The G Protein–Gated Potassium Current $I_{K,ACh}$ Is Constitutively Active in Patients With Chronic Atrial Fibrillation

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**Background**—The molecular mechanism of increased background inward rectifier current ($I_{K1}$) in atrial fibrillation (AF) is not fully understood. We tested whether constitutively active acetylcholine (ACh)-activated $I_{K,ACh}$ contributes to enhanced basal conductance in chronic AF (cAF).

**Methods and Results**—Whole-cell and single-channel currents were measured with standard voltage-clamp techniques in atrial myocytes from patients with sinus rhythm (SR) and cAF. The selective $I_{K,ACh}$ blocker tertiapin was used for inhibition of $I_{K,ACh}$. Whole-cell basal current was larger in cAF than in SR, whereas carbachol (CCh)-activated $I_{K,ACh}$ was lower in cAF than in SR. Tertiapin (0.1 to 100 nmol/L) reduced $I_{K,ACh}$ in a concentration-dependent manner with greater potency in cAF than in SR ($-\log IC_{50}$; 9.1 versus 8.2; $P<0.05$). Basal current contained a tertiapin-sensitive component that was larger in cAF than in SR (tertiapin [10 nmol/L]-sensitive current at $-100$ mV: cAF, $-6.7 \pm 1.2$ pA/pF, $n=16/5$ [myocytes/patients] versus SR, $-1.7 \pm 0.5$ pA/pF, $n=24/8$), suggesting contribution of constitutively active $I_{K,ACh}$ to basal current. In single-channel recordings, constitutively active $I_{K,ACh}$ was prominent in cAF but not in SR (channel open probability: cAF, $5.4 \pm 0.7\%$, $n=19/9$ versus SR, $0.1 \pm 0.05\%$, $n=16/9$; $P<0.05$). Moreover, $I_{K1}$ channel open probability was higher in cAF than in SR (13.4 $\pm 0.4\%$, $n=19/9$ versus 11.4 $\pm 0.7\%$, $n=16/9$; $P<0.05$) without changes in other channel characteristics.

**Conclusions**—Our results demonstrate that larger basal inward rectifier $K^+$ current in cAF consists of increased $I_{K1}$ activity and constitutively active $I_{K,ACh}$. Blockade of $I_{K,ACh}$ may represent a new therapeutic target in AF. (Circulation. 2005;112:3697-3706.)

**Key Words:** arrhythmia ■ fibrillation ■ ion channels ■ remodeling ■ signal transduction

Atrial fibrillation (AF) is the most frequent cardiac arrhythmia in the clinical setting. It is associated with shorter action potential duration (APD) and effective refractory period and a loss of rate-dependent APD adaptation that involve concomitant alterations in ion current activity. Ion channel remodeling in patients with chronic AF (cAF) includes decreased density of L-type Ca$^{2+}$ currents ($I_{Ca,L}$) and increased amplitude of the background inward rectifier $K^+$ current $I_{K1}$.

The changes in atrial electrical properties (electrical remodeling) promote induction of AF and the tendency of the arrhythmia to be sustained. The autonomic nervous system may also contribute to initiation and persistence of AF. Vagal stimulation reduces atrial APD and effective refractory period and increases dispersion of atrial repolarization. This creates an arrhythmogenic substrate for reentry of the excitation wavefront, which promotes the duration of the AF episodes.

Although it is generally accepted that vagal nerve activation contributes to initiation of AF, its precise role in cAF and the underlying molecular mechanisms are currently unknown.

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Vagally released acetylcholine (ACh) stimulates muscarinic receptors (M-receptors) and activates the atrial ACh-regulated potassium current $I_{K,ACh}$. The resulting shortening of APD in response to M-receptor stimulation is mediated by $I_{K,ACh}$ because in knockout mice lacking this channel, M-receptor stimulation did not induce AF. Therefore, increased activity of $I_{K,ACh}$ was expected to contribute to induction or perpetuation of AF. Studies in atrial biopsies from patients with cAF revealed, however, that both expression of the Kir3.1 and Kir3.4 channel subunits and activation of whole-cell $I_{K,ACh}$ in response to M-receptor stimulation were lower in AF than in sinus rhythm.

