Chronic atrial fibrillation does not further decrease outward currents. It increases them.

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Chronic atrial fibrillation does not further decrease outward currents. It increases them. Am J Physiol Heart Circ Physiol 285: H1378–H1384, 2003; 10.1152/ajpheart.00137.2003.—Rapid atrial pacing causes electrical remodeling that leads to atrial fibrillation (AF). AF can further remodel atrial electrophysiology to maintain AF. Our previous studies showed that there was a marked difference in the duration of AF in dogs that have been atrial paced at 400 beats/min for 6 wk. We hypothesized that this difference is based on the changes in the degree of electrical remodeling caused by rapid atrial pacing versus that by AF. Right atrial cells were isolated from control dogs (Con, N = 28), from dogs with chronic AF (cAF dogs, N = 13, episodes lasting at least 6 days), or from dogs with nonsustained or brief episodes of AF (nAF dogs, N = 10, episodes lasting minutes to hours). Both transient outward (I_{to}) and sustained outward K\(^+\) current (I_{sou}) densities/functions were determined using whole cell voltage-clamp techniques. In nAF cells, I_{to} density was reduced by 69% at +40 mV; from 7.1 ± 0.5 pA/pF (Con, n = 59) to 2.2 ± 0.2 pA/pF (nAF, n = 24) (P < 0.05). The voltage dependence of inactivation of I_{to} was shifted positively and decay kinetics were changed; however, recovery from inactivation was not altered in nAF cells. In contrast, I_{to} density in cAF cells was both significantly different from Con cells and larger than that in nAF cells [at +40 mV, 3.5 ± 0.3 pA/pF (cAF, n = 29), P < 0.05]. In cAF cells, recovery from inactivation and decay of I_{to} were both slow; yet, voltage dependence inactivation of I_{to} approached that of Con cells. Furthermore, “recovered” I_{to} of cAF cells was more sensitive to tetraethylammonium than currents of Con and nAF cells. I_{sou} densities of nAF and cAF cells did not differ. Both nAF and cAF cells have reduced I_{to} versus Con cells, but I_{sou} remodeling of nAF cells differed from that of cAF cells. I_{to} in cAF dogs was likely remodeled by AF per se, whereas that in nAF dogs was likely the consequence of the rapid rate in the absence of sustained AF.

**METHODS**

**Animal preparation.** This investigation conforms with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Pub. No. 85-23, Revised 1996).

Adult mongrel dogs weighing 20–25 kg were anesthetized with thiopental sodium (17 mg/kg iv) and ventilated with 1.5–2% isoflurane and 2 l/min O\(_2\). Morphine sulfate (0.15 mg/kg) was injected into the epidural space to reduce the pain after dogs awakened from anesthesia. With the use of sterile techniques, Medtronic active fixation leads were attached to the RA appendage and right ventricular free wall, tunneled subcutaneously, and then connected to a Medtronic Thera 8962 pacemaker (Minneapolis, MN). A bipolar stimulating and recording electrode was also attached to the RA appendage for the induction of AF. Complete atrioventricular conduction block was produced by injection of 0.1–0.3 ml of 40% formaldehyde into the His bundle, usually resulting in an idioventricular escape rhythm of 30–50 beats/min. The ventricular pacemaker was programmed as follows: rate, 60 beats/min (and held at 60 beats/min throughout the pacing

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protocol; pulse amplitude, 3.3–5 V; pulse width, 0.35–0.5 ms; sensitivity, 2.5 V; and refractory period, 300 ms. The dogs were given cefazolin (25 mg/kg im) prophylactically once before surgery and for 2 days after surgery. After recovery for at least 2 wk, atrial pacing was instituted (rate, 400–900 beats/min; amplitude, 2.5–4 V; pulse width, 0.2–0.4 ms; Itrrel 7424 or MINIX 8340) and maintained for 5–7 wk. At the beginning of pacing, there was no difference in RA ERP in dogs from the two different groups (RA ERP, S1-S1 = 400 ms; nAF 143 ± 12 ms vs. cAF 132 ± 8 ms, P > 0.05). Each dog was monitored intermittently in the laboratory every 3–5 days and for several hours each time.

