Regional differences in effects of 4-aminopyridine within the sinoatrial node

M. R. Boyett, H. Honjo, M. Yamamoto, M. R. Nikmaram, R. Niwa, and I. Kodama. Regional differences in effects of 4-aminopyridine within the sinoatrial node. Am. J. Physiol. 275 (Heart Circ. Physiol. 44): H1158–H1168, 1998.—4-Aminopyridine (4-AP)-sensitive transient outward current ($I_{to}$) has been observed in the sinoatrial node, but its role is unknown. The effect of block of $I_{to}$ by 5 mM 4-AP on small ball-like tissue preparations (diameter $\approx 0.3–0.4$ mm) from different regions of the rabbit sinoatrial node has been investigated. 4-AP elevated the plateau, prolonged the action potential, and decreased the maximum diastolic potential. Effects were greater in tissue from the periphery of the node than from the center. In peripheral tissue, 4-AP abolished the action potential notch, if present. 4-AP slowed pacemaker activity of peripheral tissue but accelerated that of central tissue. Differences in the response to 4-AP were also observed between tissue from more superior and inferior regions of the node. In the intact sinoatrial node, 4-AP resulted in a shift of the leading pacemaker site consistent with the regional differences in the response to 4-AP. It is concluded that 4-AP-sensitive outward current plays a major role in action potential repolarization and pacemaker activity in the sinoatrial node and that its role varies regionally.

Transitional and peripheral regions of the sinoatrial node to the surrounding atrial muscle of the crista terminalis. Although the normal function of the transitional and peripheral regions of the sinoatrial node is to conduct the action potential from the center to the atrial muscle, in response to a wide variety of interventions the leading pacemaker site shifts to the transitional or peripheral region and, therefore, these regions can have a pacemaker role (37, 40). In the center, the cells are smaller than those in the periphery and contain fewer and less well-organized myofilaments (1, 36, 38). Furthermore, in the center, the action potential upstroke is slower, the action potential overshoot is less, the action potential is longer, the maximum diastolic potential is less negative, and intrinsic pacemaker activity is slower than in the periphery (1, 26, 28). The differences in electrical activity are known to be the result of genuine regional differences in membrane properties (rather than electrotonic interactions) because the differences are also seen in both small ball-like tissue preparations isolated from different regions of the sinoatrial node (26, 28) and in single cells (19).

We distinguish between single sinoatrial node cells on the basis of cell capacitance, a measure of cell size (we assume that the small cells are from the center, whereas the large cells are from the periphery; see above) (19). Small cells have a low density of the hyperpolarization-activated current ($I_{f}$) and lack the tetrodotoxin (TTX)-sensitive Na$^+$ current ($I_{Na}$), whereas in large cells the density of both currents is high (19). The possibility that this could explain the faster intrinsic pacemaker activity of the periphery was confirmed by blocking the currents in small ball-like tissue preparations from different regions of the sinoatrial node (28, 35). Although the density of the transient component of $I_{to}$ may not be correlated with cell capacitance, the density of the sustained component is correlated and is greater in larger cells with a higher capacitance (4). This suggests that the role of $I_{to}$ will be greater in the periphery of the sinoatrial node. In the present study, we have investigated this possibility by blocking the current with 4-AP in small ball-like tissue preparations from different regions of the rabbit sinoatrial node.

METHODS

Experiments were carried out on the intact sinoatrial node and small ball-like preparations of sinoatrial node tissue. Intact sinoatrial node. 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
The heart was rapidly excised into modified Krebs-Ringer 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 right atrium was then trimmed to leave a preparation ~15 × 15 mm, which included the whole sinoatrial node and some of the surrounding atrial muscle. A typical preparation is illustrated in Fig. 1A. The preparation (endocardial surface up) was fixed in a tissue bath.

Small ball-like preparations of sinoatrial node tissue. After the sinoatrial node had been isolated as described above, four strands of tissue (0.3–0.4 mm in width and 3–4 mm in length) were cut from the sinoatrial node in a direction perpendicular to the crista terminalis. A typical position of the strands in the intact sinoatrial node is shown in Fig. 1A; the cut end of the crista terminalis is marked CT. The crista terminalis runs from top to bottom in the diagram, and its position is marked by the right branch of the sinoatrial ring bundle (RSARB), a thin flap of tissue that also marks the border between the atrial muscle and the crista terminalis. The strands were cut from the sinoatrial node in a direction perpendicular to the crista terminalis. A typical position of the strands in the tissue bath was superfused with modified Krebs-Ringer solution at 32°C. Solution flowed under the action of gravity at a rate of 20–25 ml/min through a heat exchanger into the chamber. The bath temperature was monitored using a miniature thermometer to ensure that the temperature remained at 32°C. Experiments were carried out at 32°C, because our experience is that all electrophysiological properties are stable for much longer periods (>8 h) at 32°C than at 37°C.

