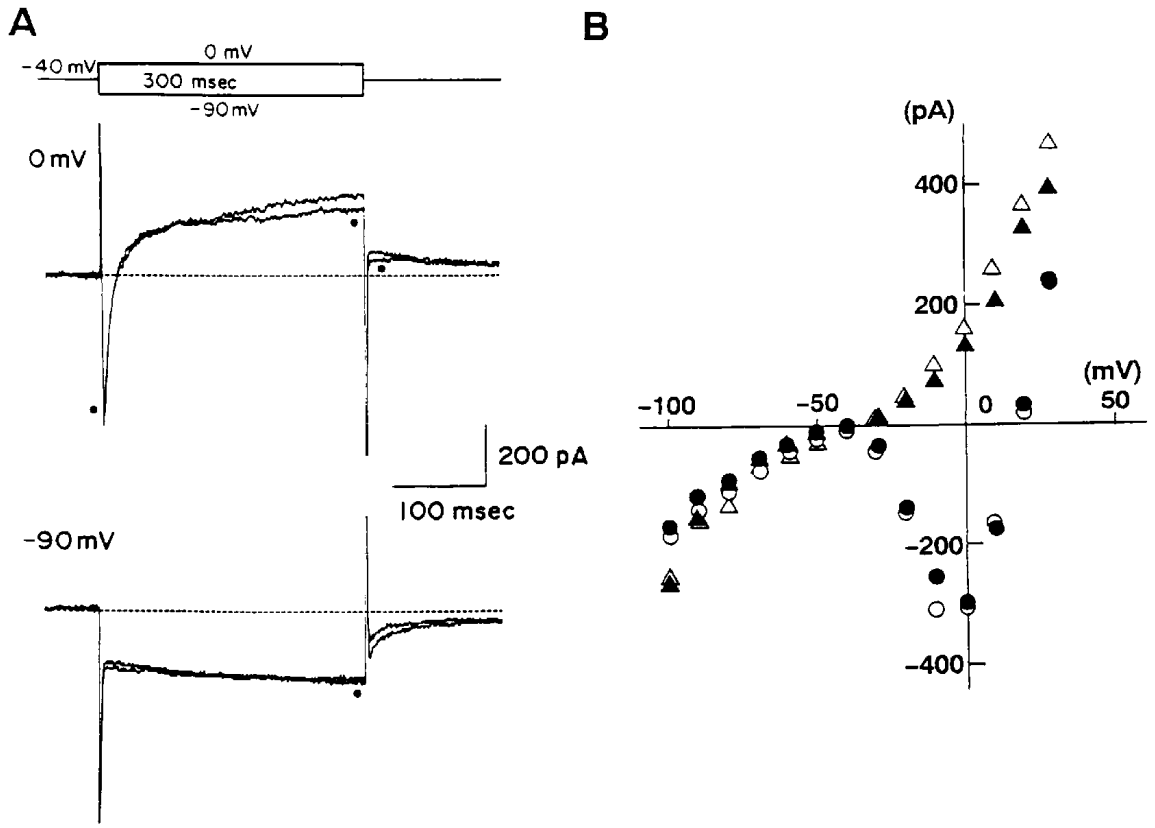

Fig. 1. Effects of 1 μM dofetilide on membrane currents in rabbit sinoatrial node cells. A: Superimposed traces in the absence and presence (●) of dofetilide. The current traces in the upper panel were elicited by a 300-msec depolarization pulse to 0 mV from a holding potential of −40 mV, while those in the lower panel were elicited by a hyperpolarizing pulse to −90 mV. The voltage protocol is shown in the upper panel. B: Effect of dofetilide on the current-voltage relationship of rabbit sinoatrial node cell. Circles (○, ●) indicate the current at an early period of the test pulse. Triangles (△, ▲) indicate the current at the end of the test pulses. Open (○, △) and closed (●, ▲) symbols indicate currents in the absence and presence of 1 μM dofetilide, respectively.

