Absence of the inhibitory G-protein, Gα$_{i2}$, predisposes to ventricular cardiac arrhythmia

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Abstract

Background—We have explored the role that inhibitory heterotrimeric G-proteins have in ventricular arrhythmia.

Methods and Results—We studied mice with global genetic deletion of Gα$_{i2}$ (Gα$_{i2}$ (−/−)) and find that they have a prolonged QT interval on the surface ECG when awake and studied with telemetry. We used in-vivo electrophysiology studies and found that the Gα$_{i2}$ (−/−) mice have a reduced ventricular effective refractory period and a predisposition to ventricular tachycardia when challenged with programmed electrical stimulation. Neither control nor mice with combined global deletion of Gα$_{i1}$ and Gα$_{i3}$ showed these abnormalities. There was no evidence for structural heart disease at this time point in the Gα$_{i2}$ (−/−) mice as assessed by cardiac histology and echocardiography. The absence of Gα$_{i2}$ thus leads to a primary electrical abnormality and we explored the basis for this. Single isolated ventricular cells studied using patch clamping showed that Gα$_{i2}$ (−/−) mice had a prolonged ventricular action potential duration but steeper action potential shortening as the diastolic interval was reduced in restitution studies. Gene expression studies showed increased expression of L-type Ca^{2+} channel subunits and patch clamping revealed an increase in these currents in Gα$_{i2}$ (−/−) mice. There were no changes in K+ currents.

Conclusion—Thus the absence of inhibitory G-protein signaling in particular that mediated via Gα$_{i2}$ is a substrate for ventricular arrhythmias.

Keywords

Inhibitory G-proteins; long QT syndrome; ventricular tachycardia; calcium channel

Introduction

Sudden cardiac death resulting from ventricular tachyarrhythmia represents a major healthcare issue. An estimated 300,000 cases per year are recorded in the United States

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stressing the importance of accurate prediction and treatment of those at particular risk [1]. Implantable cardiac defibrillators for the management of ventricular tachycardia have proven benefits, however precise calculation of those at greatest risk remains crude [2]. Pharmacological suppression of ventricular tachycardia has additionally been largely ineffective, and in some cases pro-arrhythmic [3]. Thus there is a pressing need to better understand the molecular pathways that determine the induction and persistence of ventricular tachycardia in an effort to develop treatment strategies.

The action of the sympathetic and parasympathetic systems on the heart are opposing in nature. The sympathetic system acting via β-adrenoreceptors and the stimulatory G-protein Gs is positively inotropic, chronotropic and lusitropic. In contrast, acetylcholine released from the vagus nerve acting via muscarinic receptors (largely M2) and inhibitory G-proteins is negatively chronotropic and inotropic. The ventricle expresses M2 muscarinic receptors at ~300 fmol\mg of protein [4]. The density of innervation from the parasympathetic nervous system is less in the ventricle than in supraventricular structures but is nevertheless present. At the cellular level $G_{i\backslash o}$-proteins are involved in inhibition of adenylyl cyclase, activation of PI-3 kinase and modulation of K^+ and Ca^{2+} channels [5]. The heterotrimer consists of a Gα subunit that defines the particular family and a Gβγ subunit. There are multiple isoforms of inhibitory G-proteins ($G\alpha_{i1}$, $G\alpha_{i2}$, $G\alpha_{i3}$, $G\alpha_o$ with splice variants $G\alpha_{o1}$ and $G\alpha_{o2}$) and more than one member is often expressed in a single tissue [5]. In general acetylcholine has no effect on basal L-type Ca^{2+} currents in the ventricle but it strongly antagonizes the increase on the application of a catecholamine or forskolin [6]. In contrast the α subunit of G_s activates adenylyl cyclase [7] and may also positively regulate the voltage-gated calcium channel by direct action on the α1 subunit independent of protein kinase A [8].

There is also evidence for pathological changes in the cardiovascular system occurring with an imbalance of signalling through stimulatory and inhibitory heterotrimeric G-proteins. For example, in the failing heart β_1-adrenergic receptors are down regulated and $G_{i\backslash o}$ α subunit expression, in particular $G\alpha_{i2}$, is significantly increased 9. Activation of the sympathetic nervous system whilst acutely beneficial is maladaptive in chronic heart failure. Chronic adrenergic receptor activation promotes cardiac myocyte apoptosis, ion channel remodelling and is pro-arrhythmic, partly through cAMP triggered activity [10]. This pathological process may be counteracted by signalling via cardiac $G\alpha_i$-coupled GPCR's within the heart 11. Interestingly whilst β_1 adrenoreceptor receptor overexpression results in a dilated cardiomyopathy, β_2 overexpression (which couples via both $G\alpha_s$ and $G\alpha_{i/o}$ mechanisms) has a much more modest phenotype suggesting that $G\alpha_{i/o}$ coupled signaling may be cardioprotective [10;12]. $G\alpha_{i2}$ has also been directly implicated in ventricular tachycardia induction from within pathological foci in the setting of right ventricular outflow tract tachycardia. A somatic cell mutation (F200L) from within the arrhythmia focus results in loss of function of $G\alpha_{i2}$ resulting in failed suppression of forskolin stimulated cAMP production [13]. It is known that increases in sympathetic drive can initiate ventricular tachyarrhythmias and that vagal nerve activation in an opposing fashion can terminate ventricular tachycardia and increase the ventricular fibrillation threshold [14–16]. Furthermore, lethal arrhythmias can often be precipitated by exercise and stress because of the activation of β-adrenergic pathways in defined arrhythmic syndromes and this is particularly the case in some variants of the long QT syndrome (LQT1 and LQT2)[17]. In this study we address the question of whether signalling via inhibitory heterotrimeric G-proteins is pro- or antiarrhythmic. We show that the genetic deletion of $G\alpha_{i2}$ in mice leads to a proarrhythmic substrate in part accounted for by increased L-type Ca^{2+} channel activity.