In some experiments, activation maps of the intact sinoatrial node were made by recording extracellular potentials from 90–100 sites with a pair of modified bipolar electrodes (see Fig. 10). The electrodes were positioned using a calibrated XYZ micromanipulator with 0.1-mm precision. Another pair of modified bipolar electrodes was used to record the extracellular potential from the atrial muscle as a reference signal. Each pair of modified bipolar electrodes consisted of two 100-µm stainless steel wires (one wire 1 mm shorter than the other) insulated to the tip and taped together. High-gain (50–88 dB) amplification and filtering (0.5–30 Hz band-pass filter used) of the signals from the modified bipolar electrodes resulted in a sharp negative deflection at the instant of activation of the recording site (confirmed by action potential recording by conventional glass microelectrodes). The time interval between the time of activation at the recording site and the time of activation at the reference site 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

![Diagram](image-url)
procedure cycle length was measured 10 times at 5-min intervals (from beginning to end of mapping procedure), and the mean cycle length was then calculated from the 10 values.

Intracellular action potentials were recorded from small balls of sinoatrial node tissue using conventional glass microelectrodes (resistance, 30–40 MΩ; filling solution, 3 M KCl). Action potential duration at −30 mV and spontaneous cycle length (time interval between successive spontaneous action potentials) were measured using an electronic device (24). Action potentials, action potential duration, and spontaneous cycle length were recorded using a thermal array recorder (RTA-1200, Nihon Kohden), tape (sampling rate, 5 kHz; digital magnetic tape recorder, PC-108M, Sony), and Axotape software (Axon Instruments, Burlington, CA) for later analysis. The 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. A stock solution of 0.5 M 4-AP was prepared in distilled water (pH titrated to 7.4 using HCl). This was added to modified Krebs-Ringer solution to give the required concentration of 4-AP.

Data are presented as means ± SE for the indicated number of preparations. Student’s t-test (paired or unpaired as appropriate) or a one-way analysis of variance was used to test differences for normally distributed data. For data not normally distributed, an equivalent nonparametric test was used (Mann-Whitney rank sum test, Wilcoxon signed-rank test, Kruskal-Wallis ANOVA on ranks). SigmaStat (Jandel Scientific Software) or Microsoft Excel was used. A difference was considered significant if P < 0.05. Linear regressions were carried out using SigmaStat or Fig.P (Fig.P Software).

RESULTS

Effect of 4-AP on small ball-like tissue preparations from different regions of sinoatrial node. Regional differences from periphery to center. Five millimolar 4-AP was principally used in this study (see Discussion for justification of concentration used). In experiments on small ball-like tissue preparations, when a microelectrode impalement was steady, action potentials were recorded under control conditions and then 4-AP was applied for 2 min, after which 4-AP was washed off and the tissue was allowed to recover. All effects of 4-AP shown were recorded once the preparation had reached a steady state and were reversible on washoff of 4-AP.

Action potentials recorded from tissue taken from the periphery (ball A) and center (ball D) of the sinoatrial node are shown in Fig. 2. Under control conditions, typical differences in electrical activity between the periphery and center of the sinoatrial node can be seen: in the peripheral ball, the action potential upstroke was faster, the action potential overshoot was greater, the maximum diastolic potential was more negative, and pacemaking was faster. The effects of 5 mM 4-AP are also shown in Fig. 2. 4-AP increased both the overshoot and the duration of the action potential. The increase in duration was greater in ball A from the periphery than in ball D from the center. In the peripheral ball, 4-AP decreased the maximum diastolic potential (i.e., made it more positive; Fig. 2A), but in the central ball it increased the maximum diastolic potential (Fig. 2B). Although a decrease in the maximum diastolic potential was observed in all peripheral balls (A and B) studied (n = 19), an increase in the maximum diastolic potential was seen in 6 of 13 central balls (D and E). A decrease in the maximum diastolic potential was observed in the remaining seven central balls. Finally, 4-AP altered the cycle length, increasing it in the peripheral ball (Fig. 2A) but decreasing it in the central ball (Fig. 2B).

