# Inhibition of Delayed Rectifier $K^+$ Current by Dofetilide and E-4031 Differentially Affects Electrical Cardiac Responses to Vagus Stimulation in Anesthetized Dogs

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ABSTRACT—Vagal activation influences various cardiac functions as well as occurrence of arrhythmias. Inhibition of a rapid type of delayed rectifier $K^+$ current ($I_{Kr}$) has been reported to be effective for the treatment of both ventricular and supraventricular arrhythmias. However, it is unknown how $I_{Kr}$ inhibition modulates the cardiac responses to vagal activation in situ. We analyzed the effects of $I_{Kr}$ inhibitors, dofetilide and E-4031, and a class I antiarrhythmic agent, disopyramide, on electrical cardiac responses to vagus stimulation in anesthetized dogs. Dofetilide (0.003-0.3 μmol/kg, i.v.), E-4031 (0.01-1 μmol/kg, i.v.) and disopyramide (2.9-29 μmol/kg, i.v.) prolonged sinus cycle length (SCL), right atrial effective refractory period (AERP) and ventricular effective refractory period (VERP) dose-dependently. During cervical vagus stimulation-induced prolongation of SCL, atrio-His (AH) interval and VERP and shortening of AERP, dofetilide and E-4031 inhibited the prolongation of SCL but potentiated the shortening of AERP. Dofetilide and E-4031 did not affect prolongations of AH interval and VERP. On the other hand, disopyramide inhibited all electrical cardiac responses to vagus stimulation. These results suggest that $I_{Kr}$ inhibition differentially modulate cardiac responses to vagus activation probably due to a different role of $I_{Kr}$ in each cardiac function in the heart in situ.

*Keywords*: Dofetilide, E-4031, Disopyramide, Parasympathetic nervous system, Refractory period

In recent years, much attention has been focused on agents that increase myocardial refractoriness for the treatment of both ventricular and supraventricular arrhythmias. These agents prevent arrhythmias due to reentry by prolonging myocardial action potential duration (1). Dofetilide (2, 3) and E-4031 (4, 5) are new agents that prolong the myocardial refractoriness by selectively blocking the rapidly activating component of delayed rectifier $K^+$ current ($I_{Kr}$) (6-8).

Vagal activity influences various cardiac functions such as heart rate, atrioventricular conduction and myocardial contractility (9). Thus, it is important to understand the interaction between vagal activity and $I_{Kr}$ inhibitors in the heart. In a previous study (10), we showed that E-4031 selectively inhibited the negative chronotropic response to parasympathetic nerve stimulation but had no influence on the negative dromotropic and atrial negative inotropic responses in autonomically decentralized anesthetized dogs. This suggested that E-4031 selectively inhibited the negative chronotropic response to parasympathetic stimulation at a site distal to the muscarinic receptor.

However, it is still unknown whether other $I_{Kr}$ inhibitors can also selectively inhibit the negative chronotropic response to vagus stimulation. Furthermore, it is unknown how E-4031 and other $I_{Kr}$ inhibitors affect the changes in atrial and ventricular refractoriness in response to vagus stimulation. Vagal activity influences the occurrence of both ventricular and supraventricular arrhythmias. Vagal activation shortens the atrial refractory period and sometimes worsens supraventricular arrhythmias such as atrial flutter and fibrillation (11). On the other hand, vagal activation has been reported to reduce the incidence of ventricular tachycardia and fibrillation (12). Vagal activation may influence the ability of $I_{Kr}$ inhibitors to suppress both ventricular and supraventricular arrhythmias under various autonomic activities. Thus, it is important to clarify the influences of $I_{Kr}$ inhibitors on vagally-induced changes in atrial and ventricu-

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lar refractoriness as well as other cardiac responses.

We, therefore, studied the effects of dofetilide and E-4031 on the electrical cardiac responses to vagus stimulation and compared them with the electrical cardiac effects of disopyramide, which has an antimuscarinic action (13–15).

## MATERIALS AND METHODS

### *Preparations*

Twenty-three mongrel dogs of either sex weighing 8–19 kg were used in the present study. Each dog was anesthetized with pentobarbital sodium (30 mg/kg, i.v.). A tracheal cannula was inserted, and intermittent positive pressure ventilation was started. The left femoral artery and vein were isolated and cannulated for measurement of systemic arterial blood pressure and for drug administration, respectively. The chest was opened transversely at the fifth intercostal space. Each cervical vagus nerve complex was crushed with a tight ligature, and each stellate ganglion was ligated tightly at its junction with the ansa subclavia. These maneuvers have been shown to remove almost all tonic neural activity to the heart (16).

