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The role of inhibitory G proteins and regulators of G protein signaling in the *in vivo* control of heart rate and predisposition to cardiac arrhythmias

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Inhibitory heterotrimeric G proteins and the control of heart rate. The activation of cell signaling pathways involving inhibitory heterotrimeric G proteins acts to slow the heart rate via modulation of ion channels. A large number of Regulators of G protein signalings (RGSs) can act as GTPase accelerating proteins to inhibitory G proteins and thus it is important to understand the network of RGS\G-protein interaction. We will review our recent findings on *in vivo* heart rate control in mice with global genetic deletion of various inhibitory G protein alpha subunits. We will discuss potential central and peripheral contributions to the phenotype and the controversies in the literature.

Keywords: inhibitory G protein, regulators of G protein signaling, heart, arrhythmia

INTRODUCTION

The pacemaker in the sinoatrial node (SAN) of the heart has an intrinsic rate independent of autonomic innervation and this leads to a measurement of "intrinsic" heart rate that is determined in practice by pharmacologically inhibiting the sympathetic and parasympathetic systems. At the molecular level this self-regenerating electrical activity arises from a membrane clock and\or a calcium clock dependent on intracellular calcium release events (Lakatta and DiFrancesco, 2009). This pacemaker activity can be modulated: increased sympathetic activity leads to an increase in rate and an increase in vagal tone to a decrease. The release of noradrenaline from sympathetic nerve endings or the release of adrenaline from the adrenal medulla into the circulation modulates SAN cells by binding to β-adrenoreceptors. The stimulatory G protein is activated and this leads to stimulation of adenylate cyclase and the generation of cyclic adenosine monophosphate (cAMP). Increased cAMP can directly modulate the hyperpolarization-activated cation currents (I_h/I_f) leading to increased pacemaker depolarization and rate. However, increased cAMP can also activate protein kinase A (PKA), which can modulate intracellular calcium-handling proteins such as phospholamban and the ryanodine receptor to modulate rate.

However, the main focus of this review is on the inhibitory events typified by the negative chronotropic response in the SAN to vagal nerve efferent activation. Acetylcholine (ACh) released from the vagus nerve binds to the muscarinic (M2) receptor, leading to the activation, and dissociation of inhibitory G protein heterotrimers. The resulting βγ-dimer directly activates the G protein coupled inward rectifying potassium (GIRK) channel to cause membrane hyperpolarization, slowing pacemaker depolarization, and sinus rate. Furthermore, the activation of the inhibitory Gα subunit will inhibit adenylate cyclase and thus modulate I_h/I_f as detailed above. Regulators of G protein signaling (RGS) proteins interact with the inhibitory Gα subunit to switch off this pathway through GTPase accelerating protein (GAP) activity and allow the heterotrimer to reform. This process is summarized in **Figure 1**.

It has emerged from the SHIFT trial using ivabradine, an inhibitor of I_h/I_f, in addition to standard therapy that heart rate *per se* may be an important risk factor in chronic heart failure (Swedberg et al., 2010). Furthermore, autonomic precipitants are well known for common arrhythmias such as atrial fibrillation (AF) and ventricular tachyarrhythmia (see below). Thus the focus in this review is an attempt to link physiological and pathophysiological function in the control of heart rate and rhythm with the known molecular isoforms of the inhibitory G proteins and RGSs. These proteins are widely distributed in the heart thus we will consider not only their effects on heart rate but also what electrical processes they might modulate in the atria, atrioventricular node (AVN), and ventricles. One of the key approaches is the use of genetically modified mice. However, these often have global knockout of the relevant gene and this will mean deletion of the signaling molecule in central circuits and peripheral nerves of the autonomic nervous system. Thus we review the role of inhibitory G proteins in the central circuits and peripheral limbs of the autonomic nervous system, consider the limited information available and experimental approaches by which this might be investigated.

THE MOLECULAR NUTS AND BOLTS

We initially discuss this from a cardiac perspective but the role of the nervous system is obviously important and this is addressed in another section.