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This was interpreted as an adaptation of atrial myocytes to the high beating rate by reducing $I_{K_{ACh}}$ to counteract the shortening of APD.\(^2\)

Reduced M-receptor–mediated activation of $I_{K_{ACh}}$ in AF compared with SR is associated with higher $I_{K1}$ amplitude.\(^3,4,6,7,14,15\) Although larger amplitude of $I_{K1}$ during AF is paralleled by increased mRNA and protein levels of the Kir2.1 channel subunit,\(^5,15\) the molecular basis of increased $I_{K1}$ remains incompletely understood. One possibility is that $I_{K_{ACh}}$, or the ATP-regulated inward rectifier $I_{K_{ATP}}$, which cannot be distinguished electrophysiologically at the whole-cell level, contributes to background $I_{K1}$ by developing spontaneous activity. Therefore, throughout the remainder of this report, inward rectifier current in the absence of agonists is called basal current rather than $I_{K1}$.

Theoretically, each component of the M-receptor–mediated signal transduction, including $I_{K_{ACh}}$, may develop spontaneous activity in the absence of abnormal vagal tone. For instance, dog atrial myocytes possess a constitutively active $I_{K_{ACh}}$–like current that develops higher amplitude after 1 week of atrial tachypacing.\(^16\) Hence, constitutively active $I_{K_{ACh}}$ channels may also exist in atria of AF patients.

The present study tested the hypothesis that increased $I_{K1}$ in patients with cAF consists of enhanced $I_{K1}$ and constitutively active $I_{K_{ACh}}$ current components.

**Methods**

**Human Samples**

The study was approved by the local ethics committee of the university (No. EK790799), and each patient gave written informed consent.

Right atrial appendages were obtained from 46 patients with SR and 33 patients with cAF (cAF > 6 months; Table 1).

**Electrophysiological Recordings**

Atrial myocytes were isolated with the use of our previous protocol\(^17\) and were suspended in storage solution (in mmol/L: KCl 20, KH2PO4 10, glucose 10, K-glutamate 70, \(\beta\)-hydroxybutyrate 10, taurine 10, EGTA 10, albumin 1, pH 7.4). Membrane currents were measured with standard whole-cell voltage-clamp techniques. ISO-2 software (MFK) was used for whole-cell and single-channel data acquisition and analysis.

For whole-cell measurements, borosilicate glass microelectrodes had tip resistances of 1 to 2 MΩ when filled with pipette solution (in mmol/L: K-aspartate 100, NaCl 10, KCl 40, Mg-ATP 5, EGTA 2, GTP-Tris 0.1, HEPES 10, pH 7.4). Myocytes were superfused with a solution containing the following (in mmol/L): NaCl 120, KCl 20, MgCl\(_2\) 1, CaCl\(_2\) 2, glucose 10, HEPES 10, pH 7.4 at 22°C to 24°C. Seal resistances were 4 to 8 GΩ. Series resistance and cell capacitance were compensated. Basal current was measured by applying a depolarizing ramp pulse from −100 to +40 mV (holding potential −80 mV; see inset in Figure 1A). $I_{K_{ACh}}$ was stimulated with the nonselective M-receptor agonist carbachol (CCh) (2 \(\mu\)mol/L) and was identified with the selective bee venom toxin tertiapin.\(^18\) The nature of basal current and $I_{K_{ACh}}$ as inward rectifier currents was proven in each myocyte by applying Ba\(^{2+}\) (1 mmol/L) at the end of the experiments. The myocytes were superfused with tyrode solution via a peristaltic pump throughout the experiments. Drugs were applied via an additional rapid solution exchange system (ALA Scientific Instruments). During drug-free periods, the rapid solution exchange system supplied tyrode solution only. Data were not corrected for the calculated liquid junction potential (−12 mV; software

**TABLE 1. Patient Characteristics**

<table>
<thead>
<tr>
<th></th>
<th>SR</th>
<th>cAF</th>
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<tr>
<td>Patients, n</td>
<td>46</td>
<td>33</td>
</tr>
<tr>
<td>Gender, M/F</td>
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<td>25/8</td>
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<tr>
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<tr>
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<tr>
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<tr>
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<td>15*</td>
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<tr>
<td>Lipid-lowering drugs, n</td>
<td>29</td>
<td>13*</td>
</tr>
</tbody>
</table>

CAD indicates coronary artery disease; MVD, mitral valve disease requiring valve replacement; AVD, aortic valve disease requiring valve replacement; LVEF, left ventricular ejection fraction; LVEDD, left ventricular end-diastolic diameter; LVEDP, left ventricular end-diastolic pressure; LAD, left atrial diameter; IVS, interventricular septum thickness; LVPW, left ventricular posterior wall thickness; ACE, angiotensin-converting enzyme; and AT, angiotensin receptor.