In the periphery of the intact sinoatrial node (but not in the center) and in small ball-like tissue preparations from the periphery (but not from the center), we frequently observe action potentials with notches; after the action potential upstroke there is a brief period of rapid repolarization followed by a second period of depolarization. A typical example is shown in Fig. 3. A similar notch in the action potential in the periphery of the intact sinoatrial node of the rabbit was reported by Kreitner (29). Figure 3 shows that the notch was abolished on application of 4-AP by the elimination of the early rapid period of repolarization; there was an earlier and larger secondary depolarization instead, leading to an increase in the action potential overshoot. Abolition of the action potential notch by 4-AP was observed in a total of four balls (either ball A or ball B from the periphery); in no ball did 4-AP fail to abolish the notch. In the example shown in Fig. 3, 4-AP also resulted in a large prolongation of the action potential; this is expected because the example is from a peripheral ball.

Figures 4–6 show mean data for the effect of 4-AP on action potential overshoot, maximum diastolic poten-
4-AP-sensitive current in sinoatrial node

**Fig. 3. Abolition of notch of peripheral action potential by 4-AP.** Action potentials from ball A from the periphery and balls D and E from the center are shown. Furthermore, because no differences in the response of the overshoot and maximum diastolic potential to 4-AP were detected in the superior-inferior direction, data for A and B from the periphery and balls D and E from the center are shown. Under control conditions, both the overshoot and the maximum diastolic potential were significantly greater (P < 0.001) in balls A and B from the periphery than in balls D and E from the center as reported previously (see, e.g., Ref. 28). In the peripheral balls, 4-AP significantly increased (P < 0.001) the overshoot by 3.3 ± 0.5 mV (see Fig. 4A, inset) and significantly decreased (P < 0.001) the maximum diastolic potential by 3.2 ± 0.6 mV (Fig. 4B, inset). In the central balls, the changes in the overshoot and the maximum diastolic potential were smaller and not significant (Fig. 4).

**Fig. 4. Effect of 4-AP on action potential overshoot (A) and maximum diastolic potential (MDP; B) in peripheral and central regions of sinoatrial node.** In main graphs, mean values under control conditions and in presence of 5 mM 4-AP for balls A and B from periphery and balls D and E from center are shown (balls from strands 1–4). *Different (P < 0.001) from control value for same group of balls; N.S., not significantly different from control value for same group of balls.

The central balls (Fig. 5A); this is the result of the greater increase in action potential duration in the more peripheral balls. This important finding is considered further in Discussion.

Figure 6 shows the cycle length before and after the application of 4-AP. Combined data for strands 2 and 3 are shown. Under control conditions, there was a significant gradient (ANOVA on ranks, P < 0.001) in cycle length from ball A from the periphery to ball E from the center (Fig. 6A) as reported previously (see, e.g., Ref. 28). The effect of 4-AP varied in the different balls. In the more peripheral balls 4-AP prolonged the cycle length, but in the more central balls it decreased the cycle length. In Fig. 6B, the percent change in cycle length is plotted and the significant variation (ANOVA, P < 0.001) in the change in cycle length from ball A to ball E can be seen to be a progressive one. Because of the different effects in the different balls, in the presence of 4-AP the gradient in cycle length from ball A to ball E was reduced, although it was not eliminated (Fig. 6A). To quantify this, the cycle length was plotted against distance from the RSAB (see Fig. 1) assuming that the balls were 0.35 mm in diameter (not shown).
Linear regression showed that cycle length changed by 272 ms/mm \((P < 0.001)\) under control conditions but only by 38% of this \((102 \text{ ms/mm}; P < 0.001)\) in the presence of 4-AP.

The shortening of cycle length in balls of tissue from the center of the sinoatrial node by 4-AP is the effect expected of block of outward K\(_{1}\) current, but the increase in cycle length in balls of tissue from the periphery is unexpected. There is a poor correlation between the change in cycle length and the change in maximum diastolic potential \((r^2 = 0.13)\), and, therefore, the variation in the response of cycle length was unlikely to have been the result of the differing changes in maximum diastolic potential. The differential effects of 4-AP on cycle length in the different balls of tissue could be related to the changes in action potential duration. To test this, in Fig. 7A, the percent change in cycle length is plotted against the percent change in action potential duration. There is a good correlation between the two \((r^2 = 0.67)\), which shows that when there was a large increase in action potential duration there was also an increase in cycle length. An increase in action potential duration is expected to result in a concomitant increase in cycle length, and this could explain part of the correlation. To test this further, the diastolic interval was calculated (cycle length – action potential duration). For example, in ball A from the periphery, 4-AP increased the cycle length by 91 ± 8 ms \((n = 9 \text{ preparations})\), but the diastolic interval increased by only 28 ± 5 ms \((n = 9)\). Therefore, ~70% of the increase in cycle length in response to 4-AP in ball A is attributed to the increase in action potential duration. In contrast, in ball E from the center, 4-AP still prolonged the action potential (although by a small amount) and both the cycle length and diastolic interval were shortened (by 205 ± 23 and 243 ± 22 ms, respectively; \(n = 5\) preparations). In this case, the change in cycle length obviously cannot be explained by the change in action potential duration and is more likely to be a direct effect of 4-AP as considered in DISCUSSION.

