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RESEARCH ARTICLE

Differential effects of inhibitory G protein isoforms on G protein-gated inwardly rectifying K^+ currents in adult murine atria

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Nobles M, Montaigne D, Sebastian S, Birnbaumer L, Tinker A. Differential effects of inhibitory G protein isoforms on G protein-gated inwardly rectifying K^+ currents in adult murine atria. *Am J Physiol Cell Physiol* 314: C616–C626, 2018. First published January 17, 2018; doi:10.1152/ajpcell.00271.2016.—G protein-gated inwardly rectifying K^+ (GIRK) channels are the major inwardly rectifying K^+ currents in cardiac atrial myocytes and an important determinant of atrial electrophysiology. Inhibitory G protein α-subunits can both mediate activation via acetylcholine but can also suppress basal currents in the absence of agonist. We studied this phenomenon using whole cell patch clamping in murine atria from mice with global genetic deletion of $G\alpha_{i2}$, combined deletion of $G\alpha_{i1}/G\alpha_{i3}$, and littermate controls. We found that mice with deletion of $G\alpha_{i2}$ had increased basal and agonist-activated currents, particularly in the right atria while in contrast those with $G\alpha_{i1}/G\alpha_{i3}$ deletion had reduced currents. Mice with global genetic deletion of $G\alpha_{i2}$ had decreased action potential duration. Tissue preparations of the left atria studied with a multielectrode array from $G\alpha_{i2}$ knockout mice showed a shorter effective refractory period, with no change in conduction velocity, than littermate controls. Transcriptional studies revealed increased expression of GIRK channel subunit genes in $G\alpha_{i2}$ knockout mice. Thus different G protein isoforms have differential effects on GIRK channel behavior and paradoxically $G\alpha_{i2}$ act to increase basal and agonist-activated GIRK currents. Deletion of $G\alpha_{i2}$ is potentially proarrhythmic in the atria.

atria; electrophysiology; inhibitory heterotrimeric G protein; G protein-gated potassium channel

INTRODUCTION

Inwardly rectifying K^+ (Kir) channels are widely expressed in all chambers of the heart and are important in setting the resting membrane potential. However, there is a dichotomy, with the Kir2.0 family being predominant in the ventricles and His-Purkinje system and the Kir3.0 family in atrial and nodal tissues (7, 18, 24, 45). The Kir3.0 channel family encodes G protein-gated inwardly rectifying K^+ (GIRK) channel present in neurons and neuroendocrine tissues in addition to the heart (11, 27). In the heart the channel is thought to consist largely of a heteromultimer of Kir3.1 and Kir3.4 (19). A characteristic of these channels is that they are activated by G protein-coupled receptors linked to inhibitory G proteins and specifically by the free Gβγ subunits (25, 42, 44). For example, in the sinoatrial (SA) node (SAN) activation of the channel by acetylcholine released from the vagus nerve is responsible for heart rate slowing (39, 43). Recently a study has shown a critical role for Kir3.4 in the kinetics of heart rate recovery to resting level after sympathetic activation (30).

Despite GIRK channel activation being mediated by Gβγ directly binding to domains on the channel, activation seems to occur largely via members of the inhibitory G protein family (14, 21, 22, 35). In a series of studies from different laboratories using varied approaches, a more complex model has emerged. It appears that the inhibitory G protein heterotrimer is able to directly interact with the channel, and, on activation, heterotrimer dissociation occurs in a microdomain in or around the channel subunit. The dissociated Gβγ subunit leads to activation (9, 21, 34, 38). However the Gα, as either a monomer or as part of the heterotrimer, may also play an important role leading to inhibition of channel activity, and this process may be isoform dependent (5, 6, 15). Multiple isoforms of inhibitory Gα subunits ($G\alpha_{i1}$, $G\alpha_{i2}$, $G\alpha_{i3}$ and, $G_o\alpha$) are present in atrial tissue, and their roles in modulating parasympathetic signal transduction remain unclear. The subunits $G\alpha_{i2}$ and $G\alpha_{i3}$ have been shown to mediate signaling to GIRK in embryonic stem cell-derived cardiomyocytes (42a). Our own work shows that $G\alpha_{i2}$ is important for heart rate regulation in vivo (41, 46), but this occurs via modulation in the SA node (not the atria) and might be via a mechanism independent of GIRK.

There is further complexity in that the right (RA) and left (LA) atria may be different and have gradients of channel expression (17). GIRK channels are expressed at higher levels in the RA in mice and humans. It has been proposed that the gradient of GIRK current, combined with the heterogeneous distribution of parasympathetic innervation and adenosine receptor expression in the atria, may contribute to the ability of vagal nerve stimulation to augment dispersion of atrial refractoriness (12, 13, 23, 26, 40). The purpose of this study is to define the type of G proteins involved in the signaling to GIRK in the atria, the possible role of these G proteins in atrial

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asymmetry, and how they might potentially modulate arrhythmogenesis.

MATERIALS AND METHODS

Gene-targeted mice. Mice with global deletion of $G\alpha_{i2}$ and $G\alpha_{i1}$/$G\alpha_{i3}$ (deletion of both $G\alpha_{i1}$ and $G\alpha_{i3}$) maintained on a Sv129 background were compared with wild-type littermate controls. The gene-targeting strategy, genotyping, and confirmation of relevant $G_{i/o}\alpha$ deletions have previously been described (16, 46). Mice were maintained in an animal core facility under the United Kingdom Home Office guidelines relating to animal welfare. All procedures were approved by the local animal care and use committee and performed in accord with the United Kingdom Home Office regulations (PPL 70/7665). All mice were kept in a temperature-controlled environment (21–24°C) with a 12:12-h light-dark cycle. Animals were allowed ad libitum access to standard rodent chow and drinking water. Mice on a 129/Sv background aged 3–4 mo (20–30 g) were used for this study. Both males and females were used in the study, and there was no gender discrimination. Littermate controls were obtained from the $G\alpha_{i2}$ crosses.

Quantitative real-time reverse transcription PCR. RNA was isolated from the left atria and right atria from 14-wk-old mice with global deletion of $G\alpha_{i2}$ ($n = 3$) maintained on a Sv129 background and wild-type littermate controls ($n = 3$) using the RNeasy kit (Qiagen). Briefly, hearts were removed from each group of mice [$G\alpha_{i2}{}^{+/+}$ and, $G\alpha_{i2}{}^{-/-}$] and washed with cold PBS, and left atria and right atria were isolated and immediately placed in RNALater. RNA was extracted using the RNeasy kit (catalog no. 74104; Qiagen). cDNA was synthesized using the high-capacity cDNA reverse transcription kit (4368814; Life Technologies) and quan-

