Absence of the inhibitory G-protein, Gαi2, predisposes to ventricular cardiac arrhythmia

Zia Zuberi¹* BSc MB BS MRCP PhD, Muriel Nobles¹* BSc PhD, Sonia Sebastian¹ BSc, Alex Dyson¹ BSc, Shiang Y Lim² BSc PhD, Ross Breckenridge¹ BA MB BS MRCP PhD, Lutz Birnbaumer³ BSc PhD & Andrew Tinker¹ BA MB BS FRCP PhD FMedSci

¹Department of Medicine, University College London, 5 University Street, London, WC1E 6JJ, United Kingdom. ²The Hatter Cardiovascular Institute, University College London, 67 Chenies Mews, WC1E 6HX, London. ³National Institute of Environmental Health Sciences, 111 TW Alexander Dr., Research Triangle Park, North Carolina 27709, USA.
* These authors contributed equally to this study.

Correspondence:
Professor Andrew Tinker
Department of Medicine
University College London
5 University Street
London, WC1E 6JJ.
United Kingdom
Tel: 44 (0)20 8679 6391 Fax: 020 7679 6212
E-mail: a.tinker@ucl.ac.uk

Journal Subject Codes: [106] Electrophysiology; [132] Arrhythmias-basic studies; [152] Ion channels/membrane transport
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 G\(_{\alpha i2}\) (G\(_{\alpha i2}\) (-/-)) 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 G\(_{\alpha i2}\) (-/-) 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 G\(_{\alpha i1}\) and G\(_{\alpha i3}\) showed these abnormalities. There was no evidence for structural heart disease at this time point in the G\(_{\alpha i2}\) (-/-) mice as assessed by cardiac histology and echocardiography. The absence of G\(_{\alpha i2}\) thus leads to a primary electrical abnormality and we explored the basis for this. Single isolated ventricular cells studied using patch clamping showed that G\(_{\alpha i2}\) (-/-) 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\(_{\text{2+}}\) channel subunits and patch clamping revealed an increase in these currents in G\(_{\alpha i2}\) (-/-) mice. There were no changes in K+ currents.

**Conclusion** - Thus the absence of inhibitory G-protein signaling in particular that mediated via G\(_{\alpha i2}\) is a substrate for ventricular arrhythmias.

**Key words**: 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 stressing the importance of accurate prediction and treatment of those at particular risk. Implantable cardiac defibrillators for the management of ventricular tachycardia have proven benefits, however precise calculation of those at greatest risk remains crude. Pharmacological suppression of ventricular tachycardia has additionally been largely ineffective, and in some cases pro-arrhythmic. 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. 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\textsubscript{i/o}-proteins are involved in inhibition of adenylyl cyclase, activation of PI-3 kinase and modulation of K\textsuperscript{+} and Ca\textsuperscript{2+} channels. The heterotrimer consists of a G\textalpha subunit that defines the particular family and a Gβγ subunit. There are multiple isoforms of inhibitory G-proteins (Gα\textsubscript{i1}, Gα\textsubscript{i2}, Gα\textsubscript{i3}, Gα\textgamma with splice variants Gα\textsubscript{o1} and Gα\textsubscript{o2}) and more than one member is often expressed in a single tissue. In general acetylcholine has no effect on basal L-type Ca\textsuperscript{2+} currents in the
ventricle but it strongly antagonizes the increase on the application of a catecholamine or forskolin. In contrast the α subunit of Gs activates adenylyl cyclase and may also positively regulate the voltage-gated calcium channel by direct action on the α1 subunit independent of protein kinase A.

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-adrenergic receptors are down regulated and Gi/o α subunit expression, in particular Gαi2, is significantly increased. 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. This pathological process may be counteracted by signalling via cardiac Gαi-coupled GPCR’s within the heart. Interestingly whilst β1 adrenoreceptor receptor overexpression results in a dilated cardiomyopathy, β2 overexpression (which couples via both Gαs and Gαi/o mechanisms) has a much more modest phenotype suggesting that Gαi/o coupled signaling may be cardioprotective. Gαi2 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 Gαi2 resulting in failed suppression of forskolin stimulated cAMP production. 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. 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 targeting 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 x 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<sub>600</sub>), AV nodal effective refractory period (AVNERP<sub>600</sub>), 2:1 AV block coupling interval from atrial pacing and ventricular effective refractory period (VERP<sub>600</sub>). Finally a ventricular stimulation protocol was performed whereby successive extrastimuli (up to 4 (S<sub>2</sub>-S<sub>3</sub>-S<sub>4</sub>-S<sub>5</sub>)) 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 tachycardia. 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<sup>21</sup>.

