Evaluation of the Role of I_{KACh} in Atrial Fibrillation Using a Mouse Knockout Model

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OBJECTIVES	We sought to study the role of I_{KACh} in atrial fibrillation (AF) and the potential electrophysiologic effects of a specific I_{KACh} antagonist.
BACKGROUND	I_{KACh} mediates much of the cardiac responses to vagal stimulation. Vagal stimulation predisposes to AF, but the specific role of I_{KACh} in the generation of AF and the electrophysiologic effects of specific I_{KACh} blockade have not been studied.
METHODS	Adult wild-type (WT) and I_{KACh}-deficient knockout (KO) mice were studied in the absence and presence of the muscarinic receptor agonist carbachol. The electrophysiologic features of KO mice were compared with those of WT mice to assess the potential effects of a specific I_{KACh} antagonist.
RESULTS	Atrial fibrillation lasting for a mean of 5.7 ± 11 min was initiated in 10 of 14 WT mice in the presence of carbachol, but not in the absence of carbachol. Atrial arrhythmia could not be induced in KO mice. Ventricular tachyarrhythmia could not be induced in either type of mouse. Sinus node recovery times after carbachol and sinus cycle lengths were shorter and ventricular effective refractory periods were greater in KO mice than in WT mice. There was no significant difference between KO and WT mice in AV node function.
CONCLUSIONS	Activation of I_{KACh} predisposed to AF and lack of I_{KACh} prevented AF. It is likely that I_{KACh} plays a crucial role in the generation of AF in mice. Specific I_{KACh} blockers might be useful for the treatment of AF without significant adverse effects on the atrioventricular node or the ventricles. (J Am Coll Cardiol 2001;37:2136–43) © 2001 by the American College of Cardiology

The parasympathetic nervous system acts through the vagus nerve to regulate the heart rate and the conduction properties of atrial, atrioventricular (AV) nodal and ventricular tissue. In animal models, parasympathetic stimulation has been shown to predispose the atria to fibrillation, and vagal denervation has prevented the induction of atrial fibrillation (AF) (1,2). Parasympathetic stimulation leads to a release of acetylcholine from the vagus nerve. Acetylcholine binds muscarinic receptors, triggering a heterotrimeric G-protein cascade that results in activation of the muscarinic-gated potassium channel I_{KACh}, as well as modulation of a host of other ion channels (3,4). In mammals, I_{KACh} is found in the cardiac conductive tissue, sinoatrial (SA) node, atria, AV node and cardiac Purkinje fibers (5). In some species, I_{KACh} has also been reported to be present in ventricular myocytes (6,7). Given the lack of specific pharmacologic antagonists for I_{KACh}, it has been difficult to evaluate its relative contribution to key aspects of normal cardiac function, its role in the pathogenesis of cardiac arrhythmias and the cardiac electrophysiologic effects of specific I_{KACh} blockade.

Cardiac I_{KACh} is comprised of two homologous pro-teins—GIRK1 and GIRK4 (8–10). In cell expression systems, both GIRK1 and GIRK4 are required to recapitulate the key features of I_{KACh} conductance (10,11). In the absence of GIRK4, GIRK1 does not form a functional ion channel, due to its inability to achieve cell surface expression (12–14). Given these observations, we reasoned that ablation of GIRK4 would result in the functional elimination of I_{KACh}. Previously, we described the targeted ablation of the mouse GIRK4 gene and preliminarily characterized the cardiac phenotype of this mouse line (15). Mice lacking GIRK4 (I_{KACh}-deficient knockout [KO] mice) are viable and appear normal. Molecular and electrophysiologic analyses of atrial tissue isolated from the GIRK4 KO mice demonstrated complete absence of the GIRK4 protein and I_{KACh} conductance, respectively. Although rest heart rates and PR intervals of KO and wild-type (WT) mice did not differ significantly, pharmacologic stimulation of vagal activity had a significantly decreased negative chronotropic effect in KO mice compared with WT mice. This finding indicates that I_{KACh} plays a major role in the parasympathetic regulation of heart rate. In addition, beat-to-beat fluctuation in heart rate was significantly diminished in KO mice compared with WT control mice, suggesting that I_{KACh} modulation constitutes the primary mode of heart rate control over short time frames (<2 s).

In this study, we tested the hypothesis that I_{KACh} is essential for the profibrillatory effects of vagal stimulation on the atria. Furthermore, we sought to study the potential effects of a specific I_{KACh} antagonist by comparing the cardiac electrophysiologic features of KO and WT mice.