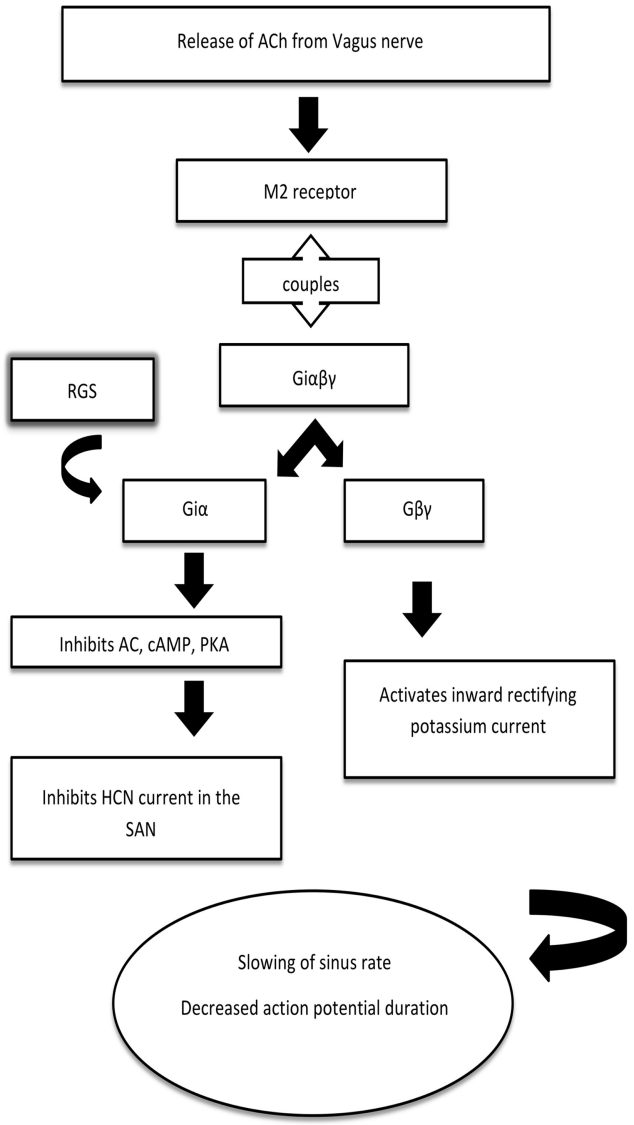

FIGURE 1 | A simplified schematic for the mechanism of heart rate regulation by inhibitory G proteins. Acetylcholine released from the vagus nerve binds to the muscarinic (M2) receptor, which is coupled to the inhibitory G protein. The βγ-dimer activates the G protein coupled inward rectifying potassium (GIRK) channel to slow the sinus rate, decrease the action potential duration and allow arrhythmia generation. Regulators of G protein signaling (RGS) interact with the α-subunit to switch off this pathway through GTPase accelerating protein activity and allow the heterotrimer to reform. AC, adenylyl cyclase; cAMP, cyclic AMP; PKA, protein kinase A; HCN, hyperpolarization-activated cyclic nucleotide-gated channel.

INHIBITORY G PROTEINS

There are four families of G proteins namely $G\alpha_{i/o}$, $G\alpha_s$, $G\alpha_{q/11}$, $G\alpha_{12/13}$. The inhibitory G proteins ($G\alpha_{i/o}$) are the most highly expressed and predominant class followed by $G\alpha_s$, $G\alpha_{q/11}$ then $G\alpha_{12/13}$. The inhibitory G proteins themselves have multiple isoforms: $G\alpha_{i1}$, $G\alpha_{i2}$, $G\alpha_{i3}$, and $G\alpha_o$. The $G\alpha_o$ ("other") isoform has two splice variants $G\alpha_{oA}$ and $G\alpha_{oB}$. $G\alpha_o$ is the predominant isoform in the brain whereas $G\alpha_{i1}$, $G\alpha_{i2}$, $G\alpha_{i3}$ are highly homologous and widely distributed in many tissues (Wettschureck and Offermanns, 2005). The inhibitory G proteins characteristically inhibit adenylate cyclase activity and lower the concentration of cAMP (Wong et al., 1991; Rudolph et al., 1996), however they also activate PI-3 kinase activity and directly regulate ion channel activity (see below).

REGULATORS OF G PROTEIN SIGNALING

Regulators of G protein signaling proteins act to effectively inhibit G protein signaling; they interact with the α-subunit and accelerate GTPase activity. Characteristically this family have a 120 amino acid conserved RGS domain, flanked by variable length N- and C-terminals. Six main subfamilies of mammalian RGSs are distinguished: R4, R7, R12, RA, RL, and RZ. The vast majority of RGSs are GAPs for inhibitory G proteins but some have activity to the $G_{q/11}$ family as well. They may also have other roles in signaling (Hollinger and Hepler, 2002). There are more than 20 mammalian RGS isoforms and many of these are expressed to some extent in the heart (Kardestuncer et al., 1998; Doupnik et al., 2001; Owen et al., 2001; Wieland and Mittmann, 2003). For both $G\alpha_{i/o}$ and RGSs the pattern of expression in conducting tissues, atria, and ventricles and in specific cell types such as myocytes and fibroblasts is not well delineated.

KEY ION CHANNELS

The current paradigm for the mechanism underlying SAN automaticity involves a complex interaction between activity of the voltage gated ion channels/exchangers in the plasma membrane and the sarcoplasmic reticulum (SR) (Lakatta and DiFrancesco, 2009). The key membrane ion channel thought to be involved in pacemaker setting is the I_h/I_f channel (DiFrancesco, 2010), and pacemaker activity is further modulated by K^+, Na^+, and Ca^{2+} currents. In addition, recent studies suggest that rhythmic local calcium releases from the SR modulated by PKA also contribute to pacemaker activity by activating inward Na^+/Ca^{2+} exchanger currents during late diastolic depolarization (Maltsev and Lakatta, 2008).

The hyperpolarization-activated cyclic nucleotide (HCN) gated cation current (I_h), also known as the "funny" current (I_f), is a non-specific cation conductance. Hyperpolarization and the binding of cAMP mediate activation of the channel. Thus inhibition of adenylate cyclase can reduce the current and slow pacemaker depolarization and heart rate. HCN4 is the major isoform present in the heart and it is present solely in conducting tissues in the healthy heart. There is not complete consensus on the relative roles of I_h/I_f and GIRK, but it is thought about half the negative chronotropic effect of muscarinic receptor activation is due to each mechanism (Wickman et al., 1998; Gehrmann et al., 2002).

