Regional differences in effects of E-4031 within the sinoatrial node

Kodama, I., M. R. Boyett, M. R. Nikmaram, M. Yamamoto, H. Honjo, and R. Niwa. Regional differences in effects of E-4031 within the sinoatrial node. Am. J. Physiol. 276 (Heart Circ. Physiol. 45): H793–H802, 1999.—Effects of block of the rapid delayed rectifier K$^+$ current (I_{K,r}) by E-4031 on the electrical activity of small ball-like tissue preparations from different regions of the rabbit sinoatrial node were measured. The effects of partial block of I_{K,r} by 0.1 µM E-4031 varied in different regions of the node. In tissue from the center of the node spontaneous activity was generally abolished, whereas in tissue from the periphery spontaneous activity persisted, although the action potential was prolonged, the maximum diastolic potential was decreased, and the spontaneous activity slowed. After partial block of I_{K,r}, the electrical activity of peripheral tissue was more like that of central tissue under normal conditions. One possible explanation of these findings is that the density of I_{K,r} is greater in the periphery of the node; this would explain the greater resistance of peripheral tissue to I_{K,r} block and help explain why, under normal conditions, the maximum diastolic potential is more negative, the action potential is shorter, and pacemaker activity is faster in the periphery.

Heart; cardiac; pacemaking

The action potential is first initiated within a small area of the sinoatrial node. In the rabbit, Bleeker et al. (2) estimated the leading pacemaker cell group to be ~5,000 cells with an area of ~0.1 mm$^2$. Because the total area of the rabbit sinoatrial node is ~12 mm$^2$ (19), this represents only ~1% of the total area. The leading pacemaker cell group is not at a fixed point within the sinoatrial node; mapping of the activation sequence in the rabbit shows pacemaker shift in response to many interventions, including neurotransmitters (19). In normal human subjects, the configuration of the P wave of the electrocardiogram changes routinely (often associated with changes in rate) (7). Changes in the P wave in the human have also been noted in exercise and myocardial infarction (8, 10). The changes in the P wave have been attributed to changes in atrial excitation, perhaps as a result of pacemaker shift (21). Intraoperative mapping in the human confirms that the leading pacemaker site is dynamic (21). The shifting of the leading pacemaker site is the result of marked heterogeneity in the electrophysiology of the sinoatrial node; in different regions of the sinoatrial node, the action potential and pacemaker activity and their response to interventions vary (2, 14). The leading pacemaker site is the site showing the fastest pacemaker activity, and, because of the heterogeneity, this depends on the prevailing conditions.

The ionic mechanisms responsible for this heterogeneity are beginning to be understood; evidence suggests that the densities of the hyperpolarization-activated current (I_p) and the 4-aminopyridine (4-AP)-sensitive transient and sustained outward current (I_{to}) are greater in the periphery of the sinoatrial node than in the center (5, 9). The tetrodotoxin (TTX)-sensitive Na$^+$ current (I_{Na}), although present in the periphery, is thought to be absent from the center (9). The putative regional differences in the currents correlate with the effects of channel blockers; I_p blockers (Cs$^+$, UL-FS-49, ZD-7288), 4-AP, and TTX exert greater effects in the periphery than in the center (4, 15–17). These differences in membrane currents help explain the regional differences in pacemaker activity in the sinoatrial node.

In the rabbit sinoatrial node, the rapid delayed rectifier K$^+$ current (I_{K,r}) plays an important role in pacemaking (18, 22). In the present study, we have investigated the effect of a blocker of I_{K,r}, E-4031, on small ball-like tissue preparations from different regions of the sinoatrial node of the rabbit. The results show that sensitivity to E-4031 varies in the different regions of the sinoatrial node.

METHODS

Experiments were carried out on small ball-like preparations of sinoatrial node tissue or on the intact sinoatrial node. Small ball-like preparations of sinoatrial node tissue. New Zealand White rabbits weighing 1.5–2 kg were anesthetized with intravenous pentobarbital sodium (30–40 mg/kg). The chest was opened, and the heart was rapidly excised into Tyrode solution at ~32°C. The right atrium was separated from the rest of the heart and opened by a longitudinal incision in the free wall to expose the endocardial surface. The atrial atrium was trimmed to leave a preparation ~15 × 15 mm square, which included the whole sinoatrial node and some of the surrounding atrial muscle. The sinoatrial node is located in the intercaval region between the superior and inferior venae cavae (Fig. 1A). Laterally, it is bounded by the atrial septum on one side and the crista terminalis and atrial appendage on the other (Fig. 1A). Conduction from the leading pacemaker site within the sinoatrial node occurs in an oblique cranial direction to the atrial muscle of the crista terminalis (conduction toward the atrial septum is blocked). Sinoatrial node tissue and atrial muscle meet on the surface...
of the crista terminalis, and this boundary is conveniently marked by the right branch of the sinoatrial ring bundle.