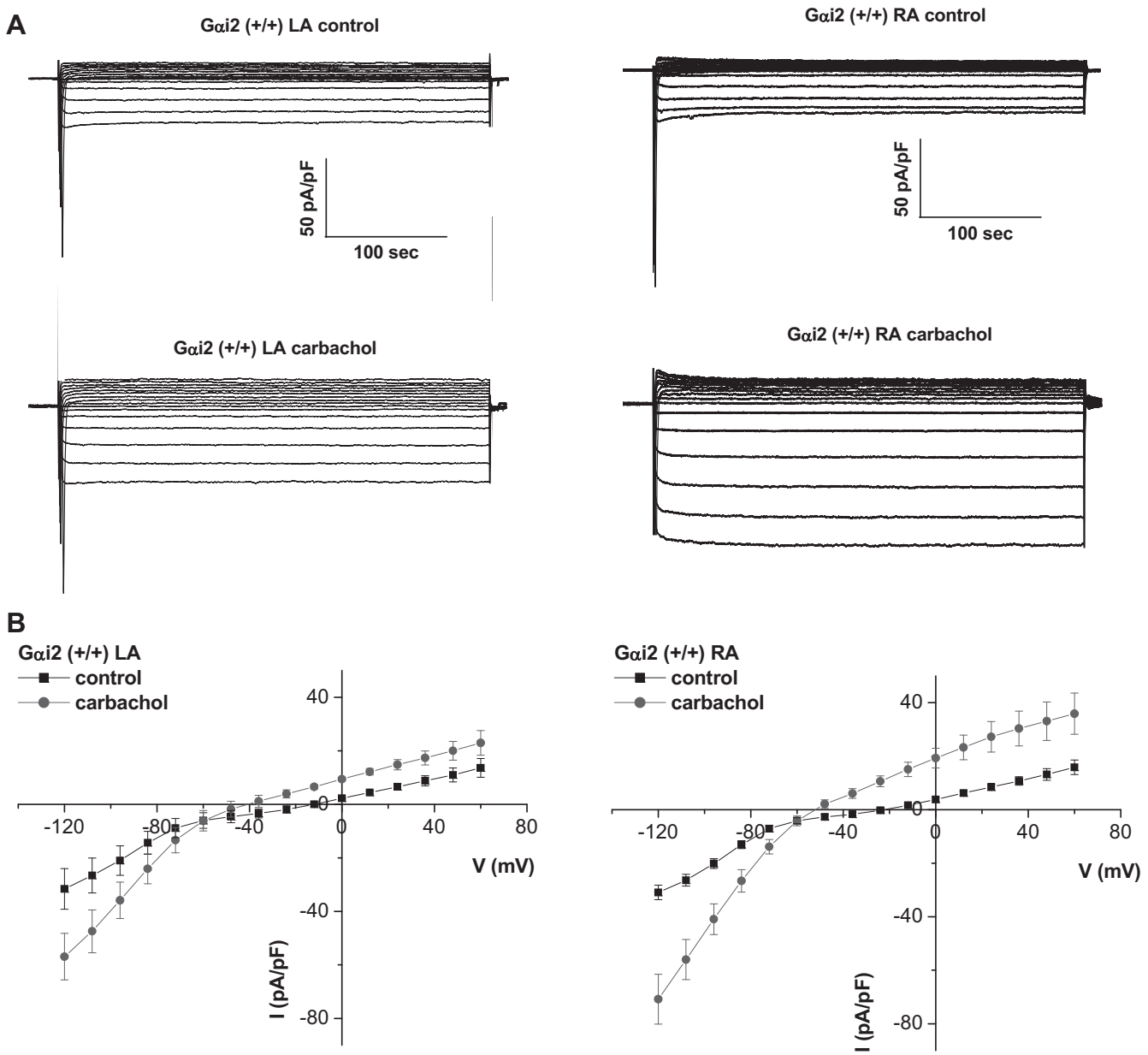

Fig. 1. GIRK currents in atrial tissue. *A*: representative traces of currents measured in atrial myocytes isolated from the left and right atria of $G\alpha_{i2}{}^{+/+}$ mice. *B*: mean current-voltage relationships. Atrial myocytes were challenged with 10 μM carbachol. GIRK currents were larger in RA compared with LA ($n = 10$, 6 mice).

tified, and 50 ng of cDNA/20 μl were used for the subsequent real-time expression assay. Real-time PCR was performed using Taqman gene expression assays (Life Technologies). All genes (Mm00434618_m1: *Kcnj3*, Mm01175829_m1: *Kcnj5*, Mm00492-379_m1: *Gnai3*, Mm01165301_m1: *Gnai1*, Mm00494677_m1: *Gnb1*, Mm00501973_m1: *Gnb4*, Mm01165191_m1: *Gng11*, Mm00-515876_m1: *Gng7*) were assayed in triplicate, and *GAPDH* was used as the housekeeping gene.

Single-cell isolation and electrophysiology. Atrial and sinoatrial cells were isolated using an adapted method for isolation of sinoatrial cardiomyocytes (29). Briefly, mice were injected with heparin, and beating hearts were removed under pentobarbital sodium (3 ml/kg) and ketamine (1 ml/kg) anesthesia. The left and right atria were excised in normal Tyrode solution containing (in mM): 140 NaCl, 5.4 KCl, 1.8 $CaCl_2$, 1 $MgCl_2$, 5 HEPES-NaOH, and 5.5 D-glucose (pH 7.4). Strips of tissues were enzymatically digested in a low-Ca^{2+} and low-Mg^{2+} solution containing (in mM): 140 NaCl, 5.4 KCl, 0.5 $MgCl_2$, 0.2 $CaCl_2$, 1.2 KH_2PO_4, 50 taurine, 5.5 D-glucose, and 5 HEPES-NaOH, pH 6.9. Collagenase type II (224 U/ml; Worthington), elastase (1.9 U/ml; Worthington), protease (0.9 U/ml; Sigma Aldrich), and bovine serum albumin (BSA, 1 mg/ml) were added. The digestion step was carried out for 20 min or 45 min for atrial and SAN tissue, respectively, under gentle mechanical agitation at 37°C. Tissue strips were then washed out and transferred to a modified "Kraftbrühe" (KB) medium containing (in mM): 70 L-glutamic acid, 20 KCl, 80 KOH, 10 D-β-hydroxybutyric acid, 10 KH_2PO_4, 10 taurine, 1 mg/ml BSA, and 10 HEPES-KOH, pH 7.4. Single myocytes were manually dissociated in KB solution by employing a fire-polished glass pipette. Finally, extracellular Ca^{2+} concentration was recovered up to 1.3 mM. A drop of cell suspension was seeded on sterilized laminin-coated coverslips. After 30–45 min, Tyrode solution containing 10% BSA was added, and cells were stored at 37°C until used in humidified

