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# Negative chronotropic and dromotropic effects of E-4031, an $I_{Kr}$ blocker, on the atrioventricular node in anesthetized dog hearts

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**Abstract**

To investigate the effect of the delayed rectifier $K^+$ current ($I_K$) on the atrioventricular (AV) node of the heart in situ, we studied the direct effects of (1-[2-(6-methyl-2-pyridyl)ethyl]-4-(methylsulfonyl-aminobenzoyl)piperidine (E-4031), an $I_{Kr}$ (a rapid type of $I_K$) blocker, on the AV junctional rate, atrio-His interval (AH interval), and right ventricular pressure, and the cardiac responses to sympathetic nerve stimulation in the anesthetized dog heart. AV junctional rhythm was induced by clamping the sinoatrial (SA) pacemaker area. E-4031 (0.01–3 $\mu$mol/kg, i.v.) attenuated the AV junctional rate dose dependently. The junctional negative chronotropic effect was less than the decrease in sinus rate induced by E-4031 in the same doses. E-4031 did not affect the junctional rate increased by sympathetic stimulation. In the paced heart, E-4031 slightly increased the AH interval but did not change right ventricular pressure responses. E-4031 attenuated neither positive dromotropic nor positive ventricular pressure responses to sympathetic stimulation. After E-4031 treatment, zatebradine (a hyperpolarization-activated current blocker) additively decreased the junctional rate and the junctional positive chronotropic responses to sympathetic stimulation. These results suggest that $I_{Kr}$ has much less effect on AV nodal pacemaker activity than on SA nodal pacemaker activity, and an $I_{Kr}$ blocker, E-4031, unlike zatebradine, does not antagonize the junctional positive chronotropic responses to sympathetic activation in anesthetized dog heart.

*Keywords:* AV node; E-4031; $K^+$ current, delayed rectifier; Zatebradine; Heart, dog

## 1. Introduction

Delayed rectifier $K^+$ current ($I_K$) as well as slow inward $Ca^{2+}$ current ($I_{Ca}$) and hyperpolarization-activated current ($I_f$) contribute to depolarization in mammalian cardiac pacemaker cells (Irisawa et al., 1993). $I_K$ exists in sinoatrial (SA) (Noma and Irisawa, 1976; DiFrancesco et al., 1979) and atrioventricular (AV) nodal cells (Noma et al., 1980; Kokubun et al., 1982). It has recently been reported that $I_K$ is composed of two currents, $I_{Kr}$ (a rapidly activated type) and $I_{Ks}$ (a slowly activated type) in guinea pig atrial and ventricular myocytes (Sanguinetti and Jurkiewicz, 1990, 1991). Isoproterenol activates $I_{Ks}$ and E-4031, a class III antiarrhythmic agent, blocks $I_{Kr}$. Sanguinetti et al. (1991) reported that isoproterenol antagonized the prolongation of the action potential and refractory period by E-4031 in guinea pig myocytes. Although $I_K$ in the SA node is one of the important currents that create a pacemaker potential (Noma and Irisawa, 1976; Irisawa et al., 1993), $I_K$ in the AV node has not been fully investigated yet. Negative chronotropic effects of class III antiarrhythmic agents including E-4031 have been reported in isolated mammalian right atria (Tande et al., 1990; Yang et al., 1991), SA nodal cells (Nishimura et al., 1990; Anumonwo et al., 1992) and anesthetized dog hearts (Wallace et al., 1991). However, there is no available report as to whether $I_K$ inhibitors affect AV junctional rhythm in the intact mammalian heart. E-4031 and zatebradine are available as $I_K$ and $I_f$ blockers, respectively, although at a high concentration they inhibit other ionic currents (Doerr and Trautwein, 1990; Goethals et al., 1993; Verheijck et al., 1995). Therefore, in the present study, to determine the effects of $I_K$ blockers on the AV node, we studied the direct effects of E-4031 on the AV junctional rate, AV conduction time and right ventricular pressure responses and the positive cardiac responses to sympathetic nerve stimulation in anesthetized dogs. To investigate the interaction between an $I_K$ blocker and a hyperpolarization-

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activated current ($I_f$) blocker, we also investigated the effects of a combination treatment with E-4031 and zatebradine, an $I_f$ blocker (Van Bogaert et al., 1990; Goethals et al., 1993) on the AV node. We have recently observed that zatebradine attenuated both the AV junctional rate directly and the junctional positive chronotropic responses of anesthetized dog heart to sympathetic nerve stimulation in the AV junctional rhythm (Yamazaki et al., 1994).

## 2. Materials and methods

### 2.1. Preparations

We used 13 mongrel dogs that weighed 8–28 kg. 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 chest was opened transversely at the fifth intercostal space. Each cervical vagosympathetic complex was crushed with a tight ligature, and each stellate ganglion was ligated tightly at its junction with ansa subclavia. These maneuvers remove almost all tonic neural activity to the heart (Levy et al., 1966).