Two bipolar epicardial electrodes were sutured on the right atrium, one on the right atrial appendage to record the electrical activity and the other on the right atrial free wall near the sinoatrial nodal area to pace the right atrial myocardium electrically. A third bipolar electrode was placed at the anterior surface of the right ventricle to electrically pace the right ventricular myocardium. A bipolar electrode catheter was inserted via the right femoral artery and positioned in the non-coronary cusp of the aortic valve to record the His bundle activity. The right atrial and His bundle electrograms, heart rate and arterial blood pressure were displayed on a thermo-writing rectigraph (RTA 1200; Nihon Kohden, Tokyo). The atrial and His electrograms were filtered with a band pass at 30–300 Hz.

Two bipolar wire electrodes were hooked in the cardiac end of each cervical vagus nerve complex to stimulate bilateral efferent vagus nerves. After setting all of the electrodes, the dogs were allowed to stabilize for at least one hour before starting electrophysiological testing.

### *Measurements*

*Sinus cycle length, atrioventricular conduction time and arterial blood pressure:* Sinus cycle length (SCL) was measured from the atrial electrogram. The atrio-His (AH) and His-ventricular (HV) intervals were measured from His bundle electrogram during atrial pacing at a 50 msec shorter cycle length than the control SCL using an electrical stimulator (SEN-7103, Nihon Kohden). All studies in an individual dog were performed at the same pacing cycle length. Pacing cycle length ranged from 400 to 650 msec.

*Refractory periods:* The effective refractory periods of the right atrium and right ventricle (AERP and VERP, respectively) were measured by the extrastimulus technique employing the programmable electrical stimulator. The right atrium or the right ventricle was paced at one and half times the late diastolic threshold voltages with a stimulus pulse duration of 1 msec. A train of 8 stimuli ($S_1$) at a constant cycle length of 350 msec was followed by a premature stimulus ($S_2$). The coupling interval ($S_1$-$S_2$) was shortened in steps of 10 msec until the premature impulse failed to produce a propagated response. Subsequently, the $S_1$-$S_2$ interval was shortened by 2-msec decrements from the prior propagated $S_1$-$S_2$ interval until S2 failed to produce a propagated response. The ERP were defined as the longest $S_1$-$S_2$ that failed to produce a propagated response.

### *Experimental protocol*

After stabilization of dogs, propranolol hydrochloride (1 mg/kg, i.v. plus an injection of 0.2 mg/kg/hr) was administered in order to block $\beta$-adrenoceptor-mediated responses. Before the treatment with each drug, the electrical cardiac responses to cervical vagus stimulation were evaluated twice at an interval of 30 min. After determination of the control electrical cardiac responses, bilateral vagus nerves were stimulated by a steady stimulation with 10 V pulse amplitude, 0.05 msec pulse duration and a frequency of 10–50 Hz using another electrical stimulator (SEN-7103, Nihon Kohden). Frequency of vagus stimulation was determined arbitrarily to produce a 25% decrease in AERP. After 2 min of vagus stimulation, we determined the electrical cardiac responses. If any of the variables were clearly different between two trials, a third trial was performed 30 min after in order to obtain two stable consecutive readings. The values obtained in the last trial were used as predrug values. Dogs in which atrioventricular block or sinus arrest was induced by vagus stimulation were excluded.

Test drugs evaluated in this study were dofetilide, E-4031 and disopyramide. Drugs were administered cumulatively with a 30-min interval between each administration. Ten minutes after treatment with a drug, we studied the effects of a drug on the electrical cardiac responses.

### *Drugs*

Compounds used in the present experiments were dofetilide (*N*-[4-(2-[2-[4-(methanesulphonamide)phenoxy]-*N*-methylethylamino]ethyl)phenyl]methanesulphonamide), E-4031 (*N*-[4-[[1-[2-(6-methyl-2-pyridinyl)ethyl]-4-piperidinyl]carbonyl]phenyl]methanesulfonamide dihy-

drochloride dihydrate), disopyramide and propranolol hydrochloride (Knoll A.G., Ludwigshafen, Germany). Dofetilide and E-4031 were generously donated by Pfizer (Sandwich, UK) and by Eisai (Tokyo), respectively. Compounds were dissolved in saline (E-4031, disopyramide and propranolol hydrochloride) or 0.1 N HCl (dofetilide).