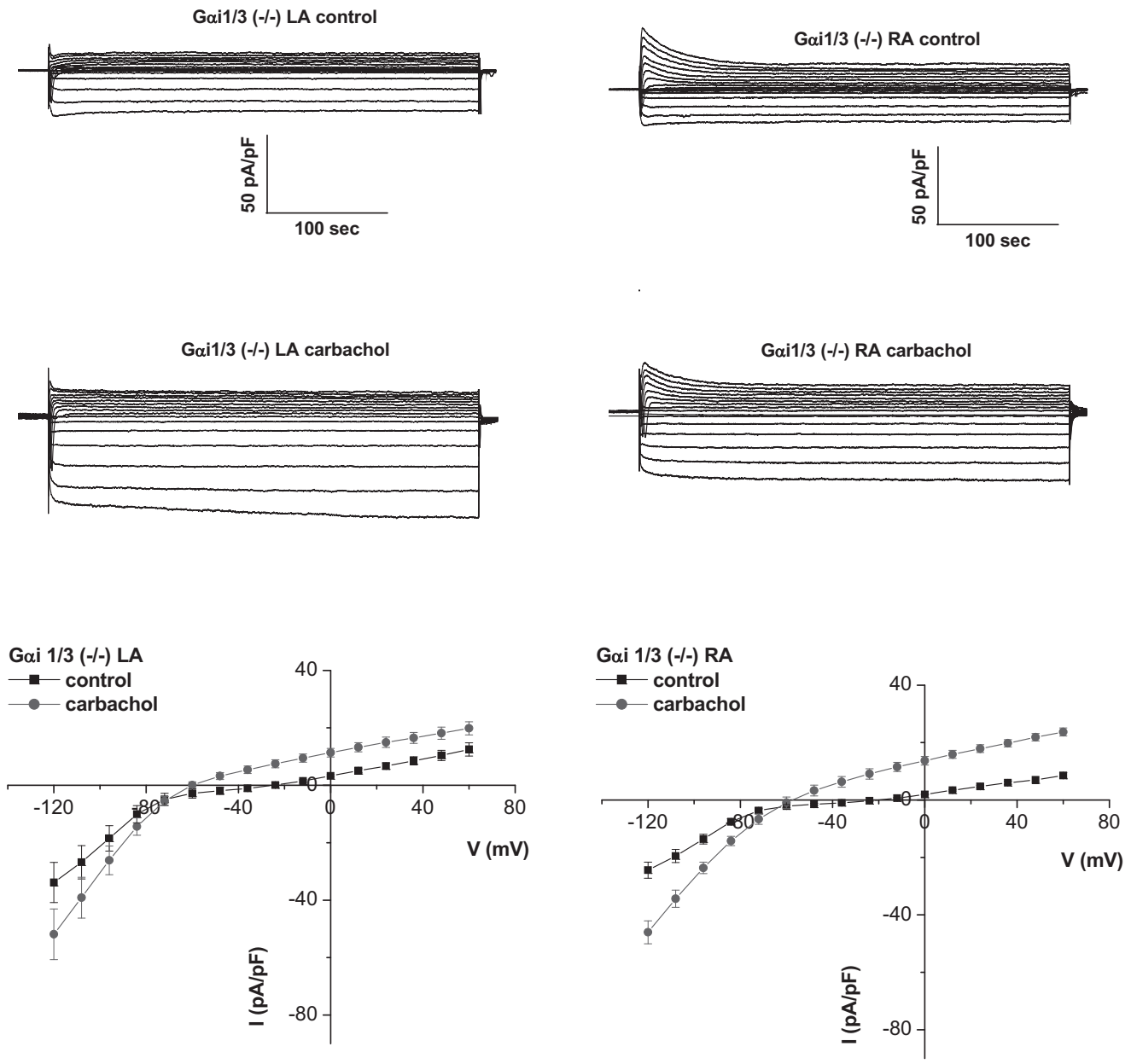

Fig. 2. Deletion of $G\alpha_{i1/3}$ affects the gradient of GIRK across the atria. Representative traces and mean current-voltage relationships of currents measured in atrial myocytes isolated from the left and right atria of $G\alpha_{i1/3}^{-/-}$ mice. There was a loss of GIRK current gradient between the LA and RA (n = 5–6, 4 mice).

5% CO_2-95% air at 37°C. All experiments were performed at room temperature.

Patch-clamp current recordings were performed with an Axopatch 200B amplifier (Axon Instruments) using fire-polished pipettes with a resistance of 3–4 MΩ pulled from filamented borosilicated glass capillaries (1.5 mm OD × 1.17 mm ID; Harvard Apparatus). Data were acquired and analyzed by using a Digidata 1322A interface (Axon Instruments) and pCLAMP software (version 10; Axon Instruments). Action potentials were recorded in the current-clamp mode. Cardiomyocytes were stimulated using a 5-ms current pulse. The resting membrane potential, the magnitude of the initial depolarization, and the action potential duration at which 50 and 90% repolarization (APD50 and APD90, respectively) occurred were measured. The cells were clamped at −60 mV in an extracellular solution containing (in mM): 135 NaCl, 5.4 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 0.33 NaH_2PO_4, 5 H-HEPES, and 10 glucose (buffered to pH 7.4 with NaOH). The intracellular solution was (in mM): 110 potassium gluconate, 20 KCl, 10 NaCl, 1 $MgCl_2$, 2 MgATP, 2 EGTA, and 0.3 Na_2GTP (buffered to pH 7.2 with KOH). The liquid junction potential was +13 mV.

Measurement of atrial electrophysiology using multielectrode arrays. Using a multielectrode array (MEA; Multichannel Systems), we investigated the effect of ablation of $G\alpha_{i2}$ on electrophysiological parameters in ex vivo atrial tissue (33). Left atria were dissected from isolated mouse hearts after mounting in a Langendorff setup and perfused with Krebs solution supplemented with 30 mM 2,3-butanedione monoxime. The tissue was then transferred to the array perfused with Krebs solution (37°C; 95% O_2-5% CO_2). Experiments were conducted in the absence and the presence of 10 nM–10 μM carbachol. Left atrial electrophysiology was assessed during electrical stimulation using a multielectrode array (MEA) system, which allows noninvasive synchronous multifocal recording of extracellular field