*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 (π x [0.5 x 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 x π 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 Goα12 (-/-) 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

\textit{Gα}_{12} (-/-) mice have spontaneous ventricular ectopy and QT interval prolongation.}

We have recently reported that \textit{Gα}_{12} (-/-) mice have impaired heart rate variability and lose the negative chronotropic response to carbachol \textsuperscript{20} probably because \textit{Gα}_{12} acts as the key molecular link between the M2 muscarinic receptor and the G-protein gated \textit{K}^+ channel in sinoatrial nodal cells. In the course of these telemetry studies in conscious and ambulatory \textit{Gα}_{12} (-/-) 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 \textit{Gα}_{12} (-/-) mice unexpectedly had QT interval prolongation despite relative tachycardia (Figure 1B) and this was reflected in a prolonged QT\textsubscript{c} \textsuperscript{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 \textit{Gα}_{11} and \textit{Gα}_{13} (Table 1).

\textit{Gα}_{12} (-/-) mice have a proarrhythmic substrate

We observed occasional and unexpected death in several \textit{Gα}_{12} (-/-) mice although we were not able to record terminal arrhythmia and this has also been previously observed \textsuperscript{22}. To investigate further this potential substrate we used \textit{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 Gαi2 (-/-) 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 Gαi1 and Gαi3 (Table II). Gαi2 (-/-) 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 Gαi2 (-/-) 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 Gαi2 (-/-) mice compared with control mice (Figure 2D).

There is a primary electrical abnormality in Gαi2 (-/-) 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 Gαi2 (-/-) 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 Gαi2 (-/-) 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 Gαi2 (-/-) it was 5.20±0.23 (n=4, P= 0.69). Finally at cardiac histological examination ventricular dimensions were normal (Supplementary data,
myocytes were not hypertrophied and there was no evidence of fibrosis in Gα12 (-/-) 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. 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 Gα12 (-/-) mice compared to control as reflected in a significantly prolonged APD50 and APD90 (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 Gα12 (-/-) 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 Gα12 (-/-) mice compared to the control mice (Figure 4E). Under basal conditions: control mice (20 to 120 ms, r²=0.88) slope=0.20±0.03, Gα12 (-/-) mice (20 to 120 ms, r²=0.93) slope=0.43±0.04, P=0.0002. In the presence of isoprenaline: control mice (20 to 120 ms, r²=0.90) slope= 0.27±0.03, Gα12 (-/-) mice (20 to 100 ms, r²=0.87) slope= 0.49± 0.07, P=0.01.

Increased L-type Ca²⁺ currents contribute to the abnormality
The observed abnormalities in repolarisation could be accounted for by increases in inward current in particular via L-type Ca\textsuperscript{2+} channels or a decrease in outward K\textsuperscript{+} 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 G\textalpha\textsubscript{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\textsuperscript{+} channels resulting in a prolonged action potential duration\textsuperscript{24}. However at the mRNA level, gene array studies comparing control and G\textalpha\textsubscript{i2} (-/-) mice showed little change in the relevant K\textsuperscript{+} 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 G\textalpha\textsubscript{i2} (-/-) mice of the calcium channel alpha subunit, CACNA1C (Ca\textsubscript{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\text{Ct}>1 corresponding to a two-fold change) was CACNA1C (Ca\textsubscript{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\textsuperscript{2+} as the charge carrier (Figure 5C and Table 3). A similar increase was present using Ba\textsuperscript{2+} as the charge carrier under basal conditions and with application of isoprenaline, in myocytes isolated from the G\textalpha\textsubscript{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 \( \text{G}_\alpha_{i2} \) (-/-) 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, \( \text{G}_\alpha_{i2} \) (but not \( \text{G}_\alpha_{i1}/\text{G}_\alpha_{i3} \)) leads to a proarrhythmic substrate in the ventricle resulting in a long QTc 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 \( \text{G}_\alpha_{i2} \) (-/-) mice but we did not study this prospectively 22. Furthermore, during short-term telemetry we did not observe a lethal ventricular arrhythmia though we did observe qualitatively an increased ventricular ectopic burden that we never saw in controls. However during minimally invasive EP testing \( \text{G}_\alpha_{i2} \) (-/-) mice had a shortened VERP, a known proarrhythmic substrate. Additionally we were able to induce VT significantly more easily in \( \text{G}_\alpha_{i2} \) (-/-) mice than controls. However not every \( \text{G}_\alpha_{i2} \) (-/-) 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 \( \text{G}_\alpha_{i2} \) (-/-) 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 \( \text{G}_\alpha_{i2} \) (-/-) 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.