Four strands of tissue (0.5 mm in width and 3–4 mm in length) were cut from the sinoatrial node in a direction perpendicular to the crista terminalis (Fig. 1A). Toward the superior vena cava a thick muscle bundle, the central pectinate muscle, projects from the crista terminalis toward the atrial appendage. The strands were cut caudal to the central pectinate muscle because the leading pacemaker site is typically located in this region (see Ref. 4 for further details). The strands were labeled 1–4 (Fig. 1A). Strand 1 was the most superior or cranial, and strand 4 was the most inferior or caudal (Fig. 1A). The peripheral part of the sinoatrial node overlaps the atrial muscle of the crista terminalis, and a razor blade was used to remove this muscle from the strands as well as the lipid tissue on the epicardial surface of the remainder of the sinoatrial node. After they had been trimmed, the strands were ~0.35 mm in width, ~0.2 mm in depth, and ~3 mm in length.

The strands were tied into a series of small balls (typically 4–6) with diameters of ~0.35 mm (Fig. 1A). A drawing of a typical strand divided into balls is shown in Fig. 1B. The ball closest to the crista terminalis included the right branch of the sinoatrial ring bundle on its surface and was labeled A (Fig. 1). The remaining balls were labeled B–F (Fig. 1). Ball A was from the periphery of the sinoatrial node, and ball F was from the center (Fig. 1A). In the intact sinoatrial node, the leading pacemaker site is normally located within the center (the leading pacemaker site is expected to be within the shaded area in Fig. 1A under normal conditions), and the

**Fig. 1. Preparations used.** A: schematic diagram of strands 1–4 cut from sinoatrial node. Strands were tied into a series of ball-like preparations, ~0.35 mm in diameter, labeled A–F. Approximate distances of strands from strand 1 and balls from right branch of sinoatrial ring bundle (RSARB) (i.e., ball A) are given. Relationship of balls to surrounding anatomic landmarks and nomenclature used in relation to balls are also indicated. B: drawing of a typical strand divided into a series of ball-like preparations. Ball A always includes part of right branch of sinoatrial ring bundle (a flap of tissue).
on the atrial muscle was measured (average time interval over 10 beats measured). The site showing the earliest activation (at which this interval was longest) was taken to be the leading pacemaker site. The time of activation of other sites with respect to the time of initiation of the action potential at the leading pacemaker site was shown as a series of isochrones at 5- to 10-ms intervals. The activation pattern was stable in all experiments reported. During the mapping procedure, cycle length was measured 10 times at 5-min intervals (from the beginning to the end of the mapping procedure), and the mean ± SE of cycle length was then calculated from the 10 values. Data were recorded using Axotape software for later analysis. Modified Krebs-Ringer solution contained (in mM) 120 NaCl, 4 KCl, 1.3 MgSO4, 1.2 NaH2PO4, 1.2 CaCl2, 25.2 NaHCO3, and 4 glucose. The solution was equilibrated with 95% O2-5% CO2 to give a pH of 7.4. E-4031 was added to the solution when required.

Data are presented as means ± SE for n preparations. SigmaStat (Jandel Scientific Software) was used for statistical analysis. Student’s t-test or the Mann-Whitney rank-sum test was used to test differences. A difference was considered significant if P < 0.05.

RESULTS

Effect of E-4031 on electrical activity in peripheral and central balls of sinoatrial node tissue. Figure 2 shows action potentials on fast and slow time bases recorded from small ball-like preparations of peripheral (ball A) and central (balls E and F) sinoatrial node tissue. Under control conditions (start of traces) the action potential upstroke was faster, the action potential peak was more positive, the action potential amplitude was greater, the action potential duration was less, the maximum diastolic potential was more negative, and the spontaneous activity was faster in the peripheral tissue. All of these differences between peripheral and central tissue are typical (15). Figure 2 shows the effect of 0.1 and 1 µM E-4031 on the peripheral and central balls. E-4031 at a concentration of 0.1 µM is sufficient to cause partial block of I_Kr, whereas 1 µM is sufficient to cause near-complete block (11, 22) (see DISCUSSION). Near-complete block of I_Kr by 1 µM E-4031 caused prolongation of the action potential followed by the cessation of spontaneous activity in all balls studied, regardless of whether they were from the periphery or center of the sinoatrial node (n = 16 preparations) (Fig. 2, bottom traces). After block of activity, the membrane potential settled at approximately −34 mV in all balls; this point is considered in more detail later in RESULTS. On washoff of E-4031, the effects of E-4031 were reversible, although over a long timescale.

