Evidence for enhanced M3 muscarinic receptor function and sensitivity to atrial arrhythmia in the RGS2-deficient mouse

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Tuomi JM, Chidiac P, Jones DL. Evidence for enhanced M3 muscarinic receptor function and sensitivity to atrial arrhythmia in the RGS2-deficient mouse. Am J Physiol Heart Circ Physiol 298: H554–H561, 2010. First published December 4, 2009; doi:10.1152/ajpheart.00779.2009.—Atrial fibrillation (AF) is the most common arrhythmia seen in general practice. Muscarinic ACh receptors (M2R, M3R) are involved in vagally induced AF. M2R and M3R activate the heterotrimeric G proteins, Gi and Gq, respectively, by promoting GTP binding, and these in turn activate distinct K+ channels. Signaling is terminated by GTP hydrolysis, a process accelerated by regulator of G protein signaling (RGS) proteins. RGS2 is selective for Gq and thus may regulate atrial M3R signaling. We hypothesized that knockout of RGS2 (RGS2/−/−) would render the atria more susceptible to electrically induced AF. One-month-old male RGS2/−/− and C57BL/6 wild-type (WT) mice were instrumented for intracardiac electrophysiology. Atrial effective refractory periods (AERPs) were also determined in the absence and presence of carbachol, atropine, and/or the selective M3R antagonist darifenacin. Susceptibility to electrically induced AF used burst pacing and programmed electrical stimulation with one extrastimulus. Real-time RT-PCR measured atrial and ventricular content of RGS2, RGS4, M2R, M3R, and M4R mRNA. AERP was lower in RGS2/−/− compared with WT mice in both the high right atrium (HRA) (30 ± 1 ms, P < 0.05) and mid right atrium (MRA) (21 ± 1 vs. 24 ± 1 ms, P < 0.05). Darifenacin eliminated this difference (HRA: 37 ± 2 vs. 39 ± 2 ms, and MRA: 30 ± 2 vs. 30 ± 1, P > 0.4). RGS2/−/− were more susceptible than WT mice to atrial tachycardia/fibrillation (AT/F) induction (11/22 vs. 1/25, respectively, P < 0.05). Muscarinic receptor expression did not differ between strains, whereas M2R expression was 70-fold higher than M3R (P < 0.01). These results suggest that RGS2 is an important cholinergic regulator in the atrium and that RGS2/−/− mice have enhanced susceptibility to AT/F via enhanced M3 muscarinic receptor activity.

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Atrial fibrillation (AF) is the most common cardiac arrhythmia seen in general clinical practice. It is characterized by abnormal, disorganized, and very rapid atrial electrical activation. AF is a common health problem in the developed world, and its prevalence increases from about 0.5% of people in their 50s to nearly 10% of those over 80 years of age (5). As the population ages, AF thus will place an increasing burden on health care resources, not only for its treatment and ongoing therapeutic management, but also because of the two most serious complications of AF, heart failure and stroke (10).

The generation of AF requires two critical components: 1) a trigger that initiates the event, and 2) a susceptible or vulnerable substrate that maintains the arrhythmia once initiated. AF can be triggered by ectopic foci firing (15) from areas such as the pulmonary vein region (13) or posterior left atrium (LA) (29). Maintenance of AF may depend on rotor formation, which is the organizing source (driver) of functional reentrant activity (spiral waves). Rotors form when a propagating electrical wave ‘‘breaks’’ on an anatomical or electrophysiological heterogeneity and, under appropriate conditions of excitability, begins to rotate (17, 41). The wavelength of excitation (which is equal to the product of the conduction velocity and the refractory period) is also important in reentry, with shorter wavelengths resulting in more stable rotors. For a review of rotors in cardiac fibrillation, see Vaquero et al. (41).