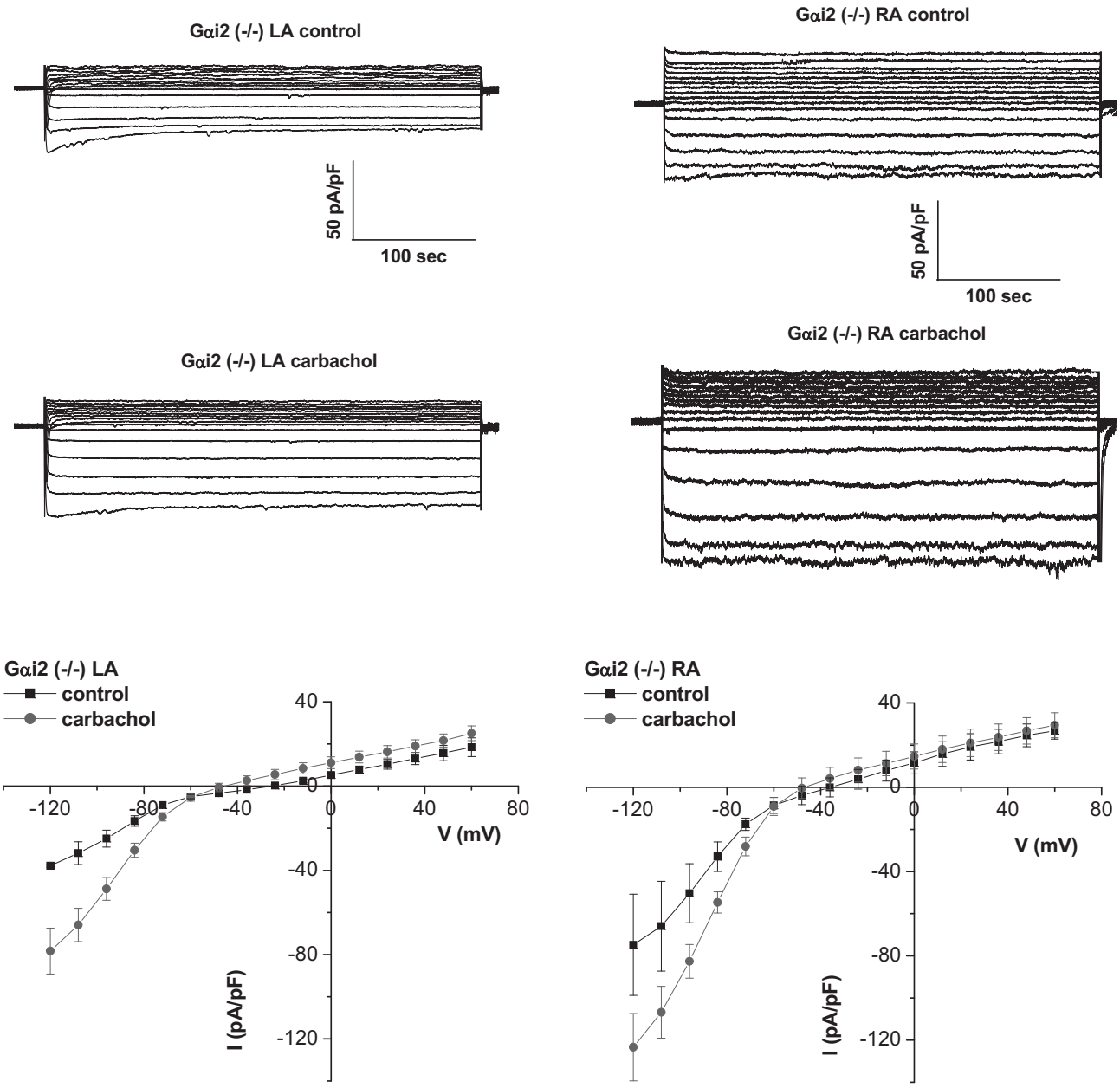

Fig. 3. Deletion of $G\alpha_{i2}$ affects the gradient of GIRK across the atria. Representative traces and mean current-voltage relationships of currents measured in atrial myocytes isolated from the left and right atria of $G\alpha_{i2}^{-/-}$ mice. Basal GIRK currents were larger in RA compared with $G\alpha_{i2}^{+/+}$ (n = 7–8, 4 mice).

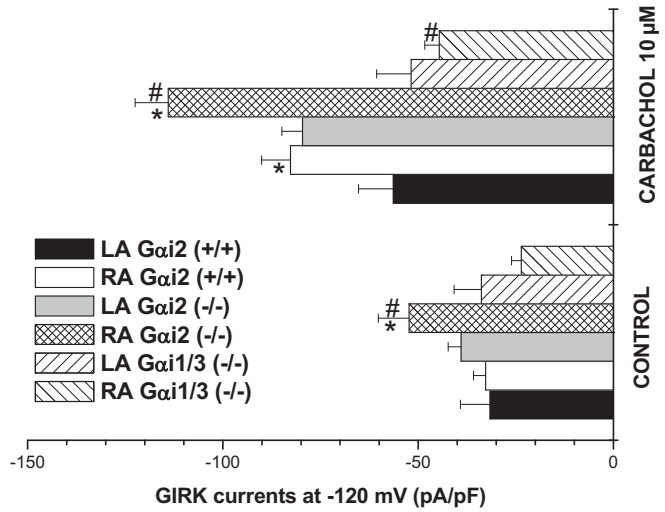

Fig. 4. Comparison of GIRK currents in the LA and RA. Bar graph showing maximum GIRK currents measured at −120 mV. In $G\alpha_{i2}{}^{+/+}$, carbachol (10 μM) led to a larger activation of GIRK currents in the RA (n = 10, 6 mice). Deletion of $G\alpha_{i2}$ led to larger basal and carbachol-activated currents in the RA (n = 6, 4 mice). Deletion of $G\alpha_{i1/3}$ led to smaller carbachol-activated currents in both LA and RA with a marked effect in the RA (n = 8, 4 mice), the consequence being a loss of gradient across the atria. *Comparison between LA and RA, #comparison between KO and WT $G\alpha_{i2}$ (+/+) and either $G\alpha_{i2}$ (−/−) or $G\alpha_{i1/3}$ (−/−).

potentials. The MEA (MEA2100; Multichannel Systems, Reutlingen, Germany) consists of 60 microelectrodes arranged in an 8 × 8 matrix, with a 20-μm electrode diameter and an interelectrode distance of 200 μm. Myocardial samples were positioned in the center of the MEA dish, held in contact with electrodes by a holder, and continuously superfused with oxygenated Krebs solution at 37°C. Baseline electrical stimulation (bipolar pulses, 2× threshold, 2 s duration, 4 Hz frequency) was applied via one of the MEA microelectrodes. Field potential data were acquired simultaneously from all 60 microelectrodes. S1–S2 train stimulation with a S1–S1 cycle length of 250 ms was used to assess atrial effective refractory period (AERP). To assess conduction properties, isolated atria were sequentially stimulated (4 Hz) from one electrode of each four edges of the array. Field potential recordings obtained in these conditions were processed using LabChart7 (ADInstruments) to define local activation time based on minimum of the derivative of field potential. Average conduction velocity (CV) was calculated by linear regression relating interelectrode distance to activation times, as previously described (33). The slope of the regression line was the average CV. Minimal wave-front cycle length was calculated for each isolated atria as AERP × CV.

Table 2. *Quantitative real-time reverse transcription PCR to measure gene expression in the RA and LA*

	LA ΔC_T		RA ΔC_T	
Gene	WT	KO	WT	KO
Gnai1	6.75 ± 0.06	6.98 ± 0.05	8.46 ± 0.35	8.15 ± 0.09
Gnai3	6.90 ± 0.18	6.65 ± 0.08	6.98 ± 0.12	7.42 ± 0.09
Gnb1	4.76 ± 0.14	5.72 ± 0.25*	5.21 ± 0.50	5.50 ± 0.07
Gnb4	7.98 ± 0.18	7.72 ± 0.07	8.75 ± 0.18	8.60 ± 0.08
Gng7	10.8 ± 0.14	11.5 ± 0.14	11.5 ± 0.51	11.7 ± 0.16
Gng11	7.04 ± 0.11	7.49 ± 0.03*	7.56 ± 0.21	7.43 ± 0.04
Kcnj3	2.86 ± 0.14	2.30 ± 0.07	3.62 ± 0.18	3.00 ± 0.05
Kcnj5	5.38 ± 0.12	4.86 ± 0.06*	5.51 ± 0.24	4.94 ± 0.07*

Values are means ± SE. Quantitative real-time reverse transcription PCR was performed as described in MATERIALS AND METHODS for the genes indicated. Measurements were performed in triplicate from $G\alpha_{i2}{}^{-/-}$ mice (n = 3 mice) and littermate controls (n = 3 mice). WT, wild type; KO, knockout. *P < 0.05 using one-way ANOVA.

Statistical analysis. The mean and SE are presented. Student's t-test or one-way ANOVA was used, with a P value <0.05 being statistically significant.