Abbreviations and Acronyms

AF	=	atrial fibrillation
AV	=	atrioventricular
ECG	=	electrocardiogram or electrocardiographic
KO	=	I_{KACh}-deficient knockout (mice)
MLC	=	myosin light chain
PCR	=	polymerase chain reaction
RNA	=	ribonucleic acid
SA	=	sinoatrial
WT	=	wild-type (mice)

We used intracardiac pacing and recording catheters to measure several variables of SA nodal, atrial, AV nodal and ventricular function in WT and KO mice under rest conditions and after administration of the muscarinic agonist carbachol.

METHODS

GIRK4 knockout mice. The generation of GIRK4 KO mice and their preliminary molecular and functional characterization have been described previously (15). The GIRK4 null allele has since been back-crossed through seven generations onto the C57BL6/J mouse strain. For the experiments described subsequently, F2 GIRK4 KO siblings were mated to produce litters consisting entirely of F3 GIRK4 KO mice. Fourteen 12-week-old GIRK4 KO mice were used for the experiments. Measurements could not be obtained in three of the GIRK4 KO mice because of technical problems with the recording equipment (n = 2) and cardiac perforation (n = 1). Fourteen gender- and age-matched WT C57BL6/J mice (Jackson Laboratories, Bar Harbor, Maine) were used as control animals.

Electrophysiologic studies. Analysis of mouse cardiac electrophysiologic variables was performed in anesthetized mice by using a modification of previously described methods (16,17). Briefly, the mice were anesthetized with intraperitoneal administration of pentobarbital (0.13 mg/g body weight). The surface six-lead electrocardiogram (ECG) was recorded from subcutaneous 25-gauge electrodes in each limb. A 2F octapolar catheter (CIBer Mouse EP Catheter, NuMed Inc., Hopkinton, New York) was introduced through the right internal jugular vein into the right ventricle. Each electrode was 0.5 mm in length, with a 0.5-mm interelectrode distance. The distal electrodes were used for sensing and pacing of the right ventricle, and the proximal electrodes were used for sensing and pacing the right atrium. The ECG channels were amplified (0.1 mV/cm) and filtered between 0.5 and 250 Hz. The ECG variables were calculated according to standard criteria. High-fidelity intracardiac electrogram signals were filtered between 5 and 400 Hz and acquired at a sampling rate of 1,000 Hz.

Sinus node function was evaluated by measuring the rest sinus cycle length and the sinus node recovery time. Sinus node function was assessed by pacing the atrium continuously for 15 s at cycle lengths of 200 and 150 ms and

measuring the duration of the return cycle. To adjust for the sinus cycle length, the sinus node recovery time was also expressed as a percentage of the sinus cycle length.

Anterograde AV nodal function was assessed from the AV interval and during incremental right atrial pacing from the sinus cycle length down to 50 ms. The minimal cycle length required to maintain 1:1 AV conduction, the Wenckebach paced cycle length and the maximal paced cycle length causing 2:1 AV block were determined. Programmed right atrial stimulation was performed at a cycle length of 150 ms to determine the AV effective refractory period. Retrograde conduction along the AV node and His Purkinje system was assessed during incremental right ventricular pacing from the sinus cycle length down to 50 ms. The minimal cycle length required to maintain 1:1 ventriculo-atrial conduction, the ventriculo-atrial Wenckebach paced cycle length and the maximal paced cycle length causing 2:1 ventriculo-atrial block were determined.

Programmed right atrial stimulation was performed at a cycle length of 150 ms to determine the AV effective refractory period. Double and triple extrastimulation techniques down to a minimal coupling interval of 10 ms were performed in an attempt to induce atrial arrhythmias. Right atrial burst pacing at rates of 40 to 80 ms was also performed to induce atrial arrhythmias.

Programmed right ventricular stimulation was performed at a cycle length of 150 ms to determine the ventricular effective refractory period. Double and triple extrastimulation techniques down to a minimal coupling interval of 40 ms were performed in an attempt to induce ventricular arrhythmias. Right ventricular burst pacing at rates of 40 to 80 ms was also performed to induce ventricular arrhythmias (16).

After all baseline electrophysiologic variables were recorded, carbachol was administered intraperitoneally at a dose of 50 ng/g. The entire protocol was repeated 5 min after administration of carbachol to assess muscarinic modulation of electrophysiologic variables (18).