The pathogenesis of AF is complex, involving not only remodeling of the atrium, but also effects mediated via the intrinsic cardiac autonomic nervous system (ICANS) (25). Subpopulations of intrinsic cardiac neurons express multiple neurotransmitters (14); however, in guinea pig posterior ganglia, choline acetyltransferase immunostaining of all neurons indicates major parasympathetic cholinergic input to the myocardium (28). Rhythm management after catheter ablation of arrhythmogenic tissue in the pulmonary veins tends to be more successful when these autonomic ganglia are knowingly or inadvertently modified (36). Recently, we demonstrated that selective surgical left atrial neuroablation blocked the ability to electrically induce AF in the anesthetized pig (20). The abrogation of parasympathetic signals may underlie the antiarrhythmic effects of ICANS manipulation, since the development of AF can be promoted by cholinergic input to the atrium. ICANS-derived ACh activates multiple muscarinic receptor subtypes present in the atria including Gαo-coupled M2 receptors (M2R) as well as Gq-coupled M3 muscarinic receptors (M3R). These receptor subtypes activate distinct potassium currents including the G protein-activated inward rectifier potassium current (GIRK3,1,3,4 or I(K,ACH)) by M2R and delayed rectifier potassium (I(K,M3)) channels via the M3R (38). Muscarinic receptors are GPCRs (G protein-coupled receptors), and as such they activate heterotrimeric G proteins by catalyzing GDP dissociation from the Gα subunit and consequently promoting GTP binding. This activating step leads to conformational changes and possibly also subunit dissociation within the G protein, thus enabling both GαGTP and the Gβγ dimer to regulate downstream effectors (44). Go proteins are turned off by their intrinsic ability to hydrolyze GTP; however, in vivo this step may be facilitated by GTPase activating proteins RGS (regulator of G protein signaling) proteins, thus shortening the duration of G protein activation. Most RGS proteins appear to be expressed in cardiac tissue (32), and seven distinct isoforms [RGS2, RGS3, RGS4, RGS6, RGS10, RGS11, RGS12] are present.
RGS19 (GAIP), and RGS17 (RGSZ2]) have been specifically localized to atrial myocytes (9). Nearly all RGS proteins are GAPs for Goq, and about half also act on Goq11 (1). RGS2 is one of the most highly expressed RGS proteins in the heart (24), and it is a uniquely selective GAP for Goq with limited potency on Goq11 (1, 7). Thus RGS2 is potentially an important regulator of atrial Goq-coupled M3 receptors.

Since RGS proteins limit GPCR signaling, we hypothesized that they may limit atrial arrhythmogenesis by keeping in check parasympathetic signals mediated via atrial muscarinic receptors. To test this, we compared RGS2−/− and wild-type mice for atrial electrophysiological properties and susceptibility to the induction of atrial tachyrhythmias. To determine whether the loss of RGS2 would enhance susceptibility to tachyarrhythmia induction, we performed in vivo electrophysiological pacing and recording studies in both strains. Overall, our results suggest that removing the inhibitory effect of RGS2 on M3-activated Goq renders the knockout mice more susceptible to electrically induced atrial tachyarrhythmia.

METHODS

Animals and preoperative procedures. The generation and genotyping of RGS2 knockout (RGS2−/−) mice have been described (Oliveira-Dos-Santos et al., Ref. 30). Mice were provided with ad libitum food and water and held on a standard (12-h:12-h) light-dark cycle. The studies were approved by the Animal Use and Care Committee of the University of Western Ontario (protocol no. 2006-121-12) and complied with the guidelines of the Canadian Council on Animal Care and the Guide to the Care and Use of Laboratory Animals published by the US National Institutes of Health (Institutional no. A5527-01). One-month-old male RGS2−/− and control C57BL/6 wild-type (WT) mice were anesthetized with an intraperitoneal injection of a mixture of ketamine (150 mg/kg) and xylazine (10 mg/kg) and fixed in a supine position over a heated water blanket. Body temperature was monitored with a YSI-402 (Yellow Springs Instruments, Yellow Springs, OH) small animal rectal probe inserted ~1 cm past the anal sphincter and maintained within the normal physiological range (36.5–38°C) (8). Hair was removed from the neck with Nair cream hair remover (Church & Dwight, Mississauga, ON, Canada). With a cut-down approach, a 23-ga Teflon endocardial tube was inserted and ligated in place to keep the airway dry and open.