*P<0.05, values from unpaired Student t test for continuous variables and from \(\chi^2\) test for categorical variables.

**Molecular Analysis of the Gβ3 Gene**

Direct activation of $I_{K_{ACh}}$ is mediated by β\(\gamma\)-subunits of G proteins. The G protein β\(\gamma\) subunit gene (G\(N\)B3) contains a C825T poly-
morphism, whereby homozygous 825T-allele carriers exhibit larger basal inward rectifier K⁺ current densities possibly because of enhanced signal transduction. To exclude confounding by homozygous 825T-allele carriers, all patients were genotyped for the C825T polymorphism. DNA extraction and genotyping were performed in a blinded manner as described. In this report the patient sample includes homozygous and heterozygous C825-allele carriers only.

**Statistical Analysis**

Throughout this report, n refers to the number of myocytes/patients, unless otherwise stated. For quantitative comparisons, average values were calculated from multiple data obtained from 1 patient. Because the number of cells investigated varied widely in each patient (unbalanced observations), we calculated means from average values for each patient.

One-way ANOVAs were applied to determine the sources of K⁺ current variation (SPSS version 12.0). Independent variables were cAF, selected clinical variables, and medication (Table 1). Differences between group means for continuous data were compared by unpaired Student t test or when patients with SR or cAF were stratified by underlying disease or medication by 1-way ANOVA and post hoc multiple comparisons tests (Bonferroni t test). The concentration-dependent effects of tertiapin on basal and CCh-activated current were evaluated with a mixed linear model (with fixed factors for rhythm status and concentration of tertiapin and random factor for subjects) followed by post hoc Tukey-Kramer t test (SAS version 9.1). To calculate the concentration of tertiapin blocking the CCh-activated Iₖ,ACh by 50% (IC₅₀) in SR and cAF, the concentration-response curve of the mean values was fitted to a 4-parameter logistic function (Prism version 4.0). Frequency data were analyzed with χ² statistics. *P*<0.05 was considered statistically significant.

**Results**

Amplitudes of basal current and Iₖ,ACh were related to selected clinical variables. Significant differences between the 2 groups were found for hyperlipidemia and left atrial diameter.
Patients with cAF more often received digitalis and diuretics, whereas lipid-lowering drugs were more frequently used in those with SR (Table 1). The patients used for the tertiapin studies showed clinical characteristics comparable to those of the other groups. With 1-way ANOVAs, AF was the only predictor of basal current and \( I_{K,ACh} \) current density (data not shown).

**Basal Current and \( I_{K,ACh} \) in Atrial Myocytes of SR and cAF Patients**

In voltage-clamped human atrial myocytes, cell capacitances averaged 80.3±4.7 pF (n=136) for SR and 89.6±4.1 pF (n=76) for cAF myocytes (P<0.05). Basal current in the absence of agonist (Figure 1A) was confirmed to be larger in cAF than in SR (at −100 mV: 22.9±2.1 pA/pF, n=76/25 [myocytes/patients] versus −12.7±1.0 pA/pF, n=136/38; P<0.05).

Application of CCh resulted in rapid initial increase (Peak) of \( I_{K,ACh} \) amplitude, which was followed by a decrease to a quasi–steady state level (Qss) despite the continuous presence of CCh (desensitization).\(^{10}\) There was no difference in desensitization of \( I_{K,ACh} \) between myocytes from SR and cAF patients (Figure 1B), the Peak/Qss ratio of \( I_{K,ACh} \) being 0.58±0.06 (n=76/25) for AF versus 0.54±0.03 (n=136/38) for SR (P=NS). As expected, the CCh-activated Peak- and Qss- \( I_{K,ACh} \) currents were smaller in cAF than in SR groups (Figure 1B, 1C).