GIRK channels (Kir3.x) are part of the family of inwardly rectifying K^+ channels. The subfamily has five members (Kir3.1–3.5) with a heterotetramer of Kir3.1 and 3.4 constituting the cardiac channel and is present in the atria and conducting tissues but has little if any expression in the ventricle (Karschin et al., 1994; Krapivinsky et al., 1995). The channel is characteristically activated by ACh binding to muscarinic (M2) receptors leading to the common designation as I_{KACh}. The increase in channel activity occurs

because of a direct interaction of the Gβγ dimer, released from the inhibitory G protein heterotrimer, with specific domains in the N- and C-termini of the channel and as such is a paradigm for βγ subunit mediated signaling (Logothetis et al., 1987). The current is also stimulated via adenosine, sphingosine-1-phosphate, and endothelin-A receptors in the heart (Ono et al., 1994, 2001; Bösche et al., 2003; Ochi et al., 2006).

The L-type calcium current is the most prominent calcium current in mammalian ventricular heart cells. The alpha subunit is $Ca_V1.2$ (also known as α1C). It is classically regulated by phosphorylation by PKA and thus will be modulated by variations in G protein coupled receptor pathways involving $G\alpha_s$ and $G\alpha_{i/o}$. For example, the activation of β1-adrenoreceptors by agonists leads to up to a fourfold increase in current and shifts the voltage dependence of activation and inactivation to more hyperpolarized potentials (McCleskey et al., 1986; Hirayama and Hartzell, 1997). Although ACh has no effect upon basal L-type calcium currents, it antagonizes their increase on application of a catecholamine (Fischmeister and Hartzell, 1986).

Other membrane and sarcolemmal ion currents modulated via the cAMP/PKA pathway can also play a role in modulation of pacemaker activity. For example, isoprenaline reduced action potential duration (APD) and increased spontaneous action potential firing rate in rabbit SAN cells by increasing the amplitude and causing a negative shift in the activation of the total delayed rectifier K^+ current (I_K) in a PKA-dependent manner (Lei et al., 2000).

REDUNDANCY IN FUNCTION

It is clear then that there are a number of isoforms of inhibitory G proteins and RGSs expressed in the heart and other tissues, which have potentially overlapping functions. Thus the question is do specific isoforms carry out unique physiological roles or does the system have significant redundancy? For example, inhibitory G proteins are characterized by their sensitivity to pertussis toxin, which ADP ribosylates the proteins and uncouples them from receptors. It is possible to engineer $G\alpha_{i/o}$ isoforms that are resistant to the toxin and thus design experimental strategies in which the isoforms can be selectively investigated. In our own work using *in vitro* cellular systems, we were able to show that only rarely would there be an absolute preference of a receptor for an inhibitory G protein isoform: most G protein coupled receptors couple well with a number of isoforms (Leaney and Tinker, 2000).

THE ROLE OF $G\alpha_{i/0}$ AND RGSS IN CONTROLLING HEART RATE

We studied the question of which inhibitory G protein isoform *in vivo* governs heart rate modulation and dynamics using mice with global KO of $G\alpha_{i2}$, $G\alpha_{i1}$, and $G\alpha_{i3}$ combined and $G\alpha_o$ (Zuberi et al., 2008). We analyzed heart rate dynamics in the time and frequency domain with implanted telemetry probes in awake and mobile mice. The influence of the autonomic system can be measured indirectly by the beat-to-beat variation of heart rate ("heart rate variability," HRV). In man R–R interval shows characteristic patterns of variability with a low frequency (LF) component (>6 s cycle length) determined by sympathetic and parasympathetic drive and a high frequency (HF) component (2.5–6 s cycle length) governed by parasympathetic input (Stauss, 2003). Such analysis has also been applied to the mouse but there is less agreement on the contribution of the different arms of the autonomic nervous system to the frequency components (Thireau et al., 2007). We found that $G\alpha_{i2}$ deficient mice were generally tachycardic with preservation of diurnal variation, had loss of HF power in HRV analysis similar to that seen with GIRK channel blocker TertiapinQ and with the muscarinic receptor blocker atropine and an attenuated bradycardic response to the muscarinic agonist carbachol. Mice with global deficiency in $G\alpha_{i1,3}$ were similar to WT but $G\alpha_o$ showed a different phenotype that is discussed below. There is one conflicting study suggesting $G\alpha_o$ and not $G\alpha_{i2}$ is necessary in muscarinic mediated parasympathetic activity which may be due to methodological issues as the mice were studied very early after probe implantation (Duan et al., 2007). In keeping with our observations, mice with a G184S knockin point mutation in the switch I region of $G\alpha_{i2}$, preventing the binding of RGS proteins and deactivation of $G\alpha_{i2}$, had marked enhancement of muscarinic agonist mediated bradycardia (Fu et al., 2007).

$G\alpha_{i2}$ and $G\alpha_o$ signaling has also been implicated in the muscarinic inhibition of β-adrenoreceptor activated voltage gated L-type calcium channels in the heart. Using ventricular myocytes isolated from global $G\alpha_{i2}$ and $G\alpha_{i3}$ knockout mice, Nagata et al. (2000) demonstrated reduced calcium channel currents with carbachol following isoproterenol stimulation in $G\alpha_{i3}$ knockout mice and littermate controls but not in $G\alpha_{i2}$ knockout mice. Using a similar experimental strategy in $G\alpha_o$ knockout mice, Valenzuela et al. (1997) demonstrated similar impaired muscarinic regulation of L-type calcium channels in ventricular myocytes lacking $G\alpha_o$ but no difference in potassium current compared to wild type controls.