Surface limb lead ECG. They should be defined at first use and used consistently thereafter. For common abbreviations that do not need to be defined, see the list published at the front of most issues of the journal. Also please note that it is against APS style to define abbreviations within titles or subheads.

Measurements were obtained using four subcutaneous 25-ga platinum electrodes (Grass Instrument, Quincy, MA) placed at the base of each limb. ECG tracings were filtered between 0.05 to 100 Hz, digitized, and sampled at 1.5 kHz, with an ECG 100 preamplifier connected to a MP100 recording system (BIOPAC Systems, Biolyx, Montreal, PQ, Canada).

In vivo intracardiac electrophysiology studies. A 2-Fr octopolar stimulation/recording/drug infusion catheter (CIB'ER Mouse, NuMED, Hopkinton, NY) used for intracardiac electrophysiological studies in the mouse. Note the 0.5-mm spacing of the electrodes. Each bracketed electrode pair was used for recording and electrical stimulation. Numbers indicate the position within the heart: 1 = right ventricle (RV), 2 = His bundle, 3 = mid right atrium (MRA), 4 = high right atrium (HRA)/superior vena cava. B: representative recording from electrode pair 2 showing the atrial, ventricular, and His deflections.

Pacing protocols. Bipolar pacing used 2-ms pulses at twice the diastolic threshold, delivered through a Grass SIU5 stimulus isolation unit, connected to a Grass S99 stimulator, programmed with a custom-built timer. Atrial (AERP), ventricular, and atrial-ventricular nodal effective refractory periods were assessed with programmed electrical stimulation (PES) (27) using a drive train of 9 stimuli (S1) at cycle lengths of 150 and 100 ms followed by delivery of an extrastimulus (S2). Atrial pacing with 1-ms decrements were used to determine the Wenckebach cycle length.

Arrhythmia induction. Both PES (19) and burst pacing (18) (2 ms pulses at 50 Hz, 400-ms burst duration) were used to determine susceptibility to atrial arrhythmia induction. Burst pacing used up to 30 bursts of pacing in both atrial locations. Atrial fibrillation in the mouse was implied to be distinguishable from atrial tachycardia based on the surface electrograms: lack of regular P waves, irregularly irregular ventricular responses, in combination with intracardiac electrograms having fractionation and/or intracardiac activation heterogeneities between the MRA and HRA.

Effects of muscarinic receptor drugs. Pharmacological agents were purchased from Sigma (Mississauga, ON, Canada), unless otherwise stated. Muscarinic receptor drugs, including the nonselective agonist carbachol (0.5 mg/kg) and the nonselective antagonist atropine (1 mg/kg), were injected intraperitonally. The M3R-selective antagonist darifenacin hydrobromide (Cedarlane Laboratories, Markham, ON, Canada; 1 mg/kg) was administered intravenously. Electrophysiological measurements were made sequentially in the absence of drug, and then in the presence of carbachol, followed either by atropine or by darifenacin and then atropine. Effects of vagal stimulation (10 V, 30 Hz, 3 s) on heart rate were measured prior to and after darifenacin and atropine administration.

RNA isolation and real-time RT-PCR. RNA was isolated by use of a Trizol kit (Qiagen, Invitrogen, Burlington, ON, Canada). RNA concentration was determined with a spectrophotometer (Beckman Coulter, Mississauga, ON, Canada). Isolated RNA was converted to cDNA by using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Carlsbad, CA). Real-time analyses were carried out using specific primers and probe sets for selected genes. The M2R−/− and wild-type livers were used as controls. The expression of the target gene was normalized to the expression of the β-actin gene.
out using Taqman gene expression Assays-on-Demand from Applied Biosystems and normalized to levels of the reference gene, GAPDH. Relative expression of M2R, M3R, M4R, RGS2, and RGS4 mRNA were determined by using 40 cycles on the ABI Prism 7900 HT sequence detector (Perkin Elmer Life Sciences, Woodbridge, ON, Canada). All samples were amplified in three parallel reactions per trial. To determine real-time amplification efficiencies and starting concentration of the amplicon, nonbaseline corrected raw output data from the ABI Prism 7900 HT sequence detector was analyzed by linear regression analysis with LinRegPCR (2009) software (34).

