

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 ± 1.2 pA/pF, $n = 16/5$ [myocytes/patients] versus SR, -1.7 ± 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.^{1,2} Ion channel remodeling in patients with chronic AF (cAF) includes decreased density of L-type Ca^{2+} currents (I_{CaL}) and increased amplitude of the background inward rectifier K^+ current I_{K1} .^{3–8} The changes in atrial electrical properties (electrical remodeling) promote induction of AF and the tendency of the arrhythmia to be sustained.¹ 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.⁹ This creates an arrhythmogenic substrate for reentry of the excitation wavefront, which promotes the duration of the AF episodes.¹ 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 $I_{K,ACH}$.¹⁰ 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.¹¹ 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

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rhythm (SR).^{6,7,12,13} 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,^{6,15} 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 protocol¹⁷ and were suspended in storage solution (in mmol/L: KCl 20, KH₂PO₄ 10, glucose 10, K-glutamate 70, β -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 M Ω 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, MgCl₂ 1, CaCl₂ 2, glucose 10, HEPES 10, pH 7.4 at 22°C to 24°C. Seal resistances were 4 to 8 G Ω . 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 μ 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 Ba²⁺ (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

	SR	cAF
Patients, n	46	33
Gender, M/F	34/12	25/8
Age, y	66.4 \pm 1.5	68.6 \pm 1.3
Body mass index, kg/m ²	27.3 \pm 0.5	27.0 \pm 0.8
CAD, n	26	11
MVD/AVD, n	8	13
CAD + MVD/AVD, n	12	9
Hypertension, n	35	25
Diabetes, n	18	13
Hyperlipidemia, n	35	15*
LVEF, %	56.9 \pm 2.4	58.2 \pm 2.4
LVEDP, mm Hg	14.4 \pm 1.1	14.1 \pm 1.8
LAD, mm	40.3 \pm 0.8	48.1 \pm 1.8*
LVEDD, mm	50.4 \pm 1.0	51.8 \pm 1.7
IVS, mm	11.6 \pm 0.4	11.8 \pm 0.5
LVPW, mm	11.0 \pm 0.3	11.6 \pm 0.3
Digitalis, n	2	12*
ACE inhibitors, n	32	22
AT1 blockers, n	2	2
β -Blockers, n	39	29
Dihydropyridines, n	5	2
Diuretics, n	17	21*
Nitrates, n	12	7
Lipid-lowering drugs, n	29	13*

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 χ^2 test for categorical variables.

JPCalc, version 2.2). To control for variability in myocyte size, the currents are expressed as densities (pA/pF).

Single-channel currents were recorded in cell-attached configuration (Axopatch 200B, Axon Instruments). The bath solution contained the following (in mmol/L): NaCl 120, KCl 20, MgCl₂ 1, CaCl₂ 2, glucose 10, HEPES 10, pH 7.4 with NaOH. Borosilicate glass microelectrodes were coated with Sylgard (Dow Corning) and had tip resistances of 3 to 8 M Ω when filled with the pipette solution (in mmol/L: KCl 140, HEPES 10, pH 7.2 with KOH). $I_{K,ACH}$ was activated by including 10 μ mol/L CCh into the pipette solution; identity of $I_{K,ACH}$ in the absence of CCh application was tested by applying 10 nmol/L tertiapin to the bath solution. The single-channel characteristics of I_{K1} and $I_{K,ACH}$ were analyzed at -120 mV. The total recording time was 20 seconds for each voltage or drug application. The open probability of the channels was calculated from the total number of openings during 20 seconds of recording time. The average open probability per 1-second tracing was used for statistical evaluation. The single-channel slope conductances of I_{K1} and $I_{K,ACH}$ were calculated from the individual current-voltage relationships by linear regression analysis.