RESULTS

Inward currents in right and left atria. With the use of a step voltage protocol, current-voltage (I–V) relationships of atrial cardiomyocytes isolated from the right atria (RA) and left atria (LA) were compared in control mice (normal genotype littermates from the $G\alpha_{i2}$ crosses) (Fig. 1). Currents were larger and showed greater inward rectification in RA than in the LA in the presence of carbachol (Figs. 1 and 2). Experiments were also performed in the presence of GTPγS in the patch pipette to activate GIRK currents in the absence of receptor stimulation. In these experimental conditions, we still obtained a larger current in RA (−59.8 ± 6.5 pA/pF, n = 5) compared with LA (−27.8 ± 2 pA/pF, n = 5) of $G\alpha_{i2}{}^{+/+}$ atrial myocytes (n = 5 mice).

In $G\alpha_{i2}{}^{-/-}$ mice, the I–V relationship of the RA was altered with larger basal and carbachol-activated inward currents in the RA (Figs. 3 and 4). In mice with the combined deletion of $G\alpha_{i1}/G\alpha_{i3}$ there were reduced carbachol-activated currents in the RA (Figs. 2 and 4). The effects of $G\alpha_{i2}$ deletion were more pronounced in the RA, leading to loss of regional difference across the atria. Kinetics of GIRK current activation by carbachol were assessed using a 20-s application of agonist, as we

Table 1. *Deletion of $G\alpha_{i2}$ or $G\alpha_{i1/3}$ and GIRK current kinetics across the atria*

	$G\alpha_{i2}{}^{+/+}$			$G\alpha_{i2}{}^{-/-}$			$G\alpha_{i1/3}{}^{-/-}$		
	LA (n = 9)	RA (n = 8)	SAN (n = 10)	LA (n = 12)	RA (n = 10)	SAN (n = 11)	LA (n = 7)	RA (n = 8)	SAN (n = 5)
I_m, pA/pF	−85 ± 8	−86.5 ± 7.8	−55.9 ± 8	−77.6 ± 6.2	−75.1 ± 7	−49.7 ± 6.2	−67 ± 4.2	−70 ± 9.5	−58.2 ± 18
GIRK, pA/pF	−106 ± 15	−162 ± 34.5	−108.3 ± 20	−87.8 ± 8.2	−128 ± 12	−118.3 ± 14.4	−92.51 ± 10	−134.8 ± 24	−175.2 ± 18
Lag + TTP, s	1.06 ± 0.06	0.84 ± 0.05	0.99 ± 0.01	0.9 ± 0.05	0.82 ± 0.04	0.96 ± 0.05	1 ± 0.05	0.68 ± 0.04	0.79 ± 0.07
τac, ms	188 ± 11.1	142 ± 11.3	162 ± 24.4	156 ± 10.8	153 ± 27	144 ± 7.2	256 ± 45	114 ± 13	143 ± 13.7
τdeac, ms	2,996 ± 458	811 ± 66.6*	607 ± 92*	2,654 ± 438	779 ± 137*	466 ± 40.3*	3,567 ± 739	824 ± 141*	493 ± 71.3*
Lag inac, s	0.68 ± 0.14	0.32 ± 0.06*	0.21 ± 0.01*	0.59 ± 0.08	0.33 ± 0.03*	0.23 ± 0.02*	0.53 ± 0.01	0.30 ± 0.07*	0.26 ± 0.01*
% des 20 s	25.8 ± 2.35	25.2 ± 3.34	20 ± 1.5	29.5 ± 3.4	27.1 ± 2.9	31 ± 3.2	22.5 ± 1.6	24.1 ± 2.7	14.1 ± 3.5
G	22.7 ± 1.3	18.7 ± 1	26.4 ± 2.7	25 ± 2.2	19.6 ± 1.3	22.8 ± 2.4	34.4 ± 3.3	22.6 ± 2.6	17.8 ± 2.2

Values are means ± SE. Cells were clamped at −60 mV and carbachol was applied for 20 ms with a fast perfusion system. Characteristics of the GIRK current kinetics are presented for the LA, RA, and SAN region for $G\alpha_{i2}{}^{+/+}$ (n = 10–12 from 4 mice), $G\alpha_{i2}{}^{-/-}$ (n = 8–10, 5 mice), and $G\alpha_{i1/3}{}^{-/-}$ (n = 5–8, 3 mice). The current inactivation characteristics [τdeac (tau of deactivation) and Lag inac (lag of inactivation)] were faster in the RA and SAN compared with the LA (*P < 0.05). I_m, basal current; Lag, time lag; TTP, time to peak; τac, tau of activation; % des 20 s, percentage of desensitization after 20 s; G, cell conductance.

have previously described (2, 3, 31). There were no major changes in activation and rapid desensitization between RA and LA and in the mice with either $G\alpha_{i2}$ or $G\alpha_{i1}/G\alpha_{i3}$ deletion (Table 1). In contrast, deactivation was slower in the RA than the LA, but this pattern was not changed in the $G\alpha_{i2}^{-/-}$ mice or mice with combined $G\alpha_{i1}/G\alpha_{i3}$ deletion (Table 1).

We examined for expression changes of relevant components in the signaling cascade in the RA and LA of $G\alpha_{i2}^{-/-}$ mice and littermate controls. Using quantitative real-time reverse transcription PCR, we measured the expression of $G\alpha_{i1}$ (Gnai1), $G\alpha_{i3}$ (Gnai3), some representative $G_s\beta\gamma$ (Gnb1, Gnb4, Gng7, and Gng11), and the GIRK channel subunits (Kcnj3 and Kcnj5), and the results are shown in Table 2. Gnb4 and Gng11 were chosen as they have potentially been associated with cardiovascular traits, in particular heart rate, in genomewide association studies (10). In general, the changes between $G\alpha_{i2}^{-/-}$ mice and littermate controls are modest even when significant. However, in $G\alpha_{i2}^{-/-}$ mice, GIRK channel subunit kcnj5 expression was increased in both atrial chambers, suggesting that some of the differences in regulation of GIRK channels in $G\alpha_{i2}^{-/-}$ mice may be related to transcriptional changes in channel expression.

Comparison with the SAN. We also isolated and patch clamped SA nodal cells. GIRK currents were of a similar magnitude as that in the RA but rectified more strongly and deactivated more rapidly after carbachol application (Fig. 5 and Table 1). The properties of the currents in the SAN were unaffected in mice with global genetic deletion of $G\alpha_{i2}$ or $G\alpha_{i1}/G\alpha_{i3}$.