A quadripolar electrode was placed on the base of the epicardial surface of the right atrial appendage to record atrial electrical activity and to pace the atrium electrically. A bipolar electrode was placed in the noncoronary cusp of the aorta via the right femoral artery to record the His-bundle electrogram. The atrial and His-bundle electrograms were passed through a band-pass filter at 30–300 Hz (Nihon Kohden AP621G, Tokyo, Japan). Right ventricular pressure was measured with a catheter-tip pressure transducer (Nihon Kohden TCP2) that was inserted into the basal portion of the right ventricle via the right external jugular vein. Arterial blood pressure was measured via the femoral artery. The electrograms, right ventricular pressure (RVP), its first derivative (dRVP/d$t$), and arterial systolic blood pressure (SBP) were recorded and displayed on a thermo-writing rectigraph (Nihon Kohden RTA 1200).

To stimulate the left ansa subclavia, a bipolar hook electrode was placed on the cardiac side of the stellate ganglion. The electrode was connected to an electrical stimulator (Nihon Kohden SEN7103). We stimulated the left cardiac sympathetic nerves with 10 V and 1 ms pulse duration at a frequency of 2 Hz for 30 s.

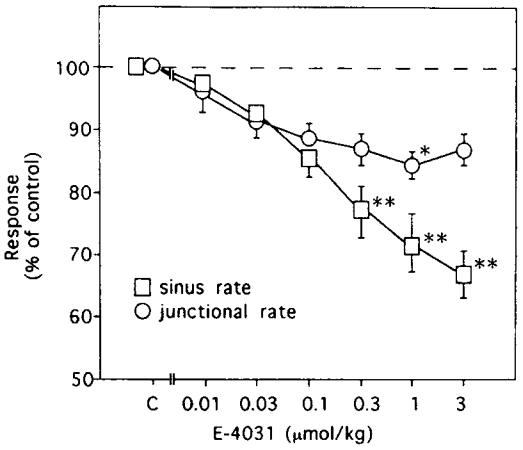


Fig. 2. Effects of E-4031 (0.01–3 μmol/kg, i.v.) on the percentage changes in junctional rate ($n = 8$) and sinus rate ($n = 5$) in junctional rhythm hearts and sinus rhythm hearts, respectively, of open-chest anesthetized dogs. Vertical bars show S.E.M. $^{*}$ $P < 0.05$, $^{**}$ $P < 0.01$ vs. control (C).

To induce the AV junctional rhythm, we clamped the sinoatrial (SA) nodal area along the crista terminalis and atrial free wall mechanically with forceps until the AV junctional rhythm appeared and drove the heart. We confirmed the AV junctional rhythm or other rhythms by His-bundle electrogram through the experiment. We paced the heart at a rate of 100–150 beats/min to maintain the cardiohemodynamics except when studying the effects of a drug on AV junctional pacemaker activity.

### 2.2. Protocol

We investigated the effects of E-4031 (0.01–3 μmol/kg, i.v.) on the AV junctional rate and the chronotropic responses to stimulation of the left ansa subclavia (LS) in 8 dogs. We also determined the effects of E-4031 on the atrio-His (AH) and His-ventricle (HV) intervals, RVP, dRVP/d$t$, and arterial systolic blood pres-

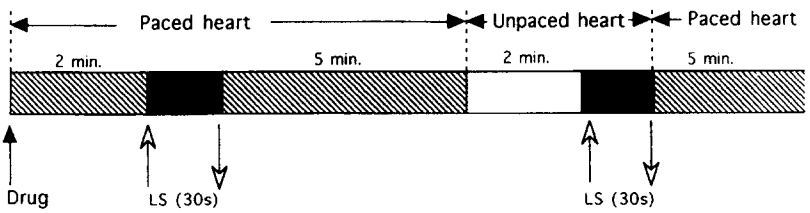


Fig. 1. Protocol for the experiment. After a 2-min control period, control responses, i.e., atrio-His bundle interval (AH), right ventricular pressure (RVP), its first derivative (dRVP/d$t$) and arterial systolic blood pressure (SBP), were determined in an atrial paced heart (shaded area) and the changes in the cardiovascular responses to stimulation of the left ansa subclavia at a frequency of 2 Hz with 1-ms pulse duration and 10 V for 30 s (closed area) were also determined. Then, 5 min after atrial pacing, we determined the AV junctional rate in the unpaced heart for 2 min (open area) and the cardiac responses to left ansa subclavia stimulation for 30 s in the unpaced heart. After cessation of sympathetic stimulation, the heart was paced again. Drugs were given cumulatively 2 min before sympathetic stimulation of the atrial paced heart.