Molecular Analysis of the G β 3 Gene

Direct activation of $I_{K,ACH}$ is mediated by $\beta\gamma$ -subunits of G proteins. The G protein β_3 subunit gene (*GNB3*) contains a C825T poly-

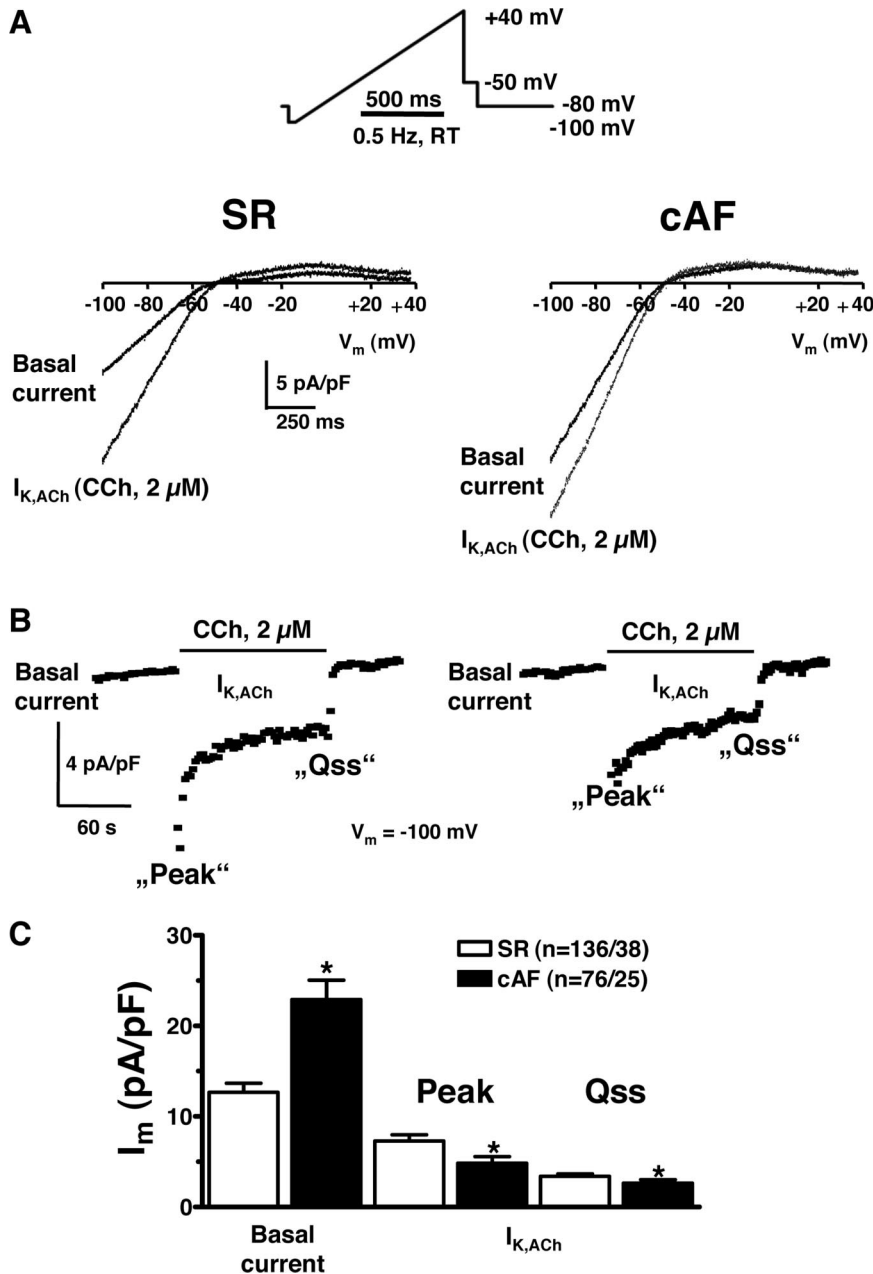


Figure 1. Inward rectifier currents in atrial myocytes from SR and cAF patients. A, Original recordings from a myocyte in SR (left) or cAF (right) under basal conditions (basal current) and in response to 2 $\mu\text{mol/L}$ CCh ($I_{K,ACh}$ was defined as CCh-sensitive current). Top, Ramp protocol. B, Time course of $I_{K,ACh}$ analyzed at -100 mV. $I_{K,ACh}$ was stimulated with 2 successive CCh applications (S_1 , S_2 , 4 minutes apart) in SR (left) and in cAF (right). During each activation, the strong initial increase (“Peak”) of $I_{K,ACh}$ faded (rapid desensitization) to a quasi-steady state level (“Qss”). C, Mean \pm SEM of basal current and $I_{K,ACh}$ from SR and cAF during S_1 only ($V_m = -100$ mV; * $P < 0.05$ vs SR). The numbers indicate number of myocytes/patients.



morphism, whereby homozygous 825T-allele carriers exhibit larger basal inward rectifier K^+ current densities possibly because of enhanced signal transduction.¹⁷ 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.⁷ 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 $I_{K,ACh}$ by 50% (IC_{50}) 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 χ^2 statistics. $P < 0.05$ was considered statistically significant.