Differential coupling of G protein subtypes to adenosine A1 and muscarinic M2 receptor mediated heart rate slowing was also demonstrated *in vitro* using RGS insensitive $G\alpha_o$ and $G\alpha_{i2}$ ES derived cardiomyocytes (Fu et al., 2006). The RGS insensitive $G\alpha_o$ homozygous knockin cells demonstrated enhanced adenosine A1 and muscarinic M2 receptor mediated bradycardic responses. In contrast, RGS insensitive $G\alpha_{i2}$ homozygous knockin cells showed enhanced responses to M2 but not A1 receptors. Blocking GIRK channels largely abolished the mutation-induced enhancement of the M2 receptor mediated response but had a minimal effect on A1 responses.

Taken together, the evidence suggests that M2 mediated GIRK channel activation is coupled preferentially to the $G\alpha_{i2}$ subunit whereas coupling to voltage gated L-type calcium channel appears to be less specific with both $G\alpha_o$ and $G\alpha_{i2}$ implicated.

There are two studies asking which individual RGS might be important for heart rate control. Cifelli et al. (2008) suggested RGS4 is likely to be the predominant RGS in the SAN. RGS4 mRNA levels were found to be higher in the SAN compared to the atrium, conscious RGS4 null mice showed an increased bradycardic response to carbachol and isolated sinus node myocytes from these mice have modified M2 receptor mediated K_{ACh} currents. More recently, RGS6 has been found to be highly expressed in the heart, in particular in the SAN and AVN. Conscious and anesthetized RGS6 null mice also show an increased bradycardic response to carbachol. Perfused hearts demonstrated a similar

phenomenon, together with the inhibition of spontaneous action potential firing rates in the SAN, as well as atrioventricular block (Yang et al., 2010). RGS6 deficient mice show impaired desensitization and slower deactivation of the GIRK current in atrial myocytes in response to agonist application (Posokhova et al., 2010), confirming that RGS6 has GAP activity. Taken together, the results indicate that RGS4 and RGS6 have an important parasympathetic modulatory role in the SAN, preventing severe bradycardia and that there is very little redundancy. It would be interesting to know if the combined knockout has a more severe phenotype.

ATRIAL FIBRILLATION

Atrial fibrillation is the most common cardiac arrhythmia and affects at least 10% of the octogenarian population (Ho et al., 1993; Benjamin et al., 1998). It is characterized by rapid, irregular, and chaotic electrical excitation of the atria, where these can beat 300 times per minute in the human. The AVN has decremental properties and prevents every beat from being conducted to the ventricle. Despite this, the ventricular rate can still be rapid resulting in symptoms of palpitations, shortness of breath, and dizziness and it can precipitate overt heart failure. There have been several proposed mechanisms of AF. Classically, it has been suggested that AF arises due to an atrial ectopic focus, a single re-entry circuit, or multiple re-entrant circuits (Nattel, 2002). A more modern variant of these mechanisms for AF involves the concept of rotors and spiral waves (Jalife, 2003). A rotor is a stable rotating pattern around a pivot point. This gives rise to a spiraling wavefront (spiral waves) into the surrounding tissue. Such a rotor is thought to be present at the pulmonary vein-left atrial junction, with spiral waves emanating into the atria, giving fibrillation. Catheter ablation producing anatomical block between the pulmonary veins and left atrium can terminate AF supporting this hypothesis (Haissaguerre et al., 1998).

It has been said that "AF begets AF" (Wijffels et al., 1995). This statement is an illustration of the remodeling that occurs once AF has commenced. Indeed permanent AF is often preceded by paroxysmal AF. Remodeling can take two forms: electrical, which occurs as a result of AF itself and structural, which is associated with congestive cardiac failure and other cardiac and extracardiac diseases predisposing to fibrosis. An increase in rate caused by AF results in increased cellular calcium loading, which threatens cell viability. Subsequently, the density of the L-type calcium channel is reduced with a corresponding increase in the inward rectifying potassium currents, leading to a decreased APD and effective refractory period (ERP). This, in turn, promotes and perpetuates the induction and maintenance of AF through multiple re-entrant circuits. These calcium-handling abnormalities impair atrial contractility and can cause atrial dilatation, which also promotes AF (Nattel et al., 2008). In contrast, patients with congestive cardiac failure are thought to develop AF secondary to a change in atrial architecture due to fibrosis, which interferes with electrical conductance. Profibrotic signaling pathways have been identified and these include angiotensin II, transforming growth factor-β_1 and platelet derived growth factor. Extracardiac disease, such as diabetes, obesity, and sleep apnea, may also be responsible for an increased risk of AF through fibrosis. Atrial stretch, which can occur secondary to mitral valve disease, is also a known trigger. Genetic factors may contribute to remodeling; gain-of-function mutations in the potassium channels and loss-of-function mutations in the L-type calcium channel can promote AF. Moreover, single nucleotide polymorphisms alongside genes contributing to atrial integrity have been associated with AF, for example, angiotensin-converting enzyme and *PITX2* (Wakili et al., 2011).