*Statistical analyses*

The data were expressed as means±S.E.M. Doses that prolonged the ERP or SCL by 20% were determined as $ED_{20}$ for each dose-response curve. Fifty percent inhibition doses ($ID_{50}$) were also determined for each dose-response curve. The responses to vagus stimulation were expressed as percentage of the predrug response. Student's *t*-test was used for paired data. One-way analysis of variance (ANOVA) was used to test the difference among the predrug or prevehicle value of each treatment group. Direct effects of drugs or effects of drugs on the responses to vagus stimulation were tested by two-way ANOVA. Drug treatment and doses of a drug were considered as a fixed factor. In the presence of a significant F value for the treatment, results at each dose level were compared with the predrug value by one-way ANOVA for repeated measurements with Scheffe's test for multiple comparisons. P values less than 0.05 were considered statistically significant.

## RESULTS

*Direct cardiac effects of dofetilide, E-4031 and disopyramide*

Cardiac control values before treatment with drugs or vehicle are shown in Table 1. Values were not significantly different among each treatment group. AH and HV intervals, AERP and mean arterial pressure (MAP) were stable throughout the duration of the study in the vehicle-treated group. SCL and VERP increased slightly by 33±9 and 8±3 msec, respectively, 150 min after the beginning of the experiment in the vehicle-treated group ($P<0.05$). Direct effects of dofetilide, E-4031 and disopyramide on electrophysiological parameters before vagus stimulation are shown in Fig. 1. Dofetilide (0.003−0.3 μmol/kg, i.v.) and E-4031 (0.01−1 μmol/kg, i.v.) prolonged AERP and VERP dose-dependently (Fig. 1: A and B). The prolongation of the AERP by dofetilide and E-4031 were greater than those of the VERP. $ED_{20}$s of dofetilide for the AERP and the VERP were 0.015±0.006 and 0.035±0.011 μmol/kg, respectively. $ED_{20}$s of E-4031 for the AERP and the VERP were 0.032±0.005 and 0.066±0.009 μmol/kg, respectively. On the other hand, the increases in AERP and VERP induced by disopyramide (2.9−29 μmol/kg, i.v.) were smaller than those by dofetilide and E-4031 (Fig. 1C).

All drugs increased the SCL dose-dependently. Dofetilide did not affect the AH interval significantly (Fig. 1A). In contrast, E-4031 at 1 μmol/kg and disopyramide at 29 μmol/kg prolonged the AH interval significantly ($P<0.05$) (Fig. 1: B and C).

Neither dofetilide nor E-4031 affected the HV interval and MAP. On the other hand, disopyramide at 29 μmol/kg prolonged the HV interval (Fig. 1C) and decreased MAP significantly ($P<0.01$).

*Effects on the electrical cardiac responses to vagus stimulation*

Before treatment with vehicle or each drug, bilateral cervical vagus stimulation increased SCL and AH interval and decreased AERP (Table 2). Vagus stimulation did not affect the HV interval, but increased the VERP significantly ($P<0.01$). Changes in response to vagus stimulation were not significantly different among each treatment group. In the vehicle-treated group, the changes in response to vagus stimulation were stable throughout the duration of the study.

Dofetilide (0.003−0.3 μmol/kg, i.v.) attenuated the negative chronotropic response to vagus stimulation dose-dependently ($P<0.001$) (Fig. 2A). $ID_{50}$ of dofetilide for the inhibition of the negative chronotropic response was 0.028±0.010 μmol/kg. Dofetilide tended to potenti-

**Table 1.** Prestimulation cardiac control values in open-chest anesthetized dogs*

| Treatment | SCL (msec) | AH (msec) | HV (msec) | AERP (msec) | VERP (msec) | MAP (mmHg) |
|---|---|---|---|---|---|---|
| Dofetilide (n=6) | 541±41 | 97±3 | 25±1 | 136±5 | 183±5 | 96±7 |
| E-4031 (n=5) | 560±35 | 98±6 | 24±1 | 155±9 | 177±5 | 109±7 |
| Disopyramide (n=6) | 518±35 | 93±2 | 22±1 | 142±9 | 163±8 | 100±3 |
| Vehicle (n=6) | 543±39 | 96±6 | 23±1 | 140±6 | 166±6 | 99±7 |

*Values are not significantly different among each treatment group. SCL indicates sinus cycle length; AH, atrio-His interval; HV, His-venticular interval; AERP, atrial effective refractory period; VERP, ventricular effective refractory period; MAP, mean arterial pressure.