Results

Amplitudes of basal current and $I_{K,ACh}$ were related to selected clinical variables. Significant differences between the 2 groups were found for hyperlipidemia and left atrial diameter.

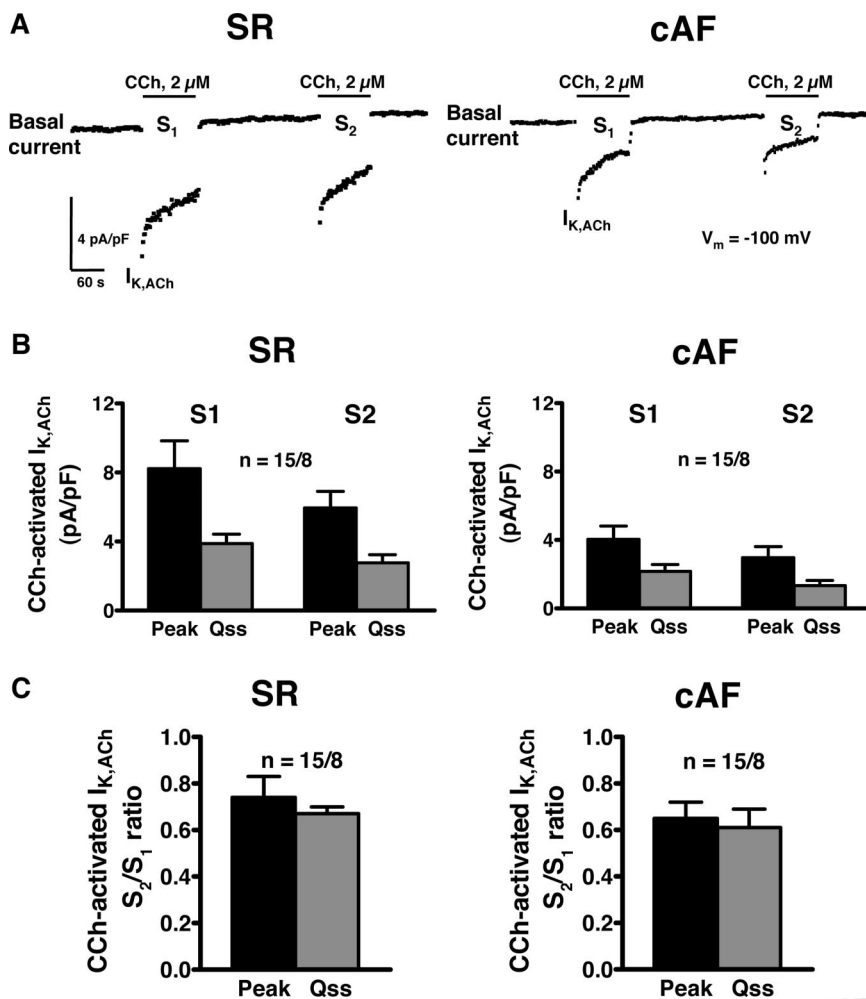


Figure 2. Repeated activation of $I_{K,ACh}$ by carbachol. A, The activation of $I_{K,ACh}$ during 2 successive CCh ($2 \mu\text{mol/L}$) applications (S_1 , S_2 , 4 minutes apart) revealed reduced responses during S_2 in SR (left) and in cAF (right). B, C, Mean \pm SEM (at -100 mV) of $I_{K,ACh}$ at Peak and Qss and of the corresponding ratios in SR and cAF, respectively. The numbers indicate number of myocytes/patients.

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 tertipatin 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 Tertipatin on Basal Current and $I_{K,ACh}$

The selective $I_{K,ACh}$ blocker tertipatin¹⁸ was used to discriminate between I_{K1} and $I_{K,ACh}$ contribution to enhanced basal current in AF. Tertipatin blocks $I_{K,ACh}$ with IC_{50} values between 8 and 30 nmol/L without any effect on I_{K1} up to 1 $\mu\text{mol/L}$.^{16,19,20}

For this set of experiments we applied CCh ($2 \mu\text{mol/L}$) twice with 4 minutes of washing in between (S_1 , S_2). S_1 served as internal control, whereas S_2 was measured in the presence of tertipatin or the nonselective M-receptor blocker atropine. Even under control conditions, $I_{K,ACh}$ was lower during S_2 than during S_1 , suggesting incomplete recovery from desensitization (Figure 2A, 2B). However, the degrees of $I_{K,ACh}$ desensitization were similar in SR and cAF myocytes (Figure 2C).