Figure 7B shows the effect of 4-AP at a range of concentrations \((0.3, 1, \text{ and } 3 \text{ mM, as well as } 5 \text{ mM as used in previous experiments})\) on action potential duration in peripheral (balls A and B) and central (balls D and E) balls. Figure 7B shows that concentrations of
4-AP < 5 mM also resulted in a prolongation of the action potential, although the magnitude of the effect was reduced compared with that with 5 mM 4-AP. At 4-AP concentrations > 0.3 mM, the prolongation of the action potential was greater in the peripheral balls, as expected. The changes in the cycle length and maximum diastolic potential were qualitatively similar at lower concentrations, although at 0.3 and 1 mM there was no decrease in cycle length in central balls and there was little discernible change in the maximum diastolic potential in both peripheral and central balls.

Rather than a direct effect on sinoatrial node cells, it is possible that the effects of 4-AP are indirect as a result of an effect on the nerve fibers within the sinoatrial node. To test this, 10^{-6} M TTX was applied to inhibit nerve fibers in four balls of tissue, two peripheral balls (A) and two central balls (D). The effects of 5 mM 4-AP were similar in the absence and presence of TTX; for example, under control conditions 4-AP increased action potential duration by 56 ± 10%, whereas in the presence of TTX it increased it by 59 ± 16% (paired t-test, P = 0.68). It is concluded that the effects of 4-AP are not indirect.

Effect of 4-AP on small ball-like tissue preparations from different regions of sinoatrial node. Regional differences in superior-inferior direction. The action potential in the sinoatrial node not only varies from the periphery to the center, it also varies from the superior to the inferior region, although less is known about the variation in this direction. The superior sinoatrial node-inferior sinoatrial node differences are important because pacemaker shift almost invariably involves a shift in the superior or inferior direction (37). In two successful experiments, a strand from the transitional-central region of the sinoatrial node was made running parallel to the crista terminalis (i.e., running from the superior part to the inferior part of the sinoatrial node; Fig. 1). The strand was tied into a series of eight small ball-like tissue preparations, each ~0.3–0.4 mm in diameter. Figure 8 is taken from one of these experiments and shows that the response to 4-AP varied in the superior and inferior regions. Figure 8A shows the effect of 4-AP on the action potential from a ball from

Fig. 7. A: relationship between change in cycle length and change in APD on application of 5 mM 4-AP. Percent change in cycle length is plotted against percent change in APD. Same data as in Figs. 5 and 6. Solid line is regression line; dashed line shows zero change in cycle length. B: dose-response curves for 4-AP. Percent change in APD is plotted against 4-AP concentration ([4-AP]). Data from balls A and B; □, data from balls D and E. Means ± SE are shown (n = 4 or 5 preparations). Data are fitted with typical dose-response curves to guide the eye.

Fig. 8. Effect of 4-AP on action potential recorded from small balls of tissue from superior and inferior regions of sinoatrial node. Superimposed action potentials from a ball from superior region (A) and a ball from inferior region (B) under control conditions and after application of 5 mM 4-AP are shown. A strand of tissue was cut from transitional-central region of sinoatrial node, parallel to crista terminalis, and then tied into a series of 8 balls. Balls recorded from were 1st (superior region) and 7th (inferior region) in strand and were separated by ~3 mm. Dashed lines show 0 mV.
the superior region (1st ball, i.e., most superior in the strand), whereas Fig. 8B shows the effect on the action potential from a ball from the inferior region (7th ball in the strand). In both balls the action potential was prolonged, but the prolongation was greatest in the ball from the more inferior region. A similar result was obtained from the second experiment. To quantify this regional difference, strands 1–4 cut perpendicular to the crista terminalis as shown in Fig. 1 were used. Mean data for action potential duration are shown in Fig. 9, A and B (data for balls C and D are shown combined). Under control conditions there was a small (but not significant) increase in action potential duration from strand 1 from the more superior region to strand 4 from the more inferior region. In all balls, 4-AP caused a significant increase (P < 0.005) in action potential duration (Fig. 9A), but the percent increase was significantly greater (ANOVA on ranks, P = 0.002) in the balls from the more inferior region (Fig. 9B). In the presence of 4-AP there was a significant gradient (ANOVA, P < 0.001) in action potential duration from the more superior to the more inferior region, unlike under control conditions.