A difference between the peripheral and central tissue emerged when I_Kr was partially blocked by the lower concentration of E-4031 (Fig. 2, top traces). In the examples shown in Fig. 2, on application of 0.1 µM E-4031 pacemaking continued in ball A from the periphery, but it again ceased in ball F from the center. Action potentials recorded at a fast time base from peripheral and central balls of tissue are shown in Fig. 3. Under control conditions, the typical differences in electrical activity can be seen. For example, in the peripheral tissue the maximum diastolic potential was more negative than in the central tissue: the maximum diastolic potential was −77 mV in ball B from the periphery and −63 mV in ball F from the center. Electrical activity after the application of 0.1 µM E-4031 is also shown in Fig. 3. In the central tissue, 0.1 µM E-4031 again abolished the action potential. In the peripheral tissue, 0.1 µM E-4031 did not abolish spontaneous activity.
although it did depolarize the membrane (maximum diastolic potential reduced), slow the action potential upstroke, prolong the action potential, and slow spontaneous activity (Fig. 3A). In the presence of 0.1 µM E-4031, the action potential in the peripheral tissue in some respects became more like that in the central tissue under control conditions.

Results from all balls studied with 0.1 µM E-4031 are summarized in Figs. 4 and 5. Figure 4A shows the action potential peak and maximum diastolic potential in balls A-E (from periphery to center) under control conditions and after the application of 0.1 µM E-4031. The data are plotted against the distance of the ball from the right branch of the sinoatrial ring bundle. Under control conditions, the action potential peak and maximum diastolic potential declined from ball A from the periphery to ball E from the center (Fig. 4A, left). In the presence of 0.1 µM E-4031, the action potential peak and maximum diastolic potential were generally reduced in all balls (the increase in the action potential peak in Fig. 3A is not typical). The reductions in the action potential peak and maximum diastolic potential were greatest in the balls from the center, and as a result the gradient from the periphery to the center in both the action potential peak and maximum diastolic potential was greater in the presence of 0.1 µM E-4031 (Fig. 4A). In Fig. 4B, the mean action potential amplitude (difference between action potential peak and maximum diastolic potential) in the presence of 0.1 µM E-4031 as a percentage of the control is shown for balls A-E. This declined from 72±8% in ball A from the periphery to 7±7% in ball E from the center. There is a significant linear correlation (P < 0.001) between the action potential amplitude in the presence of 0.1 µM E-4031 (as a percentage of control) and distance (Fig. 4B).

Figure 5 shows the effect of 0.1 µM E-4031 on the rate of spontaneous activity. The rate of spontaneous activity under control conditions and in the presence of 0.1 µM E-4031 is shown in Fig. 5A, and the rate in the presence of 0.1 µM E-4031 as a percentage of the control is shown in Fig. 5B. Under control conditions, the characteristic progressive slowing of intrinsic pacemaker activity from the periphery to the center can be seen, i.e., a progressive decrease in rate from ball A to ball E (Fig. 5A, left). In the presence of 0.1 µM E-4031, the rate of spontaneous activity was reduced in all balls, but the decrease was greatest in the central balls (Fig. 5A). Because of this, the gradient in rate from the periphery to the center in the presence of 0.1 µM E-4031 (Fig. 5A, right) was greater than that under control conditions (Fig. 5A, left). The rate of spontaneous activity in the presence of 0.1 µM E-4031, as a percentage of control, was least in the central balls (Fig. 5B). There is a significant linear correlation (P = 0.002) between the rate in the presence of 0.1 µM E-4031 (as a percentage of control) and distance from the right branch of the sinoatrial ring bundle (Fig. 5B).

Figures 4 and 5 again show that the electrical activity of the peripheral balls in the presence of 0.1 µM E-4031 in some respects was similar to that of the central balls under control conditions. Table 1 compares various measurements in ball A from the periphery under control conditions and in the presence of 0.1 µM E-4031 with that of balls E and F from the center under control conditions. In the presence of 0.1 µM E-4031, the upstroke velocity, action potential duration and amplitude, maximum diastolic potential, and rate of spontaneous activity of the peripheral ball went toward those of the central balls under control conditions.