Isolation of ribonucleic acid (RNA) and reverse transcription polymerase chain reaction (PCR). Total RNA was isolated from the atrial appendages and ventricular apices of WT adult mouse hearts using Trizol reagent, according to the manufacturer's instructions (Gibco BRL, Rockville, Maryland). Negative control RNA was obtained from NIH-3T3 cells. One microgram of total RNA was reverse transcribed using oligo(dT) primers, with the SuperScript First-Strand complementary deoxyribonucleic acid Synthesis kit (Gibco BRL). Ten percent of the reaction product was amplified by PCR using GIRK1-, GIRK4- or myosin light chain (MLC)-2a–specific primers. The PCR primers were designed to hybridize to GIRK4 or the MLC-2a coding sequence on either side of an intron. The PCR cycling variables are available on request. The PCR products were visualized by gel electrophoresis (2% agarose) followed by ethidium bromide staining.

Statistical analysis. Cardiac electrophysiologic variables were compared for WT and KO mice before and after carbachol administration by using the two-tailed Student *t* test and two-way analysis of variance with repeated measures on one factor, where appropriate. Interaction terms were used to evaluate whether the effect of carbachol differed between the genotype groups. Data are presented as the mean value ± SEM. Statistical significance was defined as p < 0.05.

RESULTS

Arrhythmia inducibility. In the absence of carbachol administration, no atrial or ventricular arrhythmias could be induced with programmed stimulation or with burst pacing in either WT or KO mice. After carbachol administration, AF lasting for a mean of 5.7 ± 11 min was induced in 10 of 14 WT mice (Fig. 1). In contrast, KO mice remained uninducible when treated with carbachol. In the WT mice, AF could be repeatedly induced using burst atrial pacing, programmed atrial stimulation, burst ventricular pacing or programmed ventricular stimulation. In susceptible mice, AF could be induced 3.1 ± 1.9 times. In one mouse, atrial arrhythmia began as a more organized-appearing tachycardia (i.e., atrial flutter), which subsequently degenerated into AF. Atropine (40 µg intraperitoneally) was administered in one mouse after 35 min of AF, and in another mouse after 66 s of AF. In these instances, AF abruptly terminated after 2 min and 45 s, respectively. Re-induction of AF was attempted after atropine administration in three mice in which AF had been induced multiple times; AF could not be re-induced in these mice. Ventricular tachyarrhythmia could not be induced in mice of either genotype under any tested condition.

Cardiac electrophysiologic variables. The cardiac electrophysiologic variables in WT and KO mice measured at baseline and after carbachol administration are summarized in Table 1. The main differences between KO and WT mice at baseline were in the sinus cycle length and ventricular effective refractory period. Mice lacking I$_{KACh}$ (i.e., KO mice) had faster rest heart rates (shorter sinus cycle lengths) than WT mice under these experimental conditions. Furthermore, the ventricular effective refractory period was longer in KO mice than in WT mice. There was no significant difference in any other measured variable.

After carbachol administration, the differences in the sinus cycle length and ventricular effective refractory period between the KO and WT mice persisted, without an interaction effect (Table 1). In addition, the KO mice displayed significantly shorter sinus node recovery times after pacing at the 150-ms cycle length (Fig. 2). After pacing at a cycle length of 200 ms, the sinus node recovery time was likewise shorter in the KO mice than in the WT mice, although at the longer cycle length, the difference

between the groups was evident only after adjusting for the post-carbachol sinus cycle length.

Changes between the baseline and post-carbachol electrophysiologic variables were compared between the genotypes (Table 1). The WT mice exhibited a greater change from baseline in sinus node recovery time, as well as a greater change in the AV interval after carbachol administration, compared with KO mice. We found it difficult to compare the atrial effective refractory periods of WT and KO mice because of the very small values and difficulty in differentiating atrial capture from stimulus artifact.

I$_{KACh}$ subunit expression in the mouse ventricle. Although I$_{KACh}$ has been recorded from human and ferret ventricular myocytes (6,7), there is little published data demonstrating the expression of GIRK1 or GIRK4 protein or messenger RNA (mRNA) in the adult mammalian ventricle. Given the significant difference observed in the ventricular effective refractory period between the two genotypes, we speculated that I$_{KACh}$ is present in the mouse ventricle. To test this hypothesis, we used PCR to attempt to amplify GIRK1- and GIRK4-specific fragments from reverse-transcribed samples of total RNA from the mouse ventricle. Both GIRK1 and GIRK4 were detected in the samples of the mouse ventricle (Fig. 3). Detection of GIRK1 and GIRK4 mRNA in the ventricle was not due to contamination of the ventricular RNA samples by atrial RNA, because mRNA encoding MLC-2a could not be detected in the ventricular RNA samples. Myosin light chain-2a is expressed in atrial but not ventricular myocytes (19).