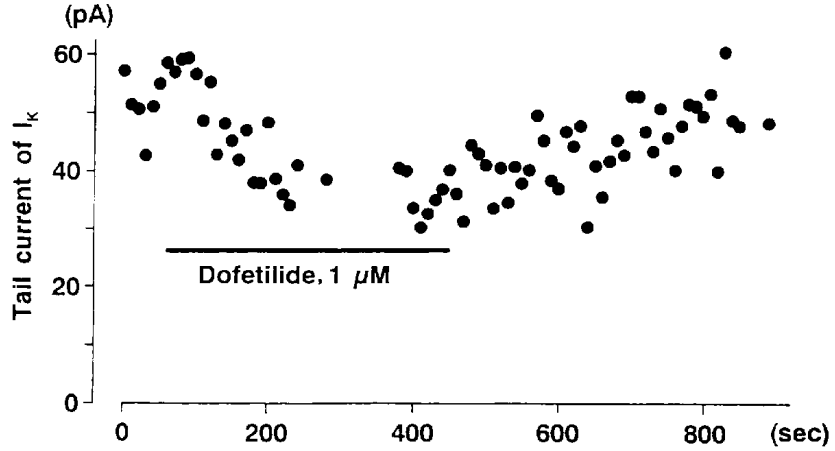

Fig. 2. Effect of dofetilide (1 μM) on the I_K tail. Dofetilide decreased the tail current elicited by the 300-msec depolarizing pulse to 0 mV at a rate of 0.1 Hz. The decrease reached a steady state in 2 min after the first exposure to the drug. The effect of dofetilide was abolished by washout.

(PC-98XA; NEC, Tokyo) for later analyses. All investigations were performed at 37±1℃. A holding potential of −30 or −40 mV was used, because the threshold of Ca^{2+} current was shifted from −30 to −40 mV in each cell.

Dofetilide (UK-68,798; donated by Pfizer Central Research, Sandwich, UK) was dissolved in 0.05 N HCl to produce a 10 mM stock solution, which was freshly prepared every day. The solvent had no effect on the membrane currents of the single cells. E-4031 (1-[2-(6-methyl-2-pyridyl)ethyl]-4-(4-methylsulfonylaminobenzoyl)pireridine) (donated by Eizai Pharmaceuticals, Tsukuba) was dissolved in distilled water to form a 10 mM stock solution.

All values are presented as means±S.E. Statistical analyses were performed with Student's paired *t*-test; $P<0.05$ was defined as significant.

RESULTS

Effects of dofetilide on membrane currents

Membrane currents of single nodal cells were elicited by 300-msec test pulses from a holding potential of −40 mV, delivered at a rate of 0.1 Hz. Figure 1 shows representative current recordings. A depolarizing test pulse to 0 mV elicited a rapid and transient inward Ca^{2+} current (I_{Ca}) and a subsequent time-dependent development of outward current (I_K, Fig. 1). Time-dependent decay of the outward current observed after repolarization to the holding potential was the result of deactivation of I_K (I_K tail). Extracellular application of dofetilide at a concentration of 1 μM decreased the I_K and I_K tail. This effect stabilized 4 min after initiation of drug treatment (Fig. 2). In five cells, 1 μM dofetilide reduced the I_K tail, elicited by a test pulse to 0 mV, by 50.2±10.2% (mean±S.E.). In all examined cells, the reduction in the I_K and I_K tail recovered within 10 min, following dofetilide washout (Fig. 2).

A decrease in I_{Ca} was observed in four cells exposed to 1 μM dofetilide, as shown in Fig. 1. However, the decrease