**RESULTS**

**Atrial effective refractory periods.** PES revealed a regional heterogeneity in refractory period, with longer AERPs in the HRA region compared with the MRA (Fig. 2), and this difference was observed in both RGS2−/− mice and WT mice (Table 1). AERPs were significantly lower in the RGS2−/− mice in both the HRA (34 ± 2 vs. 30 ± 1 ms, P < 0.05) and MRA (23 ± 1 vs. 21 ± 1 ms, P < 0.05) regions compared with WT mice (Table 1). Strain-related differences remained after carbachol administration, which reduced AERPs in both strains. In contrast, the nonselective muscarinic antagonist atropine increased AERPs to similar levels in both strains (Table 1). To determine the role of the M3R in the RGS2−/− mouse we used the selective M3R blocker darifenacin hydrobromide. Darifenacin increased the AERP from 20 ± 2 to 30 ± 1 of the MRA in the RGS2−/− mouse (n = 5, P < 0.05) and from 25 ± 1 to 30 ± 1, in WT mice (n = 6, P < 0.05), which eliminated the strain-related differences. Two-way ANOVA revealed a significant interaction between strain and drug (darifenacin), suggesting that increased signaling through the M3 receptor may underlie the lower AERPs measured in animals lacking RGS2.

Since it has been established that vagally induced bradycardia is mediated via M2R but not M3R (11), we compared the effects of darifenacin and atropine on heart rate changes following vagus nerve stimulation. Stimulating the vagus (10V, 30 Hz, 3 s) decreased heart rate by 38 ± 6.8% in WT animals. This decrease was abolished by atropine but was unaltered in the presence of darifenacin (35 ± 4.5%, n = 4, P > 0.5). This provided evidence that this dose of darifenacin did not affect M2R signaling.

**Atrial tachyarrhythmia induction.** Atrial burst pacing (Fig. 3) and PES (Fig. 4) (18) induced atrial tachyarrhythmias that demonstrated properties of AF; however, validating AF would require high-density mapping and recording from the LA,

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<th>Vehicle</th>
<th>Carbachol</th>
<th>Atropine</th>
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<tr>
<td></td>
<td>Wild Type</td>
<td>RGS2−/−</td>
<td>Wild Type</td>
</tr>
<tr>
<td>AhERP100</td>
<td>34 ± 1</td>
<td>17</td>
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<td>VERP150</td>
<td>41 ± 1</td>
<td>18</td>
<td>43 ± 1</td>
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Values are means ± SE; n (no. of mice) indicated beside value. AhERP, high atrial effective refractory period; AmERP, mid atrial effective refractory period; VERP, ventricular effective refractory period; AVNERP, atrial-ventricular effective refractory period; WCL, Wenkebach cycle length. Subscripts 100 and 150 refer to drive cycle lengths of 100 and 150 ms, respectively. *95% Confidence interval (CI) and 99% CI for difference in RGS2−/− vs. wild type within drug group.
which often is the driver of regular rhythms recorded from the RA (29). Thus these have generally been labeled atrial tachycardia/fibrillation (AT/F). PES induction is thought to mimic physiological initiations of AF, since premature atrial beats often precede AF episodes (15). On the other hand, burst pacing, although thought to be a nonphysiological provocation, will reliably induce AF via cardiac electrical instability (18). AF is very rarely induced by single extrastimuli in large animal and human studies and was thus anticipated to be even less provokable in mice, due to their small size. Thus AT/F sensitivity to single extrastimuli in the mouse implies a highly vulnerable myocardial substrate. The duration of induced arrhythmia is also important with “sustained” AF being defined in large animal and human electrophysiological studies as lasting >30 s. Hence, AT/F data were analyzed on the basis of susceptibility to both pacing modalities and were grouped into duration of the induced arrhythmia lasting <10 s, between 10 and 30 s, and >30 s.