DISCUSSION

Acetylcholine is released from the vagus nerve of the parasympathetic system and opposes many of the actions of the sympathetic nervous system. It does so by activation of the muscarinic type 2 receptor, which is coupled to the G$_i$ G protein heterotrimer. Gαi inhibits Gα's stimulation of adenylyl cyclase and, in turn, its generation of protein kinase A by the beta-adrenergic receptor. On the basis of data primarily from the guinea pig, rabbit and rat, protein kinase A phosphorylates a wealth of proteins in cardiac myocytes. Most importantly, from the perspective of this study, protein kinase A phosphorylates and increases the activity of ion channels that speed pacemaker depolarization (I$_f$; HCN), increase calcium entry (I$_{CaL}$; α1C) and shorten the action potential through one or more of the delayed rectifier K$^+$ currents (I$_{Kr}$; HERG, I$_{Ks}$; a heteromultimer of minK and KvLQT1, and I$_{Kur}$; Kv1.5). It also slows pacemaker depolarization directly by release of Gβγ, which binds and directly activates I$_{KACh}$. Thus, the variety of changes noted in the electrophysiologic variables measured in Table 1 reflect the large number of actions of acetylcholine and the degree of beta-adrenergic stimulation under the recording conditions (3).
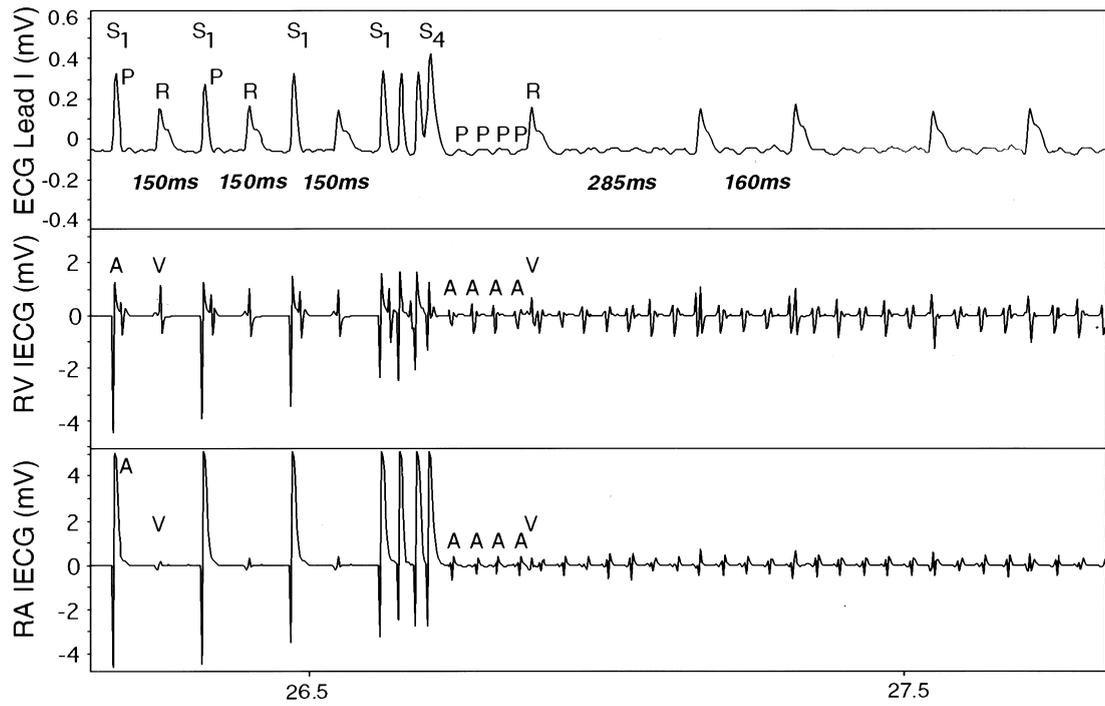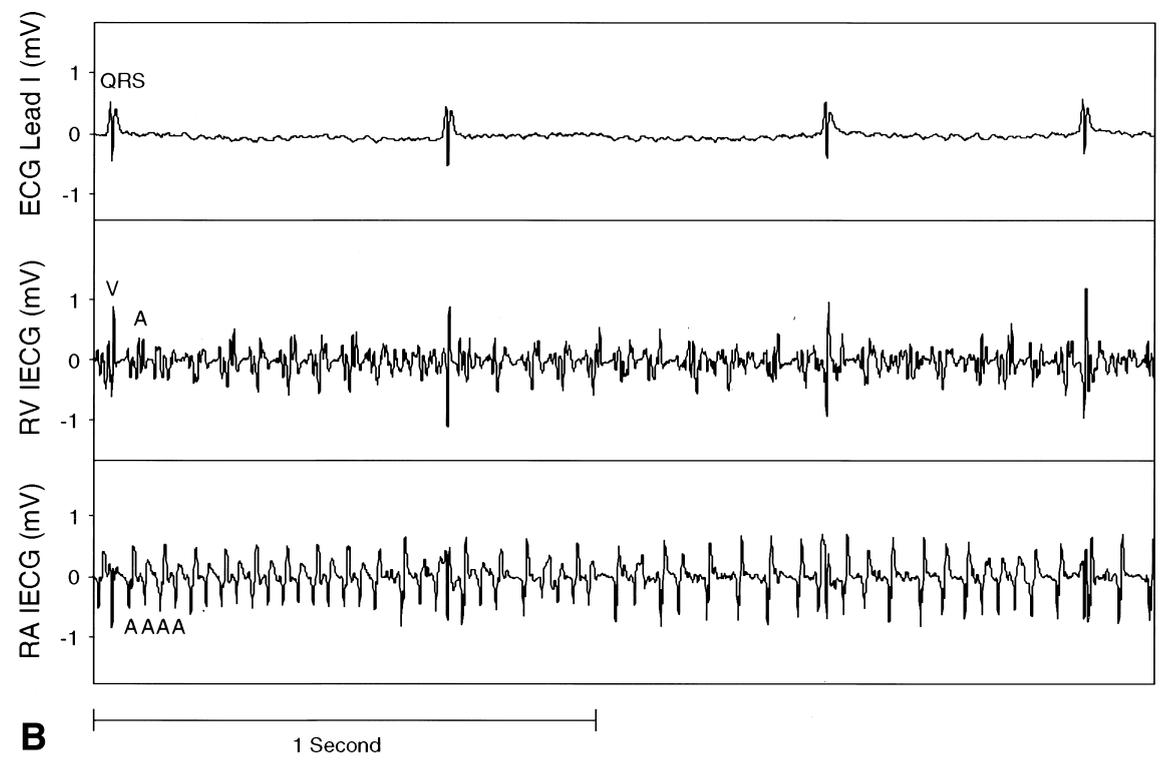
Figure 1. (A) Induction of sustained atrial tachycardia in wild-type mouse after carbachol administration. Three atrial extrastimuli are delivered following a basic drive train of 150 ms. **(B)** Sustained atrial fibrillation, with irregular atrial cycle lengths and variable ventricular response. IECG = intracardiac electrogram; mV = millivolts; RA = right atrial; RV = right ventricular.