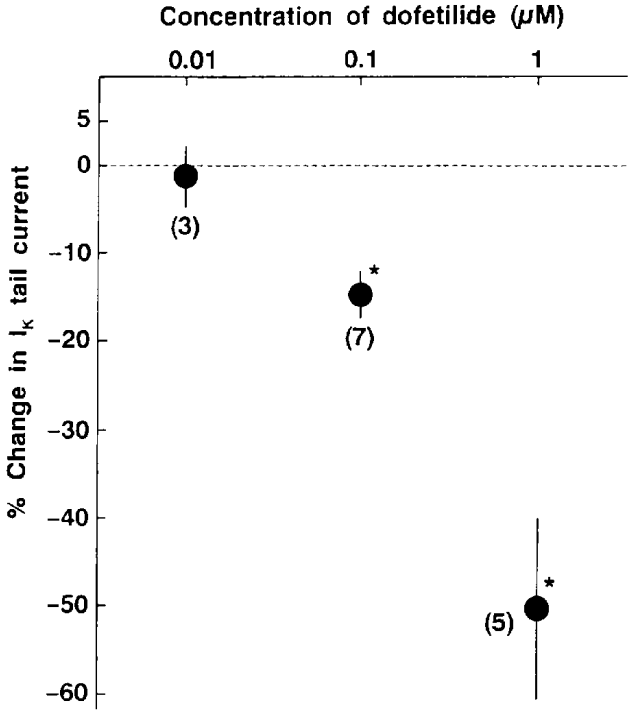

Fig. 3. Concentration-response relation between dofetilide and the decrease in tail current of I_K. Numbers in parentheses give the number of cells. Data are means±S.E. *Difference from the control is statistically significant at $P<0.05$.

in I_{Ca} did not recover after dofetilide washout. Furthermore, in another cell, I_{Ca} was unchanged by dofetilide treatment.

When a hyperpolarizing test pulse to −90 mV was applied to the cells, a time-dependent development of inward current was observed (Fig. 1). Exposure to 1 μM dofetilide slightly reduced this hyperpolarization-activated inward current (I_f) at −90 mV by 7.8±16.1% (n=5).

Figure 3 shows the concentration-response relation between dofetilide and the I_K tail. The I_K tail was elicited by a 300-msec depolarizing pulse to 0 mV. Dofetilide did not decrease the I_K tail at a concentration of 0.01 μM, but significantly decreased at 0.1 μM.

The effects of dofetilide treatment upon the voltage-dependent activation of I_K are shown in Fig. 4. Figure 4A shows the time course of the I_K tail elicited by depolarizing test pulses to −30, −20, −10, 0, 10, 20 and 30 mV from a holding potential of −40 mV. In this cell, I_{Ca} was abolished because of "run-down". Dofetilide (1 μM) decreased the I_K tail elicited by the test pulse to each potential. Figure 4B summarizes the effect of dofetilide on voltage-dependent activation of the I_K tail. It is apparent that the ability of dofetilide to block I_K is not voltage dependent.

Effects of E-4031 on membrane currents

Figure 5 shows effects of E-4031 (1 μM) on a current-voltage relationship obtained using test pulses from a holding potential of −30 mV for a duration of 300 msec. E-4031 decreased both I_K during the test pulse and the I_K tail after the test pulse (Fig. 5A). This decrease in I_K was restored by a washout of E-4031. In four cells, 1 μM E-4031 decreased the I_K tail by 35.4±9.1%. Conversely, E-4031 did not produce significant changes in I_f, as shown in Fig. 5 (A and B). The drug slightly decreased I_f by 8.5±19.6% (n.s., n=4). During this experiment, I_{Ca} also decreased.

Figure 6 shows effects of E-4031 on voltage-dependent activation of the I_K tail. E-4031 decreased the I_K tail elicited by every test potential, and therefore, the I_K blockade induced by E-4031 was not voltage-dependent.

Effects of dofetilide on spontaneous beating of sinoatrial node cells

The membrane potential of sinoatrial node cells was recorded using the current clamp version of patch clamp-