As expected, 1-mo-old WT mice were mostly insensitive to single stimulus-induced AT/F (4%, 1/25) compared with burst pacing (40%, 10/25, P < 0.05) (Fig. 3, top). Carbachol increased the susceptibility to both single extrastimulus (33%, 4/12) and burst pacing (58%, 7/12). Muscarinic receptor activation also prolonged the duration of AT/F episodes with only 2 of 12 untreated mice having sustained AT/F, whereas 5 of the 12 had sustained AT/F after carbachol administration.

The site of stimulation within the atrium also affected susceptibility to AT/F induction. For WT mice, burst pacing more readily induced AT/F from the MRA (40%, 10/25) compared with the HRA (8%, 2/25, P < 0.05) region. This is likely related to the relative AERPs, which were significantly shorter in the MRA compared with the HRA (Table 1). This electrical heterogeneity may be an important factor in providing a permissive substrate for wave break and reentry initiation in the RA.

RGS2−/− mice were more susceptible to PES induction of AT/F from the MRA (50%, 11/22) compared with the WT mice (4%, 1/25, P < 0.05) (Table 2). There was a trend for a greater percentage of RGS2−/− mice to have sustained AT/F (>30 s) with either pacing modality, but this did not reach significance (0.1>, P > 0.05) (Table 2).

Mechanism of arrhythmia (evidence for reentry). Rapid focal activity has the potential to initiate rotors due to the interaction of a high-frequency propagating wave fronts with the refractory tail of the previous wave (17, 41). Immediately after PES-initiated arrhythmia, local cycle lengths were often identical in the HRA and His bundle regions. However, sometimes at arrhythmia onset, heterogeneities in refractory periods were accompanied by a regional conduction block into the HRA (Fig. 4, top). Also, heterogeneity in local cycle lengths were observed between the HRA and His bundle regions immediately following initiation of AT/F in an RGS2−/−
mouse (Fig. 4, bottom). Immediately after induction, the local atrial electrogram from the His bundle region was shorter (22 ms) than that of the HRA (27 ms) region. However, 173 ms after initiation, local cycle length of the His bundle region converged with that of the HRA (25 ms). This pattern suggests a drifting rotor that rapidly became anchored. However, this pattern may also indicate a tachycardia with a rapid rate at onset that slows down as the driving mechanism stabilizes. The rapid onset may combine with the regional ERP heterogeneity to produce a functional conduction block/slowing that recovers at a slower rate. In this and other mice ($n$ = 4) the cycle length during AT/F (27 ms) was faster than the intrinsic AERP in the HRA region (32 ms), suggesting the possibility of electronic interactions from a rotor core causing reduced refractoriness (41).

Expression of M2, M3, and M4 receptors and RGS2 and RGS4 mRNA. Regional heterogeneity (LA vs. RA) in the expression of muscarinic receptors and/or their associated RGS proteins may contribute to arrhythmia. Therefore, we compared regional levels of mRNA encoding M2, M3, and M4 receptors, as well as RGS2 and RGS4, in 1-mo-old WT mice using the Relative Expression Software Tool (REST) (31). There was no difference in M2, M3, and M4 receptor expression in WT compared with RGS2/−/− mice (data not shown). However, M2 receptor expression in WT mice was higher in the RA compared with the LA ($P < 0.05$, ratio = 0.763), whereas M3 receptor was higher in the LA ($P < 0.05$, ratio = 1.188) (Fig. 5). M4R, RGS2, and RGS4 did not differ between the two atria. To compare the expression levels of M2, M3, and M4 muscarinic receptors, LinRegPCR analysis was used to determine both the PCR reaction efficiencies and the starting concentration of the amplicon. As expected, the M2 receptor expression was substantially higher than both M3 and M4 receptors in atria and ventricles (Fig. 6, bottom). RGS2 was more highly expressed than RGS4 in both RA and LA, whereas

Table 2. Incidence of AT/F induced by PES or burst pacing and maximum duration of induced AT/F with either PES or burst pacing induction