Materials and Methods

Experimental Animals

Mice with global deletion of $G\alpha_{i2}$ ($G\alpha_{i2}$ (–/–)) and the combined global deletion of $G\alpha_{i1}$ and $G\alpha_{i3}$ were generated by homologous recombination on a 129SvEv background. The gene targetting strategy and confirmation of selective $G\alpha_{i2}$ deletion have previously been published [18;19]. $G\alpha_{i1}/G\alpha_{i3}$ double (–/–) were obtained from $G\alpha_{i1}/G\alpha_{i3}$ double (–/–) intercrosses. $G\alpha_{i2}$ (–/–) mice were obtained from $G\alpha_{i2}$ (+/–) intercrosses. Age-matched controls ($G\alpha_{i2}$ (+/+) genotype) were generated as littermates in $G\alpha_{i2}$ (+/–) intercrosses. The genotyping details are given in Supplementary Methods. Mice were housed up to 4 per cage with free access to standard rodent chow/water with 12hr day/night cycles and in accordance with British Home Office animal welfare guidelines (PPL 70/6732). At the time of study mice (equal sex distribution) were 12–14 weeks of age, weighing 20–25g.

Gene Expression

The methods for the gene expression arrays and quantitative real time PCR are given in Supplementary Methods.

Telemetry implantation

Details of telemetry system (TEA-F20, DSI, St Pauls) implantation have previously been described [20]. Recording leads were tunnelled subcutaneously in a conventional "Lead II" ECG configuration to continuously record surface ECG after a 2 week period of surgical recovery. Single lead telemetry data was recorded continuously over 48hrs to look for evidence of spontaneous arrhythmia. In order to record standard surface ECG parameters, consecutive individual ECG complexes recorded over 2 minutes during sinus rhythm at high sampling frequency (2000Hz) were analysed (>1000 ECG complexes/mouse) using an ECG analysis extension module of CHART 4.0 software (ADIntruments, Oxford).

Histology and Isolation of cardiomyocytes

The protocols for the histological study of the heart and the isolation of single cardiomyocytes are given in the Supplementary Methods.

Single-cell electrophysiology

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 (Harvard Apparatus, 1.5 mm OD × 1.17 mm ID). Data were acquired and analysed by using a Digidata 1322A interface (Axon Instruments) and pCLAMP software (version 10, Axon Instruments). All experiments were done at room temperature. Drugs were applied by a gravity driven system. Further details are given in the Supplementary Methods.

In vivo electrophysiological and programmed ventricular stimulation protocols

Mice were anaesthetised with continuous 2.0% isoflurane gas. After needle electrodes were inserted to record surface ECG, an octapolar 1.1F ultraminature cardiac electrophysiology (EPR-800, Millar Instruments) was inserted into the right ventricular apex through the right internal jugular vein to pace the heart. Cardiac pacing using extrastimulation protocols was performed using a S88-Grass stimulator (Grass technologies, USA) to record *in vivo* cardiac electrophysiological parameters. These included: sinus node recovery time ($SNRT_{600}$), AV nodal effective refractory period ($AVNERP_{600}$), 2:1 AV block coupling interval from atrial pacing and ventricular effective refractory period ($VERP_{600}$). Finally a ventricular stimulation protocol was performed whereby successive extrastimuli (up to 4 (S_2-S_3-S_4-S_5))

were added to the basic drive train (15 paced beats at 100ms coupling interval) to try and provoke ventricular arrhythmia. If unsuccessful 0.1mg/kg intraperitoneal isoprotenerol was administered and the procedure repeated. Ventricular tachycardia (VT) was defined as at least 4 beats of pacing induced broad complex tachycardia that was qualitatively different from sinus rhythm (atrial electrograms confirmed AV dissociation during tachcayrdia. VT > 10 cycle lengths was defined as sustained ventricular tachycardia (i.e. a positive study). These definitions of VT are identical to those recently published [21].

Echocardiography

Mice were placed in the supine position and transthoracic echocardiography performed under 1.0% isoflurane anaesthesia using a commercial echocardiography machine (Vivid 7 Dimension™, GE Healthcare, Bedford, UK) with a 14 MHz probe recording at a depth of 0–1 cm. End diastolic and systolic dimensions were determined from a parasternal short-axis view by M-mode at the papillary muscle level. Measurements were taken of the internal dimension of the cavity using the leading-edge-to-leading-edge convention. Aortic blood flow velocities were determined by pulsed-wave Doppler in aortic arch before the bifurcation of the right carotid artery. The direction of blood flow was confirmed by colour Doppler. Stroke volume was determined as the product of the velocity time integral and the vessel cross-sectional area ($\pi \times [0.5 \times diameter]^2$). Prior studies in mice of this age showed the aortic diameter to be 1.34 mm, thus a cross sectional area of $(0.67)^2 \times \pi$ was assumed for all animals studied. The peak aortic blood flow velocity was measured as the average maximum velocity from 6 velocity-time traces. Heart rate was determined by measuring the time between 6 consecutive cycles from the start of each Doppler trace. Cardiac output was calculated as the product of heart rate and stroke volume.

Statistical analysis

Continuous data are presented as mean ± SEM and categorical data as percentages. The following statistical tests were used: Student's t-test to compare two groups, one way ANOVA with a Bonferroni post-hoc test to compare three groups or two way ANOVA to compare the data in Figures 4B, 4C and 5D (GraphPad Prism v4.0). Fischer's Exact test of proportions was used to compare the induction of VT between $G\alpha_{i2}$ (−/−) and control. Standard linear regression was used (GraphPad Prism v4.0) and a correlation coefficient and slope are shown for the data in Figure 4. The slopes are compared using a method equivalent to the analysis of covariance (GraphPad Prism v4.0). A p-value of <0.05 was taken to be statistically significant.