**Effects of Tertiapin on Basal Current and \( I_{K,ACh} \)**

The selective \( I_{K,ACh} \) blocker tertiapin\(^{18}\) was used to discriminate between \( I_{K1} \) and \( I_{K,ACh} \) contribution to enhanced basal current in AF. Tertiapin blocks \( I_{K,ACh} \) with IC\(_{50}\) values between 8 and 30 nmol/L without any effect on \( I_{K1} \) up to 1 \( \mu \)mol/L.\(^{16,19,20}\)

For this set of experiments we applied CCh (2 \( \mu \)mol/L) twice with 4 minutes of washing in between (\( S_1, S_2 \)) to revealed reduced responses during \( S_2 \) in SR (left) and in cAF (right). B, C, Mean±SEM (at −100 mV) of \( I_{K,ACh} \) at Peak and Qss and of the corresponding ratios in SR and cAF, respectively. The numbers indicate number of myocytes/patients.

**Figure 2.** Repeated activation of \( I_{K,ACh} \) by carbachol. A, The activation of \( I_{K,ACh} \) during 2 successive CCh (2 \( \mu \)mol/L) applications (\( S_1, S_2 \), 4 minutes apart) revealed reduced responses during \( S_2 \) in SR (left) and in cAF (right). B, C, Mean±SEM (at −100 mV) of \( I_{K,ACh} \) at Peak and Qss and of the corresponding ratios in SR and cAF, respectively. The numbers indicate number of myocytes/patients.
3D, 3E). After subtraction of the tertiapin-sensitive current component, basal current of cAF patients remained increased (Figure 3D), suggesting enhanced \( I_{K1} \) in these patients.

Tertiapin also suppressed a component of basal current in SR; however, this amounted to only \( \frac{1}{1002} \pm \frac{1}{1006} \) pA/pF at 10 nmol/L (n=24/8). Application of drug-free tyrode solution alone caused a small decrease of basal current by \( \frac{1}{1002} \pm \frac{1}{1006} \) pA/pF (n=5/4) in SR and \( \frac{1}{1002} \pm \frac{1}{1006} \) pA/pF (n=4/3) in cAF. Our results suggest constitutively active \( I_{K\,ACh} \) channels in cAF only (Figure 3A, 3C).

The cAF patients involved in the experiments with tertiapin did not have a history of vagally mediated AF. Compared with SR, they had larger left atria and a higher prevalence of diabetes, and they more frequently took diuretics (Table 1). However, basal currents were similar in cAF patients with and without valvular heart disease (28.7±4.2 pA/pF, n=21/5 versus 26.2±4.4 pA/pF, n=17/4) or diabetes (27.1±3.4 pA/pF, n=28/7 versus 29.2±7.5 pA/pF, n=10/2). The same holds true for treatment with diuretics (27.9±3.5 pA/pF, n=24/6 versus 26.9±6.2 pA/pF, n=14/3). The tertiapin (10 nmol/L)-sensitive current component was 6.4±1.5 pA/pF (n=12/3) and 6.3±1.6 pA/pF (n=12/4) in the absence of diuretics and digitalis, respectively, suggesting a lack of drug effects on constitutively active \( I_{K\,ACh} \) channels in cAF.

Next we excluded the contribution of spontaneously active M-receptors to enhanced basal current in cAF. Atropine (1 µmol/L) abolished the CCh-activated \( I_{K\,ACh} \) in both groups without effect on basal currents. Thus, tertiapin-sensitive constitutive activity of \( I_{K\,ACh} \) is independent of M-receptors (Figure 4).

Single-Channel Activity of \( I_{K1} \) and \( I_{K\,ACh} \) in SR and Chronic AF Patients

For a more direct proof of constitutive activity of \( I_{K\,ACh} \) channels in cAF, we measured single-channel activity in the cell-attached mode. Constitutive activity of \( I_{K\,ACh} \) was detected in cAF but was minute in SR (channel open probability: cAF, 5.4±0.8%, n=19/9 versus SR, 0.1±0.01%, n=16/9; \( P<0.05 \); Figure 5A to 5C). Moreover, open probability of \( I_{K1} \) was higher in cAF than in SR (13.4±0.4%, n=19/9 versus 11.4±0.7%, n=16/9; \( P<0.05 \); Figure 5D) without changes in other channel characteristics (Table 2). Exclusion of cAF patients taking diuretics or digitalis had no impact on constitutive activity of \( I_{K\,ACh} \). Open probability of \( I_{K\,ACh} \) was 6.1±0.7% (n=8/4) and 5.2±0.5% (n=9/4) in the absence of diuretics and digitalis, respectively.
Inclusion of 10 μmol/L CCh in the pipette solution caused single-channel openings in periodic bursts in all SR and cAF myocytes studied. Amplitudes and open times of constitutively active \( I_{K,ACh} \) channels were not different from those of channels activated with CCh (Table 2).