In this study, we have demonstrated that activation of I$_{KACh}$ using a cholinergic agonist, such as carbachol, predisposes the mouse atria to fibrillation. A lack of I$_{KACh}$ prevented initiation of AF, but did not alter AV nodal function significantly. A lack of I$_{KACh}$ had a favorable effect on the ventricles by prolonging the effective refractory

Table 1. Cardiac Electrophysiologic Variables of Wild-Type and I$_{KACh}$-Deficient Mice Before and After Carbachol Administration

Variable	Wild-Type Mice			I$_{KACh}$-Deficient Mice		
	−CCh	+CCh	Δ	−CCh	+CCh	Δ
Atrium						
Inducible fibrillation	0/14	10/14 (71%)		0/11	0/11*	
Ventricle						
ERP, 150 ms	48 ± 12	58 ± 13	11 ± 7	68 ± 11*	76 ± 15†	8 ± 10
Sinus node						
Sinus cycle length	293 ± 38	334 ± 70	41 ± 52	239 ± 35†	265 ± 41†	25 ± 19
SNRT, 200 ms	275 ± 90	438 ± 160	164 ± 97	243 ± 69	328 ± 106	83 ± 77‡§
SNRT/SCL, 200 ms (%)	115 ± 17	129 ± 16	14 ± 16	104 ± 15	114 ± 13‡	11 ± 14
SNRT, 150 ms	300 ± 68	406 ± 92	107 ± 68	239 ± 35	297 ± 56‡	35 ± 41‡§
SNRT/SCL, 150 ms (%)	132 ± 13	137 ± 13	5 ± 16	122 ± 11	120 ± 12‡	2 ± 6
AV node						
AV interval	50 ± 8	56 ± 9	6 ± 5	49 ± 4	50 ± 4	2 ± 4‡§
AV Wenckebach	145 ± 21	177 ± 49	31 ± 32	151 ± 13	160 ± 18	10 ± 19§
AV 2:1 block	120 ± 18	153 ± 48	33 ± 33	117 ± 9	128 ± 23	10 ± 23§
AV ERP, 200 ms	93 ± 18	134 ± 46	43 ± 38	98 ± 16	122 ± 23	23 ± 29
AV ERP, 150 ms	106 ± 20	106 ± 20	15 ± 16	109 ± 12	107 ± 10	8 ± 4
VA Wenckebach	178 ± 48	200 ± 81	50 ± 48	186 ± 41	243 ± 60	30 ± 62
VA 2:1 block	144 ± 51	144 ± 21	19 ± 14	129 ± 33	153 ± 38	15 ± 22