Figure 8 also shows the effect of 4-AP on cycle length. Cycle length was decreased in the superior ball, and it was increased in the inferior ball. Mean data are shown in Fig. 9, C and D. Under control conditions there was a small (but not significant) gradient in cycle length from strand 1 from the more superior region to strand 4 from the more inferior region. Panels C and D of Fig. 9 show that, on average, 4-AP caused a small (but not significant) decrease in cycle length in balls from strand 1 from the more superior region and a substantial and significant increase (P < 0.01) in cycle length in balls from strand 4 from the more inferior region. The variation of the effect of 4-AP on cycle length in the superior-inferior direction (Fig. 9D) is significant (ANOVA on ranks, P = 0.007).

Effect of 4-AP on intact sinoatrial node. In the intact sinoatrial node, the changes in the intrinsic pacemaker activity of different regions caused by 4-AP could result in pacemaker shift. The leading pacemaker site is the site showing the fastest pacemaker activity. Although inspection of Fig. 6A 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 transitional or central region (25). In the presence of 4-AP the intrinsic pacemaker activity of the peripheral and transitional regions is suppressed, whereas that in the center is accelerated (Fig. 6), and, therefore, the leading pacemaker site is expected to shift further toward the center (i.e., away from the atrial muscle of the crista terminalis). Furthermore, because 4-AP accelerates the intrinsic pacemaker activity of the more superior region but suppresses the intrinsic pacemaker activity of the more inferior region (Fig. 9), 4-AP is expected to shift the leading pacemaker site toward the superior region of the sinoatrial node. These predictions were tested in a series of eight experiments on the intact sinoatrial node.

Activation maps were constructed as described in METHODS under control conditions and after the application of 5 mM 4-AP for at least 40 min (to allow the preparation to reach a steady state). Although the small ball-like tissue preparations were exposed to 4-AP for a shorter time, our experience is that the intact sinoatrial node preparations always take longer to reach steady state after application of a drug than the small ball-like tissue preparations, presumably because of the more extensive tissue mass (see also Refs. 28, 35). A result is shown in Fig. 10. 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 0.7 mm from the RSARB (Fig. 10A); this is typical (1). After the application of 4-AP, there was no significant change in the cycle length (in 8 preparations, cycle length was 565 ± 30 ms under control conditions and 580 ± 29 ms in presence of 4-AP). After the application of 4-AP, the leading pacemaker site shifted in the superior direction and away from the crista terminalis (Fig. 10B). This is consistent with our predictions. In seven preparations, the leading pacemaker site shifted by 1.8 ± 0.3 mm. The shift was 1.5 ± 0.3 mm along the RSARB in the superior direction and 0.5 ± 0.3 mm away from the crista terminalis. In one other preparation, there was no pacemaker shift in the presence of 4-AP.

DISCUSSION

This study shows for the first time that 4-AP-sensitive current plays an important role in the electrical activity of the sinoatrial node and that this role varies from the periphery to the center and from the superior part of the sinoatrial node to the inferior part.

Viability of preparations used. The majority of the experiments in this study were carried out on strands of tissue divided into small balls of tissue (~0.3–0.4 mm in diameter) by ligatures. Several lines of evidence suggest that although the balls of tissue were small they were unlikely to be damaged significantly by the dissection procedure: 1) all balls studied showed stable spontaneous activity; 2) the regional differences in electrical activity of the small balls have been consistently observed in various studies in our laboratories in Japan and England (see, e.g., Refs. 28, 35) since we first reported them in 1985 (26); and 3) the electrical activity of the small balls is similar to the electrical activity recorded from similar sites in the intact sinoatrial node (26), and the electrical activity of the small balls prepared using ligatures is similar to that of small balls prepared by cutting (39).