Results

$G\alpha_{i2}$ (−/−) mice have spontaneous ventricular ectopy and QT interval prolongation

We have recently reported that $G\alpha_{i2}$ (−/−) mice have impaired heart rate variability and lose the negative chronotropic response to carbachol [20] probably because $G\alpha_{i2}$ acts as the key molecular link between the M2 muscarinic receptor and the G-protein gated K^+ channel in sinoatrial nodal cells. In the course of these telemetry studies in conscious and ambulatory $G\alpha_{i2}$ (−/−) mice, we noted that two of the six mice studied, under basal conditions, exhibited significant spontaneous ventricular ectopic activity and aberrantly conducted beats – an example is shown in Figure 1A. This prompted us to closely examine the surface ECG parameters in all of these mice. We noted that the $G\alpha_{i2}$ (−/−) mice unexpectedly had QT interval prolongation despite relative tachycardia (Figure 1B) and this was reflected in a prolonged QT_c [20] (Table 1). These abnormalities (and spontaneous ectopy) were not seen in control or a separate group of mice with the combined global genetic deletion of $G\alpha_{i1}$ and $G\alpha_{i3}$ (Table 1).

Gα_{i2} (−/−) mice have a proarrhythmic substrate

We observed occasional and unexpected death in several Gα_{i2} (−/−) mice although we were not able to record terminal arrhythmia and this has also been previously observed [22]. To investigate further this potential substrate we used *in-vivo* electrophysiological studies in anaesthetised mice. The results of these studies are shown in Table 2. In particular we noted that the minimum ventricular S1–S2 coupling interval capable of ventricular capture (VERP) was significantly shorter in Gα_{i2} (−/−) mice compared with control mice (Table 2 and Figure 2A). A reduction in VERP is pro-arrhythmic in situations of both re-entry and triggered activity. These abnormalities were not seen in control or mice with the combined global genetic deletion of Gα_{i1} and Gα_{i3} (Table II). Gα_{i2} (−/−) mice had an increased incidence and duration of induced ventricular tachycardia (Figure 2B). We were able to induce sustained VT in 7 out of 11 Gα_{i2} (−/−) mice (63.6%) compared with 2 out of 12 (16.7%) littermate controls (Figure 2C, p-value=0.023, Fisher's exact test). Additionally a significant increase in mean VT duration was seen in Gα_{i2} (−/−) mice compared with control mice (Figure 2D).

There is a primary electrical abnormality in Gα_{i2} (−/−) mice

One possibility is that mice studied at this age (12–14 weeks) have developed pathological heart disease and the arrhythmic phenotype is secondary to this. However, there was no evidence of functional impairment in contractile function or other abnormalities as assessed using echocardiography in Gα_{i2} (−/−) mice compared to control wildtype mice (Figure 3 and Supplementary Data Table S1). Furthermore, when we harvested the organs there was no evidence of gross hypertrophy when comparing the ratio of heart weight to body weight or tibial length. In control mice the ratio of heart weight (mg) to body weight (g) was 5.02±0.20 (n=4) and in Gα_{i2} (−/−) it was 5.34±0.07 (n=4, P=0.18). In control mice the ratio of heart weight (mg) tibial length (mm) was 5.37±0.34 (n=4) and in Gα_{i2} (−/−) it was 5.20±0.23 (n=4, P= 0.69). Finally at cardiac histological examination ventricular dimensions were normal (Supplementary data, Figure S1A), myocytes were not hypertrophied and there was no evidence of fibrosis in Gα_{i2} (−/−) mice (Supplementary Data, Figure S1B).

The action potential duration and its' rate dependence

The correlation of ventricular action potential depolarisation and repolarisation with the QRS-T complex on the ECG is not as well established in mice as in larger mammals [23]. Thus we isolated single cardiac ventricular myocytes and studied the action potential characteristics using patch clamping. We found that the later phases of repolarisation were significantly prolonged in Gα_{i2} (−/−) mice compared to control as reflected in a significantly prolonged APD$_{50}$ and APD$_{90}$ (Figure 4A and Table 3). There was no difference in magnitude of the initial depolarisation or the resting membrane potential between the two groups (Table 3).

We next examined the rate dependence by constructing single cell restitution curves (see Methods and Figure 4B). There were significant differences between the Gα_{i2} (−/−) mice and control mice under basal conditions and in the presence of isoprenaline (both P<0.01, Two way ANOVA) (Figure 4B and 4C). Specifically the slope of the restitution curve in its linear phase was steeper in the Gα_{i2} (−/−) mice compared to the control mice (Figure 4E). Under basal conditions: control mice (20 to 120 ms, r^2=0.88) slope=0.20±0.03, Gα_{i2} (−/−) mice (20 to 120 ms, r^2=0.93) slope=0.43±0.04, P=0.0002. In the presence of isoprenaline: control mice (20 to 120 ms, r^2=0.90) slope= 0.27±0.03, Gα_{i2} (−/−) mice (20 to 100 ms, r^2=0.87) slope= 0.49± 0.07, P=0.01.