*$p < 0.001$; †$p < 0.01$; ‡$p < 0.05$; §Indicates variable with an interaction effect. Statistical analysis was performed using two-way analysis of variance. Data are presented as the number (%) of patients or the mean value ± SD. All times are given in milliseconds.

AV = atrioventricular; CCh = carbachol; ERP = effective refractory period: 200 ms and 150 ms refer to the cycle lengths of the pacing protocols; SCL = sinus cycle length; SNRT = sinus node recovery time; VA = ventriculo-atrial; Δ refers to the difference of the change in the variable before and after carbachol administration; −CCh, +CCh and Δ columns of the I$_{KACh}$-deficient mice refer to differences in these variables between the genotypes.

period. These findings might have important therapeutic implications.

I$_{KACh}$ involvement in AF. Several studies in larger mammals have demonstrated that increased vagal tone, or adenosine administration, can promote the genesis of AF (1,20,21). Vagal denervation of the atria prevented induction of AF and eliminated heart rate variability and baroreflex sensitivity in dogs (1,2). Our findings in mice are consistent with these studies. We demonstrated that sustained AF could be induced in mice using programmed stimulation after administration of cholinergic agonists, such as carbachol. Furthermore, we showed that activation of I$_{KACh}$ is essential for the profibrillatory action of carbachol, because the absence of I$_{KACh}$ uniformly prevented initiation of AF. Activation of I$_{KACh}$ in response to vagal stimulation may decrease the threshold to AF by decreasing the atrial refractory period. Shortening of the atrial refractory period in response to cholinergic and adenosinergic agonists has been previously demonstrated (20,22,23).

It is possible that AF in humans results from heterogeneous hyperactivity of I$_{KACh}$. What could cause such heterogeneous hyperactivity of I$_{KACh}$? One possibility is heterogeneous sympathetic denervation in the atria. Phenol was used to cause heterogeneous sympathetic denervation in the atria, and sites with sympathetic denervation had significantly shorter effective refractory periods than sites without denervation (24). Our study supports the hypothesis that areas with I$_{KACh}$ hyperactivity would have shorter effective refractory periods. Such anisotropic distribution of refractory periods in the atrium could lead to AF.

I$_{KACh}$ involvement in mouse ventricular function. Ventricular effective refractory periods were longer in KO mice

than in WT mice. Our observations suggest that I$_{KACh}$ plays a significant role in the electrophysiologic properties of the mouse ventricular myocardium. Consistent with this functional observation, we detected both GIRK1 and GIRK4 mRNAs in samples of total RNA from the adult mouse ventricle, although at reduced levels compared with those in the atria (data not shown). The expression of GIRK subunits in the ventricle may be age- and species-dependent. GIRK1 was not detected in RNA from adult rat and guinea pig ventricles by Northern blot analysis, and GIRK1 and GIRK4 proteins were not detected in adult bovine ventricles by Western blot analysis (8,10). However, using in situ hybridization, GIRK1 and GIRK4 have been found to be expressed at comparable levels in the atria and ventricles in late-stage rat embryos (25). Shortening of action potential duration and stimulation of I$_{KACh}$ conductance in response to acetylcholine have been reported in ventricular myocytes from humans, ferrets and frogs, but not in ventricular myocytes from guinea pigs or chicks (6,26,27).

Prolongation of the ventricular refractory period is generally considered to be antiarrhythmic. Consistent with this finding, there was no inducible ventricular tachyarrhythmia with programmed ventricular stimulation in KO mice. This is consistent with our previous study, which did not find any spontaneous ventricular tachyarrhythmias during prolonged, continuous ECG monitoring of the KO mice. Furthermore, there was no obvious difference in the frequency of ectopic beats between the KO and WT mice. In short, a lack of I$_{KACh}$ correlates with prolongation of the ventricular effective refractory period and does not predispose mice to ventricular arrhythmias.