An imbalance in the autonomic system may increase the propensity to AF. In 18 subjects with paroxysmal AF, vagal overactivity was described as the precipitating factor. Here, each paroxysm was preceded by atrial coupling followed by a pause and accompanied by slowing of the sinus rate (Coumel et al., 1978). Changes in autonomic tone through the study of HRV has been assessed in 77 patients with paroxysmal AF (Bettoni and Zimmermann, 2002). This study showed that at least 20 min prior to the onset of paroxysmal AF, there was an initial increase in adrenergic drive followed by a shift in autonomic tone such that increased vagal tone dominated immediately before the onset of paroxysmal AF. The effects of both cholinergic and β-adrenergic stimulation and blockade were studied in the intact dog heart (Sharifov et al., 2004). ACh and catecholamines, individually and in combination, were infused into the heart via the sinus node artery. No electrical stimulation was performed. Cholinergic stimulation was found to be the main factor responsible for spontaneous AF initiation. Adrenergic tone, however, was involved in modulating the initiation and maintenance of cholinergically mediated AF. Further evidence for the role of a vagal mechanism in paroxysmal AF was demonstrated in patients undergoing circumferential pulmonary vein ablation in whom complete vagal denervation was also performed (Pappone et al., 2004). These patients had greater freedom from AF.

Activation of GIRK current results in membrane hyperpolarization, and shortening of APD and ERP. The change in atrial ERP may predispose to the induction and maintenance of AF. Thus any increased activity in inhibitory G protein signaling may predispose to AF. There is surprisingly little data mapping specific genetic modifications in this pathway to experimental supraventricular tachyarrhythmia. It is possible to induce non-sustained AF in the mouse using programmed electrical stimulation (Kovoor et al., 2001). Patch clamp studies utilizing atrial cardiomyocytes from humans (Dobrev et al., 2005) and dogs (Voigt et al., 2008) illustrate that the GIRK channel is constitutively active in AF. Furthermore, the Kir3.4 knockout mouse is resistant to AF (Kovoor et al., 2001). Overexpression of $G\alpha_{i2}$ using a viral vector has also been tried as a therapeutic strategy (Bauer et al., 2004). Injection of the construct into the AVN of animals slowed the heart rate in AF (Bauer et al., 2004). The delivery of cell penetrating peptides to the posterior left atrium to disrupt M2-$G\alpha_i$ coupling prolonged refractoriness in the left atrium and suppressed vagally induced AF (Aistrup et al., 2009). It is interesting to note that it was necessary to target both $G\alpha_{i2}$ and $G\alpha_{i3}$ here. Furthermore, it has been shown that in $G\alpha_{i2}$ knockout mice, where the hearts are studied *ex vivo*, pacemaking occurs from the atria not the SAN and there is no impairment of muscarinic receptor mediated heart rate slowing (Boknik et al., 2009). This suggests that there may be differences in the coupling profile between the M2 receptor and

GIRK channel in the atria and SAN. Once again there is little information on which RGSs might be important in the atria. Of those studied functionally, RGS6 but not RGS4 is expressed there and RGS10 mediates β-adrenergic receptor effects on the GIRK current in rat atrial cells (Bender et al., 2008). There is one report showing inducible AF in the RGS2 knockout mouse (Tuomi et al., 2010).

VENTRICULAR ARRHYTHMIA

Ventricular cardiac arrhythmias, namely ventricular tachycardia (VT) and ventricular fibrillation (VF), cause sudden cardiac death (SCD). SCD poses a significant clinical burden with an estimated 300 000 cases per year recorded in the USA (Noseworthy and Newton-Cheh, 2008). This highlights the importance in predicting risk of SCD, although precise calculation of such a risk remains challenging. Drug therapy, as in AF, can be limited by modest efficacy, significant toxicity, and can be proarrhythmic. ICDs have proven benefit but are complicated by inappropriate shocks, the need for a generator and possible lead changes and complications at the time of insertion, for example infection. Catheter ablation is invasive and is suitable for only modest numbers of patients. Therefore, there is a need to understand the molecular mechanisms responsible for VT initiation and maintenance, in order to develop successful treatments.

The etiology of VT/VF can be broadly divided into those occurring in patients with structurally normal or abnormal hearts. Normal heart VT/VF has traditionally been considered a primary electrical problem, caused by "channelopathies" including the long QT (LQT) syndromes and Brugada syndrome. In some cases, the cause of VT/VF is unknown and hence diagnosed as idiopathic VT. Structurally abnormal hearts causing VT/VF can be due to inherited conditions such as hypertrophic cardiomyopathy (HCM) and arrhythmogenic right ventricular cardiomyopathy (ARVC) or acquired cardiomyopathies, the most common of which is ischemic cardiomyopathy.

Autonomic precipitants to VT/VF is well recognized clinically. Both increased sympathetic and parasympathetic tone can lead to VT/VF depending on etiology and the underlying electrical abnormalities. For example, LQT 1 and 2 are precipitated by an increase in sympathetic tone whereas in LQT 3, it is protective (Moss and Kass, 2005). In Brugada syndrome, vagotonic agents and β adrenergic blockers can precipitate VT/VF (Kasanuki et al., 1997). In patients with idiopathic VT/VF and short coupled variant of torsades des pointes, HRV was significantly depressed prior to the onset of ventricular tachyarrhythmia suggesting vagal precipitant (Leenhardt et al., 1994). The arrhythmogenic substrate is thought to be due to the amplification of spatial dispersion of repolarization by the underlying autonomic tone, causing abnormal re-entry, or triggered activity (Verrier and Antzelevitch, 2004).