Increased L-type Ca^{2+} currents contribute to the abnormality

The observed abnormalities in repolarisation could be accounted for by increases in inward current in particular via L-type Ca2+ channels or a decrease in outward K+ currents. These could arise through changes in gene expression and concomitantly protein levels or alternatively changes in the surface expression or activity of these channels. Thus we initially ran gene expression arrays and compared Gα_{i2} (−/−) mice with controls. There is evidence for remodelling of the electrophysiological properties of cardiac myocytes in a number of pathological states. In particular, there have been reports of a decreased expression of K+ channels resulting in a prolonged action potential duration [24]. However at the mRNA level, gene array studies comparing control and Gα_{i2} (−/−) mice showed little change in the relevant K+ channel genes and in some cases their expression was even increased (Table 4). In contrast we did find a significant increase in expression (~2 fold) in Gα_{i2} (−/−) mice of the calcium channel alpha subunit, CACNA1C (Ca$_v$1.2), which underlies the L-type calcium current and SCN5A which underlies the rapidly inactivating sodium current in ventricular myocytes (Table 4). We sought to confirm the changes in gene expression using quantitative real time PCR (Figure 5A). The only ion channel gene with significant increased expression ($\Delta\Delta$Ct>1 corresponding to a two-fold change) was CACNA1C (Ca$_v$1.2).

Thus, we measured calcium currents in single ventricular myocytes using patch clamping. Representative recordings and current-voltage relationships are shown in Figure 5B. There was a significant increase in L-type calcium currents measured under basal conditions when using Ca^{2+} as the charge carrier (Figure 5C and Table 3). A similar increase was present using Ba^{2+} as the charge carrier under basal conditions and with application of isoprenaline, in myocytes isolated from the Gα_{i2} (−/−) mice compared to control (Figure 5D). In a two way ANOVA if the rows are with and without isoprenaline and the columns are the genotype then the row factor has a P= 0.0009 and the column factor P=0.02. The interaction factor is not significant (P=0.49). Nifedipine blocked the current in cells where it was tested. We also measured outward K$^+$ currents (see methods) and these were not significantly changed in the Gα_{i2} (−/−) mice compared to control (Figure 5E and Table 3). There were no differences in cell capacitance between the two groups of mice (Table 3).

Discussion

Our findings show that the absence of the inhibitory G-protein, Gα_{i2} (but not Gα_{i1}/Gα_{i3}) leads to a proarrhythmic substrate in the ventricle resulting in a long QT$_c$ interval and a predisposition to ventricular tachycardia. This is reflected at the cellular level with a prolonged ventricular action potential, most likely resulting from changes in expression and \or regulation of L-type Ca^{2+} currents. Additionally, we and others have observed unexpected sudden death in Gα_{i2} (−/−) mice but we did not study this prospectively [22]. Furthermore, during short-term telemetry we did not observe a lethal ventricular arrythmia though we did observe qualitatively an increased ventricular ectopic burden that we never saw in controls. However during minimally invasive EP testing Gα_{i2} (−/−) mice had a shortened VERP, a known proarrhythmic substrate. Additionally we were able to induce VT significantly more easily in Gα_{i2} (−/−) mice than controls. However not every Gα_{i2} (−/−) mouse developed VT and it is worth noting that the 129\Sv strain is relatively resistant to ventricular arrhythmia [25]. Furthermore, in a proportion of the Gα_{i2} (−/−) mice cAMP responses are relatively well preserved [26;27].

We performed studies to investigate potential mechanisms for the long QT interval on the ECG and the prolonged action potential duration in single cell patch clamping studies. The latter is an important observation as these measurements were made in the absence of extracellular signalling and agonists for these pathways. This contrasts with the *in-vivo*

situation where tonic regulation of ion channel currents via signalling pathways will also make an important net contribution to cardiac cell excitability. It is worth emphasising that the *in-vivo* phenotype of the $G\alpha_{i2}$ (−/−) mice will also be significantly influenced by changes in L-type calcium channel regulation. The absence of muscarinic receptor regulation of this current in $G\alpha_{i2}$ (−/−) mice has already been established and potentially *in-vivo* this will considerably exacerbate the increase in the calcium current as the heart is continuously exposed to varying vagal and sympathetic drive [28]. The loss of the muscarinic regulation of $Ca_v1.2$ will ensure that Ca^{2+} currents are larger for any given level of sympathetic-to-vagal balance. However our data suggest that the change in action potential duration is due to a long-term change in channel density at the membrane due to changes in expression or trafficking. Our studies revealed an increase in calcium channel expression and currents in the $G\alpha_{i2}$ knockout mice. Previous studies have seen no significant change though there was a trend towards this in the data and the experimental conditions were different [28]. That defects in L-type calcium channels might be associated with a long QT_c is very much an emerging theme. Timothy syndrome is characterised by a mutations in CACNA1C and prolonged QT_c, predisposition to sudden death, autism and a variety of defects in other tissues [29]. RAD, a ras related GTPase, is important in governing $Ca_v1.2$ channel trafficking. Overexpression of a dominant negative construct in mice and guinea pigs led to a doubling of L-type calcium current (a magnitude of change comparable to ours) and severe prolongation of the QT_c together with an arrhythmogenic phenotype [30]. Finally, recent large scale human genetic studies have shown linkage of variations in the population QT_c interval to variants within the NOS1AP gene that encodes an adaptor protein for nitric oxide synthase 1 [31]. Subsequent electrophysiological studies have revealed that the protein can regulate the expression of calcium currents in cardiac myocytes [32]. The exact link between inhibitory G-protein signalling and ion channel gene expression is unclear and we are actively investigating this. For example, it is known that the promoter for the $\alpha1C$ calcium channel contains a cAMP response element [33] and this pathway involving activation of CRE binding protein is an obvious candidate.