Heightened vagal tone has been shown to have an
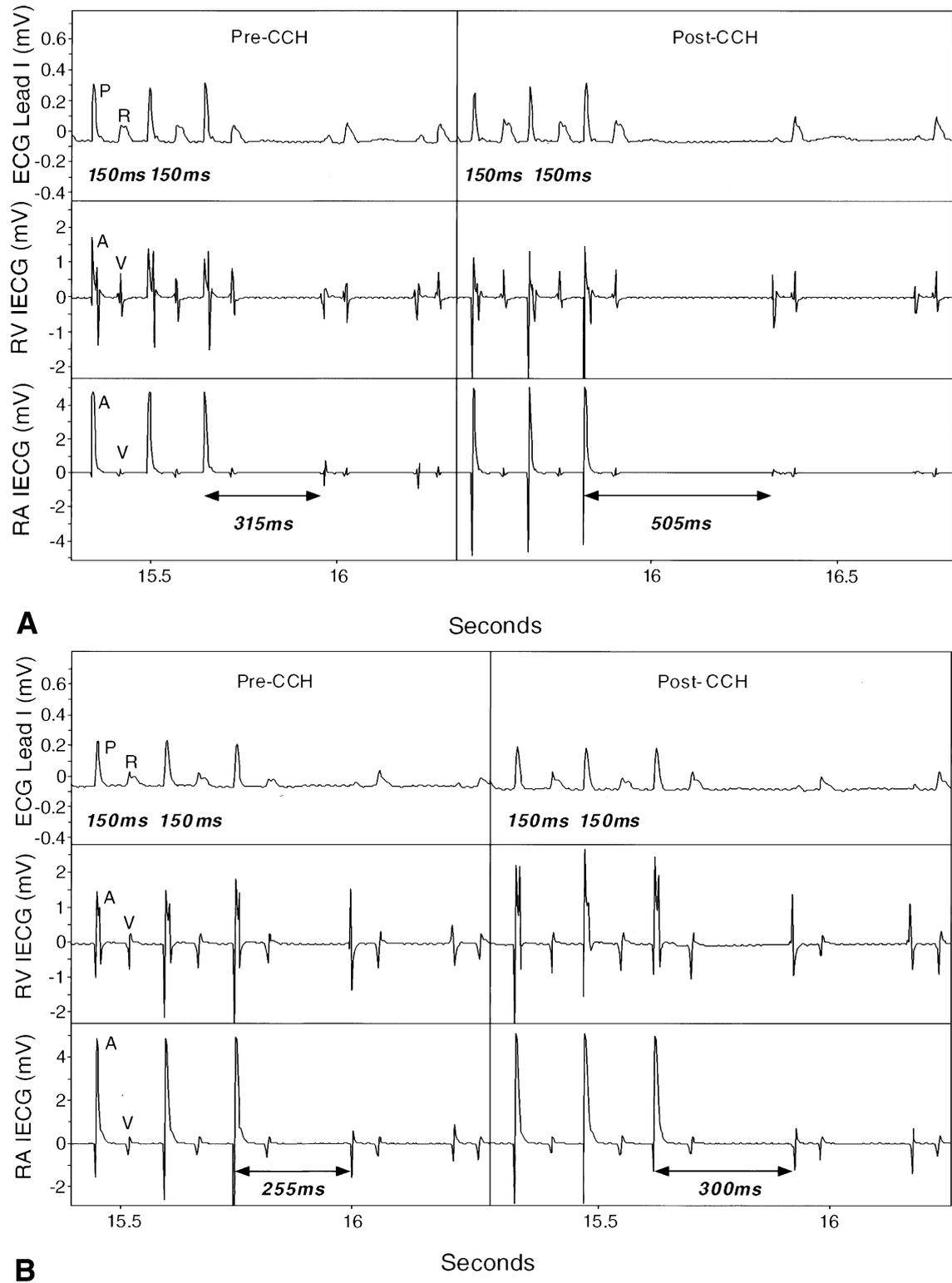
Figure 2. Sinus node recovery times in wild-type **(A)** and I$_{KACh}$-deficient **(B)** mice after pacing for 30 s at a cycle length of 150 ms. **(A)** The sinus node recovery times were 315 ms at baseline and 505 ms after carbachol administration. **(B)** The sinus node recovery times were 255 ms at baseline and 300 ms after carbachol. Pre-CCH = before administration of carbachol; Post-CCH = after administration of carbachol; RA IECG = right atrial intracardiac electrogram; RV IECG = right ventricular intracardiac electrogram.