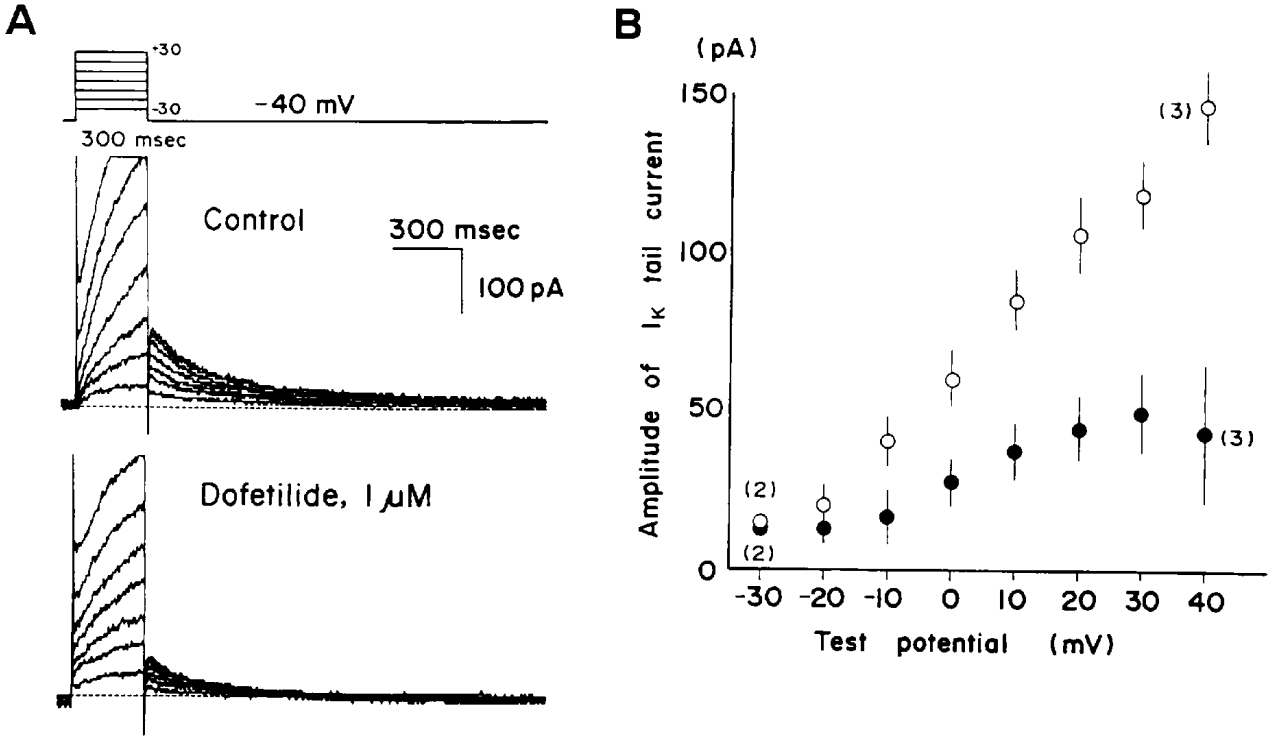

Fig. 4. Effect of dofetilide on the voltage-dependent activation of the I_K tail. A: Impact of 1 μM dofetilide on the time course of deactivation of I_K (I_K tail) elicited by 300-msec depolarizing pulses. The voltage protocol is shown in the upper panel. In this cell, I_{Ca} was abolished because of "run-down". B: Dofetilide (1 μM) decreased the I_K tail elicited by the depolarizing pulse to each potential in a range from −40 to +40 mV. Symbols (○, ●) indicate mean values of 5 cells except for the data at −30 and 40 mV, which are with the cell number in parentheses. Vertical bars represent S.E. of the mean. ○, control; ●, 1 μM dofetilide.