Table 1
Control cardiac parameters before and during stimulation of the left ansa subclavia and responses to E-4031 in 8 anesthetized dogs

| | E-4031 ( μmol/kg) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Control | 0.01 | 0.03 | 0.1 | 0.3 | 1 | 3 |
| JR (/min) | 46 ± 6.2 | 43 ± 3.9 | 42 ± 3.8 | 40 ± 3.5 | 39 ± 3.3 | 38 ± 3.7 | 40 ± 3.7 |
| ΔJR (/min) | 73 ± 6.7 | 71 ± 7.0 | 70 ± 6.9 | 66 ± 6.7 | 63 ± 6.5 | 61 ± 6.8 | 64 ± 7.2 |
| AH (ms) | 110 ± 5.9 | 111 ± 6.6 | 114 ± 5.9 | 116 ± 6.5 | 118 ± 6.9 | 122 ± 5.9 | 123 ± 6.8 |
| ΔAH (ms) | −31 ± 5.4 | −33 ± 6.1 | −34 ± 5.9 | −36 ± 6.5 | −39 ± 6.9 | −42 ± 6.4 | −43 ± 7.2 |
| HV (ms) | 30 ± 1.9 | 30 ± 1.9 | 30 ± 1.9 | 30 ± 1.9 | 30 ± 1.9 | 30 ± 1.9 | 30 ± 1.9 |
| ΔHV | ND | ND | ND | ND | ND | ND | ND |
| RVP (mm Hg) | 19 ± 1.0 | 19 ± 1.0 | 20 ± 1.2 | 20 ± 1.1 | 20 ± 1.2 | 21 ± 1.5 | 20 ± 1.1 |
| ΔRVP (mm Hg) | 8.5 ± 1.7 | 8.6 ± 1.6 | 8.4 ± 1.5 | 8.9 ± 1.5 | 9.3 ± 1.5 | 9.1 ± 1.7 | 8.9 ± 2.0 |
| dRVP/dt (mm Hg/s) | 181 ± 9 | 187 ± 13 | 187 ± 13 | 193 ± 15 | 206 ± 1.7 | 206 ± 17 | 193 ± 14 |
| ΔdRVP/dt (mm Hg/s) | 256 ± 49 | 256 ± 40 | 256 ± 34 | 250 ± 34 | 256 ± 38 | 256 ± 38 | 293 ± 51 |
| SBP (mm Hg) | 119 ± 7.4 | 125 ± 6.2 | 128 ± 5.5 | 127 ± 6.2 | 123 ± 7.7 | 123 ± 7.7 | 113 ± 5.9 |
| ΔSBP (mm Hg) | 25 ± 6.1 | 21 ± 4.8 | 22 ± 4.9 | 23 ± 4.9 | 26 ± 7.4 | 26 ± 7.4 | 30 ± 7.5 |

Data are shown as means ± S.E.M. Data for JR were obtained from 8 experiments with junctional rhythm hearts of anesthetized dogs and the other data were obtained in 8 atrial paced hearts. JR, junctional rate; AH, atrio-His interval; HV, His-ventricular interval; RVP, right ventricular pressure; dRVP/dt, first derivative of RVP; SBP, systolic blood pressure; Δ, changes in response to sympathetic stimulation; ND, not determined.

sure and the positive cardiac responses to LS in atrial pacing hearts. First, we studied the direct cardiac effects of E-4031 2 min after a drug treatment and the effects of E-4031 on the positive cardiac responses to LS at 2 Hz for 30 s in the atrial paced heart (Fig. 1). After 5-min atrial pacing, we ceased atrial pacing and 2 min later, we determined the direct effects of E-4031 on the positive cardiac responses to LS for 30 s. Then, we paced the heart again for 5 min before the next drug treatment.

To investigate the effects of zatebradine in E-4031-treated hearts, we studied the cardiac effects of zatebradine (0.3, 1 and 3 μmol/kg, i.v.) after treatment with E-4031 in 8 AV junctional rhythm hearts.

We previously confirmed the reproducibility of the AV junctional pacemaker activity without drug treatment in the same protocol as used for the drug treatment (Yamazaki et al., 1994).

We also investigated the effects of E-4031 (0.01–3 μmol/kg, i.v.) on sinus rate in 5 spontaneously beating hearts of open-chest anesthetized dogs.

### 2.3. Drugs

Drugs used in the present experiments were (1-[2-(6-methyl-2-pyridyl)ethyl]-4-(4-methylsulfonyl-aminobenzoyl)piperidine (E-4031), generously donated by Eisai, Tokyo, Japan and zatebradine generously donated by Nippon Boehringer Ingelheim, Hyogo, Japan.

### 2.4. Statistical analysis

All data were expressed as means ± S.E.M. The data were analyzed by one-way or two-way analysis of variance and Bonferroni's method for multiple comparisons. $P$ values less than 0.05 were considered statistically significant.