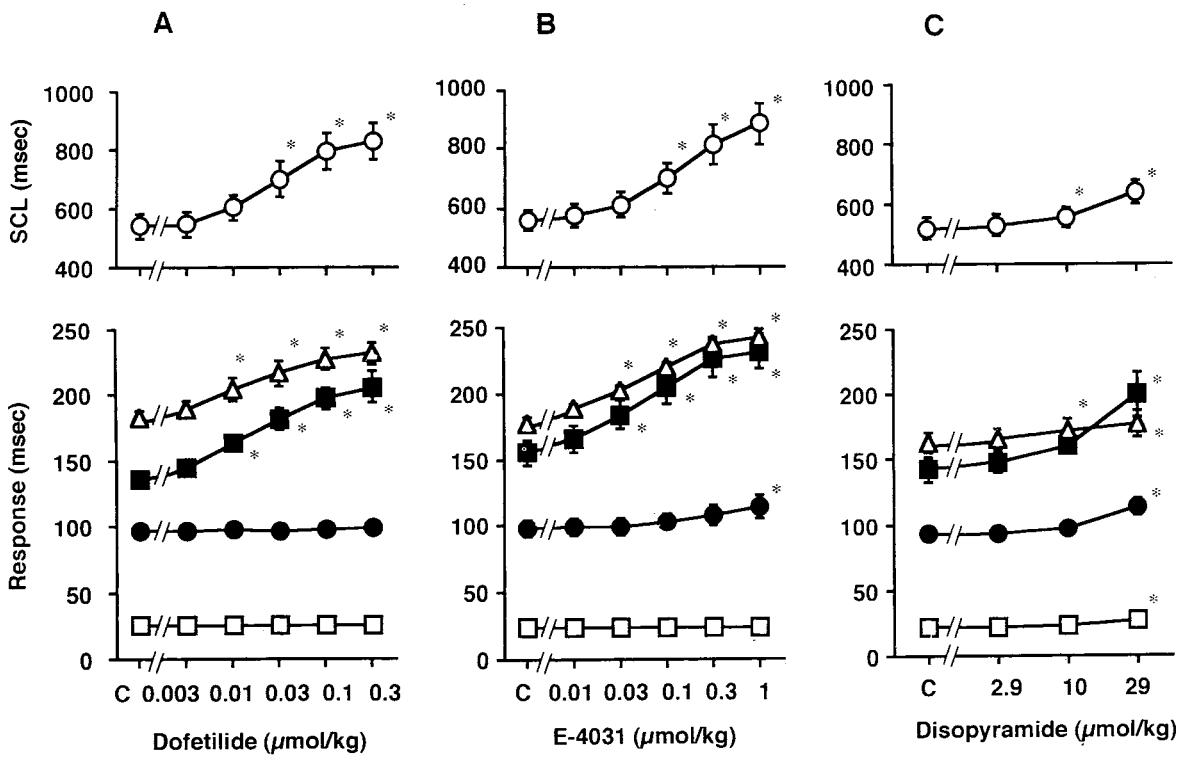


**Fig. 1.** Effects of dofetilide at doses of 0.003 to 0.3 μmol/kg, i.v. (A); E-4031 at doses of 0.01 to 1 μmol/kg, i.v. (B); and disopyramide at doses of 2.9 to 29 μmol/kg, i.v. (C) on electrophysiological parameters before vagal stimulation in autonomically decentralized hearts of open-chest anesthetized dogs. Open circles show sinus cycle length (SCL); closed circles, atrio-His (AH) interval; open squares, His-ventricular (HV) interval; closed squares, atrial effective refractory period (AERP); open triangles, ventricular effective refractory period (VERP); C, control. AH interval was measured during atrial pacing at constant cycle length. AERP and VERP were measured with a basic cycle length of 350 msec. Vertical bars show S.E.M.s. *$P<0.05$ vs control.