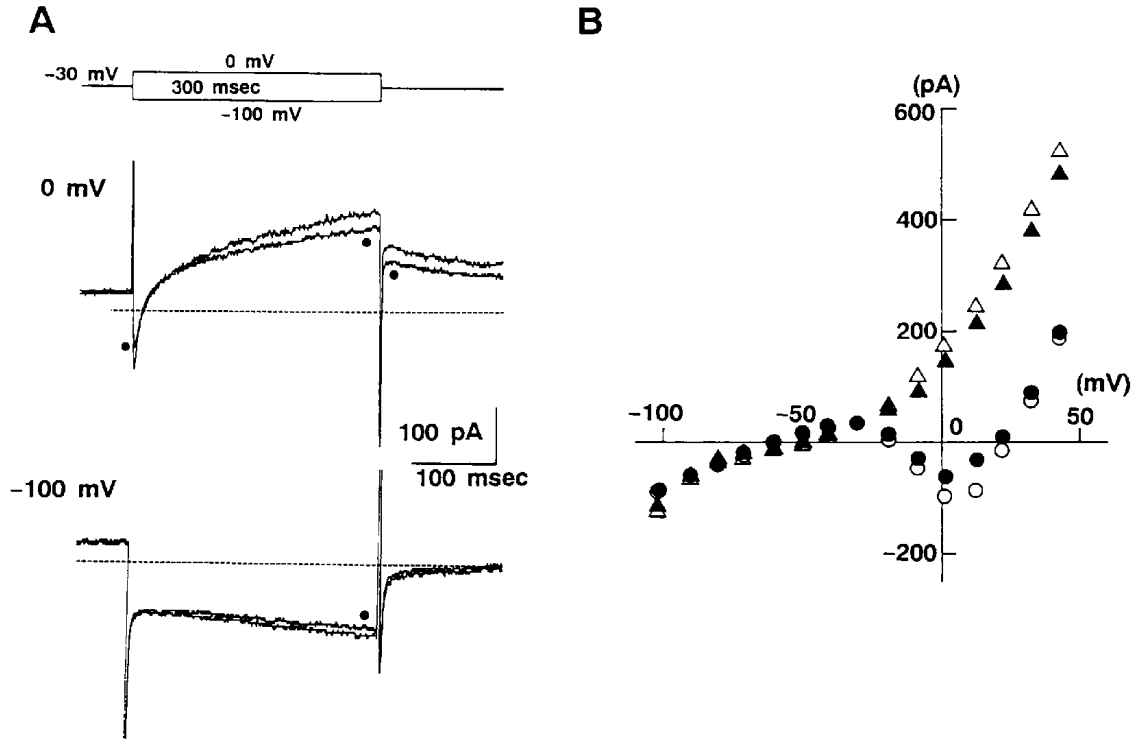

Fig. 5. Effects of 1 μM E-4031 on membrane currents in rabbit sinoatrial node cells. A: Superimposed traces in the absence and presence (●) of E-4031. The current traces in the upper panel were elicited by a 300-msec depolarizing pulse to 0 mV from a holding potential of −30 mV, while those in the lower panel were elicited by a hyperpolarizing pulse to −100 mV. The voltage protocol is shown in the upper panel. B: Effect of E-4031 on the current-voltage relationship of rabbit sinoatrial node cell. Circles (○, ●) indicate the current at an early period of the test pulse. Triangles (△, ▲) indicate the current at the end of the test pulses. Open (○, △) and closed (●, ▲) symbols indicate currents in the absence and presence of 1 μM E-4031, respectively.

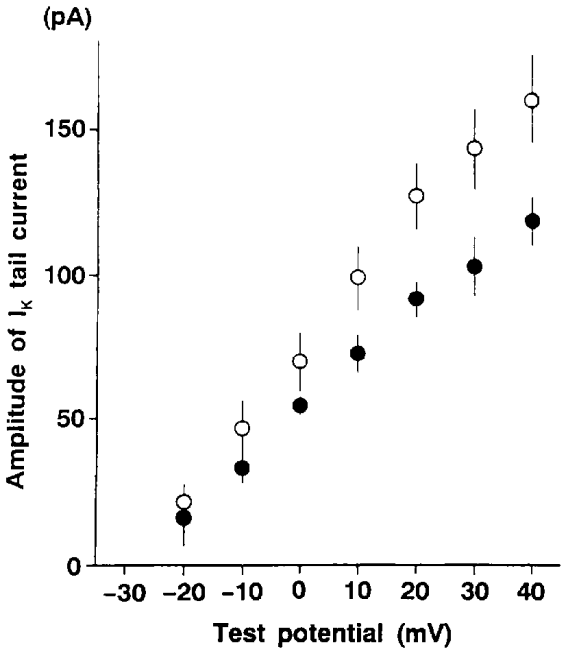

Fig. 6. Effect of E-4031 on the voltage-dependent activation of I_K. Each point is representative of the mean values of three cells. Vertical bars represent S.E. of the mean. ○, control; ●, 1 μM E-4031.

ing. These cells exhibited a spontaneous excitation and action potential configuration accompanied by delayed diastolic potential (pacemaker potential), as shown in Fig. 7A. Dofetilide (1 μM) reduced the firing rate of the nodal cells (Fig. 7B) and halted spontaneous firing beyond 2 min after the start of drug perfusion. The drug depolarized the maximum diastolic potential (MDP) and reduced the slope of pacemaker potential. A similar change was observed in two further cells. The MDP was -43.8 ± 3.2 mV and -32.5 ± 0.7 mV, in the absence and presence of dofetilide, respectively. These findings indicate that dofetilide was associated with a strong negative chronotropic effect in isolated sinoatrial nodal cells.