Single-cell action potentials. It might be predicted that an increased GIRK current in $G\alpha_{i2}^{-/-}$ mice might lead to a shortened action potential duration and atrial effective refrac-

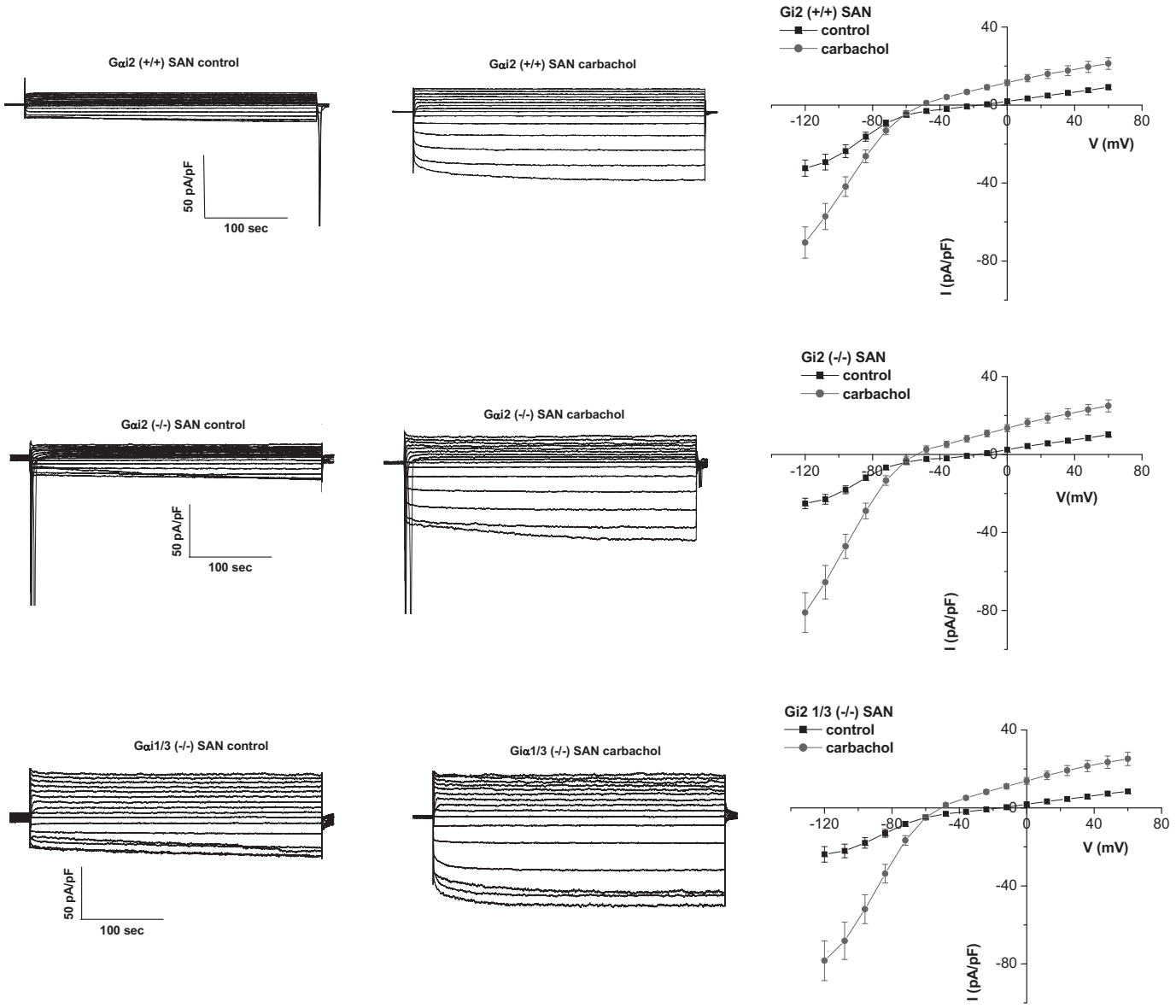

Fig. 5. GIRK current in the SAN. *Left*: representative traces of GIRK currents in control and after activation with 10 μM carbachol. Comparison is made between $G\alpha_{i2}^{+/+}$ (n = 18, 8 mice), $G\alpha_{i2}^{-/-}$ (n = 15, 5 mice), and $G\alpha_{i1/3}^{-/-}$ (n = 16, 7 mice). *Right*: mean current-voltage relationships. Atrial myocytes were challenged with 10 μM carbachol. GIRK currents were not affected by $G_i\alpha$ deletion.

tory period. We compared atrial action potentials in the RA and LA myocytes in control and $G\alpha_{i2}^{-/-}$ mice and found that RA atrial myocytes from RA $G\alpha_{i2}^{-/-}$ mice had a shorter APD90 than control RA myocytes. A similar trend was observed in the LA myocytes, although this was not statistically significant (Fig. 6).

Carbachol (10 μM) led to shortening of APD with a more pronounced effect in the RA. The decrease of the APD90 reached 61 ± 3% in $G\alpha_{i2}^{+/+}$ LA (n = 9, n = 3 mice) and 76 ± 3% in $G\alpha_{i2}^{+/+}$ RA (n = 8, n = 3 mice, P = 0.006). In $G\alpha_{i2}^{-/-}$ murine atrial myocytes, carbachol decreased APD90 by 57 ± 7% in the LA (n = 7, n = 3 mice) and by 50 ± 8% in the RA (n = 6, n = 3 mice, not significant).

Tissue electrophysiology. We performed an analysis of the tissue electrophysiology in isolated LA using a multielectrode array. The analysis of intact RA was complicated by the intrinsic pacemaking activity. In the $G\alpha_{i2}^{-/-}$ LA, there was a shortened effective refractory period (ERP) and no alteration in conduction velocity in comparison to $G\alpha_{i2}^{+/+}$ LA, resulting in a significant decrease in potential path length for reentry (Fig. 7). A similar relative decrease of left atrial ERP was observed in the presence of carbachol (10 nM to 10 μM) between $G\alpha_{i2}^{-/-}$ and control, with no significant difference in log EC_{50}, i.e., −6.9 ± 0.4 vs. −7.1 ± 0.4, respectively. Carbachol did not alter left atrial CV (n = 7, Fig. 7).

DISCUSSION

Our main findings are that in murine atria specific isoforms of inhibitory G proteins have defined roles in controlling GIRK channel function. Specifically, $G\alpha_{i2}$ suppresses the basal and agonist-induced activity of GIRK, whereas $G\alpha_{i1}$ and/or $G\alpha_{i3}$ mediate muscarinic activation of the current. Our experiments are in agreement with previous work showing larger GIRK currents in the RA and the SAN regions compared with the LA region (12, 13, 23, 26, 40). Deletion of $G\alpha_{i2}$ accentuated this chamber asymmetry, but it was attenuated in mice with global genetic deletion of $G\alpha_{i1}$ and $G\alpha_{i3}$. In keeping with the changes in GIRK currents, the global genetic deletion of $G\alpha_{i2}$ resulted in a shortened action potential duration, reduced tissue atrial effective refractory period, and reduced minimum wave front cycle length.

These findings complement our previous work in which we have investigated heart rate regulation in various G protein α-subunit knockout mice (41, 46). Specifically, mice with global and SAN-specific deletion of $G\alpha_{i2}$ were tachycardic with impaired high-frequency responses in heart rate variability studies. It is worth stating that the majority of studies reported here were conducted in the atria and reveal differences between the SAN and across the atria in GIRK channel signaling and G protein dependency. Our observations reported here in the SAN show preserved signaling via muscarinic receptors to GIRK channels with deletion of both $G\alpha_{i2}$ and combined $G\alpha_{i1}\backslash G\alpha_{i3}$. This suggests that GIRK channel-independent mechanisms may be important in determining the in vivo phenotype in $G\alpha_{i2}$ knockout mice. Specifically, the loss of negative coupling to adenylate cyclase, fall in cAMP, and effects on the hyperpolarization-activated cyclic nucleotide-gated channel and/or modulation of protein kinase A regulating the "calcium clock" may be important (20).