Nature of 4-AP-sensitive current. Ito is blocked by 4-AP with EC50 values of 0.2–0.5 mM (6, 41). Kv4.2 and Kv4.3 channels are probably responsible for Ito, although it has been suggested that Kv1.4 is responsible (6, 10, 15). Ultrarapid delayed rectifier K+ currents (IK,ur) are also blocked by 4-AP, with EC50 values ranging from 5 µM to 1 mM (44). Kv1.5 and possibly Kv3.1 channels may be responsible for such currents (13, 14, 44). In sinoatrial node cells, 4-AP blocks a transient outward current (8, 33) and, in addition, a sustained outward current (4, 21). Here it is assumed that the sustained current is a nonactivating component of Ito. However, it is possible that it is a separate current (possibly an IK,ur). We have recently cloned a Kv4.2 channel from a rabbit sinoatrial node cDNA library, and the channel when expressed in Xenopus oocytes is similar (but not identical) to IK,ur in the rabbit sinoatrial node (E. Conley, J. Hancox, and M. R. Boyett, unpublished observations). In addition, using immunocytochemistry we have shown the presence of Kv1.5 in the guinea pig sinoatrial node (11). The concentration of 4-AP used in the present study is sufficient to block any of the currents above. The dose dependence of the effect of 4-AP on action potential duration (Fig. 7B) is comparable to that of Ito.

4-AP can affect other currents. In skeletal muscle, 4-AP blocks the ATP-sensitive K+ current (IK,ATP), with an EC50 value of 3.3 mM at 0 mV (7). However, IK,ATP is not expected to be present in the sinoatrial node under normal conditions. In sheep cardiac Purkinje fibers, 4-AP blocks the background inward rectifying K+ current (IK,1) (42), but this current is absent from the sinoatrial node (20). Finally, another K+ current, the muscarinic K+ current (IK,ACh), is reported to be activated by 4-AP (34). In our experiments on rabbit sinoatrial node cells, 4-AP only blocked a transient outward current and a sustained outward current during depolarizing pulses (4); it had no effect on the holding current at −60 mV (see also Ref. 21). This suggests that in our experiments IK,ATP, IK,1, and IK,ACh (if present) were not being affected, because at −60 mV (with a normal extracellular K+ concentration), IK,ATP, IK,1, and IK,ACh are expected to be large and a change in one of the currents should have resulted in a discernible change in whole cell current. If 4-AP does generally block IK,1, it is curious that a depolarization of the resting membrane is not a characteristic feature reported (see, e.g., Ref. 9). Furthermore, in our experiments there was no 4-AP-sensitive tail current after depolarizing pulses (4); this confirms that 4-AP did not block the rapid or slow delayed rectifying K+ currents (IK,A and IK,S, respectively). Finally, 4-AP has been shown to affect the hyperpolarization-activated current Ii (42); it shifts the Ii activation curve in the depolarizing direction, although it also partly blocks the current. This effect of Ii is expected to hasten pacemaker activity and perhaps to cause a decrease in the maximum diastolic potential but to have no effect on action potential duration.
Role of 4-AP-sensitive current in sinoatrial node. In atrial, Purkinje, and ventricular tissue, block of 4-AP-sensitive current can greatly slow the initial rapid phase of repolarization (phase 1) after the action potential peak, abolish the action potential notch, elevate the action potential plateau, and prolong the action potential (3, 9, 17). Block of \( I_{\text{to}} \) by 4-AP can explain all of the actions of 4-AP. However, block of \( I_{\text{Kur}} \) alone is known to produce a prolongation of the action potential, and, therefore, block of \( I_{\text{Kur}} \) could contribute to the prolongation of the action potential in the presence of 4-AP (see, e.g., Ref. 44). In the present study, 4-AP produced all of the above effects in the sinoatrial node: it slowed phase 1 repolarization and abolished the action potential notch (if present), elevated the plateau, and prolonged the action potential (Figs. 2-5). Like previous authors, we suggest that these 4-AP-dependent changes are the result of the block of \( I_{\text{to}} \) (and possibly \( I_{\text{Kur}} \)); they cannot be the result of a change in \( I_{\text{KATP}} \), \( I_{\text{K1}} \), \( I_{\text{ach}} \), or \( I_{\text{f}} \) for the reasons discussed above. The results obtained suggest that the 4-AP-sensitive current plays a more important role in the periphery of the sinoatrial node, because the notch and its abolition by 4-AP were only observed in the periphery (Fig. 3), there was only a significant increase in the action potential overshoot by 4-AP in the periphery (Fig. 4A), and the increase in action potential duration was significantly greater in the periphery (Fig. 5B). There are several reasons why the effects of 4-AP were greater in the periphery. First, the density of \( I_{\text{to}} \) may be greater in the periphery. We have previously shown (4) that the density of the sustained component of \( I_{\text{to}} \) is greater in larger sinoatrial node cells presumably from the periphery of the sinoatrial node. Second, because the diastolic potentials are more negative in the periphery of the sinoatrial node compared with those in the center, the voltage-dependent inactivation of \( I_{\text{to}} \) during diastole is expected to be less in the periphery (and, consequently, greater \( I_{\text{to}} \) will be activated during the action potential in the periphery). There is another reason why the action potential notch may only be evident in the periphery. In the center, \( I_{\text{to}} \) may activate during the slow upstroke of the action potential and, therefore, activation of the current will not lead to a notch, whereas in the periphery activation of \( I_{\text{to}} \) will follow the rapid upstroke and may lead to a notch.