We also examined the rate dependence of action potential duration and found that as the test interval decreased the action potential shortened more steeply in the $G\alpha_{i2}$ (−/−) mice than in controls. This is inherently proarrhythmic as it may lead to repolarisation alternans a known marker for ventricular arrhythmia [34]. It also may help to rationalise how a shortened VERP and prolonged QT interval can both be present. Thus at normal heart rates the action potential duration (and QT_c interval) are relatively prolonged in the $G\alpha_{i2}$ (−/−) mice. However at higher rates the slope of the restitution curve in the linear phase is steeper in these mice and thus action potential duration shortens more resulting ultimately in a reduced VERP. The decrease in VERP would support re-entrant circuits by shortening the potential path length. We have not seen evidence for early or delayed afterdepolarisations in these experiments and thus think there is not a prominent substrate for triggered activity. Both of these can occur because of abnormalities in Ca^{2+} handling [35]. For example administration of an L-type Ca^{2+} channel agonist can lead to early afterdepolarisations [36]. However, myocardial contractility as measured using echocardiography in our study was not different. Furthermore other investigators have examined Ca^{2+} transients and contractility in $G\alpha_{i2}$ (−/−) mice and found no differences under control conditions or after the addition of isoprenaline [37]. We saw no evidence for structural heart disease in these mice but we only examined this at a relatively young age and it is not clear what happens as the mice age.

There are debates as to what can be learnt from murine models [38] but what are the potential pathophysiological implications of our observations? There has been a substantial focus on the role of aberrant signalling in heart failure via β-adrenergic receptors particularly in light of the beneficial outcomes of β-blockers on survival [11;39]. It has been established that there is an enhanced expression of $G\alpha_{i2}$ in heart failure [9]. Our results would suggest that

increased expression may be a potential protective mechanism against malignant ventricular tachyarrhythmias. More broadly, parasympathethic modulation of cardiac rhythm is considered protective against ventricular arrhythmia [40]. In contrast parasymapthetic attenuation manifesting as a reduced heart rate variability signal is a powerful predicitor of sudden cardiac death in heart failure [41] and a number of other cardiac pathologies [42]. Interestingly pravastatin treatment has been reported to selectively upregulate $G\alpha_{i2}$ expression and increase high frequency (HF) power [43]. We have previously described increased heart rate and selective loss of HF power in mice deficient for $G\alpha_{i2}$ (but not $G\alpha_{i1}$, $G\alpha_{i3}$ or $G\alpha_o$) 20. In the setting of hypertrophic cardiomyopathy, an increased of expression of $G\alpha_{i1}$ has been proposed to be proarrhythmic [44]. Our studies failed to reveal any significant abnormalities in mice with combined global genetic deletion of $G\alpha_{i1}$ and $G\alpha_{i3}$. Intriguingly, such remodelling may only become important in certain disease settings and different inhibitory G-protein isoforms may exhibit differential effects under these conditions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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A Control

B
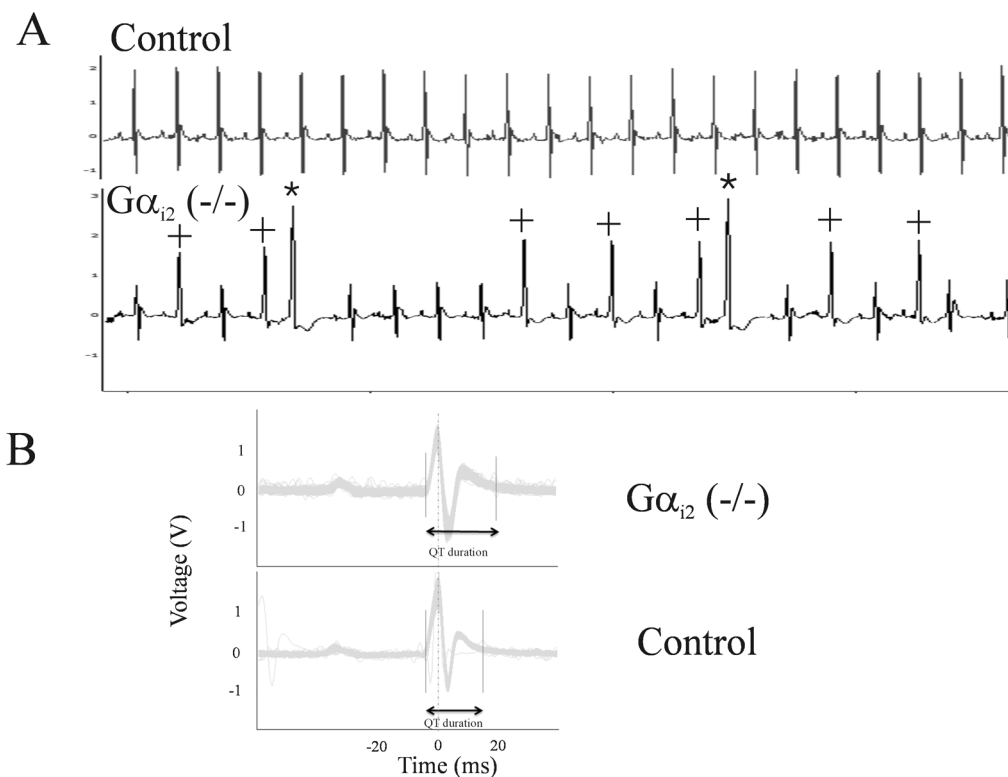
Figure 1. Single lead II surface ECG characteristics recorded from conscious freely moving mice under basal conditions using implantable telemetry