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<th>Wild Type</th>
<th>RGS2−/−</th>
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<tr>
<td><strong>Inducibility</strong></td>
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<tr>
<td>PES</td>
<td>4% (1/25)</td>
<td>50% (11/22)</td>
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<tr>
<td>Burst (50 Hz, 400 ms pulse)</td>
<td>40% (10/25)</td>
<td>64% (14/22)</td>
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<tr>
<td><strong>Duration</strong></td>
<td></td>
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<tr>
<td>No response</td>
<td>60% (15/25)</td>
<td>36% (8/22)</td>
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<tr>
<td>&lt;10 s</td>
<td>20% (5/25)</td>
<td>23% (5/22)</td>
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<tr>
<td>10-30 s</td>
<td>16% (4/25)</td>
<td>18% (4/22)</td>
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<tr>
<td>&gt;30 s</td>
<td>4% (1/25)</td>
<td>23% (5/22)</td>
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$\chi^2$ Analysis of discrete values revealed significant differences in atrial tachycardia/fibrillation (AT/F) susceptibility with programmed electrical stimulation (PES) ($P < 0.05$).
there was greater expression of RGS2 in the atrium than the ventricle (Fig. 6, top).

Effects of body temperature. Previously, with the exception of rate-corrected QT interval, all electrophysiological parameters were found to be prolonged at lower body temperatures (3). We extended these results to determine the effect of low body temperature (33.5°C) on AERP and AT/F susceptibility. At 33.5°C, AERPs were prolonged in both the HRA (34 ± 3 vs. 41 ± 3, P < 0.05) and MRA (24 ± 3 vs. 29 ± 3, P < 0.05) regions. The prolongation of AERPs reduced the susceptibility to AT/F induction. Maintenance of body temperature is important since previous studies have stated that AT/F cannot be induced in mice without carbachol (22). Our studies clearly show that AT/F can be induced without carbachol administration, and this difference is likely due to having maintained body temperature constant at 37°C throughout the previous studies.

**DISCUSSION**

This study, to our knowledge, demonstrates for the first time a role for RGS proteins in atrial arrhythmia. The RGS2−/− mice were more susceptible to PES-induced AT/F, and there was a greater percentage of RGS2−/− mice with sustained AT/F. The greater susceptibility to AT/F was likely due to the abbreviated AERP in RGS2−/− mice. This strain-dependent difference was maintained in the presence of carbachol, whereas atropine abolished the strain-dependent differences. These findings suggest an alteration in muscarinic receptor-gated K+ flux evidently due to an increase in muscarinic receptor response per se. The observed phenotypic difference cannot easily be attributed to increased parasympathetic release of ACh, since chronic telemetry showed that although mean arterial pressure was increased by ~10 mmHg in RGS2−/− animals, heart rate was unchanged, indicating a resetting of the baroreceptor reflex (12). It follows that any observed cardiac electrophysiological phenotype would be due to the direct effect of RGS2 loss in the atria, rather than a baroreflex-mediated enhancement of vagal activity. Consistent with other studies, we found that RGS2 was highly expressed in the atria of mice. Since RGS2 is a selective GAP for Goq, while having limited potency on Gox, (1), we hypothesized that Gox-
tible to PES-induced AT/F, which, although not diagnostic in itself, is thought to more readily induce reentry than triggered activity (16). 2) Myocardial regions with large heterogeneities in electrical properties are often more vulnerable to reentry (2), which can result from unidirectional conduction block in regions with longer refractoriness (4). In the present study the atrial ERP was significantly longer in the HRA compared with the MRA region, and AT/F was more easily induced by pacing the MRA. 3) Computer modeling has been used to determine electrical and structural heterogeneities between the crista terminalis and RA as being key mechanisms underlying the initiation of reentry (4). Rapid pacing in this area results in unidirectional conduction blockage toward the crista terminalis and the initiation of reentry (4). Consistent with this concept, we observed activation heterogeneities between the MRA and HRA region during and at the onset of AT/F (Fig. 4, top).