The loss of the muscarinic regulation of Ca\(_v\)1.2 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. 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 mutation in CACNA1C and prolonged QT\(_c\), predisposition to sudden death, autism and a variety of defects in other tissues.

RAD, a ras related GTPase, is important in governing Ca\(_v\)1.2 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. 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.
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 \(\alpha_{1C}\) 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 \(\text{G}_{\alpha_{12}}\) \((-/-)\) 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 QTc interval) are relatively prolonged in the \(\text{G}_{\alpha_{12}}\) \((-/-)\) 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 \(\text{Ca}^{2+}\) handling \(^{35}\). For example administration of an L-type \(\text{Ca}^{2+}\) 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 \(\text{Ca}^{2+}\) transients and contractility in \(\text{G}_{\alpha_{12}}\) \((-/-)\) 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 \(\beta\)-adrenergic receptors particularly in light of the beneficial outcomes of \(\beta\)-blockers on survival \(^{11,39}\). It has been established that there is an enhanced expression of \(\text{G}_\alpha_{i2}\) in heart failure \(^{9}\). Our results would suggest that increased expression may be a potential protective mechanism against malignant ventricular tachyarrhythmias. More broadly, parasympathetic modulation of cardiac rhythm is considered protective against ventricular arrhythmia \(^{40}\). In contrast parasympathetic attenuation manifesting as a reduced heart rate variability signal is a powerful predictor 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 \(\text{G}_\alpha_{i2}\) 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 \(\text{G}_\alpha_{i2}\) (but not \(\text{G}_\alpha_{i1}\), \(\text{G}_\alpha_{i3}\), or \(\text{G}_\alpha_{o}\)) \(^{20}\). In the setting of hypertrophic cardiomyopathy, an increased of expression of \(\text{G}_\alpha_{i1}\) has been proposed to be proarrhythmic \(^{44}\). Our studies failed to reveal any significant abnormalities in mice with combined global genetic deletion of \(\text{G}_\alpha_{i1}\) and \(\text{G}_\alpha_{i3}\). Intriguingly, such remodelling may only become important in certain disease settings and different inhibitory G-protein isoforms may exhibit differential effects under these conditions.

**Funding Sources:** This work was supported by the Wellcome Trust, Medical Research Council and the British Heart Foundation, and by the Intramural Research Program of the NIH (project Z01-ES101643 to LB).

**Conflict of Interest Disclosures:** None
References


16. Brack KE, Patel VH, Coote JH, Ng GA. Nitric oxide mediates the vagal protective effect on ventricular fibrillation via effects on action potential duration restitution in the rabbit heart. J.Physiol 2007;583:695-704.


Keating MT. Ca(V)1.2 calcium channel dysfunction causes a multisystem disorder including arrhythmia and autism. Cell 2004;119:19-31.


TABLE 1 – Mean ECG parameters

<table>
<thead>
<tr>
<th></th>
<th>Control (n=6)</th>
<th>Gα12 (-/-) (n=6)</th>
<th>Gα113 (-/-, -/-) (n=6)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-R interval</td>
<td>109±4 ms</td>
<td>92.4±4.1 ms (*)</td>
<td>121±4 ms</td>
<td>0.0006</td>
</tr>
<tr>
<td>PR interval</td>
<td>36.9±1.4 ms</td>
<td>33.9±0.6 ms</td>
<td>35.6±1.1 ms</td>
<td>0.18</td>
</tr>
<tr>
<td>QRS duration</td>
<td>10±0.7 ms</td>
<td>9.8±0.4 ms</td>
<td>10.7±0.7 ms</td>
<td>0.57</td>
</tr>
<tr>
<td>QTc</td>
<td>63.4±2.3</td>
<td>81.1±3.7 (*) †</td>
<td>50.1±5.3</td>
<td>0.0004</td>
</tr>
</tbody>
</table>

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 Gα12 (-/-) and control with Gα113 (-/-, -/-). 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