Effect of E-4031 on resting potential. Figure 6A (left bars) shows the mean value of the maximum diastolic potential in balls A and B from the periphery and balls D, E, and F from the center under control conditions. The significant difference (P < 0.001) between the two is 10.9 mV and is shown in Fig. 6B. Figure 6A (right bars) also shows the membrane potential after near-complete block of IKr by 1 µM E-4031. The membrane potential is the resting potential, because 1 µM E-4031 abolished activity in tissue from all regions of the sinoatrial node (Fig. 2). There is no significant difference (P = 0.32) between the resting potentials in the presence of 1 µM E-4031 in the peripheral and central balls (Fig. 6). Figure 6A (middle bars) also shows the resting potential of the peripheral and central balls...
when the action potential and pacemaker activity were terminated by the application of 6 µM nifedipine and 3 µM TTX; block of both the L-type Ca$^{2+}$ current ($I_{Ca}$) and $I_{Na}$ is required to stop spontaneous activity in all balls (15). The resting potentials were significantly greater ($P = 0.003$) when spontaneous activity was terminated by the use of TTX and nifedipine rather than 1 µM E-4031 (Fig. 6A). The resting potential of the periphera l balls when spontaneous activity was terminated by nifedipine and TTX was significantly greater than

![Graph A](image1.png)  
**Fig. 4.** Differences in response to 0.1 µM E-4031 within sinoatrial node in peripheral-central direction: action potential amplitude of balls A–E. A: mean values for action potential peak (top bars) and maximum diastolic potential (bottom bars) under control conditions (left) and in presence of 0.1 µM E-4031 (right). B: action potential amplitude (as percentage of control) in presence of 0.1 µM E-4031. Data are plotted against distance of ball of tissue from RSARB. Data for ball E (at 1.4 mm) include 1 value for ball F. Data are from strands 1 to 4. Number of preparations for all panels: 12, 12, 13, 13, and 7 (left to right).

![Graph B](image2.png)  
**Fig. 5.** Differences in response to 0.1 µM E-4031 within sinoatrial node in peripheral-central direction: rate of spontaneous action potentials of balls A–E. A: mean values for rate of spontaneous action potentials under control conditions (left) and in presence of 0.1 µM E-4031 (right). B: rate of spontaneous action potentials (as percentage of control) in presence of 0.1 µM E-4031. Data are plotted against distance of ball of tissue from RSARB. Data for ball E (at 1.4 mm) include 1 value for ball F. Data are from strands 1 to 4. Number of preparations for all panels: 12, 12, 13, 13, and 7 (left to right).
that of the central balls (Fig. 6A). Figure 6B shows the significant differences (P ≤ 0.009) between the peripheral and central balls in maximum diastolic potential (10.9 mV or 15% of the value in peripheral balls) and resting potential when activity was terminated by TTX and nifedipine (6.4 mV or 13%), as well as the small difference (3 mV or 8%; not significant) between the peripheral and central balls in the resting potential when activity was terminated by near-complete block of I_{Kr} by 1 μM E-4031.

Differences in response to E-4031 in superior-inferior direction. As well as differences in the response to 0.1 μM E-4031 in the transverse direction (i.e., from periphery to center), differences in the longitudinal direction (i.e., from the superior part of the sinoatrial node to the inferior part) were observed. The difference was observed in balls B, C, and D (i.e., all balls apart from the most peripheral and most central). For example, in the case of strand 1 from the more superior part of the sinoatrial node, 2 of 10 balls (20%) were stopped by 0.1 μM E-4031; in the case of strand 2, 3 of 11 balls (27%) were stopped; in the case of strand 3, 3 of 9 balls (33%) were stopped; and in the case of strand 4 from the more inferior part of the sinoatrial node, 6 of 8 balls (75%) were stopped. Figure 7A shows the effect of 0.1 μM E-4031 on ball 1D (i.e., ball D in strand 1) from the more superior part of the sinoatrial node. Although

### Table 1. Comparison of electrical activity of ball A from periphery under control conditions and in presence of 0.1 μM E-4031 with that of balls E and F from center under control conditions