Initiation of rotors is suggested to be key in maintaining AF (41). Once initiated, rotors have the potential to drift until they anchor around tissue heterogeneities (i.e., scars, coronary arteries, bands of connective tissue, etc.) (41). Drifting rotors exhibit Doppler-like shifts in tachycardia cycle lengths such that the coupling interval is shorter in an area it drifts toward and longer in regions from which it moves away (16). After anchoring, cycle lengths in the two regions should be identical. Because of the small size of the mouse heart and the left atrial predominance of AF mechanisms, one could speculate that anchoring should occur rapidly with Doppler shifts observed relatively infrequently in the RA. However, we observed this complex activation pattern in an RGS2−/− mouse (Fig. 4, bottom). Rotors also enhance the repolarization of excited myocardium (reducing AERP) up to 1 cm away because of a strong electrotonic current flow into the unexcited core (16, 41). For this mouse, the local cycle length in the HRA during arrhythmia was faster than what would be allowed by the intrinsic ERP (32 ms), further strengthening the argument for a reentrant mechanism in AF in the RGS2−/− mouse. Nevertheless, the suggestion of reentry supporting AF in the mouse requires techniques such as optical mapping to document the mechanism.

Both M2 and M3 muscarinic receptor-mediated K+ currents may be involved in the development of AF. In the canine heart, M2R-regulated Ik,ACh comprises >60% of the total muscarinic-mediated outward K+ current (37). However, with AF due to ventricular tachypacing-induced congestive heart failure, the contribution of Ik,M3 increased to ~50% of the total outward K+ current (37). Ik,ACh channels also can participate in AF, given that GIRQ3.4 knockout (GIRQ4−/−) mice, which lack functional Ik,ACh channels, appear to be completely resistant to carbachol-induced AF (22).

RGS protein regulation of M2R function likely contributes to AF. RGS4 was identified as an important regulator of Ik,ACh channels in the SA node; however, little RGS4 was detected in the surrounding RA (6). Consistent with this observation, we found >150-fold higher expression of RGS2 than RGS4 in the mouse atria. Future studies may reveal a role for other RGS proteins in atrial arrhythmia.

Clinical relevance. With the aging of the population there is an increasing drive to identify novel treatments for AF. This will require increased understanding of the signaling pathways and molecular regulators involved in arrhythmia induction, perpetuation, and atrial remodeling. The clinical relevance is highlighted by the success of ACE inhibitors in preventing fibrosis and development of an AF substrate (26). Mutations in or altered expression/function of a variety of RGS proteins could be involved in AF mechanisms in patients, and indeed changes in RGS2 have been identified in several human cardiovascular phenotypes. Targeting RGS proteins may be important for drug development (33). Also, our data suggest that selective M3R blockade, alone or in combination with other antiarrhythmic agents, may be useful for patients with AF. However, in mice oral darifenacin exerted only transient binding to cardiac muscarinic receptors (45), and so its use may be limited.

Limitations. Although these results indicate a role for RGS2 and the M3 muscarinic receptor in promoting AF in the mouse, additional studies will need to directly determine the role of Ik,M3 activity. In the mouse, it may be difficult to distinguish atrial fibrillation from atrial tachycardia, particularly with an arrhythmia of short duration. However, by using standard clinical criteria for atrial fibrillation (lack of regular P waves, irregularly irregular ventricular responses), often it was possible to demonstrate characteristics of atrial fibrillation in the mouse. Nevertheless, this is difficult particularly with arrhythmias of short duration. Atrial vulnerability may also be due to a balance of autonomies (20, 36). RGS2−/− mice have also been shown to have reduced renal sympathetic nerve activity compared with WT mice (40). Although the lack of a heart rate difference between RGS2−/− and WT mice found in their earlier study (12) does not assist in establishing the role of the sympathetic nervous system in atrial susceptibility, the role of the balance between sympathetic and M3 responses remains to be determined. RNA expression provides an index of the relative importance of the proteins but may or may not parallel Bmax values of radioligand assessment of M2, M3, or M4 protein expression, distribution and function. One-month-old mice were chosen to avoid potential complexity due to the chronic effects of the hypertensive phenotype associated with RGS2−/− in the mouse. It is also recognized that it may not be possible to directly extrapolate from mouse experiments to the human.

GRANTS

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DISCLOSURES

No conflicts of interest are declared by the author(s).

REFERENCES