<table>
<thead>
<tr>
<th></th>
<th>Control (n=12)</th>
<th>Gα12 (-/-) (n=11)</th>
<th>Gα113 (-/-, -/-) (n=4)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SNRT600</td>
<td>149±9 ms</td>
<td>160±3</td>
<td>164±21 ms</td>
<td>0.50</td>
</tr>
<tr>
<td>AVNERP600</td>
<td>51.6±3 ms</td>
<td>52.1±3.2 ms</td>
<td>59.7±6.1 ms</td>
<td>0.78</td>
</tr>
<tr>
<td>2:1 block</td>
<td>55.1±2.5 ms</td>
<td>48.9±1.3 ms</td>
<td>56.7±0.7 ms</td>
<td>0.049</td>
</tr>
<tr>
<td>VERP600</td>
<td>38.2±2.4 ms</td>
<td>29.8±2.2 ms (*)</td>
<td>41±4.5 ms</td>
<td>0.023</td>
</tr>
<tr>
<td>VT stim</td>
<td>2/12</td>
<td>7/11 (p=0.03)</td>
<td>1/4 (p=0.61)</td>
<td>*</td>
</tr>
<tr>
<td>VT duration</td>
<td>205±141 ms</td>
<td>963±308 ms (*)</td>
<td>90±90 ms</td>
<td>0.04</td>
</tr>
</tbody>
</table>

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 Gα12 (-/-) and control with Gα113 (-/-, -/-). † 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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>WT</th>
<th>Gαi2 (/-)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell capacitance</td>
<td>104 ± 4.4 pF, n=37</td>
<td>99 ± 4.3 pF, n=35</td>
<td>0.42</td>
</tr>
<tr>
<td>Em</td>
<td>-64.8 ±2.3 mV, n=16</td>
<td>-64.4±2.5 mV, n=11</td>
<td>0.91</td>
</tr>
<tr>
<td>Δ</td>
<td>100.6 ± 2.6 mV, n=16</td>
<td>101.4 ±5.5 mV, n=11</td>
<td>0.89</td>
</tr>
<tr>
<td>APD50</td>
<td>9.9 ±1 ms, n=16</td>
<td>16.9 ± 2.2 ms, n=11</td>
<td>0.004</td>
</tr>
<tr>
<td>APD90</td>
<td>41.2 ± 5.6 ms, n=16</td>
<td>72 ±7.1 ms, n=11</td>
<td>0.002</td>
</tr>
<tr>
<td>Ica</td>
<td>-3.9 ± 1 pA/pF, n=7</td>
<td>-7.8 ± 1.1 pA/pF, n=8</td>
<td>0.022</td>
</tr>
<tr>
<td>τ Ica</td>
<td>38.2 ± 5.9 ms, n=7</td>
<td>16.3 ± 2.3 ms, n=8</td>
<td>0.003</td>
</tr>
<tr>
<td>IK peak</td>
<td>52.5 ± 5.2 pA/pF, n=8</td>
<td>38.2 ± 6.6 pA/pF, n=5</td>
<td>0.12</td>
</tr>
<tr>
<td>IK plateau</td>
<td>13.9 ± 2.3 pA/pF, n=8</td>
<td>11.7 ± 2.2 pA/pF, n=5</td>
<td>0.53</td>
</tr>
<tr>
<td>IK τ1</td>
<td>142 ± 15.1 ms, n=8</td>
<td>166.6 ± 32.3 ms, n=5</td>
<td>0.45</td>
</tr>
<tr>
<td>IK τ2</td>
<td>1122 ± 91.5 ms, n=8</td>
<td>1150 ± 126 ms, n=5</td>
<td>0.86</td>
</tr>
</tbody>
</table>

Electrophysiological parameters recorded in single cell studies from ventricular myocytes of control and Gαi2 (/-) 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 \( \gamma_2 \) and littermate controls.