<table>
<thead>
<tr>
<th></th>
<th>Ball A, Control Conditions</th>
<th>Ball A, 0.1 μM E-4031</th>
<th>Balls E and F, Control Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upstroke velocity, V/s</td>
<td>55 ± 7</td>
<td>6 ± 4</td>
<td>7 ± 2</td>
</tr>
<tr>
<td>Action potential peak, mV</td>
<td>15 ± 2</td>
<td>14 ± 5</td>
<td>4 ± 4</td>
</tr>
<tr>
<td>Action potential duration, ms</td>
<td>97 ± 5</td>
<td>163 ± 16</td>
<td>131 ± 12</td>
</tr>
<tr>
<td>Action potential amplitude, mV</td>
<td>92 ± 2</td>
<td>67 ± 8</td>
<td>66 ± 6</td>
</tr>
<tr>
<td>Maximum diastolic potential, mV</td>
<td>−76 ± 2</td>
<td>−53 ± 9</td>
<td>−62 ± 3</td>
</tr>
<tr>
<td>Spontaneous rate, Hz</td>
<td>2.8 ± 0.2</td>
<td>1.6 ± 0.2</td>
<td>1.9 ± 0.1</td>
</tr>
<tr>
<td>Cycle length, ms</td>
<td>370 ± 20</td>
<td>603 ± 32</td>
<td>545 ± 32</td>
</tr>
</tbody>
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Values are means ± SE; n = 11/12 (ball A) or 7 (balls E and F).

Fig. 6. Comparison of maximum diastolic potentials (MDP) and resting potentials (RP) in peripheral and central balls of sinoatrial node tissue. A: mean values of MDP and RP when spontaneous activity was arrested with 6 μM nifedipine (Nif) and 3 μM tetrodotoxin (TTX), and RP when spontaneous activity was arrested with 1 μM E-4031 in peripheral balls (balls A and B; filled bars) and central balls (balls D, E, and F; open bars). Balls are from strands 1 to 4. Number of preparations: 24, 20, 9, 5, 9, and 7 (left to right). B: difference (Δ) in MDP and in RP between peripheral and central balls (calculated from mean values in A). *Significantly different from value from peripheral balls (P < 0.01). NS, not significant.

Fig. 7. Effect of 0.1 μM E-4031 on spontaneous action potentials recorded from balls from more superior (A) and more inferior (B) parts of sinoatrial node. Action potentials under control conditions and in presence of E-4031 are shown superimposed. A: strand 1, ball D. B: strand 4, ball B.
Figs. 4 and 5 show that ball D is sensitive to 0.1 µM E-4031, in this example it was resistant (Fig. 7A). Figure 7B shows the effect of partial block of I_{K, r} by 0.1 µM E-4031 on ball 4B (i.e., ball B in strand 4) from the more inferior part of the sinoatrial node. Although Figs. 4 and 5 show that ball B is resistant to 0.1 µM E-4031, in this example it was sensitive (Fig. 7B). These results suggest that the inferior part of the sinoatrial node is more sensitive to E-4031 than the superior part. This is confirmed by Fig. 8, which shows mean action potential amplitude and rate of spontaneous activity in the presence of 0.1 µM E-4031 (as a percentage of control) for strands 1–4 (data have been plotted against the distance of the strand from strand 1). Data for balls B, C, and D have been combined. There is a significant linear correlation (P < 0.05) between action potential amplitude in the presence of E-4031 (as a percentage of control) and distance (Fig. 8A), as well as the rate of spontaneous activity in the presence of 0.1 µM E-4031 (as a percentage of control) and distance (Fig. 8B). This confirms that tissue from the inferior part of the sinoatrial node is more sensitive to 0.1 µM E-4031 than tissue from the superior part.

Because the effects of E-4031 varied with the position of the tissue in both the superior-inferior and peripheral-central directions, a multiple linear regression was used, i.e., the action potential amplitude or rate of spontaneous activity was correlated with both distance of the strand from strand 1 and distance of the ball from the right branch of the sinoatrial ring bundle. With a multiple linear regression, the rate of spontaneous activity in the presence of 0.1 µM E-4031 changes by $-63\%$/mm ($P < 0.005$) in the central direction. With a multiple linear regression, the rate of spontaneous activity in the presence of 0.1 µM E-4031 changes by $-47\%$/mm ($P < 0.05$) in the central direction.

Effect of E-4031 on intact sinoatrial node. In the intact sinoatrial node, the changes in the intrinsic pacemaker activity of different regions caused by E-4031 could result in pacemaker shift. The leading pacemaker site is the site showing the fastest pacemaker activity. Although inspection of Fig. 5A suggests that the periphery will be the leading pacemaker under control conditions, this is not the case, because in the intact sinoatrial node the periphery is suppressed by the atrial muscle and the leading pacemaker site is in the central region (13). In the presence of E-4031, the intrinsic pacemaker activity of the central region is suppressed to a greater extent than that of the periphery (Fig. 5), and, therefore, the leading pacemaker site is expected to shift away from the center (i.e., toward the atrial muscle of the crista terminalis). Furthermore, because E-4031 suppresses the intrinsic pacemaker activity of the more inferior region of the sinoatrial node to a greater extent than that of the more superior region (Fig. 8), E-4031 is expected to shift the leading pacemaker site toward the superior region of the sinoatrial node. These predictions were tested in a series of experiments on the intact sinoatrial node.

