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_{sust}) 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_{sust} densities of nAF and cAF cells did not differ. Both nAF and cAF cells have reduced I_{to} versus Con cells, but I_{sust} remodeling of nAF cells differed from that of cAF cells. I_{sust} 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.

ATRIAL FIBRILLATION (AF) is the most common chronic cardiac arrhythmia in human subjects. The canine/goat rapid pacing-induced model has been widely used to study AF, given its structural and functional similarities to long-lasting AF in patients (4, 10, 14). Nattel and colleagues (6, 18), using the rapid paced canine model, have reported that the extent of atrial ion channel remodeling correlated with the duration of rapid atrial pacing. That is, with an increment of the duration of rapid pacing, Na^+ current, L-type Ca^{2+} current (I_{Ca,L}), and transient outward K^+ current (I_{to}) progressively decreased with no detectable changes in kinetic properties. In these studies, the remodeled atrial cells had shortened action potential (AP) durations (APDs) and effective refractory periods (ERPs), and animals had nonsustained (<45 min) episodes of AF.

We have shown that similar to the APs of dogs that are paced but have nonsustained AF (nAF), APs of right atrial (RA) fibers from dogs with chronic AF (cAF; >6 days) are also reduced in duration (9). Importantly, although the AP phenotype is reasonably similar in nAF versus cAF RA cells (9), there are significant differences in the remodeling I_{Ca,L} between cells from nAF and cAF dogs (17). This suggests to us that persistence of AF involves a remodeling process different from that resulting from rapid atrial pacing.

Therefore, the purpose of this study was to determine whether I_{to} and sustained outward K^+ current (I_{sust}) of RA cells from nAF dogs are altered and whether any further changes occur in RA cells from cAF dogs.

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; Itrel 7424 or MINIX 8340) and maintained for 5–7 wk. At the beginning of pacing, there was no difference in 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–ms voltage step to test potentials (Vt) from –60 mV to –110 mV was delivered with increasing interpulse coupling intervals (IPI) from 5 to 5,000 ms. The degree of recovery at each IPI was determined by normalizing Itot at each IPI by the Itot at IPI = 5,000 ms. The time course of recovery was estimated by fitting data to a biexponential function using a simplex algorithm.

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

RESULTS

Effects of persistence of AF on Itot and Isus: Representational current traces of Itot 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 Itot and Isus density–voltage relations for Con cells (n = 59), nAF cells (n = 24), and cAF cells (n = 29). Itot density was significantly reduced in nAF and cAF cells. For example, at +40 mV, Itot 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, Itot 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 Itot, voltage-dependent and kinetic properties were determined and compared. A double-pulse protocol was used to assess the voltage dependence of inactivation of Itot. 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 Itot 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 $11.3 \pm 2.4\%$, $19.3 \pm 6.4\%$, and $42.5 \pm 7.8\%$ 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 $I_{sus}$ component 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

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**Table 1. Time course of recovery from inactivation of $I_{to}$**

<table>
<thead>
<tr>
<th>Group</th>
<th>$\tau_f$, ms</th>
<th>$\tau_s$, ms</th>
<th>$A_s$%</th>
<th>$I_{to,max}$, pA/pF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con</td>
<td>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</td>
<td>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</td>
<td>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>
</tbody>
</table>

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; $A_s$%, amplitude of the slow-component time constant normalized to total amplitude; $I_{to,max}$, 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-

Fig. 5. Effects of tetraethylammonium (TEA; 5 mM) on $I_{to}$ and $I_{sus}$. A and B: TEA-sensitive currents at $+40$ mV determined from a constant paced protocol (inset) in a subset of Con ($n = 14; N = 10$), nAF ($n = 8; N = 4$), and cAF ($n = 8; N = 4$) cells. *$P < 0.05$ vs. Con; +$P < 0.05$ vs. nAF. C–E: original current recordings of a Con (C), nAF (D), and cAF (E) cell in the absence (before TEA; top) and presence of TEA (+TEA; middle) and TEA-sensitive currents (bottom).

Fig. 6. $I_{to}$-V (A) and $I_{sus}$-V (B) in the presence of TEA for a subset of Con, nAF, and cAF cells. The voltage-clamp protocol used was similar to that shown in Fig. 1. *$P < 0.05$ vs. Con.
mals), $I_{to}$ density did not further decrease; rather, it increased. Moreover, the voltage-dependent and kinetic properties of this “recovered $I_{to}$” in cAF cells differed from that of nAF and Con cells. Our results of $I_{to}$ 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, $I_{sus}$ 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 $I_{sus}$ in AF (3, 15), whereas others have shown a reduction in $I_{sus}$ (12, 2).

The mechanisms by which $I_{to}$ 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 $I_{to}$ is associated with hypertrophy (but see Refs. 11 and 13). However, cAF cells with large capacitances did not show a further decrease in $I_{to}$ but, unexpectedly, an increase over that in nAF cells. In cAF cells, it is likely that both a significantly long time course of $I_{to}$ recovery from inactivation and slow decay of peak $I_{to}$ contributed to the observed reduced $I_{to}$ versus Con cells. Thus not only might the number of channels that comprise the composite $I_{to}$ decrease, but the fundamental nature of channels contributing to $I_{to}$ 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 $I_{to}$ were reported, they concluded that a decrease in the number of $I_{to}$ channels contributed to the reduction in $I_{to}$ density in nAF dogs. In the presence of TEA, we found there to be no difference between $I_{to}$ in nAF and cAF animals (Fig. 6). Yet we report here that in cAF RA cells, composite $I_{to}$ 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 $I_{sus}$ is small in our Con RA cells. This is dissimilar to the findings of others (5, 18, 19), where a prominent current, $I_{k,surt}$ (~8 pA/pF at +40mV), was defined. Notably, Yue et al. (18) reported that $I_{k,surt}$ did not vary in their rapid paced AF model. We show in this report that, whereas $I_{sus}$ 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 $I_{to}$ 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, $I_{Klow}$ (16), is upregulated (8, 20).

This is the first report of the effects of long-term AF on $I_{to}$ and $I_{sus}$ in the rapid atrial pacing dog model. The process of $I_{to}$ 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 $I_{sus}$ were not studied. Whereas $I_{sus}$ does not differ among cells from different groups, individual components of $I_{sus}$ 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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