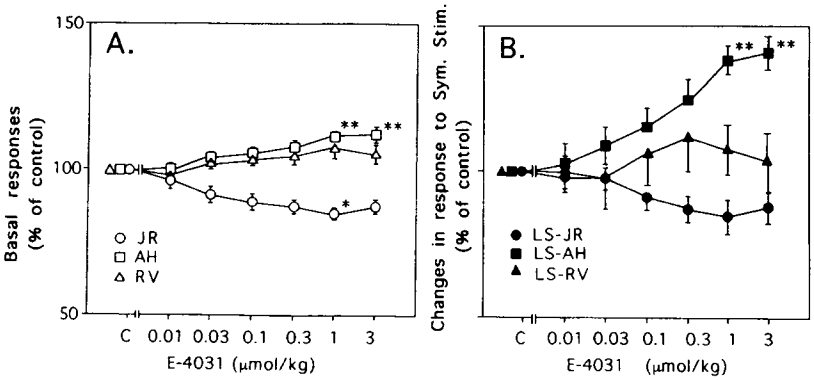


Fig. 3. Effects of E-4031 on the percentage changes in atrioventricular (AV) junctional rate, AH interval and right ventricular pressure (A) and the changes in response to left ansa subclavia stimulation (LS) (B) in 8 autonomically decentralized hearts of open-chest anesthetized dogs. JR (open circle), junctional rate; LS-JR (closed circle), increase in junctional rate in response to LS; AH (open square), atrio-His interval; LS-AH (closed square), increase in AH interval in response to LS; RV (open triangle), right ventricular pressure; LS-RV (closed triangle), increase in RV in response to LS. Vertical bars show S.E.M. * $P < 0.05$, ** $P < 0.01$ vs. control (C).

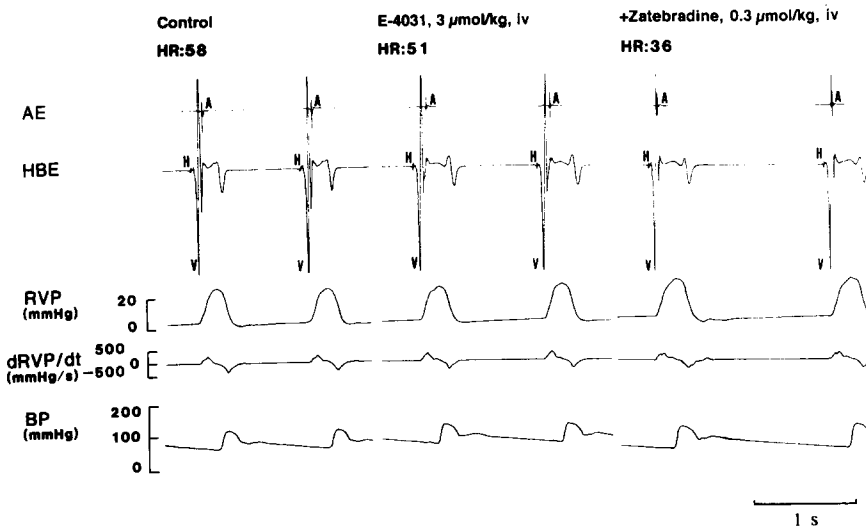


Fig. 4. Effects of E-4031 at a dose of 3 μmol/kg, i.v. and combination treatment with zatebradine at a dose of 0.3 μmol/kg, i.v. on AV junctional pacemaker activity, right ventricular pressure (RVP), its first derivative (dRVP/d$t$), and arterial blood pressure (BP) in a junctional rhythm heart of an open-chest anesthetized dog. AE, atrial electrogram; HBE, His bundle electrogram; HR, heart rate (beats/min); A, atrial electrogram from the base of the atrial appendage; H, His bundle electrogram from His bundle electrode; V, ventricular electrogram from His bundle electrode.

## 3. Results

### 3.1. Effects of E-4031 on AV junctional rate and sinus rate

Table 1 shows the basal AV junctional rate and increase in junctional rate in response to sympathetic stimulation in 8 AV junctional rhythm hearts of anesthetized dogs.

E-4031 in doses of 0.01–3 μmol/kg, i.v. decreased the AV junctional rate dose dependently ($P < 0.001$) in 8 AV junctional rhythm hearts of anesthetized dogs (Fig. 2). E-4031 at a dose of 1 μmol/kg, i.v. decreased the AV junctional rate to 85 ± 5.9% of the control. E-4031 also decreased the sinus rate dose dependently ($P < 0.001$) in 5 spontaneously beating hearts of anesthetized dogs (Fig. 2). 1 μmol/kg, i.v., of E-4031 decreased the sinus rate to 72 ± 4.8% ($P < 0.01$) of the control sinus rate. The decrease in junctional rate induced by E-4031 was less than that in sinus rate expressed as percentage change.

E-4031 tended to attenuate the positive chronotropic responses to sympathetic stimulation slightly but the attenuation was not significant (Fig. 3B).