Activation maps were constructed as described in METHODS under control conditions and after the application of 1 µM E-4031. A result is shown in Fig. 9. The isochrones show the extent of propagation of the action potential in a given time (in ms) after the action potential was first initiated at the leading pacemaker site (0-ms isochrone); the set of isochrones shows the sequence of activation. Under control conditions, spontaneous excitation first occurred at a site 0.9 mm from the right branch of the sinoatrial ring bundle (Fig. 9A); this is typical (2). After the application of E-4031, the mean cycle length was changed from 580 ± 4 to 759 ± 5 ms, and the leading pacemaker site shifted (the original leading pacemaker site is shown by a filled circle and highlighted by an arrow in the righthand diagram) in the superior direction and toward the crista terminalis (Fig. 9B). This is consistent with our predictions. In four preparations, the leading pacemaker site shifted by 1.6 ± 0.5 mm. The shift was composed of a 1.4 ±
that, in rabbit sinoatrial node cells, 0.1 µM E-4031 had no effect on I niece value not reported) and 1 µM E-4031 blocked ~96% of I . In the study of Ito and Ono (11) on rabbit sinoatrial node cells, 0.27 mM E-4031 caused half-maximal inhibition of I. In the study of Verheijck et al. (22) on rabbit sinoatrial node cells, 1 µM E-4031 (highest concentration used in present study) had no effect on I _Ca_ and 10 µM E-4031 had no effect on I . In the study of Ito and Ono (11) on rabbit sinoatrial node cells, it was concluded that 3 mM E-4031 (a higher concentration than that used in the present study) only blocked I , and had no effect on I _Ca_ and I . In ventricular cells, I _Ca_ has also been reported to be insensitive to E-4031 (20). In summary, all available evidence suggests that E-4031 at the concentrations used is a selective blocker of I , although it remains a possibility that it is having another unknown action.

**DISCUSSION**

It is already known that block of I abolishes pacemaker activity in the sinoatrial node (18, 22). The present study shows that the sensitivity to E-4031 varies in different regions of the sinoatrial node: the sensitivity of central tissue is greater than that of the peripheral tissue, and the sensitivity of tissue from the more inferior part is greater than that of tissue from the more superior part.

Selectivity of E-4031. Verheijck et al. (22) reported that, in rabbit sinoatrial node cells, 0.1 mM E-4031 caused partial block of I (precise value not reported) and 1 mM E-4031 blocked ~96% of I . In the study of Ito and Ono (11) on rabbit sinoatrial node cells, 0.27 mM E-4031 caused half-maximal inhibition of I . In the study of Verheijck et al. (22) on rabbit sinoatrial node cells, 1 mM E-4031 (highest concentration used in present study) had no effect on I _Ca_ and 10 mM E-4031 had no effect on I . In the study of Ito and Ono (11) on rabbit sinoatrial node cells, it was concluded that 3 mM E-4031 (a higher concentration than that used in the present study) only blocked I , and had no effect on I _Ca_ and I . In ventricular cells, I _Ca_ has also been reported to be insensitive to E-4031 (20). In summary, all available evidence suggests that E-4031 at the concentrations used is a selective blocker of I , although it remains a possibility that it is having another unknown action.

**Action of E-4031.** In the studies of Ono and Ito (18) and Verheijck et al. (22) on single cells from the rabbit sinoatrial node, 0.1 mM E-4031 decreased the maximum diastolic potential and action potential amplitude, increased the action potential duration, and slowed or stopped spontaneous activity; 1 and 10 mM E-4031 always stopped spontaneous activity (18, 22). The effects of 0.1 and 1 mM E-4031 in the present study were the same. The decrease in the maximum diastolic potential and increase of action potential duration are likely to be the direct effects of the block of I . In contrast, the decrease of the action potential upstroke velocity and overshoot are likely to be indirect effects resulting from voltage-dependent inactivation of the inward currents (I and I ) responsible for the action potential upstroke. The decrease in spontaneous activity caused by E-4031 is again likely to be an indirect effect perhaps caused by the increase in action potential duration, decrease in the takeoff potential as a result of the inactivation of I and I (Figs. 3 and 7), and reduced activation of I during the pacemaker potential as a result of the depolarization.