Bradycardia itself *per se* may precipitate polymorphic VT by causing prolongation of APD and development of early afterdepolarizations, leading to triggered activity in the form of ventricular premature beats (El-Sherif et al., 1988). Hence any perturbation of $G\alpha_{i/o}$ signaling in the conduction system which leads to bradycardia could in theory increase susceptibility to ventricular arrhythmia.

In patients with ischemic cardiomyopathy induced VT/VF and heart failure, the picture is somewhat different. Sympathetic nervous system activation is beneficial acutely in heart failure but detrimental in the long term. Such a mechanism promotes apoptosis of cardiac myocytes, ion channel remodeling, and is proarrhythmic (DeGeorge et al., 2008). Cardiac $G\alpha_{i/o}$ signaling pathways may counteract this process (Zheng et al., 2005). Indeed, in chronic heart failure, β_1-adrenoceptors are downregulated whereas $G\alpha_{i2}$ is significantly increased (Eschenhagen et al., 1992). Overexpression of β_1-adrenoceptors in transgenic mice results in a dilated cardiomyopathy but a more modest phenotype is seen with β_2-adrenoceptor overexpression. β_2-adrenoceptors are coupled to $G\alpha_{i/o}$ and $G\alpha_s$, suggesting a cardioprotective role for $G\alpha_{i/o}$ (Foerster et al., 2003; DeGeorge et al., 2008).

In heart failure patients with ICDs, a loss in HRV was noted immediately before an episode of VT and this was found to be an independent predictor of SCD (Pruvot et al., 2000). Parasympathetic activation can terminate VT and increase VF threshold whereas increases in sympathetic activation can precipitate ventricular arrhythmias (Kolman et al., 1975; Waxman and Wald, 1977; Brack et al., 2007). Taken together, there is good evidence that increased vagal tone protects against the development of heart failure and VT/VF precipitated by ischemic cardiomyopathy. Certainly β-blocker therapy improves prognosis in those with heart failure (Poole-Wilson et al., 2003).

To explore the role of $G\alpha_{i2}$ and predisposition to cardiac ventricular arrhythmias, we performed *in vivo* cardiac electrophysiological studies on global $G\alpha_{i2}$ knockout mice and compared them with wild type littermates and combined $G\alpha_{i1}$ and $G\alpha_{i3}$ knockout controls (Zuberi et al., 2010). Mice with global genetic deletion of $G\alpha_{i2}$ were found to have a reduced ventricular effective refractive period and propensity for VT induced with programmed electrical stimulation compared to controls. Conscious $G\alpha_{i2}$ knockout mice had a prolonged QT interval and single ventricular myocytes studied with patch clamping showed a steep restitution curve but prolonged APD. Increased levels of message for the L-type calcium channel was found on gene expression studies, with confirmation of an increase in this current on patch clamping. No structural heart disease was noted on histology or echocardiography. These findings suggest that the absence of $G\alpha_{i2}$ is a substrate for VT. Interestingly, Dizayee et al. (2011) studied ventricular myocytes isolated from $G\alpha_{i2}$ and $G\alpha_{i3}$ knockout mice and found reduced L-type calcium channel current density in $G\alpha_{i2}$ knockouts compared to littermate controls but increased in $G\alpha_{i3}$ knockouts where there was a compensatory increase in $G\alpha_{i2}$ expression. The differences between our results and theirs may be accounted for by strain differences in the mice (129\Sv versus C57\Black) Clinically, pravastatin, given to patients in order to lower cholesterol, selectively upregulates $G\alpha_{i2}$ and increases HF power in HRV analysis (Welzig et al., 2003).

REGULATION OF THE HEART BY THE AUTONOMIC NERVOUS SYSTEM

The autonomic nervous system maintains homeostasis and coordinates physiological responses in the body via its two functional limbs: the sympathetic and parasympathetic nervous systems. The