DISCUSSION

In sinoatrial node cells, three major time- and voltage-dependent currents (I_{Ca}, I_K and I_f) have been described (7). The study reported here demonstrates that dofetilide selectively blocks I_K and therefore performs as a pure class III agent in rabbit sinoatrial nodal cells. Although there was some indication that dofetilide might also in-

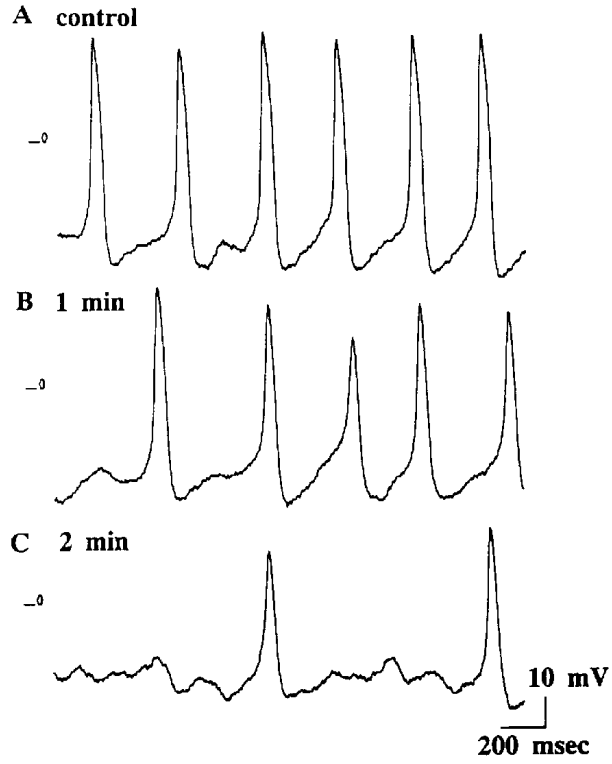

Fig. 7. Effect of 1 μM dofetilide on spontaneous firing action potentials of rabbit sinoatrial node cells. Dofetilide abolished spontaneous firing within 2 min of the start of its application.

duce blockade of I_{Ca}, the lack of reversibility of this phenomenon on washout suggested that this was attributable to "run-down", a phenomenon well-recognized to occur in isolated cells during patch clamping. Therefore, in agreement with data generated in guinea pig ventricular cells by Gwilt et al. (5) and supported by Siara et al. (11), it was concluded that dofetilide does not block I_{Ca} in nodal cells.

Interestingly, I_K in rabbit nodal cells seemed to be less sensitive to dofetilide than that in other cardiac cellular preparations. In this study, 0.01 μM dofetilide did not decrease the nodal I_K, and 1 μM dofetilide caused a 50% decrease in the nodal I_K. On the other hand, it has been reported that 30 nM and 2 μM dofetilide abolished this current in rabbit (6) and guinea pig ventricular cells (5), respectively. It is possible that I_K channels in sinoatrial node cells are structurally and/or functionally different from those in ventricular cells. Therefore, the apparent difference in sensitivity between atrial and ventricular preparations may indicate that dofetilide will suppress ventricular arrhythmias at lower concentrations and therefore with less effect upon heart rate.

The negative chronotropic effect of dofetilide (2–4) has been attributed to the I_K blocking action of this agent since, under circumstances where I_K is decreased, action potential duration is prolonged and the slope of the pacemaker potential is less steep. Recently, it has been reported that E-4031 inhibits I_K and then slows the spontaneous excitation in rabbit sinoatrial nodal cells (12). In the present study, it has also been demonstrated that 1 μM E-4031 also blocks the I_K of sinoatrial node cells, reducing nodal I_K more weakly than 1 μM dofetilide. Therefore, it seems likely that all class III agents that block I_K will potentially also be associated with a negative chronotropic effect.