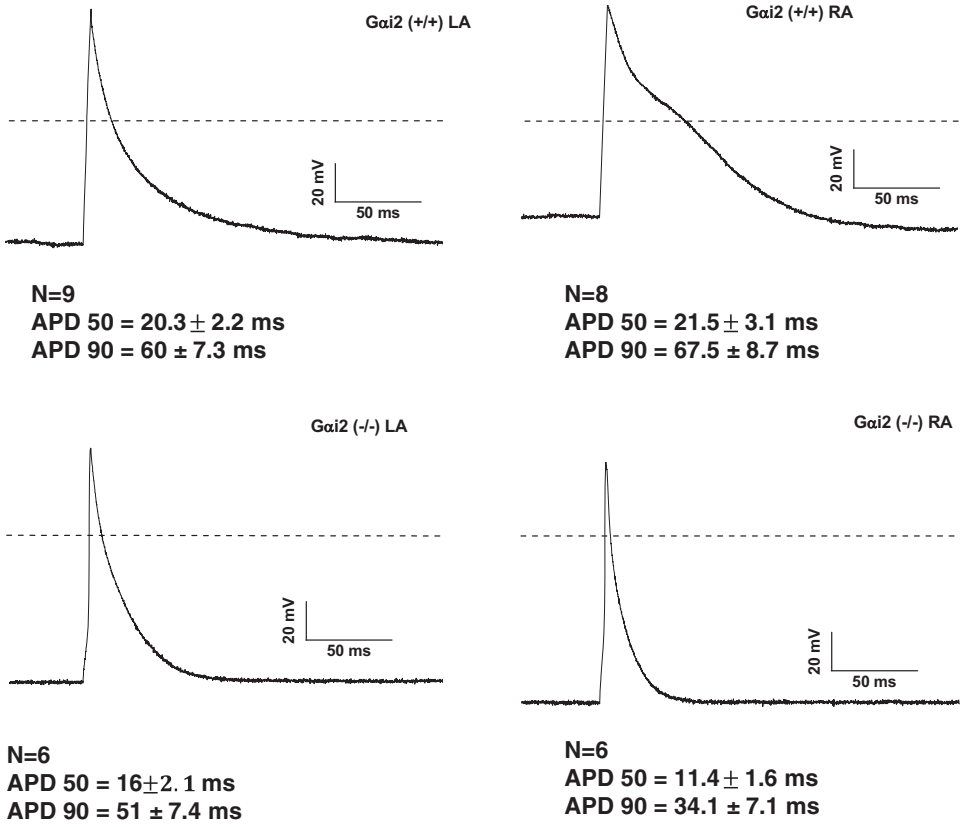

Fig. 6. Consequence of the deletion of $G\alpha_{i2}$ on the action potential of single cardiomyocytes. Single cardiomyocyte action potential (AP) was measured after stimulation of cells by a 5-ms pulse after pacing at 1 Hz for 60 s. In $G\alpha_{i2}^{+/+}$, the APD90 were longer in the RA (n = 8–9, 3 mice), although the mean values did not reach significance during analyses with t-test due to the variability of the data values. In $G\alpha_{i2}^{-/-}$ (n = 6–7, 3 mice), both APD50 and APD90 were significantly reduced in the right atria.

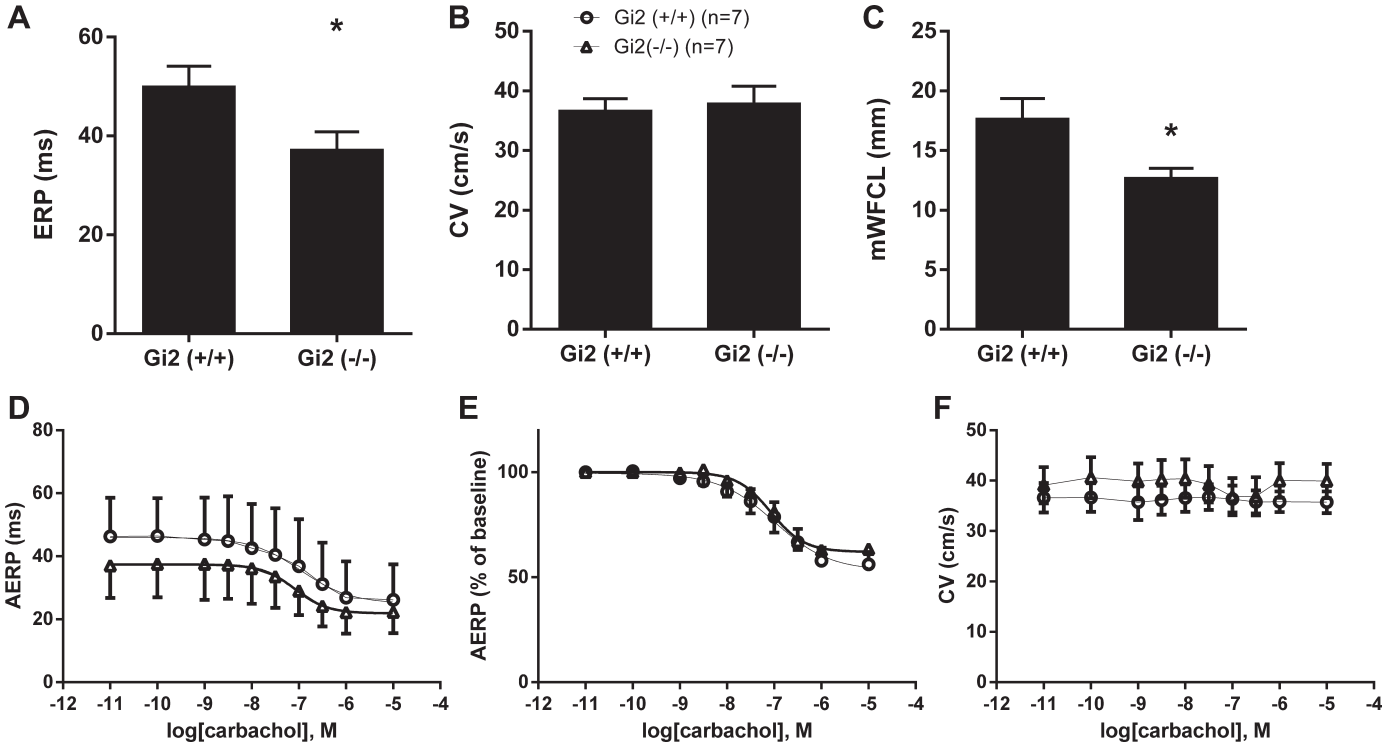

Fig. 7. Electrophysiology of isolated right and left atria. *A–C*: atrial effective refractory period (ERP; *A*), conduction velocity (CV; *B*), and minimum wave front cycle length (mWFCL; *C*) in left atrial tissue deficient in $G\alpha_{i2}{}^{-/-}$ mice compared with $G\alpha_{i2}{}^{+/+}$. *$P < 0.05$. *D–F*: dose response for carbachol on left atrial AERP (*D*), relative AERP to baseline (*E*), and CV (*F*) in $G\alpha_{i2}{}^{-/-}$ mice compared with $G\alpha_{i2}{}^{+/+}$. Experiments were performed with 7 mice in each group.