At the time of terminal study, dogs were anesthetized with pentobarbital (30 mg/kg) and the hearts were removed. Only sections of the RA free wall were excised for myocyte studies to eliminate the heterogeneity in ion channel function that has been reported for normal canine atria (5). RA trabeculae were removed from adjacent tissue for cellular electrophysiological studies (9).

Three groups of dogs were studied. Dogs in nAF (N = 10) had been paced, but when AF was induced, it was short lived. A nAF dog was defined as an animal that had AF <6 days duration (usually <24 h). nAF animals could have multiple episodes of AF. Pacemakers of nAF dogs were stopped on the day of death. The second group of dogs was those in cAF (N = 13). These dogs had been paced as above, but when AF occurred, it persisted for at least 6 days. cAF dogs were killed during AF. Age-matched animals (2–5 yr, N = 28) were used for control (Con) RA free wall cells.

**Myocyte preparation.** Single calcium-tolerant atrial cells were dispersed from the RA sections using our previously described method (17). Briefly, the tissue was rinsed twice in a Ca2+-free solution containing (in mM) 115 NaCl, 5 KCl, 35 sucrose, 10 dextrose, 10 HEPES, and 4 taurine (pH 6.95) to remove blood. It was then trituated in 20 ml of enzyme-containing solution (collagenase type II from Worthington Biochemical, 0.13 mg/ml, 36–37°C) for 30 min, after which the solution was decanted and discarded. The second trituration was discarded after 30 min. The next six to seven triturations were each done for 15 min. Each time, the solution was centrifuged at 500 rpm for 3 min to collect the supernatant and dispersed cells. Resuspension solution was changed every 30 min for solutions containing increasing concentrations of Ca2+ (from 0 to 0.5 mM). With this procedure, the living atrial cell yield was ~30–40%. Only rod-shaped cells with staircase ends, clear cross-striations, and surface membranes free from blebs were used for study.

**Whole cell voltage-clamp recording.** Ito and Isus were recorded using the whole cell configuration of voltage-clamp techniques. Borosilicate glass microelectrodes (outer diameter, 1.5 mm) had resistances from 1 to 2 MΩ when filled with the internal solution containing (in mM) 140 KCl, 1 MgCl2, 10 EGTA, 5 MgATP, 5 creatine phosphate, 0.2 GTP, and 10 HEPES (pH 7.2) with KOH. To measure Ito, atrial cells were externally superfused with a Na+-free solution containing (in mM) 144 N-methyl-D-glucamine-Cl, 5.4 KCl, 1 MgCl2, 2.5 CaCl2, 0.5 CdCl2, and 10 HEPES (pH 7.4) at 30–31°C. Na+ currents were suppressed with the use of this Na+-free solution, and Ica,l was blocked with 0.5 mM Cd2+. Membrane currents associated with Na+/Ca2+ exchange were eliminated by the absence of external Na+. Ito was elicited by a 210-mA test pulse to 400 ms; a holding potential of −60 mV at 0.1 Hz after a 10-ms prepulse to −90 mV. The amplitude of Ito was determined as the difference between the peak of Ito and the current level at the end of the pulse or as noted. Isus was taken as the amplitude of the current at the end of the test pulse relative to the zero-current level (7, 12). Ito was fit with a double- and/or single-exponential function to estimate the time constants of decay. The steady-state inactivation relationship was determined using a double-pulse protocol: a 500-ms prepulse to various conditioning potentials (Vc) between −90 to +20 mV, followed by a 210-ms test pulse to +60 mV. Peak current elicited at each test pulse was expressed as a fraction of the current at Vc = −90 mV. A Boltzmann equation was used to fit normalized data to obtain the half-maximal voltage and slope factor for each cell. The time course of recovery from inactivation was evaluated using a paired-pulse protocol: two identical 210-ms pulses from a holding potential of −80 to +40 mV were delivered with increasing interpulse coupling intervals (IPI) from 5 to 5,000 ms. The degree of recovery at each IPI was determined by normalizing Ito at each IPI by the Ito at IPI = 5,000 ms. The time course of recovery was estimated by fitting data to a biexponential function using a simplex algorithm.