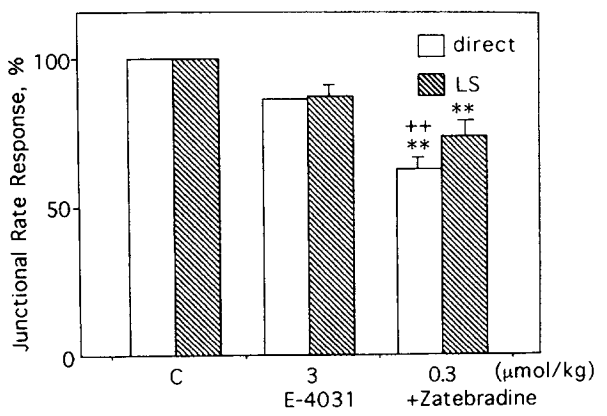


Fig. 5. Effects of zatebradine at a dose of 0.3 μmol/kg, i.v. on the AV junctional rate and the positive chronotropic response to stimulation of the left ansa subclavia in 8 open-chest anesthetized dogs after E-4031 at a dose of 3 μmol/kg, i.v. Open columns (direct) and shaded columns (LS) present the direct effects of drugs on the AV junctional rate and on the increases in junctional rate induced by stimulation of the left ansa subclavia, respectively. Vertical bars show S.E.M. $^{**}P < 0.01$ vs. control (C). $^{++}P < 0.01$ vs. the value of the effect of E-4031 (3 μmol/kg).

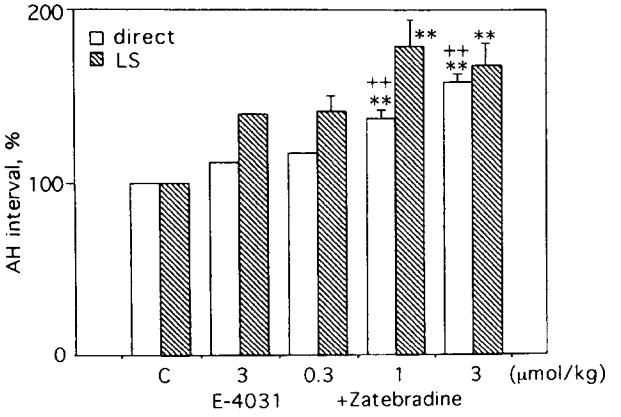


Fig. 6. Effects of zatebradine on the AH interval and the decreases in AH interval in response to stimulation of the left ansa subclavia in 8 open-chest anesthetized dogs after E-4031 at a dose of 3 μmol/kg, i.v. Open columns (direct) and shaded columns (LS) present the direct effects of drug on the AH interval and on the decreases in AH interval induced by stimulation of the left ansa subclavia, respectively. Vertical bars show S.E.M. $^{**}P < 0.01$ vs. control (C). $^{++}P < 0.01$ vs. value of the effect of E-4031 (3 μmol/kg).

### 3.2. Effects of E-4031 on AVCT, RVP, dRVP / dt and SBP

Table 1 shows basal cardiac parameters and changes in response to sympathetic stimulation in 8 atrial paced hearts of anesthetized dogs.

In atrial paced hearts, E-4031 in doses of 0.01–3 μmol/kg, i.v. prolonged the AH interval directly ($P < 0.001$) (Fig. 3A). E-4031 did not prolong the HV interval. E-4031 affected neither the right ventricular pressure, its first derivative nor systolic blood pressure in atrial paced hearts (Fig. 3A) and in junctional rhythm hearts (data not shown).

When sympathetic nerve stimulation shortened the AH interval in atrial paced hearts, E-4031 (0.01–3 μmol/kg, i.v.) augmented the positive dromotropic response ($P < 0.001$) (Fig. 3B). E-4031 at a dose of 3 μmol/kg augmented the positive dromotropic response to LS to 141 ± 11.8% of the control. However, E-4031 did not affect the positive pressure responses to LS in atrial paced hearts (Fig. 3B) and in junctional rhythm hearts (data not shown).

### 3.3. Effects of zatebradine on E-4031-treated hearts

After E-4031 treatment, to study the effects of a combination treatment with E-4031 and zatebradine on AV junctional rhythm, zatebradine (0.3, 1 and 3 μmol/kg, i.v.) was given to anesthetized dogs. After E-4031, at a dose of 3 μmol/kg i.v., had decreased the junctional rate to 86 ± 2.5% of the control rate, zatebradine at a dose of 0.3 μmol/kg i.v. further decreased the junctional rate to 63 ± 5.4% of the control rate ($P < 0.001$) (Figs. 4 and 5). Zatebradine (0.3 μmol/kg, i.v.) also attenuated ($P < 0.01$) the positive chronotropic response to sympathetic nerve stimulation of the junctional rhythm heart of the anesthetized dog (Figs. 4 and 5). Zatebradine at doses of 1 and 3 μmol/kg, i.v. changed the junctional rhythm to atrial rhythm in all cases.

Zatebradine (0.3–3 μmol/kg, i.v.) further prolonged ($P < 0.01$) the AH interval after E-4031 had increased it (Figs. 3 and 6). Zatebradine did not augment the decrease in AH interval in response to sympathetic stimulation after E-4031 had augmented this decrease (Fig. 6).