ate the shortening of the AERP in response to vagus stimulation, although the potentiation was not significant. Dofetilide at 0.1 μmol/kg augmented the shortening of the AERP from 32±3 to 59±8 msec. $ED_{20}$ of dofetilide for the potentiation of the shortening of the AERP in response to vagus stimulation was 0.015±0.006 μmol/kg. Dofetilide did not influence the increase in the AH interval in response to vagus stimulation. Although dofetilide did not influence the increase in the VERP (data not shown), standard errors for percent response of the VERP were large because of the small increase in VERP before drug treatment.

E-4031 (0.01−1 μmol/kg, i.v.) attenuated the negative chronotropic response to vagus stimulation in a dose-dependent manner ($P<0.01$) (Fig. 2B). $ID_{50}$ of E-4031 for the inhibition of the negative chronotropic response was 0.053±0.024 μmol/kg. E-4031 at 1 μmol/kg potentiated the shortening of the AERP from 41±2 to 91±13 msec ($P<0.05$). $ED_{20}$ of E-4031 for the potentiation of the shortening of the AERP in response to vagus stimulation

**Table 2.** Percent changes in response to vagal stimulation before treatment with each drug in open-chest anesthetized dogs*

| Treatment group | SCL (%) | AH (%) | HV (%) | AERP (%) | VERP (%) | MAP (%) |
|---|---|---|---|---|---|---|
| Dofetilide (n=6) | 40.7±6.8 | 35.7±8.1 | 0 | −23.0±1.8 | 3.2±0.9 | −7.3±1.6 |
| E-4031 (n=5) | 48.8±7.9 | 46.1±8.0 | 0 | −26.6±1.4 | 6.5±2.1 | −5.0±2.6 |
| Disopyramide (n=6) | 68.5±5.8 | 41.9±7.7 | 0 | −23.1±2.5 | 5.1±0.9 | −8.9±1.1 |
| Vehicle (n=6) | 50.3±9.4 | 35.0±5.4 | 0 | −23.3±2.1 | 5.1±1.1 | −11.6±2.2 |

*Percent changes are not significantly different among each treatment group. SCL indicates sinus cycle length; AH, atrio-His interval; HV, His-ventricular interval; AERP, atrial effective refractory period; VERP, ventricular effective refractory period; MAP, mean arterial pressure.

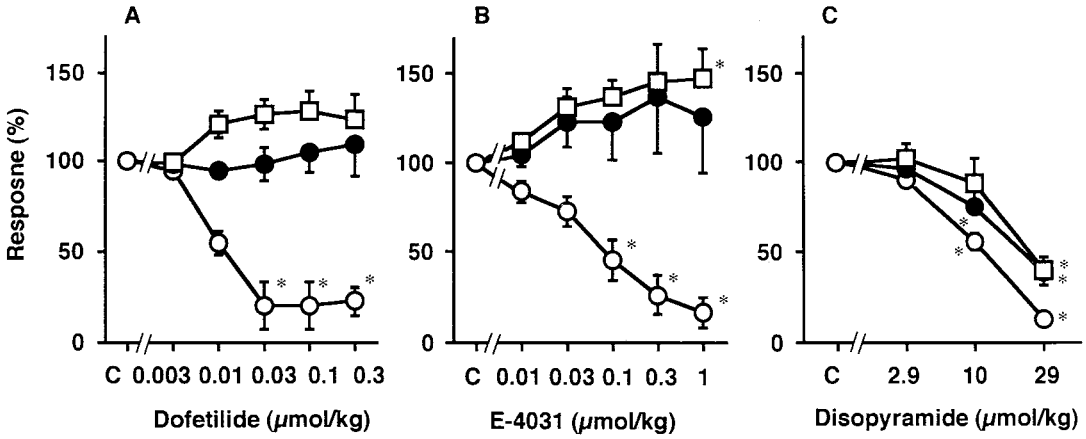


**Fig. 2.** Effects of dofetilide at doses of 0.003 to 0.3 μmol/kg, i.v. (A); E-4031 at doses of 0.01 to 1 μmol/kg, i.v. (B); and disopyramide at doses of 2.9 to 29 μmol/kg, i.v. (C) on the negative chronotropic (open circles), dromotropic (closed circles) responses and shortening of AERP (open squares) in response to vagus stimulation in autonomically decentralized hearts of the open-chest anesthetized dogs. Data are shown as the percentage change of the control response (C) to stimulation. Control responses are shown in Table 2. Vertical bars show S.E.M.s. *$P<0.05$ vs control.

was 0.026±0.008 μmol/kg. E-4031 did not influence the increase in the AH interval or VERP in response to vagus stimulation.