Ito and Isus were normalized by the membrane capacitance of each cell (in pF) and expressed as current density (pA/pF).

Average cell capacitance was 50 ± 1.9 pF in Con cells (n = 21), 60 ± 6.5 pF in nAF cells (n = 13, P < 0.05 vs. Con), and 123 ± 6.1 pF in cAF cells (n = 45, P < 0.05 vs. Con and nAF).

**Statistics.** Group data are presented as means ± SE; N is the number of dogs and n is the number of cells. Statistical comparisons between groups were made using ANOVA/Bonferroni’s or a Student’s t-test. P < 0.05 was considered significant.

**RESULTS**

**Effects of persistence of AF on Ito and Isus.** Representative original current tracings of Ito and Isus from canine RA cells from Con, nAF, and cAF dogs are shown in Fig. 1, A–C. Data were obtained using the pulse protocol shown in Fig. 1D, inset. Figure 1, D and E, shows average Ito and Isus density-voltage relations for Con cells (n = 59), nAF cells (n = 24), and cAF cells (n = 29). Ito density was significantly reduced in nAF and cAF cells. For example, at +40 mV, Ito averaged 7.1 ± 0.3 pA/pF in Con cells compared with 2.2 ± 0.2 pA/pF in nAF cells (P < 0.05 vs. Con) and 3.5 ± 0.3 pA/pF in cAF cells (P < 0.05 vs. Con). Interestingly, Ito density in cAF cells was significantly greater than that of nAF cells (P < 0.05). In contrast, Isus densities were similar in all three groups. At +40 mV, mean current densities were 3.9 ± 0.2 pA/pF in Con cells, 4.3 ± 0.7 pA/pF in nAF cells, and 4.4 ± 0.4 pA/pF in cAF cells.

To evaluate possible mechanisms involved in the AF-related changes in Ito, voltage-dependent and kinetic properties were determined and compared. A double-pulse protocol was used to assess the voltage dependence of inactivation of Ito. The results are illustrated in Fig. 2. Figure 2, A–C, displays original current tracings from RA cells isolated from Con, nAF, and cAF dogs. Figure 2D shows the average “steady-state” inactivation relations of Ito in Con, nAF, and cAF cells. In both nAF and cAF cells, curves were shifted to more positive voltages versus Con. Mean values for half-maximum inactivation voltage were −34.9 ± 1.3, −21.6 ± 1.9, and −29.1 ± 1.9 mV in Con, nAF, and cAF cells, respectively (P < 0.05, nAF vs. cAF). The slope factor averaged −7.3 ± 0.3, −11.0 ± 1.2, and −11.7 ± 0.9 mV in Con, nAF, and cAF cells (P < 0.05).
$I_{to}$ decay was analyzed by curve-fitting data obtained at the +40-mV test pulse (protocol in Fig. 1D, inset). Figure 3, A–C, shows the current tracings from Con, nAF, and cAF RA cells. Seventy-six percent of Con cell data was fit using a biexponential function. In contrast, 88% of nAF cells were best fit using a monoexponential function. However, 54% of cAF cell data was fit using biexponential functions, suggesting that the additional
remodeling of the \( I_{to} \) channel is heterogeneous in cAF cells. Compared with Con cells, cAF cells showed a significant increase in the slow time constant (\( \tau_s \)) of decay of \( I_{to} \) (\( P < 0.05 \)), with no change in the fast time constant (\( \tau_f \)) (Fig. 3D). Compared with the \( I_{to} \) decay of nAF cells, the decay in cAF cells was faster than that in nAF cells (\( P < 0.05 \)). Thus, while nAF and cAF RA cells have a decrease of overall \( I_{to} \), the kinetics of \( I_{to} \) decay differ in the two cell groups. Recovery from inactivation was studied using a double-pulse protocol (Fig. 4). A biexponential function provided best fit of data describing the recovery kinetics of \( I_{to} \). \( \tau_f \) of recovery were similar in all cell groups, but \( \tau_s \) differed (Table 1). Thus a slow, second component of recovery from inactivation may contribute to the “recovered” \( I_{to} \) of cAF cells.