A. Evidence of unprovoked spontaneous ventricular ectopic activity was observed in 2/6 $G\alpha_{i2}$ (−/−) mice. No ventricular ectopics were observed in any littermate controls or in mice with the combined knockout of $G\alpha_{i1}$ and $G\alpha_{i3}$. The morphology of ectopic beats (highlighted by an asterix) suggests a ventricular origin. The complexes (highlighted by a +) are either ventricular in origin or are aberrantly conducted sinus beats. B. Representative signal averaged ECG recording from a $G\alpha_{i2}$ (−/−) (TOP) mouse showing evidence of QT prolongation compared with control (BOTTOM). Signal recorded and averaged from 900 successive sinus beats per mouse.
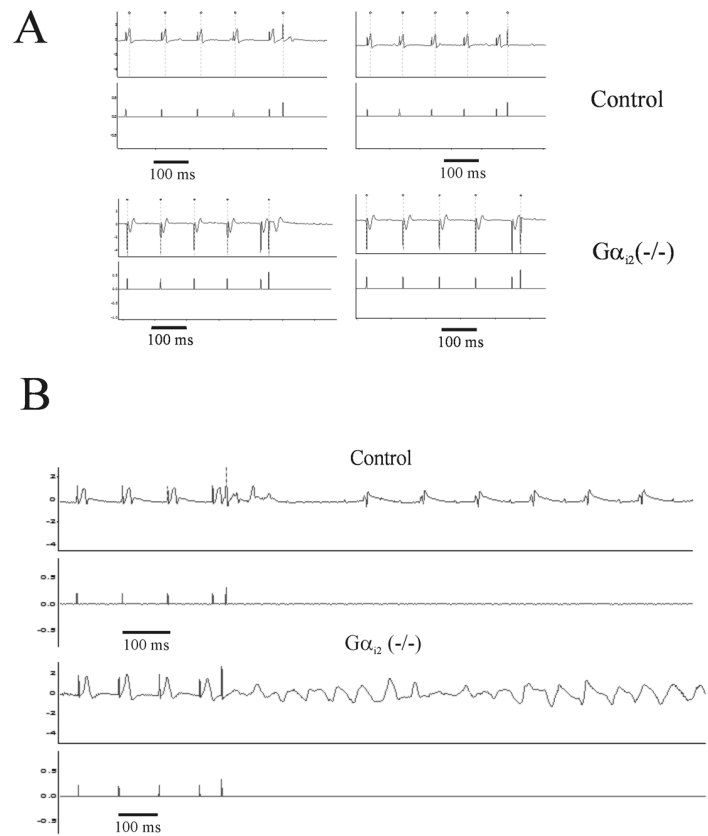
Figure 2. In vivo murine cardiac EP data and VT induced by programmed electrical stimulation
A. The measurement of VERP and this was significantly reduced in $G\alpha_{i2}$ (−/−) mice. B.
Example of programmed electrical stimulation with one extrastimulus (S2) demonstrating
VT induction. Control (TOP) shows non sustained VT induction at very short S1–S2
coupling. $G\alpha_{i2}$ (−/−) (Bottom) mice develop sustained VT using a similar induction
protocol.
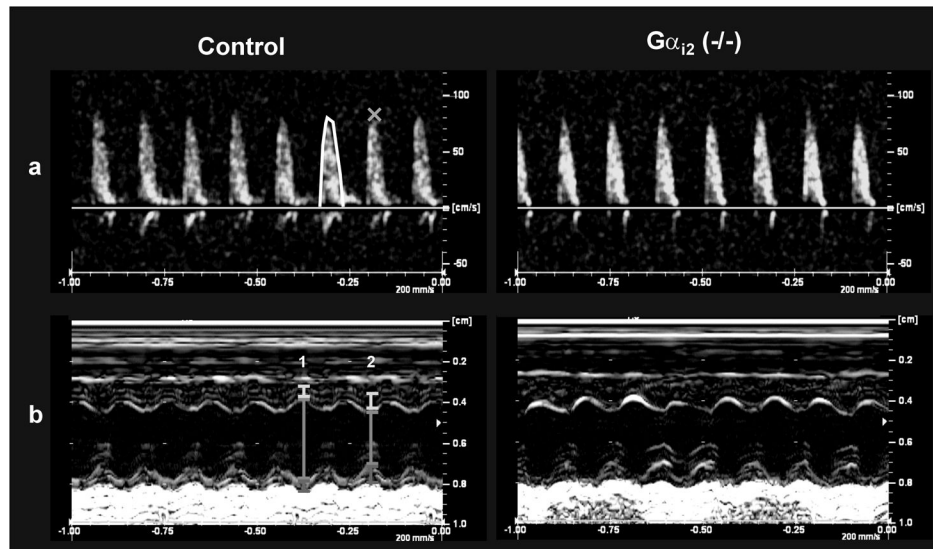
Figure 3. Echocardiography in wild type and Gα$_{i2}$ (−/−) mice
A. Pulsed-wave Doppler in the aortic arch. Velocity-time integral represents the area under each envelope (used to calculate stroke volume). Peak blood flow velocity is denoted by the orange cross (**x**). B. Parasternal short axis view of the left ventricle during M-mode. Dimensions of the anterior wall (yellow bars), left ventricle (green bars) and posterior wall (red bars) during diastole (1) and systole (2).
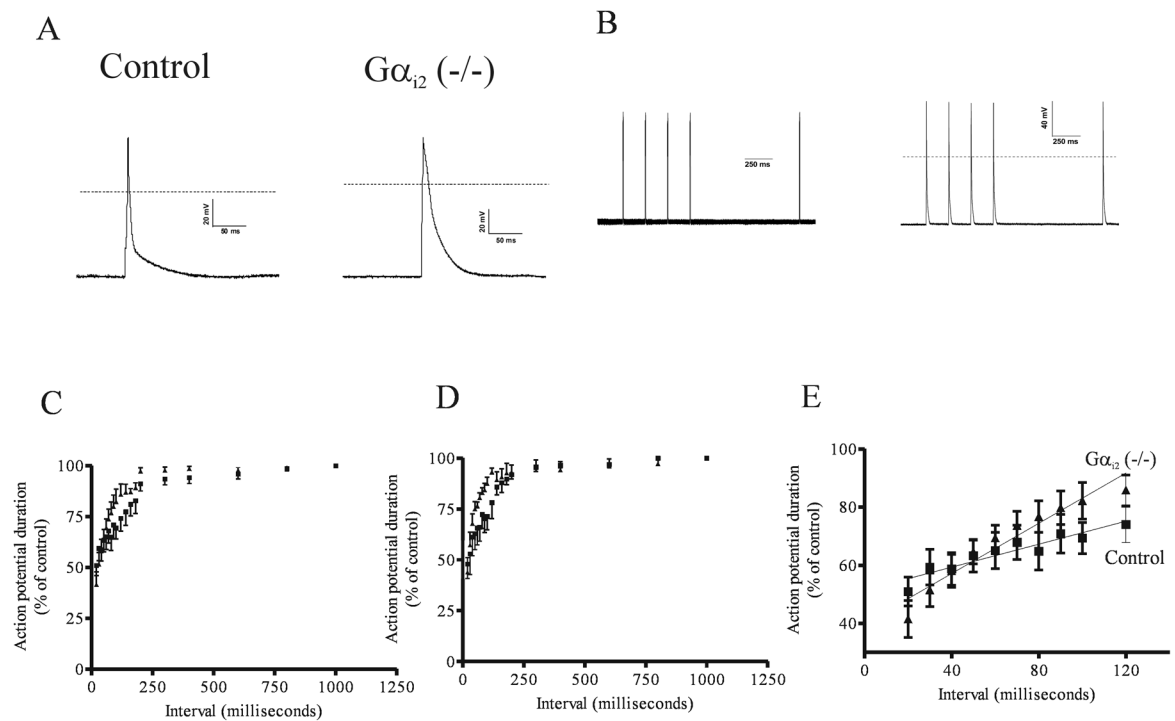
Figure 4. The action potential duration and its' rate dependence in control and Gα$_{i2}$ (−/−) mice