<table>
<thead>
<tr>
<th>Gene</th>
<th>( \gamma_2 (-/-) ) (n=3)</th>
<th>Control (n=4)</th>
<th>Adjusted P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Voltage gated K(^+) channel</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KCNA5</td>
<td>6.0±0.2</td>
<td>5.2±0.5</td>
<td>0.20</td>
</tr>
<tr>
<td>KCNB1</td>
<td>3.3±0.2</td>
<td>3.6±0.1</td>
<td>0.21</td>
</tr>
<tr>
<td>KCND2</td>
<td>7.4±0.7</td>
<td>7.2±0.1</td>
<td>0.72</td>
</tr>
<tr>
<td>KCND3</td>
<td>5.5±0.2</td>
<td>5.2±0.1</td>
<td>0.23</td>
</tr>
<tr>
<td>KCNH2*</td>
<td>7.7±0.2</td>
<td>6.7±0.2</td>
<td>0.01</td>
</tr>
<tr>
<td>KCNQ1*</td>
<td>7.5±0.3</td>
<td>6.7±0.1</td>
<td>0.03</td>
</tr>
<tr>
<td>KCNIP2</td>
<td>8.5±0.5</td>
<td>7.9±0.2</td>
<td>0.16</td>
</tr>
<tr>
<td><strong>Inward Rectifier K(^+) channel</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KCNAB2</td>
<td>4.4±0.01</td>
<td>4.4±0.05</td>
<td>0.85</td>
</tr>
<tr>
<td>KCNJ2</td>
<td>6.9±0.5</td>
<td>6.8±0.1</td>
<td>0.80</td>
</tr>
<tr>
<td>KCNJ3</td>
<td>6.9±0.3</td>
<td>6.8±0.1</td>
<td>0.82</td>
</tr>
<tr>
<td>KCNJ11*</td>
<td>7.1±0.2</td>
<td>6.3±0.3</td>
<td>0.03</td>
</tr>
<tr>
<td>ABCC9</td>
<td>10.0±0.3</td>
<td>9.3±0.2</td>
<td>0.07</td>
</tr>
<tr>
<td><strong>Twin Pore K(^+) channel</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KCNK1</td>
<td>3.7±0.05</td>
<td>3.9±0.4</td>
<td>0.80</td>
</tr>
<tr>
<td>KCNK3*</td>
<td>8.2±0.1</td>
<td>7.8±0.1</td>
<td>0.03</td>
</tr>
<tr>
<td>KCNK6</td>
<td>5.8±0.1</td>
<td>5.7±0.2</td>
<td>0.52</td>
</tr>
<tr>
<td><strong>Na(^+) channel</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCN5A*</td>
<td>8.5±0.3</td>
<td>7.5±0.4</td>
<td>0.02</td>
</tr>
<tr>
<td>SCN1B</td>
<td>5.9±0.1</td>
<td>5.8±0.2</td>
<td>0.89</td>
</tr>
<tr>
<td><strong>Ca(^{2+}) channel</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CACNA1C*</td>
<td>9.1±0.3</td>
<td>8.0±0.2</td>
<td>0.02</td>
</tr>
<tr>
<td>CACNB2</td>
<td>7.9±0.2</td>
<td>7.5±0.2</td>
<td>0.13</td>
</tr>
<tr>
<td><strong>Misc</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SLC8A1/NCX1</td>
<td>9.1±0.2</td>
<td>8.7±0.3</td>
<td>0.34</td>
</tr>
</tbody>
</table>

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.
Figure Legends

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 Gαi2 (-/-) mice. No ventricular ectopics were observed in any littermate controls or in mice with the combined knockout of Gαi1 and Gαi3. 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 Gαi2 (-/-) (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 Gαi2 (-/-) 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. Gαi2 (-/-) (Bottom) mice develop sustained VT using a similar induction protocol.

Figure 3. Echocardiography in wild type and Gαi2 (-/-) 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 Gαi2 (-/-) mice. A. Ventricular action potentials in control and Gαi2 (-/-) 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 ▲ = Gαi2 (-/-) mice). D. Restitution curve in the presence of 10 μmol\L isoprenaline (◼ = wild type mice ▲ = Gαi2 (-/-) mice). E.
Regression analysis of the linear portion of the restitution curve under basal conditions (□ = wild type mice ▲ = Gαi2 (-/-) mice).

**Figure 5.** *Electrophysiological properties in control and Gαi2 (-/-) mice.* A. Quantitative real-time PCR data comparing transcript expression between control and Gαi2 (-/-) mice (four mice in each group with each assay performed in triplicate). The data are expressed as ΔΔCt i.e. – (ΔCt(Gαi2 (-/-) - ΔCt(control)). ΔCt is measured relative to the house keeping gene GAPDH. Data are shown as mean±SEM. *=p<0.05 and NS=not significant. ΔΔCt 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 Gαi2 (-/-) mice show a significant 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 Gαi2 (-/-) mice. The mean current parameters are summarised in Table 3 but there were no significant differences between the two groups.
Absence of the Inhibitory G-protein, Go12, Predisposes to Ventricular Cardiac Arrhythmia
Zia Zuberi, Muriel Nobles, Sonia Sebastian, Alex Dyson, Shaing Y. Lim, Ross Breckenridge, Lutz
Birnbaumer and Andrew Tinker

Circ Arrhythm Electrophysiol. published online May 21, 2010;
Circulation: Arrhythmia and Electrophysiology is published by the American Heart Association, 7272 Greenville Avenue,
Dallas, TX 75231
Copyright © 2010 American Heart Association, Inc. All rights reserved.
Print ISSN: 1941-3149. Online ISSN: 1941-3084

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://circep.ahajournals.org/content/early/2010/05/21/CIRCEP.109.894329