On the other hand, disopyramide (2.9−29 μmol/kg, i.v.) attenuated the negative chronotropic, dromotropic responses and the shortening of the AERP in response to vagus stimulation in a dose-dependent manner (Fig. 2C). $ID_{50}$ for the chronotropic response, dromotropic response and the shortening of the AERP were 10.4±1.2, 20.6±2.9 and 20.9±2.9 μmol/kg, respectively. Disopyramide attenuated the prolongation of the VERP in response to vagus stimulation dose-dependently; $ID_{50}$ was 9.0±2.3 μmol/kg.

## DISCUSSION

The major findings in this study were that dofetilide and E-4031 did not block changes in AERP and VERP in response to vagus stimulation and that both drugs rather potentiated shortening of the AERP in response to vagus stimulation, whereas they blocked the negative chronotropic response in anesthetized dogs.

### *Direct cardiac effects*

Dofetilide (2) and E-4031 (4, 5) have been reported to increase myocardial action potential duration by blocking delayed rectifier potassium current ($I_K$) without any influence on inward sodium or calcium current. E-4031 and dofetilide selectively block the rapidly activating component of $I_K$ ($I_{Kr}$) (6−8). In the present study, dofetilide and E-4031 increased AERP and VERP as well as SCL dose-dependently (Fig. 1). Both drugs prolonged AERP more than VERP. These results agree with those obtained from in vitro (2, 5, 17, 18) and in vivo (3, 19, 20) experiments.

In this study, high doses of E-4031 (0.3, 1 μmol/kg) significantly prolonged the AH interval. On the other hand, dofetilide did not change the AH interval. Spinelli et al. (20) also reported that the AH interval was increased by E-4031 but not affected by dofetilide in anesthetized dogs. It is unknown why the effects on AH interval between dofetilide and E-4031 are different. Verheijck et al. (21) showed that E-4031 at a high dose of 10 μM slightly reduced L-type calcium current ($I_{Ca\text{-}L}$) in rabbit sinoatrial (SA) nodal cells. On the other hand, Gwilt et al. (2) showed that dofetilide had no effect on calcium current in guinea pig ventricular cells. Thus, the prolongation of AH interval by E-4031 may be due to the reduction of $I_{Ca\text{-}L}$.

E-4031 and dofetilide did not affect HV interval but disopyramide increased it, reflecting the lack of the effect of E-4031 and dofetilide on inward sodium current of the His-Purkinje fiber.

### *Effects on cardiac responses to vagal stimulation*

In a previous study (10), we showed that E-4031 selectively attenuated the negative chronotropic response to cervical vagus stimulation or stimulation of intracardiac parasympathetic nerves to SA nodal area in the anesthetized dog heart. The present study showed that dofetilide as well as E-4031 attenuated the negative chronotropic responses to vagus stimulation (Fig. 2), suggesting that inhibition of $I_{Kr}$ probably attenuates a negative chronotropic response to vagus stimulation selectively in the dog heart.