## 4. Discussion

In the present study, E-4031 decreased the AV junctional rate in a dose-dependent manner but did not attenuate the increases in AV junctional rate in response to sympathetic nerve stimulation in an autonomically decentralized heart of the anesthetized dog. The junctional negative chronotropic effect was less than the decrease in sinus rate induced by E-4031. E-4031 and zatebradine, an $I_f$ inhibitor, additively decreased the junctional rate. These results suggest that $I_{Kr}$ inhibition by E-4031 decreases the AV junctional rate much less than the SA nodal rate in the dog heart, and that $I_{Kr}$ and $I_f$ inhibitors work additively on AV nodal pacemaker activity.

E-4031 attenuates the sinus node pacemaker activity in the rabbit sinus node (Nishimura et al., 1990), isolated guinea pig atrium (Wettwer et al., 1991) and anesthetized dog heart (Katoh et al., 1988). However, in chloralose-anesthetized dogs, E-4031 does not affect sinus pacemaker activity (Wallace et al., 1991). In the present study, E-4031 decreased the sinus rate by approximately 30% in spontaneously beating, autonomically decentralized hearts of pentobarbital anesthetized dogs and attenuated the AV junctional rate by approximately 15% in junctional rhythm hearts (Figs. 2 and 3). That is, the effects of E-4031 on the AV junctional rate were less than those on the sinus rate in the dog heart in situ. E-4031 blocks a rapid type of delayed rectifier $K^+$ current ($I_{Kr}$) (Sanguinetti and Jurkiewicz, 1990, 1991). It has been reported to block $I_K$ without affecting $I_{Ca}$ and the $Na^+$ current in guinea pig heart (Wettwer et al., 1991), although E-4031 at higher concentrations blocked $I_{Ca}$ in sinoatrial nodal myocytes of the rabbit heart (Verheijck et al., 1995). The delayed rectifier $K^+$ current ($I_K$) is one of the SA nodal pacemaker currents (Irisawa et al., 1993). Therefore, we suggest that $I_{Kr}$ participates in the pacemaker activity of the AV nodal cells in the anesthetized dog heart but its role in junctional pacemaker activity is less than in SA nodal pacemaker activity.

It has been reported that the distribution of the two components of $I_K$, $I_{Kr}$ and $I_{Ks}$, is tissue- and species-dependent (Anumonwo et al., 1992; Colatsky et al., 1990). $I_{Kr}$ is dominant in rabbit SA and AV nodal cells and $I_{Ks}$ is present in guinea pig SA nodal cells (Furukawa et al., 1989; Anumonwo et al., 1992; Sanguinetti and Jurkiewicz, 1990, 1991). E-4031 inhibited $I_{Kr}$ voltage dependently in guinea pig ventricular myocytes (Krafte and Volberg, 1994). The diastolic membrane potential of the SA nodal cell is less negative than that of the AV nodal cell. It may be possible, therefore, that E-4031 depresses SA nodal pacemaker activity more effectively than AV nodal pacemaker activity in the dog heart in situ.

Isoproterenol activates $I_{Ks}$ and prevents the prolongation of the action potential duration and refractory period induced by E-4031 in guinea pig myocytes (Sanguinetti et al., 1991). $I_{Ks}$ is a major component in the presence of isoproterenol. However, E-4031 attenuated the positive chronotropic responses to sympathetic nerve stimulation slightly but not significantly in the dog heart (Fig. 3B). Thus, it is suggested that E-4031 does not antagonize the increase in junctional rate induced by activation of the sympathetic nerves in the dog heart.

In the atrial paced heart, E-4031 slightly prolonged the atrio-His (AH) interval but it did not modify the His-ventricle (HV) interval in the autonomically decentralized heart of the anesthetized dog. The positive dromotropic response to sympathetic nerve stimulation was augmented by E-4031, that is, the AH interval prolonged by E-4031

was antagonized by sympathetic nerve stimulation. β-Adrenoceptor stimulation by isoproterenol antagonizes the prolongation of the action potential or the refractory period induced by E-4031 in guinea pig heart tissues (Sanguinetti et al., 1991). These results suggest that E-4031 slightly prolongs the AH interval and that sympathetic nerve activation antagonizes the negative dromotropic effect of E-4031 indirectly in the dog heart in situ. On the other hand, E-4031 prolongs the intra-atrial conduction time at a short pacing cycle length in anesthetized dogs (Inoue et al., 1991; Shimizu et al., 1993), although E-4031 has no effect on conduction time or conduction velocity of the ventricle in dog heart (Lynch et al., 1990; Katoh et al., 1990). Thus, although E-4031 affects conduction in various cardiac tissues differentially, E-4031 may only slightly affect the AV node in the dog heart in situ. That is, $I_{Kr}$ has a minor role in the AV node in the dog heart.