Data Supplement (unedited) at:
http://circep.ahajournals.org/content/suppl/2010/05/21/CIRCEP.109.894329.DC1.html

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in
Circulation: Arrhythmia and Electrophysiology can be obtained via RightsLink, a service of the Copyright Clearance
Center, not the Editorial Office. Once the online version of the published article for which permission is being
requested is located, click Request Permissions in the middle column of the Web page under Services. Further
information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation: Arrhythmia and Electrophysiology is online at:
http://circep.ahajournals.org//subscriptions/
Supplementary Methods and Data

Methods

Genotyping

Genomic tail snip DNA was isolated and analyzed by PCR using the primers sets: i1Ex2F1: 5'-GAATC TGGAA AGAGT ACCAT TGTGA-3', i1Ex3R1: 5'-GTCTC CGAAG TCGAT TTTCA ACCTC-3' (WT) and NeoR3 : 5'-GATTG TCTGT TGTGC CCAGT CATAG-3' (KO) for Gαi1(-/-) genotype; i2F8 5'-GATCA TCC GAGA TGGCT ACTCA GAAG-3', i2F14 5'-CAGGA TCATC CATGA AGATG GCTAC-3', i2R7 5'-CCCCT CTCAC TCTTG ATTTG CTACT GACAC-3' (WT), NeoR2 5'-GCACAT CAAAAC CGAGG ACTTA CAGAA C-3' (KO) for the Gαi2 (-/-) genotype; i3Ex7R1: 5'-TTCAT GCTTT CATGC ATTCG GTTC-3', i3Ex6F1: 5'-GTGGC CAAAG ATCCG AACGA A-3' (WT) and NeoF1 : 5'-TGCCG AGAAA GTATC CATCA TG-3' (KO) for the Gαi3 (-/-) genotype.

Gene Expression

The gene expression arrays and statistical analysis were carried out by a central service at University College, London (http://www.ich.ucl.ac.uk/services_and_facilities/lab_services/microarray_centre/). 100ng of total RNA was converted to single stranded (sense strand) DNA using the whole transcript sense target labeling kit (cat 901178, Affymetrix). The DNA was fragmented and end labeled with biotinylated nucleotides before hybridising to the Affymetrix Mouse Gene 1.0 ST arrays. Arrays were then washed and stained using the Hybridisation wash and stain kit (900720, Affymetrix) and scanned on the Affymetrix Scanner 3000. Raw data was summarised and normalised using the RMA algorithm implemented in the Affymetrix
Expression Console software. LIMMA (Linear Models for Microarray Analysis) \(^2\) was used to identify differentially expressed genes. LIMMA applies a modified t-test to each probe set which uses an empirical Bayes approach for estimating sample variances. The moderated t-statistic calculated by LIMMA is more robust than the ordinary t-statistic with small sample sizes. The p-values were corrected for multiple-testing using the Benjamini-Hochberg correction and a corrected p-value threshold of 0.05 was used to select differentially expressed genes.

Quantitative real-time PCR was performed using Taqman gene expression assays (Applied Biosystems) using either inventoried or made to order assays. We used the protocol which is given on their website (http://www3.appliedbiosystems.com/cms/groups/mcb_support/documents/generaldocuments/cms_041280.pdf). Briefly hearts were removed from each group of mice, washed with cold PBS and immediately placed in liquid nitrogen. They were ground in liquid nitrogen with a pestle and mortar and then RNA was extracted using RNeasy kit (cat no. 74104 Qiagen). cDNA was synthesized using High capacity cDNA reverse transcription Kit (4368814 Applied Biosystems). cDNA was quantified and 50ng/\(\mu\)l of DNA/20ul was used for the subsequent real time expression assay. All genes were assayed in triplicates and GAPDH was used as the house keeping gene. The assays from Applied Biosystems were are follows KCND2 (inventoried, Mm00498065 m1, 85 bp), KCND3 (made to order, Mm01302127 m1, 85 bp), KCNH2 (made to order, Mm01134905 m1, 90 bp), KCNQ1 (inventoried, Mm00434641 m1, 85 bp), KCNA4 inventoried, Mm01336166 m1, 132 bp), KCNJ2 (inventoried, Mm00434616 m1, 81 bp), KCNJ11 (inventoried, Mm00440050 s1, 85 bp), KCNB1 (inventoried, Mm00492791 m1, 73 bp), SCN5A (made to order, Mm01342505, 85 bp), SCN1B (inventoried, Mm00441210 m1, 86 bp), CACNA1C (made to order,
Mm01188838 m1, 88 bp) and CACNB2 (made to order, Mm01333544 m1, 82 bp). The samples were run on an Eppendorf Mastercycler Real PLEX qPCR system.