The results obtained also suggest that 4-AP-sensitive current plays a greater role in the inferior part of the sinoatrial node, because the prolongation of the action potential caused by 4-AP was greater in the inferior part compared with that in the superior part (Fig. 9). If the peripheral-central and superior-inferior differences are caused by differences in the expression of an ion channel, then the regional differences in ion channel expression are complex.

In the sinoatrial node, in addition to the classical effects of 4-AP, 4-AP also affected the maximum diastolic potential and pacemaking (Figs. 2, 4B, and 6). Generally, 4-AP decreased the maximum diastolic potential (Figs. 2 and 4B). The decrease suggests that 4-AP-sensitive current contributes to the maximum diastolic potential. In sinoatrial node cells, the activation threshold of \( I_{\text{to}} \) is about \(-70 \text{ mV} \) and, therefore, it is possible that there will be activation of 4-AP-sensitive current during diastole (4). Figure 4B shows that the decrease in the maximum diastolic potential was greater in the periphery of the sinoatrial node; this is consistent with the other evidence summarized above showing that the role of 4-AP-sensitive current is greater in the periphery. However, another explanation of the depolarization is that it is the result of the 4-AP-induced depolarizing shift in the \( I_{\text{f}} \) activation curve (42).

In some central balls, 4-AP increased the maximum diastolic potential (Fig. 2B). This is perhaps an indirect consequence of the block of the 4-AP-sensitive current; the elevation and prolongation of the plateau caused by 4-AP is expected to enhance the activation of delayed rectifying K\(^+\) currents (\( I_{\text{Kf}} \) and \( I_{\text{Kr}} \)), which in turn will increase the maximum diastolic potential. The increase in maximum diastolic potential was only observed in central balls, in which the increase in action potential duration was small; perhaps if the role of the 4-AP-sensitive current is slight (manifested as a small increase in action potential duration), the direct effect of the decrease in 4-AP-sensitive current on the maximum diastolic potential is slight and the indirect effect of the increase in the delayed rectifying K\(^+\) currents dominates.

In the central balls, 4-AP accelerated pacemaker activity (Figs. 2 and 6), and Figs. 2B and 8A show that this was the result of an acceleration of the pacemaker potential. This could be a direct effect of the block of 4-AP-sensitive current flowing during diastole, the evidence for which is considered above. If correct, this shows that 4-AP-sensitive current helps control the pacemaker potential and pacemaker activity. Alternatively, the acceleration in rate could again be the result of the 4-AP-induced depolarizing shift in the \( I_{\text{f}} \) activation curve (42). In some central balls, 4-AP resulted in an increase of the maximum diastolic potential, whereas in the majority it resulted in a decrease; the change in cycle length in the central balls was not correlated with the change in maximum diastolic potential.

If the role of 4-AP-sensitive current is greater in the periphery, the acceleration of pacemaker activity by 4-AP may be expected to be greater in the periphery, whereas the reverse was true. In peripheral balls, 4-AP prolonged the cycle length rather than shortening it (Figs. 2A and 6). The prolongation of the cycle length is likely to be another indirect effect of 4-AP; as shown in Effect of 4-AP on small ball-like tissue preparations from different regions of sinoatrial node. Regional differences from periphery to center (see Fig. 7A and related text), much of this was the result of the marked increase in action potential duration in the peripheral balls.