antifibrillatory effect on the ventricles (28,29), and impaired parasympathetic activity has been associated with an increased risk of sudden death in both experimental and

clinical studies (29–35). Our finding that mice lacking I$_{KACh}$ were not at an increased risk of ventricular fibrillation suggests that alterations in parasympathetic activity are
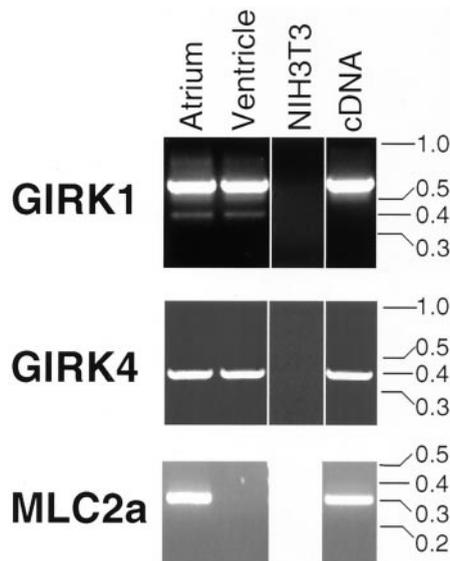
Figure 3. GIRK1 and GIRK4 are expressed in both the atrium and ventricle of the mouse. Total ribonucleic acid (RNA) from the atrium and ventricle of the adult mouse was amplified by reverse transcription-polymerase chain reaction (PCR) using primers specific for GIRK1, GIRK4 or myosin light chain (MLC)-2a. RNA from NIH-3T3 cells was used as a negative control agent, and complementary deoxyribonucleic acid (cDNA) for mouse GIRK1, GIRK4 and MLC-2a was used as a positive control agent in PCR.

unlikely to affect susceptibility to ventricular fibrillation through I_{KACh}.

I_{KACh} involvement in sinus node function. Mice lacking I_{KACh} exhibited significantly shorter sinus cycle lengths than did WT mice treated with carbachol. This is consistent with findings from our previous study, which demonstrated that after vagal stimulation or administration of the adenosine receptor agonist, GIRK4 KO mice had significantly shorter sinus cycle lengths than did WT mice (15). On the basis of these findings, we argued that I_{KACh} mediates a substantial portion of the heart rate decrease occurring after vagal stimulation or adenosine administration.

In the present study, we found that KO mice have faster heart rates than do WT mice at baseline, whereas in our previous study, we found no significant difference in the resting heart rate between WT and KO mice (15). This discrepancy is likely due to the different conditions under which the experiments were performed. Mice in the first study were implanted with 8-g radiotransmitters, and heart rate was recorded while the animals were conscious and mobile. The presence of the transmitter may have triggered sympathetic stimulation and negated a difference in rest heart rate between the GIRK4 KO and WT mice. Indeed, other groups using the implantable transmitters have reported elevated rest heart rates (36,37). In the present study, heart activity was recorded under general anesthesia. The anesthesia likely ameliorated some of the sympathetic effects, accentuating a small difference in the mean heart rate.

We also observed a difference between WT and KO mice in the time to recovery of sinus node function after rapid atrial pacing in the presence of carbachol. In mice lacking

I_{KACh}, the sinus node recovered faster than that in WT mice. The inhibitory effect of I_{KACh} on myocyte depolarization may delay the spontaneous firing of sinus node cells after a period of rapid pacing.

Specific I_{KACh} antagonist as a possible antiarrhythmic agent. It will be interesting to see whether specific I_{KACh} antagonists, if developed, will be useful in the treatment of AF. Such a drug is likely to have favorable antiarrhythmic effects in the ventricles, because a lack of I_{KACh} caused prolongation of the ventricular effective refractory period in the GIRK4 KO mice. Specific I_{KACh} antagonists are unlikely to have significant effects on the AV node, as shown by the lack of change in the AV nodal function in the KO mice. However, specific I_{KACh} antagonists might result in faster rest heart rates because of the effect on sinus node function. Torsade de pointes has been reported in humans with potassium channel blockers, such as sotalol (38) and dofetilide (39). Specific I_{KACh} antagonists also need to be evaluated in humans for a tendency to induce torsade de pointes.

Study limitations. The data included this study are from observations made in anesthetized mice and may not represent the physiologic situation in conscious humans. The rate of atrial tachycardia induced in mice is much faster than that in humans. This is likely due to the shorter action potential duration and effective refractory period in mice compared with humans.

Acknowledgments

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