Application of tertipatin (0.1 to 100 nmol/L) during S_2 reduced the S_2/S_1 ratio in a concentration-dependent manner, with greater potency in cAF than in SR ($-\log IC_{50}$: 9.1 for AF versus 8.2 for SR; $P<0.05$; Figure 3A to 3C). In addition, tertipatin concentration-dependently impaired basal current in cAF, yielding a tertipatin-sensitive component of -6.7 ± 1.2 pA/pF at 10 nmol/L ($n=16/5$; Figure

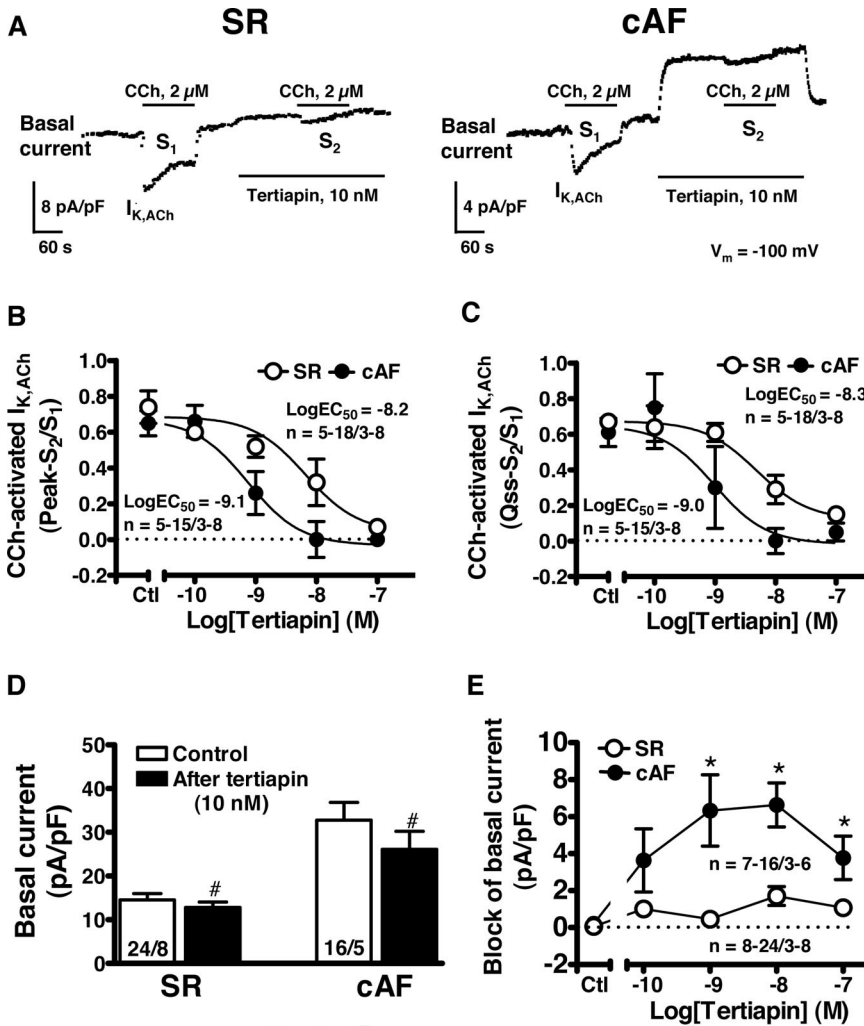


Figure 3. Effects of the selective $I_{K,ACH}$ blocker tertiapin on basal current and CCh-activated $I_{K,ACH}$. A, Effects of tertiapin (10 nmol/L) applied during S_2 with S_1 serving as internal control in SR and cAF. B, C, Concentration-dependent effects of tertiapin on the S_2/S_1 ratio of $I_{K,ACH}$ at Peak (left) and Q_{ss} (right) in SR and cAF patients. D, E, Concentration-dependent block of basal current with tertiapin in SR and cAF patients. Each point in B, C, and E represents values (mean±SEM; at -100 mV) from n independent experiments. Numbers within the figures and columns indicate number of myocytes/patients. * $P < 0.05$ vs corresponding values in SR; # $P < 0.05$ vs pre-drug control.