Peripheral-central differences in sinoatrial node in sensitivity to E-4031. In the present study, the effects of the almost complete block of I by 1 mM E-4031 were the same throughout the sinoatrial node (Fig. 2); this shows that, in both the periphery and center, I is important for pacemaking (for reasons considered in Action of E-4031). However, the effects of partial block of I by 0.1 mM E-4031 varied and were greater in the center of the sinoatrial node compared with the periphery (Figs. 3–5).

There are various possible explanations of this finding. 1) The sensitivity of I to E-4031 may vary in the different regions of the sinoatrial node. Although this is an unlikely explanation, it is a possibility.

2) The sensitivity of the tissue to block of I may vary in the different regions of the sinoatrial node. It is possible that the density of background inward current is greater in the center of the sinoatrial node compared with the periphery; this could explain why the maximum diastolic potential is more positive in the center compared with the periphery (Fig. 6), although it could not explain why the action potential is longer and the pacemaker activity is slower in the center. In this case, block of a smaller fraction of I in central tissue compared with peripheral tissue would be required to stop spontaneous activity. However, a difference in the density of background inward current would be expected to result in a difference in the resting potential as well as the maximum diastolic potential, and such a difference would be expected to occur regardless of whether spontaneous activity was terminated by E-4031 or by nifedipine and TTX; although a significant differ-
ence in the resting potential was observed after block of spontaneous activity by TTX and nifedipine, a significant difference was not observed after block by E-4031 (Fig. 6).

3) The density of $I_{K_{r}}$ may vary in the different regions of the sinoatrial node. It is possible that the density of $I_{K_{r}}$ is lower in the center of the sinoatrial node than in the periphery; this could help explain why the action potential is longer, the maximum diastolic potential is more positive, and pacemaker activity is slower in the center than in the periphery. A lower density of $I_{K_{r}}$ in the center of the sinoatrial node could explain the greater sensitivity of central tissue to 0.1 $\mu$M E-4031 compared with that of peripheral tissue. It may appear paradoxical that because the density of $I_{Na}$, $I_{to}$, and $I_{f}$ is lower in the periphery of the sinoatrial node (5, 9), block of the currents produces smaller effects on electrical activity in the center (4, 15, 17) and yet a smaller density of $I_{K_{r}}$ in the center would mean that block of the current (by E-4031) produces a greater effect. This is a consequence of the different roles of the currents in electrical activity. $I_{Na}$, $I_{to}$, and $I_{f}$ are not required for spontaneous activity to persist; spontaneous activity can continue after complete block of the currents. Block of the currents results in changes in electrical activity, and the magnitude of the changes is proportional to the normal density of the currents. However, $I_{K_{r}}$ is required for spontaneous activity to persist (as indicated by the fact that complete block of $I_{K_{r}}$ abolishes spontaneous activity). It follows from this that a minimal density of $I_{K_{r}}$ is required to sustain spontaneous activity. If the reserve of $I_{K_{r}}$ above this minimum is less in central tissue (in other words the density of $I_{K_{r}}$ is lower in central tissue) compared with that in peripheral tissue, a smaller fraction of $I_{K_{r}}$ will need to be blocked in central tissue to abolish spontaneous activity.

If the density of $I_{K_{r}}$ is higher in the periphery of the sinoatrial node, as well as helping to explain why the maximum diastolic potential is more negative in the periphery, it could also explain why the resting potential is significantly more negative in the periphery when spontaneous activity is stopped by TTX and nifedipine but not by E-4031 (Fig. 6). Some additional observations are in favor of this hypothesis. First, using models of central and peripheral action potentials in the sinoatrial node, we have shown that a greater density of $I_{K_{r}}$ in the periphery can explain the differing effects of E-4031 in the periphery and center (H. Zhang, M. R. Boyett, A. V. Holden, I. Kodama, and H. Honjo, unpublished observations). Second, using an anti-ERG antibody, Brahmanjoti et al. (6) studied the distribution of the ERG protein (the channel protein responsible for $I_{K_{r}}$) in the ferret heart. Figure 2, panel 15, of Brahmanjoti et al. (6) shows that in the intercalated region distant from the crista terminalis (where the center of the sinoatrial node is found in the rabbit at least) little ERG protein was detected, whereas in the intercalated region next to the crista terminalis (where the periphery of the sinoatrial node is found in the rabbit) the ERG protein was abundant. If the distribution of the ERG protein is the same in the rabbit sinoatrial node, it may explain the results obtained in the present study. Although these observations are in favor of the hypothesis, a proper test of the hypothesis must await the direct measurement of the density of $I_{K_{r}}$ in voltage-clamp experiments in cells from the different regions of the sinoatrial node.