Exposure of myocytes from SR patients with tertiapin (10 nmol/L) reduced open probability of CCh-activated \( I_{K,ACh} \) (Figure 6A, 6C), which is in agreement with previous results obtained in rabbit atrial myocytes.20 Consistent with the reduction of whole-cell basal current shown in Figure 3C, open probability of constitutively active \( I_{K,ACh} \) during cAF was lower in the presence of 10 nmol/L tertiapin without concomitant effects on \( I_{K1} \) (Figure 6B, 6D).

Discussion

In the present study we demonstrate that in addition to enhanced subunit expression, increased open probability of \( I_{K1} \) channels contributes to higher basal inward rectifier K\(^+\) current in patients with cAF. We provide evidence for involvement of agonist-independent constitutive activity of \( I_{K,ACh} \) channels from several observations: (1) the selective \( I_{K,ACh} \) inhibitor tertiapin reduced basal current in cAF but not in SR; (2) single-channel measurements demonstrated spontaneous openings of \( I_{K,ACh} \) channels in myocytes from cAF patients only; and (3) the open probability of constitutively active single \( I_{K,ACh} \) channels during cAF was lower in the presence of tertiapin. Evidence for agonist-independent constitutive activity of \( I_{K,ACh} \) was provided by the lack of block of these channels by the M-receptor antagonist atropine. Our results suggest that in cAF the higher basal inward rectifier K\(^+\) current results from increased expression and open probability of \( I_{K1} \) and constitutively active \( I_{K,ACh} \) channels, which may contribute to the perpetuation of the arrhythmia.
Comparison With Previous Studies

At the whole-cell level, several studies reported higher amplitude of basal inward rectifier $K^+$ current in cAF than in SR patients.\textsuperscript{3,4,6,7,14,15} Pacing-induced tachycardia in dogs also results in higher $I_{K1}$ amplitude,\textsuperscript{16,21} suggesting that the increase in $I_{K1}$ is a consequence of the arrhythmia. Because expression of the corresponding channel subunits at mRNA and protein levels followed the increase in current amplitude,\textsuperscript{6,15} it was concluded that modified channel subunit transcription and/or translation is the only mechanism of increased $I_{K1}$ during human cAF. Our results at the single-channel level, however, showed that open probability of $I_{K1}$ is slightly but significantly higher in cAF than in SR, suggesting that the molecular basis of increased $I_{K1}$ is more complex and involves not only increased channel subunit expression but also additional modifications of channel regulation. The molecular mechanisms of enhanced open channel probability of $I_{K1}$ are currently unknown but may involve impaired phosphorylation-dependent regulation. The $K_{2,\alpha}$ channel subunits of $I_{K1}$ possess phosphorylation sites for different kinases, and increased channel subunit phosphorylation results in lower $I_{K1}$ amplitude.\textsuperscript{22,23} Because cAF is associated with higher activity of the counterbalancing type 1 and type 2A serine/threonine protein phosphatases,\textsuperscript{6} it cannot be excluded that the expected stronger channel dephosphorylation contributes to higher $I_{K1}$ activity in AF. Further studies are needed to identify the molecular mechanisms of modified $I_{K1}$ regulation in cAF patients.