**Histology**

Hearts were removed, rinsed in PBS, fixed overnight in 4% paraformaldehyde in PBS, and dehydrated in a methanol series. Dehydrated hearts were cleared with histoclear, wax embedded and sectioned at 7μm thickness. Sections were then stained with H+E or Masson’s trichrome (both Sigma), and photographed. Five hearts of each genotype were examined. To grossly compare if there was cardiac hypertrophy we measured the ratio of the heart weight (mg) to the total body weight (g) and the ratio of the heart weight (mg) to tibial length (mm).

**Isolation of cardiomyocytes**

Mice were injected with heparin sodium (250 IU) and anaesthetised with a combination of ketamine/xylazine/atropine. The hearts were rapidly excised, cannulated and perfused with buffer containing (in mmol\(\text{L}^{-1}\)) 113 NaCl, 4.7 KCl, 0.6 KH2PO4, 0.6 Na2HPO4, 1.2 MgSO4.7H2O, 12 NaHCO3, 10 KHCO3, 30 taurine, 10 HEPES, 11 glucose and 10 2,3-butanedione monoxime, saturated with 95% O\(_2\)-5% CO\(_2\) at 37°C. The hearts were perfused at 3 ml/min with perfusion buffer for 4 min, then with digestion buffer (perfusion buffer containing 0.9 mg/ml collagenase (Worthington type II), 0.125 mg/ml hyaluronidase and 12.5 μmol/L CaCl\(_2\)) for 10 min. The ventricles were then cut into several pieces and agitated in digestion buffer at 37°C with oxygenation for 10 min twice. The supernatant was collected and 5% foetal calf serum was added. After centrifugation at 600 rpm for 3 min, the cell pellet was suspended in 10 ml of perfusion buffer containing 12.5 μmol/L CaCl\(_2\) and the calcium concentration was gradually restored to 1 mmol/L over 20 min. The myocytes were
re-centrifuged at 600 rpm for 3 min, the cell pellet was suspended in culture medium (M-199 medium containing 2 mg/ml bovine serum albumin, 0.66 mg/ml creatine, 0.62 mg/ml taurine, 0.32 mg/ml carnitine hydrochloride, 10 units/ml penicillin, 10 μg/ml streptomycin and 25 μmol/L blebbistatin) and seeded onto sterilised laminin-coated coverslips for 60 min in humidified 5% CO₂-95% air at 37°C. Myocytes were then gently washed once with blebbistatin-free culture medium to removed unattached cells use on the same day of isolation.

**Electrophysiology**

Action potential recordings: Action potentials were recorded in the current clamp mode. Ventricular myocytes were stimulated using a 5 ms current pulse. The resting membrane potential (Em), the magnitude of the initial depolarisation (Δ) and the action potential duration at which 50 and 90% repolarisation occurred (APD₅₀ and APD₉₀ respectively) were measured. The cells were clamped at -80 mV in an extracellular solution containing (mM): NaCl 135, KCl 5.4, CaCl₂ 2, MgCl₂ 1, NaH₂PO₄ 0.33, H-HEPES 5, Glucose 10 (buffered to pH 7.4 with NaOH). The intracellular solution was (mM): K gluconate 110, KCl 20, NaCl 10, MgCl₂ 1, MgATP 2, EGTA 2, Na₂GTP 0.3 (buffered to pH 7.2 with KOH).

Ventricular cell restitution protocol: Cells were stimulated using a 5 ms current pulse. A pacing train of 4 stimulations separated by 200 ms (S1) was followed by a single stimulus (S2). The interval between S1-S2 ranged from 1000 ms to 20 ms. Our methods were adapted from published protocols. The same extracellular and intracellular solutions were used as described above for action potential recordings.

Calcium currents. Calcium currents were measured using calcium ions as the charge carrier. Prior to whole cell configuration, ventricular myocytes were bathed in a solution...
containing (mM): NaCl 140, KCl 6, MgCl2 1, CaCl2 2, glucose 10, H-HEPES 5 (buffered to pH 7.4 with NaOH). When the whole-cell configuration was obtained the extracellular solution was switched to a solution containing (mM): CaCl2 2, TEA-Cl 140, Cs-Cl 6, MgCl2 1, glucose 5, H-HEPES 5 (buffered to pH 7.4 with TEA-OH). The junction potential was measured to be around 3 mV, and was not corrected. The intracellular solution was (mM): CsCl2 125, MgCl2 1, EGTA 10, Na2GTP 0.3, H-HEPES 20 (buffered to pH 7.2 with CsOH). Currents were evoked during 400 ms depolarizing voltage steps to potential between -100 mV and +40 mV from a holding potential of -80 mV. Maximum currents were measured.