Dofetilide has been previously reported to induce a negative chronotropic effect on both rabbit sinoatrial node preparations (4) and guinea pig right atria (3), with a maximal response in the latter preparation (20%) being observed with 1 μM dofetilide. In this study, however, it was observed that exposure to this concentration of dofetilide resulted in the arrest of spontaneous firing of rabbit sinoatrial node cells. The discrepancy between this and the previous findings may have been the result of differences in cell preparations, with the previous data being generated in a multicellular system, while the current study was conducted in single cell preparations. It might, equally, have been the result of the I_{Ca} "run-down" phenomenon, which could contribute to an exaggeration of the negative chronotropic effects associated with exposure to dofetilide. It is likely that the weak recovery of spontaneous firing was related to I_{Ca} "run-down". Furthermore, the failure to restore spontaneous firing in all but one of three experiments, in which only partial recovery was observed, does not indicate that dofetilide exerts a deleterious effect upon these cells, since washout resulted in recovery of the I_K and the I_K tail in all cases.

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REFERENCES

1 Vaughan Williams EM: A classification of antiarrhythmic action reassessed after a decade of new drugs. J Clin Pharmacol **24**, 129–147 (1984)

2 Gwilt M, Blackburn KJ, Burges RA, Higgins AJ, Milne AA and Solca AM: Electropharmacology of dofetilide, a new class III agent, in anaesthetized dogs. Eur J Pharmacol **215**, 137–144 (1992)

3 Yang T, Tande PM and Refsum H: Negative chronotropic effect of a novel class III antiarrhythmic drug, UK-68,798, devoid of β-blocking action on isolated guinea pig atria. Br J Pharmacol **103**, 1417–1420 (1991)

4 Montero M, Beyer T, Brachmann J and Kubler W: Electrophysiological effects of the new class III antiarrhythmic agent UK-68,798 in isolated rabbit sinus node and atrium. Z Kardiol **80**, Supp 3, 55 (1991)

5 Gwilt M, Arrowamith JE, Blackburn KJ, Burges RA, Cross

PE, Dalrymple HW and Higgins AJ: UK-68,798: a novel, potent and highly selective class III antiarrhythmic agent which blocks potassium channels in cardiac cells. J Pharmacol Exp Ther **256**, 318–324 (1991)

6 Carmeliet E: Voltage- and time-dependent block of the delayed K^+ current in cardiac myocytes by dofetilide. J Pharmacol Exp Ther **262**, 809–817 (1992)

7 Irisawa H, Brown HF and Giles W: Cardiac pacemaking in the sinoatrial node. Physiol Rev **73**, 197–227 (1993)

8 Sanguinetti MC and Jurkiewicz NK: Two components of cardiac delayed rectifier K^+ current: differential sensitivity to block by class III antiarrhythmic agents. J Gen Physiol **96**, 195–215 (1990)

9 Hagiwara N, Irisawa H and Kameyama M: Contribution of two types of calcium currents to the pacemaker potentials of rabbit sino-atrial node cells. J Physiol (Lond) **395**, 233–253 (1988)

10 Hamill OP, Marty A, Neher E, Sakmann B and Sigworth FJ: Improved patch clamp techniques for high-resolution current recording from cells and cell-free membrane patches. Pflugers Arch **391**, 85–100 (1981)

11 Siara J, Villena P, Beyer T, Kuebler W and Bachmann J: Selective effect of the class III agent, dofetilide, in potassium currents in guinea-pig cardiomyocytes. J Am Coll Cardiol **23**, 283A (Abstract) (1994)

12 Verheijck EE, van Ginneken ACG, Bourier J and Bouman LN: Effects of delayed rectifier current blockade by E-4031 on impulse generation in single sinoatrial nodal myocytes of the rabbit. Circ Res **76**, 607–615 (1995)