Consistent with previous studies,\textsuperscript{4,6,7} we found that the $M$-receptor–mediated activation of $I_{K,ACb}$ is smaller in cAF than in SR patients. Moreover, reduced channel subunit expression at the mRNA and protein level corresponds well to the lower activation of $I_{K,ACb}$ in cAF.\textsuperscript{6,12,13,15} Nevertheless, we clearly demonstrated that $I_{K,ACb}$ becomes constitutively active when cAF develops. The selective $I_{K,ACb}$ blocker tertiapin concentration-dependently inhibited whole-cell CCh-activated $I_{K,ACb}$ with higher potency in AF than in SR. Tertiapin blocks native $I_{K,ACb}$ channels with IC_{50} values between 8 and 30 nmol/L,\textsuperscript{16,19,20} without significant effects on other ion channels.\textsuperscript{19,20} The effective concentration range of tertiapin found in the present study (Figure 3) is in good agreement with the reported potency. In human atria, however, tertiapin unmasked a robust component of constitutively active $I_{K,ACb}$ in cAF and a substantially smaller one in SR. Recent findings in dog atria and pulmonary veins detected a tertiapin-sensitive current component contributing to basal inward rectifier current, and consistent with our results, pacing-induced

<table>
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<tr>
<th>Current</th>
<th>Rhythm Status n</th>
<th>Amplitude, pA</th>
<th>$\tau_o$, ms</th>
<th>No. of Openings (1 s Tracing)</th>
<th>$P_o$ %</th>
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<td>SR 16/9</td>
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<td>7.6±0.2</td>
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<td>$I_{K,ACb}$</td>
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<td>10.2±1.1*</td>
<td>5.4±0.7*</td>
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</table>

$\tau_o$ indicates time constant for open time distributions; $P_o$, channel open probability (both at holding potential of $-120$ mV); n, No. of myocytes/patients.

*P<0.05 vs corresponding SR group.
tachycardia was associated with higher whole-cell current density of this current component. Thus, the development of constitutively active $I_{K,ACH}$ currents in human cAF is probably a consequence of atrial remodeling contributing to the perpetuation rather than the initiation of the arrhythmia.

The molecular basis of constitutively active $I_{K,ACH}$ is currently unknown. At the single-channel level, basal $I_{K,ACH}$ activity in cAF resulted from a higher number of channel openings and subsequent increase in channel open probability without concomitant changes in basic current properties. The basic properties of $I_{K1}$ and $I_{K,ACH}$ were in good agreement with previous results. Thus, the reduced channel subunit expression in cAF is probably part of the modified $I_{K,ACH}$ regulation and can be interpreted as an adaptation on the higher frequency of $I_{K,ACH}$ openings in these patients.

The regulation of $I_{K,ACH}$ is complex, suggesting several putative mechanisms for constitutive activity of $I_{K,ACH}$. Because the latter was not affected by blocking M-receptors with atropine, basal activity of $I_{K,ACH}$ is an agonist-independent process. Increased agonist-independent dissociation of Ga- and Gβγ-subunits of inhibitory G proteins appears unlikely because in dog atrial myocytes neither pertussis toxin treatment nor absence of GTP affected the tertiapin-sensitive component of basal current.

Evidence for constitutive activity of atrial $I_{K,ACH}$ channels was provided by earlier studies that showed that agonist-independent activation of $I_{K,ACH}$ requires ATP. Interestingly, consistent with the results from atria of dogs with tACH, pertussis toxin treatment failed to prevent the ATP activation of $I_{K,ACH}$. Because activation of $I_{K,ACH}$ requires ATP, modified phosphorylation-dependent channel regulation may contribute to the development of constitutively active $I_{K,ACH}$ in cAF. Consistent with this idea, recent publications have shown that in atrial myocardiium Kir3.1 forms a macromolecular complex, allowing for local regulation of $I_{K,ACH}$ function. The atrial GIRK1 macromolecular complex is composed of the catalytic subunits of PKA, PKC, and CaMKII and the protein phosphatases PP1 and PP2A. Thus, the qualitative and quantitative composition of the GIRK1 macromolecular complex may change during AF, resulting in abnormal regulation of $I_{K,ACH}$. Further work is needed to verify these hypotheses.

**Study Limitations**

In the present investigation we studied regulation of $I_{K1}$ and $I_{K,ACH}$ only in isolated myocytes in vitro. The situation in multicellular preparations and in vivo is more complex. Thus, our data should be extrapolated with caution to the in situ atria. It is well known that the atria are heterogeneous tissues. Therefore, our data obtained in right atrial appendages may not necessarily reflect alterations in the rest of the atria. Digitalis, diuretics, and lipid-lowering drugs were more frequently prescribed in cAF. Thus, we cannot exclude that drugs affect density of $I_{K1}$ and $I_{K,ACH}$. Finally, our current recordings were performed at room temperature only. Their regulation may differ at physiological temperature.