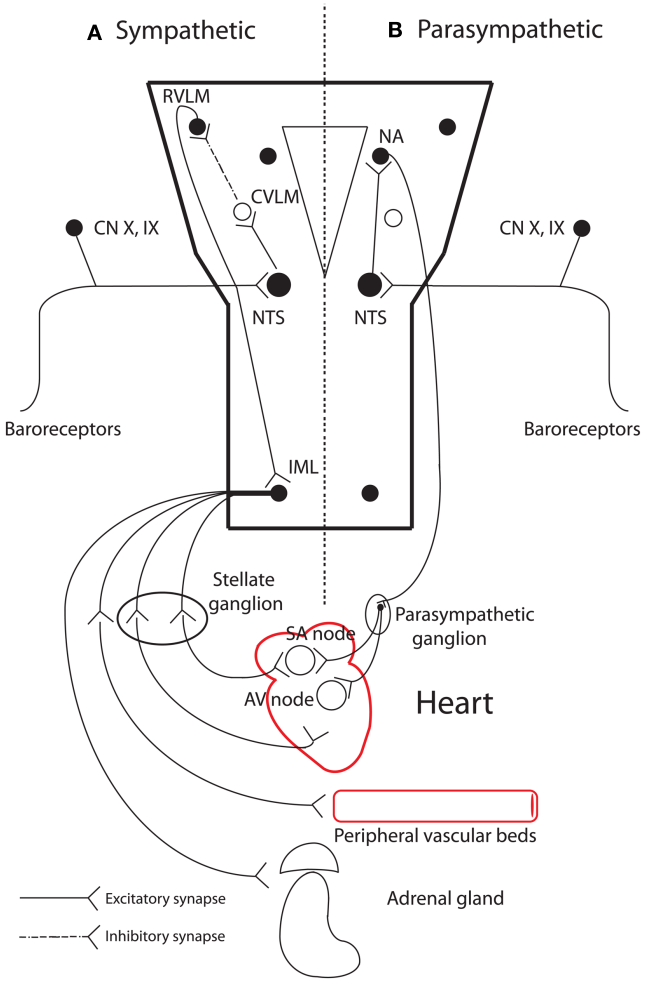

FIGURE 2 | The main peripheral and neural centers involved in autonomic cardiovascular regulation. (A) Sympathetic baroreflex arm: sensory neurons in the periphery transmit baroreceptor information via a glutamatergic synapse to the nucleus tractus solitaries (NTS) in the medulla. NTS neurons project to and excite neurons in the caudal ventrolateral medulla (CVLM). CVLM neurons in turn project and inhibit (via a GABAergic synapse) presympathetic neurons in the rostral ventrolateral medulla (RVLM). Spinally projecting presympathetic RVLM neurons make direct connections with sympathetic preganglionic neurons in the intermediolateral (IML) spinal column, which projects sympathetic outflow to effector organs. **(B)** Parasympathetic control of heart rate: NTS neurons receive baroreceptor and chemoreceptor information and relay via a glutamatergic pathway to the nucleus ambiguous (NA). Cardioinhibitory neurons in NA then project to the heart via the vagus nerve, which mediates its effect via muscarinic receptors in the heart. Adapted from Pilowsky and Goodchild (2002). *GABA*, gamma-Aminobutyric acid; SA, sinoatrial; AV, atrioventricular.

heart is regulated in this manner within the cardio-respiratory system (**Figure 2**). Afferent nerves convey baroreceptor and chemoreceptor inputs to the cardio-respiratory centers within the brainstem and generally opposing reflex responses are mediated by the sympathetic and parasympathetic efferent to the heart, blood vessels, and other effector organs such as the adrenal glands. Both sympathetic and parasympathetic systems are made up of central and peripheral components. The central component consists of cell groups in the brain and spinal cord, and myelinated preganglionic neurons. It is responsible for maintenance of autonomic tone, and integration of afferent inputs to generate appropriate autonomic reflexes. Much of the basic structure involved in the reflex control of the cardiovascular system is contained within the medulla. Integrated and behaviorally appropriate responses also require reciprocal connections between the medulla, pons, midbrain, and hypothalamus (Spyer, 1994). The peripheral component consists of ganglia and unmyelinated postganglionic afferent and efferent fibers innervating the heart and associated effector organs (e.g., blood vessels). It is increasingly recognized that the pattern of cardiac innervation peripherally may be important in cardiac arrhythmogenesis (Verrier and Antzelevitch, 2004).

As described above, the murine models used to study (patho)physiology are often global knockouts or knock-ins. It is conceivable (and probably very likely) that there are contributions to the phenotype from the effects on inhibitory G protein signaling in the nervous system. $G\alpha_{i1}$, $G\alpha_{i2}$, and $G\alpha_{i3}$ are ubiquitously expressed but $G\alpha_o$ protein expression is restricted mainly to the central and peripheral nervous systems, endocrine cells, and cardiomyocytes (Wettschureck and Offermanns, 2005). Expression of $G\alpha_o$ is greatest in the brain where it is estimated to constitute around 1% of all membrane proteins (Sternweis and Robishaw, 1984). Mice lacking the α subunit of G_o are smaller and weaker than their littermates, have greatly reduced life expectancy and display numerous neurological abnormalities including hyperalgesia, tremors, seizures, and increased motor activity with an extreme turning behavior (Jiang and Bajpayee, 2009).

GIRK channels are widely distributed in neuronal and endocrine tissues and play key roles in generating late inhibitory postsynaptic potentials and modulating hormone release in addition to their cardiac functions. In the central nervous system, GIRK channels hyperpolarize neurons in response to activation by neurotransmitters including adenosine, somatostatin, 5-HT, and opioids, and is composed of heterotetramers of Kir3.1, Kir3.2, and Kir3.3 and homotetramers of Kir3.2 (Lüscher et al., 1997; Lüscher and Slesinger, 2010). Pharmacological investigations of GIRK channels and studies in animal models suggest that GIRK activity has an important role in physiological responses, including pain perception and memory modulation. Abnormal GIRK function has been implicated in altering neuronal excitability and cell death, which may be important in the pathophysiology of diseases such as epilepsy, Down's syndrome, Parkinson's disease, and drug addiction (Lüscher and Slesinger, 2010). There is limited study of GIRK's role in autonomic traffic. Feldman and colleagues found reduced BP depressor effects of the I_1-imidazoline receptor (I_1R) selective ligands administered intracisternally in rabbits pretreated with TertiapinQ suggesting that the central sympatho-inhibitory effects of I_1R ligands are mediated by GIRK channels (Yoro Sy et al., 2008).