Physiological importance of 4-AP-sensitive current and its region dependence. From the center of the sinoatrial node through the periphery of the node to the atrial muscle there is a gradient in action potential duration, with the action potential in the center being
the longest and the action potential in the atrial muscle being the shortest. This gradient is observed in the intact sinoatrial node (35) and also in the small balls of sinoatrial node tissue (Fig. 5A; see also Ref. 28). We suggest that this is a protective mechanism. Because the action potentials more distant on the conduction path are shorter than those earlier in the conduction path, repolarization occurs in the same direction as depolarization and occurs last in the center of the sinoatrial node. This will prevent reexcitation arrhythmias by preventing reexcitation of the sinoatrial node by the atrial muscle. A similar gradient in action potential duration prevents reexcitation at other points in the excitation pathway: of the atrial muscle of the crista terminalis by the atrial muscle of the atrial appendage (43), of the Purkinje fibers by the ventricular muscle, and of the ventricular subendocardium by the ventricular subepicardium. Figure 5A shows that the gradient in action potential duration from the center to the periphery was abolished by 4-AP, and therefore, the 4-AP-sensitive current must be responsible for it. It is interesting that Ito is responsible for other region-dependent differences in the action potential (9, 30, 43) and contributes to remodeling of electrical activity in development (22), hypertrophy (see, e.g., Ref. 31), heart failure (23), and the phenomenon of cardiac memory (16).

Figure 6A shows that 4-AP reduced the regional difference in pacemaker activity (difference in cycle length in balls A and E is smaller in presence of 4-AP). Regardless of the reasons for the changes in cycle length in the presence of 4-AP (see Role of 4-AP-sensitive current in sinoatrial node for a discussion of possibilities), the result shows that 4-AP-sensitive current, as well as I, and INa (see Regional differences in role of membrane currents in sinoatrial node), must be responsible for the regional differences in pacemaker activity. The regional differences in pacemaking are expected to be important physiologically, because they will make the sinoatrial node more robust. An intervention that may adversely affect one region may be better tolerated by another. Ito is modified by a variety of different interventions, e.g., α-adrenergic agonists (5). Because our data show that 4-AP-sensitive current is able to alter, directly or indirectly, pacemaker activity (Fig. 6), it is possible that these interventions may be able to alter pacemaker activity via an effect on Ito.

The maximum diastolic potential is greater in the periphery of the sinoatrial node, and Fig. 4B shows that 4-AP-sensitive current is involved in the difference, because the difference was smaller in the presence of 4-AP.

Regional differences in role of membrane currents in sinoatrial node. The greater importance of 4-AP-sensitive current in the periphery compared with the center of the sinoatrial node is only one of a number of regional differences in membrane currents to emerge. Above it is argued that pacemaking is faster in the periphery than in the center partly because the action potential is shorter in the periphery (because of 4-AP-sensitive current). However, there are at least two other reasons for the faster pacemaking in the periphery. 1) I, plays a greater role in pacemaking in the periphery, possibly as a result of a greater density of I, (29, 35). 2) Although the Ca²⁺-current (ICa) is involved in pacemaking in the center, the TTX-sensitive INa is involved in the periphery, possibly because INa is absent in the center but is large in the periphery (19). (ICa and INa are involved in pacemaking by triggering the action potential, and pacemaking is faster when INa is responsible because the threshold for INa is more negative than that of ICa.) Because INa is present in the periphery but not the center, the action potential upstroke is faster in the periphery than in the center. Guo et al. (18) reported that the sustained inward current (Ist) is present in central sinoatrial node cells but is absent from peripheral cells; the significance of this is not known. Finally, partial block of the rapid delayed rectifying K⁺-current (IKr) by E-4031 has greater effects on the center of the sinoatrial node (I. Kodama, M. R. Boyett, M. R. Niknaram, H. Honjo, and R. Suzuki, unpublished observations); this suggests that the density of IKr may be less in the center (and, therefore, the center is more sensitive to partial block of the current), and, if correct, this difference in IKr would also contribute to the regional difference in action potential duration and maximum diastolic potential. All of the published information concerning regional differences in membrane currents within the sinoatrial node is concerned with differences between the periphery and center. Evidence for a greater role of 4-AP-sensitive current in the inferior part of the sinoatrial node from the present study is the only information about differences in the superior-inferior direction, although we have previously shown (27) that the response to vagal stimulation not only varies in the peripheral-central direction but also varies in the superior-inferior direction.

Although reported space constants of the rabbit sinoatrial node are variable (12), Bonke (2) reported a mean value of 465 μm. This is small relative to the size of the sinoatrial node of the rabbit (see, e.g., Fig. 1). Therefore, the regional differences seen in small balls of tissue are also expected to be seen in the intact sinoatrial node, and this is indeed the case (see, e.g., Ref. 1).

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REFERENCES