**Potential Clinical Implications**

Our results show that the molecular basis for the higher amplitude of basal inward rectifier current in AF involves increased Kir2.x channel subunit expression and channel open probability (present study). Two-dimensional computer simulations of human atrial tissue showed that $I_{K1}$ affects spiral wave dynamics. An increase of $I_{K1}$ during cAF resulted in reduced APD and hyperpolarization of the resting membrane potential, which is consistent with the shorter APD and the more negative resting membrane potential of multicellular trabeculae from patients with cAF. The changes in APD and resting membrane potential were associated with higher availability of the Na⁺ current ($I_{Na}$) and enhanced excitability, leading to faster rotation frequencies and stabilization of functional reentry. Most important, reduction of $I_{K1}$ in the model was associated with APD prolongation and depolarization, resulting in decreased rotation frequency and spiral wave termination. Consistent with these findings, mice with overexpression of the Kir2.1 channel subunit Kir2.1 and humans with a gain-of-function mutation of this channel develop cAF. Thus, blockade of $I_{K1}$ may be a viable antiarrhythmic option.

In analogy to $I_{K1}$, constitutively active $I_{K,ACH}$ may contribute to stabilization of functional reentry because in ACh-induced AF in sheep, the higher activation frequencies in left atria are associated with larger density of $I_{K,ACH}$. In vivo, vagal nerve stimulation abbreviates APD and increases APD heterogeneity and atrial vulnerability to tACH rhythm, which perpetuate AF. In knockout mice lacking the Kir3.4 channel subunit of $I_{K,ACH}$, M-receptor stimulation did not induce AF, suggesting that the effects of vagal nerve activation are mediated by $I_{K,ACH}$. Thus, the agonist-independent constitutive activation of $I_{K,ACH}$ during cAF is expected to increase atrial vulnerability to tACH rhythm and to sustain AF. Because some antiarrhythmic drugs such as amiodarone, flecainide, quinidine, and verapamil, which effectively terminate AF, are also inhibitors of $I_{K,ACH}$, it cannot be excluded that their effectiveness in AF results in part from blockade of $I_{K,ACH}$ and that at least in some patients with AF the inhibition of constitutively active $I_{K,ACH}$ may have an antiarrhythmic affect.

Finally, there is evidence of circulating M₂ autoantibodies in AF patients, which may exert a tonic activation of M-receptor signal transduction. Furthermore, the ACh concentrations in the synaptic clefts could be elevated because of reduced activity of its degrading enzyme acetylcholine esterase. Because atropine had no effect on basal current in cAF, the development of constitutively active $I_{K,ACH}$ channels occurs in a receptor-independent manner. Thus, it is likely that the elevated synaptic ACh concentrations and the presence of M₂ autoantibodies and constitutively active $I_{K,ACH}$ channels reinforce each other in promoting AF. Further studies are needed to test these hypotheses.
Conclusions
We demonstrated that the molecular basis of higher basal inward rectifier K\(^+\) current in patients with cAF involves increased density of \(I_{\text{K1}}\) and development of constitutively active \(I_{\text{K1,AC3}}\). Our results provide a mechanistic insight into the regulation of \(I_{\text{K1}}\) and \(I_{\text{K1,AC3}}\) in cAF that may help to design new therapeutic options for AF.

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Disclosures
None.

References

**CLINICAL PERSPECTIVE**

Vagal nerve stimulation can provoke atrial fibrillation (AF) requiring activation of the acetylcholine-gated potassium channels. The resulting $I_{K,ACh}$ current shortens action potential duration, increases repolarization heterogeneity, and enhances atrial vulnerability to tachyarrhythmia. Chronic AF is associated with changes in electrophysiological properties that promote initiation and maintenance of the arrhythmia. These electrical remodeling processes involve $I_{K,ACh}$, although the underlying molecular mechanisms are unknown. Here we applied voltage-clamp techniques to analyze background inward rectifier potassium current and $I_{K,ACh}$ in chronic AF. We found that $I_{K,ACh}$ shows strong activity despite the absence of acetylcholine or analogous pharmacological stimulation. This receptor-independent, constitutive activity of $I_{K,ACh}$ in chronic AF may be of considerable clinical relevance because it could serve as a therapeutic target for termination of AF and maintenance of SR after cardioversion.