The regulation of neurotransmitter release at presynaptic terminals is an important mechanism underlying the modulation of synaptic transmission in the nervous system. Inhibitory regulation of neurotransmitter release via inhibitory G proteins is mediated

by various G protein coupled receptors like α2-adrenoceptors, μ- and δ-opioid receptors, GABA-B receptors, adenosine A1, or endocannabinoid CB1-receptors. A major mechanism by which these G proteins mediate the inhibition of transmitter release is the direct inhibitory modulation of the action potential-evoked Ca^{2+} entry to the presynaptic terminal by the Gβγ subunit, which is required to trigger neurotransmitter release. N- and P/Q-type calcium channels that are concentrated at nerve terminals as well as R-type calcium channels have been shown to be inhibited via $G\alpha_{i/o}$-coupled receptors (Dolphin, 2003).

There is evidence showing selectivity of inhibitory G protein coupling to voltage gated Ca^{2+} channels. Neuropeptide Y (NPY) and bradykinin both inhibit N-type Ca^{2+} channels *in vitro*, and pertussis toxin treatment could completely abolish this inhibition. Addition of purified bovine $G\alpha_o$ subunits effectively restored the NPY modulation of Ca^{2+} currents while purified $G\alpha_{i1}$ or $G\alpha_{i2}$ had little or no effect. Interestingly, the bradykinin inhibitory effects on Ca^{2+} currents in pertussis toxin-treated cells was only partially restored by the addition of either purified $G\alpha_o$ or $G\alpha_{i2}$, even at high concentrations. Complete restoration required a combination of both $G\alpha_o$ and $G\alpha_{i2}$ proteins suggesting that $G\alpha_o$ and $G\alpha_{i2}$ proteins may couple to distinct subpopulations of bradykinin receptors since $G\alpha_o$ nor $G\alpha_{i2}$ protein alone cannot compensate the loss of the other (Ewald et al., 1989). $G\alpha_o$ antibodies in the superior cervical ganglion has also been shown to reduce calcium current inhibition by noradrenaline in superior cervical ganglion neurons (Caulfield et al., 1994). Signaling via $G\alpha_o$ has also been implicated in vesicular glutamate transporter within the central nervous system (Winter et al., 2005) and GAP43 receptor signaling for neuronal pathfinding (Strittmatter et al., 1990).

In vivo experiments manipulating the expression of inhibitory G proteins and its subtypes either directly with knockout models or indirectly by manipulation of their regulatory proteins have shed limited light on their physiological importance and relative contribution to the peripheral and central control of autonomic heart rate regulation. Methodologically, autonomic tone *in vivo* has been quantified by indirect methods such as catecholamine sampling and analysis of HRV. The baroreceptor reflex is the prototypical cardiovascular reflex studied and is commonly quantified by computation of baroreflex set point and gain from ambulatory BP recordings, although more direct methods have been described (Ma et al., 2002; Young and Davisson, 2011).

Interestingly, mice overexpressing RGS insensitive $G\alpha_{i2}$ were tachycardic during the daytime, have an enlarged heart and hyperdynamic echo profile. Along with reduced viability, growth retardation, and multiple neurological deficit, the cardiovascular phenotype observed was attributed to increased central sympathetic tone (Huang et al., 2006). To study the cardiac specific effect of enhanced $G\alpha_{i2}$ signaling, isoproterenol-stimulated beating isolated perfused hearts were studied and showed the expected enhancement of muscarinic mediated bradycardia (Fu et al., 2007).

Mice deficient in RGS2 were found to be hypertensive but with normal HR suggestive of resetting of baroreceptor sensitivity. There was an associated increase in urinary catecholamine secretion and reduction in both LF and HF power of HRV suggesting increased central sympathetic tone (Gross et al., 2005). This along with data on the RGS insensitive $G\alpha_{i2}$ mice described above implies that overactivity of inhibitory G proteins may contribute to increased central sympathetic tone. In our study of global $G\alpha_o$ knockout mice (Zuberi et al., 2008), in addition to the neurological abnormalities previously described, we found the mice to be tachycardic with loss of diurnal rhythm and fairly selective loss of the LF component of HRV with preserved total power. However, carbachol still had a negative chronotropic effect. This is suggestive of increased sympathetic tone, likely to be of central origin.

Significant progress has been made in our understanding of the autonomic nervous system and the effects upon cardiac function. Nonetheless, further study of the chronic alterations in the autonomic nervous system and their cardiac consequences may prove useful in the prevention of cardiac disease and provide valuable treatment modalities (Brodde and Michel, 1999).

CONCLUSION AND FUTURE DIRECTIONS

It is clear that inhibitory G proteins and their associated RGS proteins play a critical role in autonomic signaling at multiple levels. Their role in mediating the parasympathetic tone to the heart is well established. What is unclear is their role in autonomic traffic in the central autonomic circuits. This will need further elucidation to dissect the role of autonomic tone in cardiac pathophysiology. What is also unclear is what role the autonomic nervous system and the specific signaling molecules have in generating cardiac arrhythmia. Mice with global G protein deletion often have reduced viability and are difficult to work with and it can be unclear at which level signaling is contributing to the phenotype. Use of more refined temporal and spatial conditional deletion strategies would circumvent some of the problems and help define the relative role of inhibitory G proteins and its subtypes in the central and peripheral autonomic control of the heart.

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