A. Ventricular action potentials in control and Gα$_{i2}$ (−/−) mice. Representative traces of an action potential measured after stimulation of the cells by a 5 ms pulse after pacing at 1 Hz for 60 seconds. B. An illustration of the protocol for restitution with a 1000 millisecond test interval. C. Restitution curve under basal conditions (■ = wild type mice ▲ = Gα$_{i2}$ (−/−) mice). D. Restitution curve in the presence of 10 μmol\L isoprenaline (■ = wild type mice ▲ = Gα$_{i2}$ (−/−) mice). E. Regression analysis of the linear portion of the restitution curve under basal conditions (■ = wild type mice ▲ = Gα$_{i2}$ (−/−) mice).
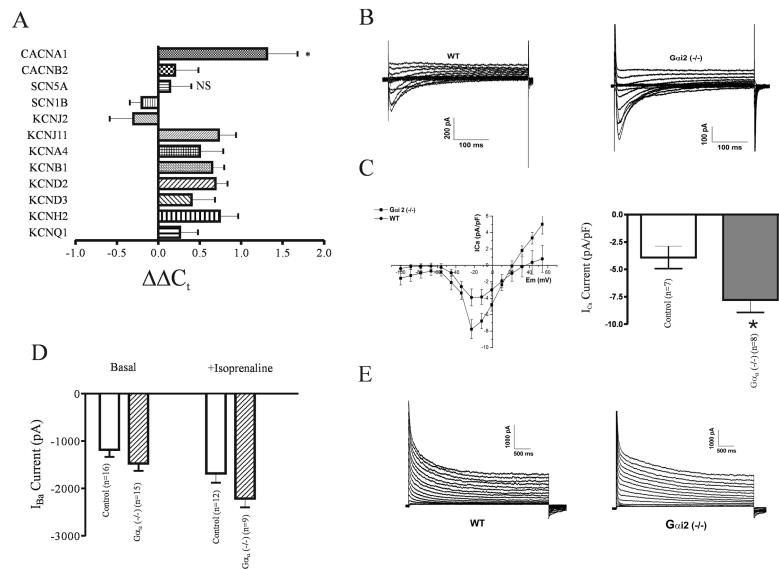
Figure 5. Electrophysiological properties in control and Gα$_{i2}$ (−/−) mice
A. Quantitative real-time PCR data comparing transcript expression between control and
Gα$_{i2}$ (−/−) mice (four mice in each group with each assay performed in triplicate). The data
are expressed as $\Delta\Delta C_t$ i.e. $-(\Delta C_t(G\alpha_{i2} (-/-) - \Delta C_t(control))$. ΔC_t is measured relative to
the house keeping gene GAPDH. Data are shown as mean±SEM. *=p<0.05 and NS=not
significant. $\Delta\Delta C_t$ of 1 is equivalent to two fold increase and 2 a four fold increase etc. B.
Representative traces of calcium current in calcium solutions (see methods) C. Mean
current-voltage relationships and a bar graph of current density. The Gα$_{i2}$ (−/−) mice show
asignificant increase in basal current density (Table 3). D. In Ba^{2+} containing solutions a
similar increase was observed in basal and isoprenaline induced currents. E. Representative
recordings of outward K$^+$ currents in control and Gα$_{i2}$ (−/−) mice. The mean current
parameters are summarised in Table 3 but there were no significant differences between the
two groups.