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 -1.7 ± 0.5 pA/pF at 10 nmol/L ($n=24/8$). Application of drug-free tyrode solution alone caused a small decrease of basal current by -1.5 ± 0.6 pA/pF ($n=5/4$) in SR and -1.6 ± 1.2 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, $5.4 \pm 0.8\%$, $n=19/9$ versus SR, $0.1 \pm 0.01\%$, $n=16/9$; $P < 0.05$; Figure 5A to 5C). Moreover, open probability of I_{K1} was higher in cAF than in SR ($13.4 \pm 0.4\%$, $n=19/9$ versus $11.4 \pm 0.7\%$, $n=16/9$; $P < 0.05$; 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 $6.1 \pm 0.7\%$ ($n=8/4$) and $5.2 \pm 0.5\%$ ($n=9/4$) in the absence of diuretics and digitalis, respectively.



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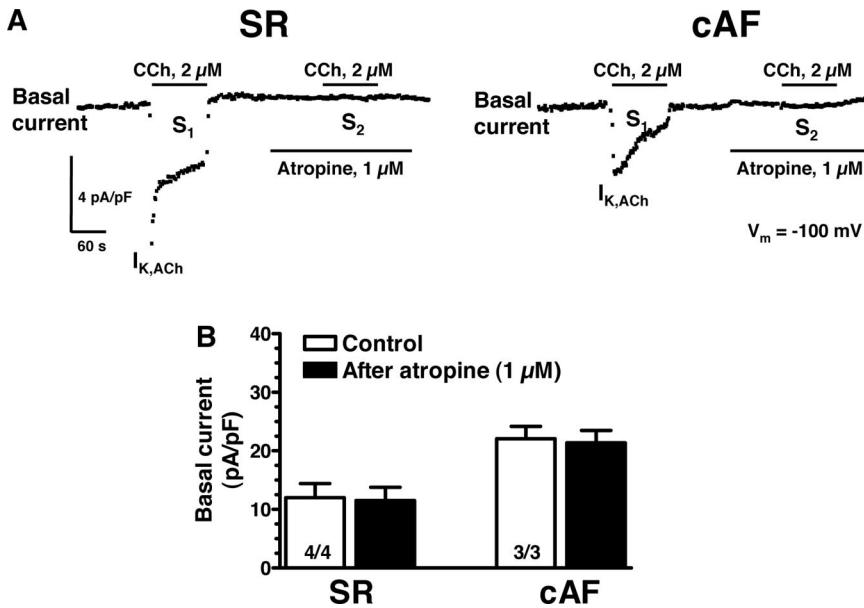


Figure 4. Effect of the nonselective M-receptor antagonist atropine on basal current and CCh-activated $I_{K,ACh}$ in SR and cAF. A, Atropine ($1 \mu\text{mol/L}$) applied during S_2 abolished CCh-activated $I_{K,ACh}$. B, Atropine did not affect basal current in SR and cAF (mean \pm SEM at -100 mV). Numbers within the columns indicate number of myocytes/patients.

Inclusion of $10 \mu\text{mol/L}$ 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.