In the present study, after treatment with E-4031, zatebradine, a hyperpolarization-activated current ($I_f$) blocker (Van Bogaert et al., 1990; Goethals et al., 1993; DiFrancesco, 1994) additively decreased the AV junctional rate (Figs. 4 and 5). Although zatebradine blocked the slow inward $Ca^{2+}$ current in rabbit SA nodal cells and cardiac myocytes (Doerr and Trautwein, 1990), it has been reported that zatebradine had little effect on the slow inward $Ca^{2+}$ current in rabbit SA node cells (Goethals et al., 1993) and, at a high dose, it decreases contractile force in isolated perfused dog left ventricle (Furukawa et al., 1993). Zatebradine slightly inhibits $I_K$ in isolated cardiac tissues (BoSmith et al., 1993; Goethals et al., 1993). Therefore, zatebradine may inhibit $I_f$ with minor inhibitory effects on $I_K$ and $I_{Ca}$. Additionally, zatebradine attenuated the increases in AV junctional rate in response to sympathetic nerve stimulation but E-4031 did not (Figs. 3–5). Therefore, we suggest that $I_{Kr}$ and $I_f$ regulate AV junctional rate independently in the dog heart in situ. Verapamil attenuates the increases in junctional rate in response to sympathetic stimulation (Yamazaki et al., 1994) as well as sinus rate itself and the increases in sinus rate in spontaneously beating hearts (Furukawa et al., 1995). Together these reports and our results suggest that $I_{Ca}$, $I_{Kr}$ and $I_f$ regulate sinus rate and junctional rate differently in the dog heart in situ. Another consequence of these possible different pacemaker mechanisms in both tissues may be that pharmacological interventions with the same drug may have different results.

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## References

Anumonwo, J.M.B., L.C. Freeman, W.M. Kwok and R.S. Kass, 1992, Delayed rectification in single cells isolated from guinea pig sinoatrial node, Am. J. Physiol. 262, H921.

BoSmith, R.E., I. Briggs and N.C. Sturgess, 1993, Inhibitory actions of ZENECA ZD7288 on whole-cell hyperpolarization activated inward current (If) in guinea-pig dissociated sinoatrial node cells, Br. J. Pharmacol. 110, 343.

Colatsky, T.J., C.H. Follmer and C.F. Starmer, 1990, Channel specificity in antiarrhythmic drug action: mechanism of potassium channel block and its role in suppressing and aggravating cardiac arrhythmia, Circulation 82, 2235.

DiFrancesco, D., 1994, Some properties of UL-FS 49 block of the hyperpolarization-activated current (if) in sino-atrial node myocyte, Pflügers Arch. 427, 64.

DiFrancesco, D., A. Noma and W. Trautwein, 1979, Kinetics and magnitude of the time-dependent potassium current in the rabbit sinoatrial node, Pflügers Arch. 381, 271.

Doerr, T.H. and W. Trautwein, 1990, On the mechanism of the "specific bradycardic action" of verapamil derivative UL-FS 49, Naunyn-Schmied. Arch. Pharmacol. 341, 331.

Furukawa, T., Y. Tsujimura, K. Kitamura, H. Tanaka and Y. Habuchi, 1989, Time- and voltage-dependent block of delayed $K^+$ current by quinidine in rabbit sinoatrial and atrioventricular nodes, J. Pharmacol. Exp. Ther. 251, 756.

Furukawa, Y., S. Takayama, S. Sawaki, Y. Inoue and S. Chiba, 1993, A bradycardic agent, UL-FS 49, increases atrial force and decreases ventricular force in isolated, perfused heart preparations of dogs, J. Pharmacol. Exp. Ther. 265, 801.

Furukawa, Y., H. Nakano, T. Oguchi, M. Kasama, Y. Hoyano and S. Chiba, 1995, Selective inhibition by zatebradine and discrete parasympathetic stimulation of the positive chronotropic response to sympathetic stimulation in anesthetized dogs, J. Pharmacol. Exp. Ther. 272, 744.

Goethals, M., A. Raes and P.P. van Bogaert, 1993, Use-dependent block of the pacemaker current $I_f$ in rabbit sinoatrial node cells by zatebradine (UL-FS 49). On the mode of action of sinus node inhibitors, Circulation 88, 2389.

Inoue, H., T. Yamashita, M. Usui, A. Nozaki, S. Saihara and T. Sugimoto, 1991, Effects of pentisomide and E-4031 on canine atrial flutter due to reentry: a comparative study with disopyramide and propafenone, J. Cardiovasc. Pharmacol. 18, 137.

Irisawa, H., H.F. Brown and W. Giles, 1993, Cardiac pacemaking in the sinoatrial node, Physiol. Rev. 73, 197.

Katoh, H., K. Nomoto, K. Sawada and T. Shoji, 1988, Electrophysiologic, antiarrhythmic and hemodynamic effects of E-4031, a novel class III antiarrhythmic agent in intact and infarcted dogs, Eur. Heart J. 9 (Suppl.), 232.