**Effects of TEA on outward currents in Con, nAF, and cAF cells.** The effects of TEA were determined in a subset of cells from each group using two protocols. In the initial constant pacing protocol (holding potential = \(-60\) to \(+40\) mV, every 15 s; see Fig. 5, A and B, inset), 5 mM TEA (3–4 min) inhibited 13.2 ± 2.4%, 19.3 ± 6.4%, and 42.5 ± 7.8% of drug-free \( I_{to} \) at \(+40\) mV in Con, nAF, and cAF cells, respectively (\( P < 0.05 \), Con vs. cAF and nAF vs. cAF). Furthermore, when the magnitudes of the TEA-sensitive currents were compared, cAF cells had \( I_{to} \) and \( I_{sus} \) components larger than those in nAF and Con cells (Fig. 5, A and B). Second, from cells where current-voltage protocols (see Fig. 1) were completed in both the absence and presence of TEA (Fig. 5, C–E), TEA-insensitive currents were measured and compared. Figure 6 shows that \( I_{to} \) remaining in the presence of TEA (TEA-insensitive currents) did not differ between nAF and cAF cells, but nAF \( I_{sus} \) remained significantly reduced versus Con cells. Thus TEA-sensitive transient/sustained currents

<table>
<thead>
<tr>
<th>( \tau_f, \text{ms} )</th>
<th>( \tau_s, \text{ms} )</th>
<th>( A_f, % )</th>
<th>( I_{to,\text{max}}, \text{pA/pF} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con 47.7 ± 5.3</td>
<td>326.5 ± 38.8</td>
<td>40.7 ± 6.2</td>
<td>9.21 ± 0.95</td>
</tr>
<tr>
<td>nAF 41.7 ± 9.7</td>
<td>598.5 ± 88.1</td>
<td>46.1 ± 6.9</td>
<td>3.23 ± 0.82*</td>
</tr>
<tr>
<td>cAF 69.8 ± 8.5</td>
<td>1,433.2 ± 282.5*</td>
<td>53.1 ± 12.0</td>
<td>4.33 ± 0.46*</td>
</tr>
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Values are means ± SE. \( \tau_f \) and \( \tau_s \), average fast and slow time constants, respectively, of best fits of recovery curves for cells in each group; \( A_f \%), amplitude of the slow-component time constant normalized to total amplitude; \( I_{to,\text{max}} \), transient outward \( K^+ \) current (\( I_{to} \)) density at interpulse coupling interval = 5,000 ms; cAF, chronic atrial fibrillation. *\( P < 0.05 \) vs. control (Con); †\( P < 0.05 \) vs. nonsustained atrial fibrillation (nAF).
contribute greatly to the increase in outward currents of RA cells from cAF animals.