On the other hand, dofetilide and E-4031 did not block

changes in AERP and VERP in response to vagus stimulation (Fig. 2). The $I_{Kr}$ inhibitors rather potentiated the shortening of AERP in response to vagus stimulation. Disopyramide blocked all examined cardiac responses to vagus stimulation. The vagolytic effects of disopyramide and quinidine are due to direct interaction of the drug with muscarinic receptors in guinea pig and canine myocardium (15, 22), although quinidine also inhibits $I_{K(ACh)}$ itself and/or G protein (23). The differential effects of E-4031 and dofetilide on cardiac responses to vagus stimulation suggest that these drugs, different from disopyramide, do not act on muscarinic receptors and that these drugs differentially modulate cardiac responses to vagus activation probably due to a different role of $I_{Kr}$ in each cardiac function. Rasmussen et al. (24) reported that dofetilide did not interact with muscarinic receptors. $ED_{20}$s for potentiation of the shortening of the AERP in response to vagus stimulation (dofetilide, 0.015 $\mu$mol/kg; E-4031, 0.026 $\mu$mol/kg) were similar with $ED_{20}$s for direct effects on AERP (0.015 $\mu$mol/kg and 0.032 $\mu$mol/kg, respectively). These results suggest that potentiation by dofetilide and E-4031 of the shortening of the AERP in response to vagus stimulation is related to the effects of these drugs on $I_{Kr}$. Another way of interpretation of our results is that vagus stimulation reverses a significant proportion of the prolonging effect of $I_{Kr}$ blockade, rather than to say that these drugs potentiate the AERP response to vagus stimulation. On the other hand, Mori et al. (25) showed that *d,l*-sotalol blocked muscarinic receptors and that E-4031 and MS-551 blocked not only muscarinic receptors, but also $I_{K(ACh)}$ and/or G protein in guinea pig atrial myocytes. The reason for the discrepancy between the results of our study and those of the in vitro study is unknown. In the in vitro study, the carbachol-induced $I_{K(ACh)}$ was inhibited by a dose of 1 – 100 $\mu$mol/l of E-4031, which increased the action potential by more than 40%. On the other hand, in our experiments, E-4031 increased AERP less than 30%. Thus, it is possible that the difference in doses of drugs in addition to the species difference of $I_{Kr}$ caused the discrepancy.

In this study, vagally-induced shortening of AERP overcame the prolongation of AERP by dofetilide or E-4031. This is because the augmentation of the outward potassium conductance by vagus stimulation functionally antagonized the reduction of potassium conductance by these drugs. Furthermore, it has been reported that the effects of dofetilide and E-4031 on $I_{Kr}$ are voltage-dependent; the $I_{Kr}$ blocking effect is greater at more positive potential (26, 27). Resting membrane potential is hyperpolarized only a little by vagus stimulation. Thus, it is likely that vagally-induced shortening of the action potential duration decreases the action potential duration prolonged by dofetilide or E-4031 in the atrial myocardium. It is well-known that vagal activation sometimes worsens supraventricular arrhythmia such as atrial flutter or fibrillation, and this is in part through the shortening of the AERP. Thus, it is possible that $I_{Kr}$ inhibitors may be not so effective on atrial flutter or fibrillation occurring in conditions of enhanced vagal activity.

Our study showed that vagus stimulation slightly prolonged the VERP (Table 2). Although previous studies have shown that vagal activation prolongs VERP, it is still unknown whether vagal activation prolongs VERP directly or by opposing sympathetic activity (28, 29). In our experiments, bilateral stellate ganglia were ligated and propranolol was administered. Thus, influence of sympathetic activity should be excluded totally. Therefore, at least under the conditions of our experiments, vagus stimulation prolonged the VERP directly.

In the present study, dofetilide and E-4031 had no significant effect on the increase in VERP in response to vagus stimulation, although standard errors of means were large because changes in VERP were very small. Prolongation of VERP by $I_{Kr}$ inhibitors and by vagal activation are additive and without an interaction (Figs. 1 and 2). Therefore, it is suggested that an $I_{Kr}$ inhibitor and vagal activation may act protectively against ventricular arrhythmia even in the absence of sympathetic activation.

Furthermore, it has been shown that the ability of $I_{Kr}$ inhibitors to prolong repolarization is enhanced at long cycle length (1, 30). This phenomenon, called "reverse" use-dependence, might promote proarrhythmia by excessively prolonging the action potential duration at slow heart rates. However, results of the present study suggest that on vagal activation excessive prolongation of action potential duration by $I_{Kr}$ inhibitors may be partially attenuated through inhibition of the negative chronotropic response to vagal activation by these drugs.

*Clinical implications*

The results of our study showed that the effect of $I_{Kr}$ inhibitors on the vagal control of the heart was different from that of disopyramide. Dofetilide or E-4031 might attenuate vagally-induced bradycardia. They have no influence on vagally-induced prolongation of VERP and may be protective against excessive prolongation of VERP through attenuation of the negative chronotropic response. In contrast, shortening of AERP in response to vagal activation was rather potentiated by E-4031 or dofetilide, different from disopyramide. These findings may have implications for the treatment of atrial flutter or fibrillation during enhanced vagal activity.

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