Katoh, H., S. Ogawa, I. Furuno, Y. Sato, K. Saeki and Y. Nakamura, 1990, Electrophysiologic effects of E-4031, a class III antiarrhythmic agent, on re-entrant ventricular arrhythmias in a canine 7-day-old myocardial infarction model, J. Pharmacol. Exp. Ther. 253, 1077.

Kokubun, S., M. Nishimura, A. Noma and H. Irisawa, 1982, Membrane currents in the rabbit atrioventricular node cell, Pflügers Arch. 393, 15.

Krafte, D.S. and W.A. Volberg, 1994, Voltage dependence of cardiac delayed rectifier block by methanesulfonamide class III antiarrhythmic agents, J. Cardiovasc. Pharmacol. 23, 37.

Levy, M.N., M.L. Ng and H. Zieske, 1966, Functional distribution of the peripheral cardiac sympathetic pathways, Circ. Res. 19, 650.

Lynch, J.J., L.A. Heaney, A.A. Wallance, J.R. Gehret, H.G. Selnick and R.B. Stein, 1990, Suppression of lethal ischemic ventricular arrhythmias by the class III agent E-4031 in a canine model of previous myocardial infarction, J. Cardiovasc. Pharmacol. 15, 764.

Nishimura, M., N. Sato, H. Tanaka, Y. Habuchi and Y. Watanabe, 1990, A novel class III agent E-4031 suppresses automaticity in the rabbit sinoatrial node by blocking the delayed rectifying $K^+$ current, Circulation 82, III-527.

Noma, A. and H. Irisawa, 1976, A time- and voltage-dependent potassium current in the rabbit sinoatrial node cell, Pflügers Arch. 366, 251.

Noma, A., H. Irisawa, S. Kokubun, M. Nishimura and Y. Watanabe, 1980, Slow current systems in the AV node of the rabbit heart, Nature 285, 228.

Sanguinetti, M.C. and N.K. Jurkiewicz, 1990, Two components of cardiac delayed rectifier $K^+$ current. Differential sensitivity to block by class III antiarrhythmic agents, J. Gen. Physiol. 96, 195.

Sanguinetti, M.C. and N.K. Jurkiewicz, 1991, Delayed rectifier $K^+$ current is composed of two currents in guinea pig atrial cells, Am. J. Physiol. 260, H393.

Sanguinetti, M.C., N.K. Jurkiewicz, A. Scott and P.K.S. Siegl, 1991, Isoproterenol antagonizes prolongation of refractory period by the class III antiarrhythmic agent E-4031 in guinea pig myocytes, Circ. Res. 68, 77.

Shimizu, A., M. Kaibara, O.A. Centurion, G. Kapuku, T. Hirata, M. Fukatani, K. Yano and K. Hashiba, 1993, Electrophysiologic effects of a new class III antiarrhythmic agent, E-4031, on atrial flutter, atrial refractoriness and conduction delay in a canine sterile pericarditis model, J. Cardiovasc. Pharmacol. 21, 656.

Tande, P., H. Bjornstad, T. Yang and H. Refsum, 1990, Rate-dependent class III antiarrhythmic action, negative chronotropy and positive inotropy of a novel IK blocking drug, UK-68,798; potent in guinea-pig but no effect in rat myocardium, J. Cardiovasc. Pharmacol. 16, 401.

Van Bogaert, P.P., M. Goethals and C. Simoens, 1990, Use- and frequency-dependent blockade by UL-FS 49 of the if pacemaker current in sheep cardiac Purkinje fibers, Eur. J. Pharmacol. 187, 241.

Verheijck, E.E., A.C.G. Van Ginneken, J. Bourier and L.N. Bouman, 1995, Effects of delayed rectifier current blockade by E-4031 on impulse generation in single sinoatrial nodal myocytes of the rabbit, Circ. Res. 76, 607.

Wallace, A.A., R.F. Stupienski, III, L.M. Brookes, H.G. Selnick, D.A. Claremon and J.J. Lynch, Jr., 1991, Cardiac electrophysiological and inotropic actions of new and potent methanesulfonanilide class III antiarrhythmic agents in anesthetized dogs, J. Cardiovasc. Pharmacol. 18, 687.

Wettwer, E., G. Scholtysik, A. Schaad, H. Himmel and U. Ravens, 1991, Effects of the new class III antiarrhythmic drug E-4031 on myocardial contractility and electrophysiological parameters, J. Cardiovasc. Pharmacol. 17, 480.

Yamazaki, K., Y. Furukawa, M. Kasama, H. Nakano, H. Imamura, Y. Sasaki and S. Chiba, 1994, Effects of zatebradine, an $I_f$ inhibitor on the subsidiary pacemakers in anesthetized dog heart, Jpn. Heart J. 35 (Suppl.), 279.

Yang, T., P. Tande and H. Refsum, 1991, Negative chronotropic effect of a novel class III antiarrhythmic drug, UK-68,798, devoid of $\beta$-blocking action on isolated guinea-pig atria, Br. J. Pharmacol. 103, 1417.