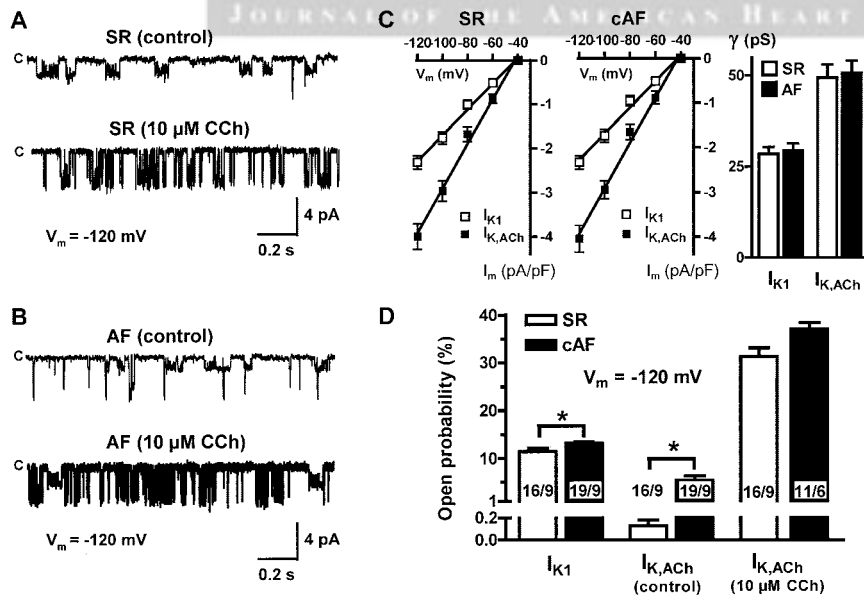


Figure 5. Characteristics of I_{K1} and $I_{K,ACh}$ single-channel activities in atrial myocytes from SR and cAF patients. $I_{K,ACh}$ was activated by inclusion of $10 \mu\text{mol/L}$ CCh in the pipette. A, B, Representative unitary I_{K1} , constitutively active $I_{K,ACh}$, and CCh-activated $I_{K,ACh}$ currents recorded in cell-attached patch conditions from a myocyte from SR and cAF patient, respectively. The myocyte from the cAF patient exhibits both I_{K1} and constitutively active $I_{K,ACh}$ currents, whereas the latter is a rare event in the myocyte from the SR patient. C, Current-voltage relationships of I_{K1} and $I_{K,ACh}$ and their single-channel slope conductances in SR and cAF, respectively. Holding potential (V_m) is expressed as voltage deviation from resting membrane potential. γ indicates single-channel conductance. D, Channel open probability of I_{K1} , constitutively active $I_{K,ACh}$, and CCh-activated $I_{K,ACh}$ currents (mean \pm SEM at -120 mV). Numbers within the columns indicate number of myocytes/patients. * $P < 0.05$ vs corresponding values in SR.

TABLE 2. Single-Channel Characteristics of I_{K1} and Constitutively Active $I_{K,ACH}$ in Myocytes From SR and cAF Patients

Current	Rhythm Status	n	Amplitude, pA	τ_o , ms	No. of Openings (1 s Tracing)	P_o , %
I_{K1}	SR	16/9	2.5±0.07	7.8±0.3	35.2±4.4	11.4±0.7
	cAF	19/9	2.4±0.07	7.6±0.2	44.5±3.1	13.4±0.4*
$I_{K,ACH}$	SR	16/9	3.9±0.11	1.9±0.2	1.1±0.2	0.13±0.05
	cAF	19/9	3.8±0.10	2.4±0.2	10.2±1.1*	5.4±0.7*

τ_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.

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.^{3,4,6,7,14,15} Pacing-induced tachycardia in dogs also results in higher I_{K1} amplitude,^{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,^{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 Kir2.x channel subunits of I_{K1} possess phosphorylation sites for different kinases, and higher channel subunit phosphorylation results in lower I_{K1} amplitude.^{22,23} Because cAF is associated with higher activity of the counterbalancing type 1 and type 2A serine/threonine protein phosphatases,⁸ 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,^{4,6,7} we found that the M-receptor-mediated activation of $I_{K,ACH}$ 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 $I_{K,ACH}$ in cAF.^{6,12,13,15} Nevertheless, we clearly demonstrated that $I_{K,ACH}$ becomes constitutively active when cAF develops. The selective $I_{K,ACH}$ blocker tertiapin concentration-dependently inhibited whole-cell CCh-activated $I_{K,ACH}$, with higher potency in AF than in SR. Tertiapin blocks native $I_{K,ACH}$ channels with IC_{50} values between 8 and 30 nmol/L^{16,19,20} without significant effects on other ion channels.^{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 $I_{K,ACH}$ 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