The electrical activity of tissue from the periphery in the presence of 0.1 $\mu$M E-4031 was in some respects similar to that of tissue from the center under control conditions (Table 1). Thus, in the peripheral tissue after partial block of $I_{K_{r}}$ by 0.1 $\mu$M E-4031, the action potential was no longer shorter, the maximum diastolic potential was no longer more negative, and the spontaneous activity was no longer faster than that of central tissue. It is possible that after partial block of $I_{K_{r}}$ in the periphery, the density of $I_{K_{r}}$ was more similar to that in the center and, therefore, the action potential duration, maximum diastolic potential, and rate of spontaneous activity were more similar to those in the center. However, a difference in the density of $I_{K_{r}}$ (if one exists) is not the only reason for the differences between the periphery and center; there is evidence that the density of $I_{to}$ is greater in the periphery and, after block of the current by 4-AP, the difference in action potential duration is no longer significant and the gradient in the maximum diastolic potential between the periphery and center is reduced (4). This suggests that the shorter action potential and more negative maximum diastolic potential in the periphery may be the result of greater densities of two K$^{+}$ currents, $I_{to}$ and $I_{K_{r}}$. $I_{K_{r}}$ also is not the only reason for the faster intrinsic spontaneous activity of the periphery of the sinoatrial node; evidence suggests that the densities of $I_{f}$ and $I_{Na}$ are greater in the periphery and that block of either $I_{f}$ or $I_{Na}$ eliminates or even reverses the difference in the speed of spontaneous activity between the periphery and center (15, 17).

Table 1 shows that in the presence of 0.1 $\mu$M E-4031 the upstroke velocity of the action potential in the peripheral tissue became like that in the central tissue. This was probably the result of the inactivation of $I_{Na}$ normally responsible for the action potential upstroke in the periphery. Once $I_{Na}$ was inactivated, $I_{Ca}$ would have been responsible for the action potential upstroke, as it is in the center under normal conditions (15). In the center, evidence suggests that $I_{Na}$ is absent, rather than being inactivated by the low diastolic potentials (1, 9). Although Table 1 shows that the electrical activity of the peripheral tissue became more like that of the center under control conditions when the peripheral tissue was exposed to 0.1 $\mu$M E-4031, the action potential peak did not become more similar and the action potential peak remained more positive than that in the central tissue. This suggests that the smaller overshoot of the action potential in the center of the sinoatrial node is not directly or indirectly related to $I_{K_{r}}$.

Although the intrinsic pacemaker activity of the periphery is faster than that of the center, in the intact sinoatrial node under normal conditions the leading pacemaker site is located in the center of the sinoatrial...
node, because the pacemaker activity of the periphery is suppressed electronically by the adjacent, more polarized atrial muscle. In the presence of E-4031, the leading pacemaker site is expected to shift to the site, the spontaneous activity of which is least affected (i.e., the cycle length of which is least increased) by E-4031. On the basis of the data in Figs. 5 and 8, the leading pacemaker site is expected to shift to the periphery of the sinoatrial node next to or on the crista terminalis and to the superior part of the sinoatrial node. Consistent with the prediction above, on application of 1 μM E-4031, the leading pacemaker site shifted 0.5 ± 0.1 mm toward the crista terminalis and 1.4 ± 0.4 mm in the superior direction (Fig. 9). E-4031 (1 μM), when used with small balls of tissue, stopped spontaneous activity even in small balls of tissue from the periphery (Fig. 2). It is likely that in the intact sinoatrial node, a higher concentration of E-4031 was tolerated, because the atrial muscle helped to keep the sinoatrial node polarized after block of I_{K,R}.

Superior-inferior differences in the sinoatrial node in sensitivity to E-4031. Despite the importance of superior-inferior differences within the sinoatrial node (pacemaker shift almost invariably involves a shift in the superior or inferior direction; see Ref. 19), less is known of the differences in this direction. On average, action potential duration is longer and spontaneous activity is slower in the inferior part of the sinoatrial node (3). The inferior part of the sinoatrial node showed a greater sensitivity to 0.1 μM E-4031 in the present study; as for the peripheral-central differences in E-4031 sensitivity, there are various possible reasons for this. One of these is a reduction in the density of I_{K,R} in the inferior part; this could help explain the longer action potential and slower pacemaking in the inferior part of the sinoatrial node. The longer action potential in the inferior part of the sinoatrial node is not the result of a lower density of I_{Na}(as it may in part be in the center), because block of I_{Na} by 5 mM 4-AP causes a greater prolongation of the action potential in the inferior part of the sinoatrial node than in the superior part (it is feasible, therefore, that the density of I_{Na} is greater in the inferior part) (4).

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