Calcium currents. Calcium channel currents were also measured with Ba2+ as the charge carrier. For recording the I_{Ba}, external Na+ was substituted by isomolar tetraethylammonium (TEA), and extracellular Ca2+ by Ba2+. The composition of the extracellular solution was (mM): TEA 135, KCl 5.2, BaCl2 2, MgCl2 1, NaH2PO4 0.33, H-HEPES 5, Glucose 10 (pH 7.4 buffered with TEA-OH). The intracellular solution was the same as the one used for AP recordings. Membrane currents were elicited with a series of 400 ms depolarising pulses to + 60 mV from a holding potential of – 80 mV in 10 mV increments. Maximum currents were measured.

Potassium currents. Voltage-gated K+ currents were evoked during a 4.5 s depolarizing voltage step to a test potential between -40 mV and +65 mV from a holding potential of -70 mV. Voltage steps were given in 10 mV increments. The extracellular solution contained (mM): NaCl 135, KCl 5.4, CaCl2 1, MgCl2 1, CoCl2 2, NaH2PO4 0.33, H-HEPES 10, Glucose 5 (pH 7.4 buffered with NaOH). The intracellular solution was the same as the one used for AP recordings.

**Figure Legend**
Figure S1. Cardiac histology in wild type and Gαi2 (-/-) mice. No detectable histological difference between hearts from Gαi2 null (ko in figure) and control (wt in figure) mice. (A, B) Representative low power image of H+E stained short-axis sections through wild-type and Gαi2 null hearts respectively. Section at mid-papillary level. Five hearts of each genotype were examined. (C,D) High power images of left ventricular free wall of wild-type and Gαi2 null hearts, H+E stained. (E, F) Masson’s trichrome staining of wild-type and Gαi2 null hearts, showing blue collagen stain in blood vessels (arrowed). Note low levels of collagen staining in both hearts. Scale bar; 100μm.

Reference List


(6) Xu H, Guo W, Nerbonne JM. Four kinetically distinct depolarization-activated K+ currents in adult mouse ventricular myocytes. J Gen Physiol 1999 May;113(5):661-78.
Table S1. Myocardial function as assessed by echocardiography.

(LV; left ventricle).

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Gα2 (-/-)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stroke Volume (μl)</td>
<td>43.0 (±2.0)</td>
<td>42.7 (±2.0)</td>
<td>0.92</td>
</tr>
<tr>
<td>Heart rate (1/min)</td>
<td>477 (±16)</td>
<td>479 (±8)</td>
<td>0.94</td>
</tr>
<tr>
<td>Cardiac Output (ml/min)</td>
<td>20.5 (±1.3)</td>
<td>20.4 (±0.8)</td>
<td>0.93</td>
</tr>
<tr>
<td>Peak Velocity (m/s)</td>
<td>0.79 (±0.02)</td>
<td>0.78 (±0.04)</td>
<td>0.73</td>
</tr>
<tr>
<td>LV End Diastolic Dimension (cm)</td>
<td>0.38 (±0.01)</td>
<td>0.38 (±0.01)</td>
<td>0.88</td>
</tr>
<tr>
<td>LV End Systolic Dimension (cm)</td>
<td>0.27 (±0.01)</td>
<td>0.27 (±0.02)</td>
<td>0.84</td>
</tr>
<tr>
<td>Ejection Fraction (%)</td>
<td>58 (±2.0)</td>
<td>55 (±4.0)</td>
<td>0.51</td>
</tr>
<tr>
<td>Diastolic Anterior Wall Dimension (cm)</td>
<td>0.065 (±0.003)</td>
<td>0.065 (±0.003)</td>
<td>1</td>
</tr>
<tr>
<td>Systolic Anterior Wall Dimension (cm)</td>
<td>0.08 (±0.00)</td>
<td>0.08 (±0.00)</td>
<td>0.62</td>
</tr>
<tr>
<td>Diastolic Posterior Wall Dimension (cm)</td>
<td>0.07 (±0.00)</td>
<td>0.07 (±0.00)</td>
<td>0.62</td>
</tr>
<tr>
<td>Systolic Posterior Wall Dimension (cm)</td>
<td>0.09 (±0.01)</td>
<td>0.09 (±0.00)</td>
<td>0.47</td>
</tr>
</tbody>
</table>