TABLE 1

Mean ECG parameters

	Control (n=6)	Gα$_{i2}$ (–/–)(n=6)	Gα$_{i1\backslash 3}$ (–/–, –/–) (n=6)	P value
R-R interval	109±4 ms	92.4±4.1 ms (*)	121±4 ms	0.0006
PR interval	36.9±1.4 ms	33.9±0.6 ms	35.6±1.1 ms	0.18
QRS duration	10±0.7 ms	9.8±0.4 ms	10.7±0.7 ms	0.57
QTc	63.4±2.3	81.1±3.7 (*) [†]	50.1±5.3	0.0004

All observations are mean ± S.E.M. One way ANOVA with a Bonferroni post hoc test. Significant in post-hoc test = *. The post-hoc test compares control with Gα$_{i2}$ (–/–) and control with Gα$_{i1}\backslash 3$ (–/–, –/–). QTc =QT/((R-R/100)^0.5).

[†] - n=5 as the program was unable to measure the QT interval in one mouse.

TABLE 2

Mean Electrophysiology Parameters

	Control (n=12)	$G\alpha_{i2}$ (−/−)(n=11)	$G\alpha_{i1\backslash3}$ (−/−, −/−) (n=4)	P value
$SNRT_{600}$	149±9 ms	160±3	164±21 ms	0.50
$AVNERP_{600}$	51.6±3 ms	52.1±3.2 ms (n=10 [†])	59.7±6.1 ms (n=3 [†])	0.78
2:1 block	55.1±2.5 ms	48.9±1.3 ms	56.7±0.7 ms	0.049
$VERP_{600}$	38.2±2.4 ms	29.8±2.2 ms ([*])	41±4.5 ms	0.023
VT stim	2/12	7/11 (p=0.03)	1/4 (p=0.61)	*
VT duration	205±141 ms	963±308 ms ([*])	90±90 ms	0.04

All observations are mean ± S.E.M. One way ANOVA with a Bonferroni post hoc test. Significant in post-hoc test = *. The post-hoc test compares control with $G\alpha_{i2}$ (−/−) and control with $G\alpha_{i1}\backslash3$ (−/−, −/−).

[†] we were not able to determine this parameter in one mouse.

[*] Calculated using Fishers Exact Test not one way ANOVA and p-value given in relevant column.

TABLE 3

Mean Single Cell Electrophysiology Parameters

	WT	Gαi2 (−/−)	P Value
Cell capacitance	104 ± 4.4 pF, n=37	99 ± 4.3 pF, n=35	0.42
Em	−64.8 ±2.3 mV, n=16	−64.4±2.5 mV, n=11	0.91
Δ	100.6 ± 2.6 mV, n=16	101.4 ±5.5 mV, n=11	0.89
APD50	9.9 ±1 ms, n=16	16.9 ± 2.2 ms, n=11	0.004
ADP90	41.2 ± 5.6 ms, n=16	72 ±7.1 ms, n=11	0.002
Ica	−3.9 ± 1 pA/pF, n=7	−7.8 ± 1.1 pA/pF, n=8	0.022
τ Ica	38.2 ± 5.9 ms, n=7	16.3 ± 2.3 ms, n=8	0.003
IK peak	52.5 ± 5.2 pA/pF, n=8	38.2 ± 6.6 pA/pF, n=5	0.12
IK plateau	13.9 ± 2.3 pA/pF, n=8	11.7 ± 2.2 pA/pF, n=5	0.53
IK τ1	142 ± 15.1 ms, n=8	166.6 ± 32.3 ms, n=5	0.45
IK τ2	1122 ± 91.5 ms, n=8	1150 ± 126 ms, n=5	0.86

Electrophysiological parameters recorded in single cell studies from ventricular myocytes of control and Gα¡2 (−/−) mice. The following parameters are presented: cell capacitance, resting membrane potential (Em), depolarisation during AP (Δ), 50 % and 90% repolarisation time (APD50, APD90), calcium current with calcium as a carrier (Ica), tau of deactivation of Ica (τ Ica), potassium current measured with 4.5 s pulse at the peak and plateau phases (IK peak, IK plateau), fast and slow inactivation constants of the K^+ currents (IK τ1, IK τ2).

Table 4

Differential gene expression in the major ion channels involved in the cardiac ventricular action potential in $G\alpha_{i2}$ (−/−) and littermate controls.

Gene	$G\alpha_{i2}$ (−\−) (n=3)	Control (n=4)	Adjusted P value
Voltage gated K$^+$ channel			
KCNA5	6.0±0.2	5.2±0.5	0.20
KCNB1	3.3±0.2	3.6±0.1	0.21
KCND2	7.4±0.7	7.2±0.1	0.72
KCND3	5.5±0.2	5.2±0.1	0.23
KCNH2*	7.7±0.2	6.7±0.2	0.01
KCNQ1*	7.5±0.3	6.7±0.1	0.03
KCNIP2	8.5±0.5	7.9±0.2	0.16
Inward Rectifier K$^+$ channel			
KCNAB2	4.4±0.01	4.4±0.05	0.85
KCNJ2	6.9±0.5	6.8±0.1	0.80
KCNJ3	6.9±0.3	6.8±0.1	0.82
KCNJ11*	7.1±0.2	6.3±0.3	0.03
ABCC9	10.0±0.3	9.3±0.2	0.07
Twin Pore K$^+$ channel			
KCNK1	3.7±0.05	3.9±0.4	0.80
KCNK3*	8.2±0.1	7.8±0.1	0.03
KCNK6	5.8±0.1	5.7±0.2	0.52
Na$^+$ channel			
SCN5A*	8.5±0.3	7.5±0.1	0.02
SCN1B	5.9±0.1	5.8±0.2	0.89
Ca^{2+} channel			
CACNA1C*	9.1±0.3	8.0±0.2	0.02
CACNB2	7.9±0.2	7.5±0.2	0.13
Misc			
SLC8A1\NCX1	9.1±0.2	8.7±0.3	0.34

Expression data is presented as Mean±S.D. on a logarithmic (base 2) scale. The method for the calculation of the adjusted p value is given in the Supplementary Methods. Therefore an expression differential of 1.0 represents a twofold change in relative expression between groups. Significant differential expression is highlighted in grey.