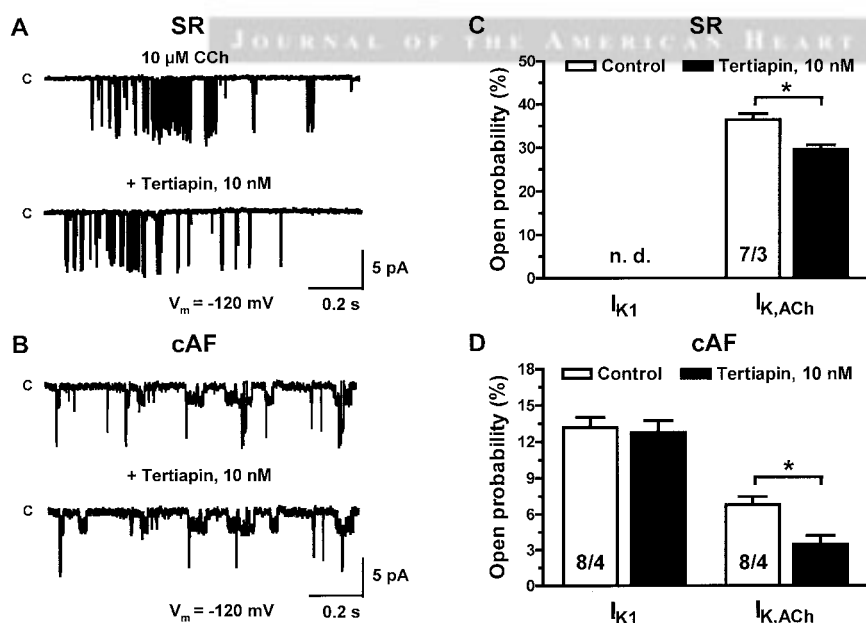


Figure 6. Effects of tertiapin on I_{K1} and $I_{K,ACH}$ single-channel activity in SR and cAF myocytes. In SR, $I_{K,ACH}$ was activated by inclusion of 10 μ mol/L CCh in the pipette. A, C, Channel open probability of CCh-activated $I_{K,ACH}$ in SR was compared before (control) and after exposure of tertiapin (10 nmol/L, mean±SEM at -120 mV). In the presence of CCh, open probability of I_{K1} was not analyzed (n.d. indicates not determined). B, D, Channel open probabilities of I_{K1} and constitutively active $I_{K,ACH}$ in cAF before (control) and after exposure to tertiapin (10 nmol/L, mean±SEM at -120 mV). Numbers within the columns indicate number of myocytes/patients. c indicates closed channel state. * $P < 0.05$ vs corresponding controls.

tachycardia was associated with higher whole-cell current density of this current component.¹⁶ 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.^{24,25} Thus, the reduced channel subunit expression in cAF^{6,12,13,15} 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 $G\alpha$ - and $G\beta\gamma$ -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.¹⁶

Evidence for constitutive activity of atrial $I_{K_{ACh}}$ channels was provided by earlier studies^{26,27} that showed that agonist-independent activation of $I_{K_{ACh}}$ requires ATP. Interestingly, consistent with the results from atria of dogs with tachypacing,¹⁶ pertussis toxin treatment failed to prevent the ATP activation of $I_{K_{ACh}}$.²⁶ Because activation of $I_{K_{ACh}}$ requires ATP,^{26–28} 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 myocardium Kir3.1 forms a macromolecular complex, allowing for local regulation of $I_{K_{ACh}}$ function.^{28,29} The atrial GIRK1 macromolecular complex is composed of the catalytic subunits of PKA, PKC, and CaMKII and the protein phosphatases PP1 and PP2A.^{28,29} 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^{6,15} and channel open probability (present study). Two-dimensional computer simulations of human atrial tissue showed that I_{K1} affects spiral wave dynamics.³⁰ 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.⁶ 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.³⁰ Consistent with these findings, mice with overexpression of the I_{K1} channel subunit Kir2.1³¹ and humans with a gain-of-function mutation of this channel develop cAF.³² 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 $I_{K_{ACh}}$.³³ In vivo, vagal nerve stimulation abbreviates APD and increases APD heterogeneity and atrial vulnerability to tachyarrhythmia,³⁴ which perpetuate AF.⁹ In knockout mice lacking the Kir3.4 channel subunit of $I_{K_{ACh}}$, M-receptor stimulation did not induce AF,¹¹ 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 tachyarrhythmia and to sustain AF. Because some antiarrhythmic drugs such as amiodarone, flecainide, quinidine, and verapamil, which effectively terminate AF,³⁵ are also inhibitors of $I_{K_{ACh}}$,^{36–38} 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 M_2 autoantibodies in AF patients, which may exert a tonic activation of M-receptor signal transduction.³⁹ Furthermore, the ACh concentrations in the synaptic clefts could be elevated because of reduced activity of its degrading enzyme acetylcholine esterase.⁴⁰ 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 M_2 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_{K1} and development of constitutively active $I_{K_{ACH}}$. Our results provide a mechanistic insight into the regulation of I_{K1} and $I_{K_{ACH}}$ in cAF that may help to design new therapeutic options for AF.

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Disclosures

None.

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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.



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