Inhibitory G protein α-subunits and GIRK channel function. GIRK channels are traditionally viewed as an example of a canonical effector activated by Gβγ subunits. However, it is clear that Gα subunits play a role. For example, we demonstrated in heterologous systems that channel activation seemed to be preferentially activated via inhibitory rather than stimulatory G proteins (21). Furthermore, studies have shown that the inhibitory G protein heterotrimer can bind to the channel complex (6, 9, 37). This interaction may have important functional consequences, namely that it suppresses basal current activity (6, 34). In other studies the Dascal laboratory demonstrated that there may be isoform differences in the nature of this behavior between $G\alpha_{i1}$ and $G\alpha_{i3}$ (15). One issue with a portion of this work is that the conclusions often depend on overexpression of engineered components in heterologous expression systems. It is unclear whether these kinds of effects occur in native settings with physiological levels of G protein and channel expression. In this study in native atrial myocytes, we show that deletion of $G\alpha_{i2}$ leads to an unexpected increase in basal and agonist-activated currents. One interpretation of this finding is that inhibitory G protein α-subunits do indeed have an ability in vivo to negatively regulate GIRK currents. However, our data reveal another potential mechanism, namely transcriptional changes in GIRK channel subunit expression engendered by $G\alpha_{i2}$ deletion. Specifically, in the $G\alpha_{i2}{}^{-/-}$ mice, expression levels for kcnj3 and kcnj5 mRNA, in a statistically significant fashion for the latter, were increased compared with littermate controls, and the magnitude of these effects was comparable to the changes in GIRK current density observed. In contrast, combined deletion of $G\alpha_{i1}$ and $G\alpha_{i3}$ impairs the magnitude of muscarinic activation, suggesting that one of $G\alpha_{i1}$ and $G\alpha_{i3}$ or both are important for mediating the agonist-induced response. Although we studied single isolated cardiac cells ex vivo, it is still possible that extracardiac effects could lead to long-lasting changes in myocyte biology.

We also examined if deletion of $G\alpha_{i2}$ had effects on the expression of other components in the signaling cascade, namely Gnai1, Gnai3, Gnb1, Gnb4, Gng7, and Gng11. These experiments have substantial practical complications, since there are 5 G protein β-genes and 14 G protein γ-genes. We selected four to examine for compensatory changes in part determined from genomewide association studies in heart rate and our own unpublished studies (10). Although there were some changes, these were modest in magnitude (possible decreases in expression of gnb1 and gng11 in the LA but no significant change in gnb4, gng7, gnai1, or gnai3). Furthermore, in the functional studies in $G\alpha_{i2}{}^{-/-}$ mice, carbachol led to increased agonist-induced current activation, suggesting Gβγ expression was not limiting for signal transduction.

Regional differences. There were regional differences in the nature and coupling profile of GIRK currents in supraventricular tissues. GIRK currents were larger in the right atrium and SAN, and these differences were accentuated in the right atrium by global genetic deletion of $G\alpha_{i2}$. Kinetic analysis also showed that GIRK current inactivation is faster in the RA and the SAN regions compared with the left atrium. This fast inactivation of the GIRK currents in the RA and pacemaker regions could reflect differential expression of regulators of G protein signaling that can increase the hydrolysis rate of GTP-bound and active G protein α-subunits (4, 33, 36). Furthermore, GIRK currents were more outwardly rectifying in the SA node and this could contribute to their importance in recovery

of heart rate after exercise as explored recently in GIRK4 knockout mice as they may play a more significant role at depolarized potentials (30).

Another interesting finding is that the molecular details of the signaling system differ between closely related regions of the heart. Hence, although $G\alpha_{i2}$ and $G\alpha_{i1}/G\alpha_{i3}$ seem to have roles in inhibition and activation, respectively, in the atria, this pattern does not exist in the SA node. Indeed, a significant amount of redundancy is suggested, since GIRK channel activation was little affected in either $G\alpha_{i2}$ or $G\alpha_{i1}/G\alpha_{i3}$ knockout mice. This suggests that the specifics of the signaling can be tissue and region dependent. G protein deletion had no effect on the kinetics of signaling, suggesting that this was predominantly determined by other factors such as the expression of regulators of G protein signaling.

G protein deletion and predisposition to arrhythmia. The increase in GIRK currents in mice with global genetic deletion of $G\alpha_{i2}$ might lead to more general effects on single-cell and tissue-level electrophysiology. Indeed, the increase in GIRK currents was sufficient to decrease the action potential duration. Furthermore, in whole left atrial preparations, $G\alpha_{i2}$ shortened the atrial effective refractory period without an effect on conduction velocity, leading to a decreased minimum wave front cycle length. This change would be potentially proarrhythmic. We have also previously observed that $G\alpha_{i2}$ deletion in the ventricle and silencing of the vagal input increase the predisposition to ventricular arrhythmia (28, 47). The mechanism is different with an effect on calcium channel regulation and expression (47). It is also known that GIRK4 knockout mice are resistant to the induction of atrial fibrillation, whereas RGS6 knockout mice with increased GIRK channel activity are predisposed (18, 36). Other investigators have observed in the dog that $G\alpha_{i2}$ and/or $G\alpha_{i3}$ knockdown using cell-permeable peptides may suppress vagally mediated atrial fibrillation when delivered into the posterior left atrium (1). The authors did not examine the specifics of the mechanism and whether it was related to GIRK channel activation.

Action potential duration has been shown to decrease with increasing distance from the SAN region (26, 32). In contrast, GIRK currents are larger in the right atrium than left atrium, and this suggests there are other important electrophysiological determinants of the variation in action potential duration across the atria. The shortened action potential duration in the left atrium is potentially important as it may allow the support of higher-frequency rotors in and around the pulmonary veins (40) although in this study the authors found higher GIRK currents in the the left versus right atrium of sheep. Despite this lack of consensus, suppression of GIRK channel activity in the left atrium abrogates reentry and atrial fibrillation (8).

Conclusions. Although not directly addressing the issue, our studies are compatible with the long-standing view that $G\beta\gamma$ subunits are important for GIRK channel activation. However, they do reveal layers of complexity in how the $G\alpha$ heterotrimeric G protein subunit might shape this response. A body of work, which we discuss above, has suggested various ways by which this might occur, including direct protein-protein interaction between G protein heterotrimer components and channel domains. However, much of this work is accomplished by expressing components, often at nonphysiological levels, in model cell systems. Here we examine native signaling in various chambers and regions of the heart using mice with global genetic deletion of $G\alpha$ subunits. Our overall conclusion is that there is much plasticity in the system, with the exact importance of a specific $G\alpha$ subunit being dependent on tissue region expression. $G\alpha$ subunits may directly suppress GIRK currents in native systems, but this could be accounted for by additional effects on channel transcription. These observed phenomena may result from variations in $G\alpha$ subunit expression or compartmentation with the channel in different cardiac regions, and these are topics for future investigation.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

M.N., D.M., and S.S. performed experiments; M.N. and D.M. analyzed data; M.N., D.M., S.S., and A.T. interpreted results of experiments; M.N. and D.M. prepared figures; M.N., D.M., and A.T. drafted manuscript; M.N., D.M., and A.T. edited and revised manuscript; M.N., D.M., S.S., L.B., and A.T. approved final version of manuscript.

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