**DISCUSSION**

We have shown that the degree of remodeling of $I_{to}$ is related to AF duration in dogs. Similar to others (18), we show that after a period of rapid atrial pacing, brief episodes of AF (defined here as nAF) are accompanied by a marked decrease in $I_{to}$ density. Furthermore, we show that this is accompanied by changes in the voltage-dependent and kinetic properties of $I_{to}$. However, in animals with long-term episodes of AF (cAF ani-
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mals), Ito density did not further decrease; rather, it increased. Moreover, the voltage-dependent and kinetic properties of this “recovered Ito” in cAF cells differed from that of nAF and Con cells. Our results of Ito changes in cAF cells are consistent with human AF-induced remodeling of K⁺ currents (2) and smaller than those of Refs. 3 and 12. In contrast, Isus density did not differ among the three groups, similar to the findings of Yue et al. (18). In human AF, some groups reported no changes in Isus in AF (3, 15), whereas others have shown a reduction in Isus (12, 2).

The mechanisms by which Ito changes with rapid atrial pacing or sustained AF are presently unknown. Interestingly, cell capacitance increased by 54% with the duration of AF, suggesting that modest RA hypertrophy occurred in cAF animals. In ventricular cells, a decrease in Ito is associated with hypertrophy (but see Refs. 11 and 13). However, cAF cells with large capacitances did not show a further decrease in Ito, but, unexpectedly, an increase over that in nAF cells. In cAF cells, it is likely that both a significantly long time course of Ito recovery from inactivation and slow decay of peak Ito contributed to the observed reduced Ito versus Con cells. Thus not only might the number of channels that comprise the composite Ito decrease, but the fundamental nature of channels contributing to Ito changes in the RA cells of the cAF dog. Yue et al. (18) found a downregulation of Kv4.3 mRNA and protein levels in their canine rapid paced model, which is similar to the nAF model in this study. Because no voltage-dependent or kinetic properties of remaining Ito were reported, they concluded that a decrease in the number of Ito channels contributed to the reduction in Ito density in nAF dogs. In the presence of TEA, we found there to be no difference between Ito in nAF and cAF animals (Fig. 6). Yet we report here that in cAF RA cells, composite Ito is increased in density over nAF cells. This is most likely due to an increase in TEA-sensitive outward currents in cAF cells (Fig. 5). Note that Isus is small in our Con RA cells. This is dissimilar to the findings of others (5, 18, 19), where a prominent current, Ikurđ (−8 pA/pF at +40 mV), was defined. Notably, Yue et al. (18) reported that Ikurđ did not vary in their rapid paced AF model. We show in this report that, whereas Isus did not differ between Con, nAF, and cAF cells, TEA-insensitive currents did.

The nature of the augmented TEA-sensitive current in cAF cells was not the focus of this study. However, because of its TEA sensitivity and the time course of these currents, it may be that in cAF cells where TEA-insensitive Ito is reduced, there is an adaptive augmentation of currents through Kv2 or Kv3 K⁺ channel proteins. In recent studies using mice genetically modified such that certain K⁺ channels are functionally knocked out or suppressed [e.g., Kv1DN mice (20) and Kv.DN2 mice (1)], the TEA-sensitive current component encoded by Kv2.1, IKslow2 (16), is upregulated (8, 20).

This is the first report of the effects of long-term AF on Ito and Isus in the rapid atrial pacing dog model. The process of Ito remodeling during persistent AF as seen in this study suggests that the chronic electrical remodeling changes may facilitate the persistence of AF. Combined with our previous study (17) showing differences in inward currents in RA cells from nAF and cAF dogs, we suggest that pharmacological agents effective in terminating nAF may differ from those effective in sustained cAF.

Limitations. Not all currents were evaluated in this report. In particular, the relative contribution of other time-dependent and -independent currents to Isus were not studied. Whereas Isus does not differ among cells from different groups, individual components of Isus may. Furthermore, we have not included changes in ion channel function in cells from other regions of the remodeled atria. Finally, within the cAF group, animals had AF of variable durations, but all were >6 days. At this time, we did not subgroup the cAF group by duration but rather focused this study on differences between nAF and cAF animals.

DISCLOSURES

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delayed rectifier K currents in dogs with chronic complete atrio-