Altered Heart Rate and Sinoatrial Node Function in Mice Lacking the cAMP Regulator Phosphoinositide 3-Kinase-γ

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Abstract—Ablation of the enzyme phosphoinositide 3-kinase (PI3K)γ (PI3Kγ−/−) in mice increases cardiac contractility by elevating intracellular cAMP and enhancing sarcoplasmic reticulum Ca2+ handling. Because cAMP is a critical determinant of heart rate, we investigated whether heart rate is altered in mice lacking PI3Kγ. Heart rate was similar in anesthetized PI3Kγ−/− and wild-type (PI3Kγ+/+) mice. However, IP injection of atropine (1 mg/kg), propranolol (1 mg/kg), or both drugs in combination unmasked elevated heart rates in PI3Kγ−/− mice, suggesting altered sinoatrial node (SAN) function. Indeed, spontaneous action potential frequency was ~35% greater in SAN myocytes isolated from PI3Kγ−/− mice compared with PI3Kγ+/+ mice. These differences in action potential frequency were abolished by intracellular dialysis with the cAMP/protein kinase A antagonist Rp-cAMP but were unaffected by treatment with ryanodine to inhibit sarcoplasmic reticulum Ca2+ release. Voltage-clamp experiments demonstrated that elevated action potential frequencies in PI3Kγ−/− SAN myocytes were more strongly associated with cAMP-dependent increases in L-type Ca2+ current (I_{Ca,L}) than elevated hyperpolarization-activated current (I_h). In contrast, I_{Ca,L} was not increased in working atrial myocytes, suggesting distinct subcellular regulation of L-type Ca2+ channels by PI3Kγ in the SAN compared with the working myocardium. In summary, PI3Kγ regulates heart rate by the cAMP-dependent modulation of SAN function. The effects of PI3Kγ ablation in the SAN are unique from those in the working myocardium. (Circ Res. 2007;101:1274-1282.)

Key Words: ion channels ▪ electrophysiology ▪ action potentials ▪ arrhythmia

Genetic knockout of phosphoinositide 3-kinase (PI3K)γ (PI3Kγ−/−) in mice results in reduced phosphodiesterase activity, increased intracellular cAMP levels, and enhanced ventricular contractility.1–5 We have previously demonstrated that increased contractility in ventricular myocytes isolated from PI3Kγ−/− mice is attributable to elevated sarcoplasmic reticulum (SR) Ca2+ levels and increased SR Ca2+ release without changes in L-type Ca2+ current (I_{Ca,L}).4,6 These results establish that the regulation of cAMP by PI3Kγ in ventricular myocytes occurs in a subcellular compartment containing the SR, but not L-type Ca2+ channels.7

Heart rate is determined by the electrical properties of specialized myocytes located in the sinoatrial node (SAN).8,9 The spontaneous electrical activity of SAN myocytes results from slow diastolic depolarizations that increase the membrane potential toward the threshold for eliciting action potentials (APs). The activity of several currents involved in the regulation of spontaneous AP firing in SAN myocytes are modulated by cAMP, including the hyperpolarization-activated current (I_h), I_{Ca,L}, a delayed rectifier K+ current (I_{Kd}) and a Na+–Ca2+ exchange current (I_{Na/Ca}) that is driven by SR Ca2+ release.8–14 Because PI3Kγ has emerged as an important regulator of cAMP in the myocardium, and because cAMP is a critical modulator of SAN pacemaker cell activity, we sought to evaluate the role of PI3Kγ in heart rate regulation using transgenic mice lacking PI3Kγ. Our results indicate that PI3Kγ modulates the intrinsic activity of the SAN in a cAMP-dependent fashion and that the effects of PI3Kγ ablation in the SAN are unique compared with the working myocardium of the atria and ventricles. Some of these data have been presented in abstract form.15

Materials and Methods

Animals

In the present study, male wild-type (PI3Kγ+/+) and PI3Kγ−/−16 littermate mice aged 10 to 14 weeks were used. All experimental procedures were in accordance with the regulations of The Canadian Council on Animal Care and were approved by the University of Toronto animal care facility.

In Vivo Heart Rate Measurements

Heart rate was measured in anesthetized mice using a pressure catheter (Millar Instruments) inserted into the aorta via the carotid artery, as described in the online data supplement at http://circres.ahajournals.org.
Isolation of Mouse SAN and Right Atrial Myocytes

The procedures for isolating single pacemaker myocytes from the SAN, as well as working right atrial myocytes from the mouse have been described and are available in the online data supplement. Single SAN and right atrial myocytes were used for patch-clamp studies using standard solutions and electrophysiological protocols, which are detailed in the online data supplement.

Results

Consistent with previous reports, no differences in heart rate were observed between PI3K+/+ and PI3K−/− mice in control conditions (Figure 1). To assess whether the intrinsic electrical properties of the SAN differed between PI3K+/+ and PI3K−/− mice, heart rate was also measured following selective blockade of the autonomic nervous system. Figure 1A shows that administration of atropine (1 mg/kg) to block parasympathetic nervous system activity caused heart rate to increase (P<0.05) from 544.1±22.2 to 589.9±17.5 beats per minute (bpm) over 20 minutes in PI3K+/+ mice and from 526.4±17.3 to 635.4±14.3 bpm over shorter periods (5 minutes) in PI3K−/− mice. After atropine treatment, heart rate was higher (P<0.05) in PI3K−/− than PI3K+/+ mice.

Treatment with propranolol (1 mg/kg; Figure 1B) to block sympathetic nervous system activity reduced (P<0.05) heart rate in PI3K+/+ (511.2±9.8 to 343.3±16.5 bpm) and PI3K−/− (523.4±15.5 to 406.9±13.0 bpm) mice. Following propranolol treatment, heart rate was greater (P<0.05) in PI3K−/− than PI3K+/+ mice. Combined treatment with atropine and propranolol (Figure 1C) produced heart rate changes that were comparable to the effects of propranolol alone.

Because the results above suggest that PI3Kγ modulates the intrinsic pacemaker properties of the SAN, we directly assessed the electrical properties of isolated SAN myocytes. Spontaneous APs could be continuously recorded for at least 20 minutes and, as summarized in Figure 2, occurred at a
higher frequency ($P<0.05$) in PI3K$^{-/-}$ myocytes (173±8 bpm) than PI3K$^{+/+}$ myocytes (129±6 bpm). The relative differences in SAN myocyte firing rate between PI3K$^{+/+}$ and PI3K$^{-/-}$ were similar to the heart rate differences observed in mice following autonomic blockade, although the absolute rates differed because of temperature differences between the studies. These changes in AP frequency were associated with increases ($P<0.05$) in the slope of the diastolic depolarization (DD) from 25.1±1.5 mV/sec in PI3K$^{+/+}$ myocytes to 41.7±1.4 mV/sec in PI3K$^{-/-}$ myocytes, without changes ($P=0.62$) in the maximum diastolic potential between genotypes (supplemental Table I). Because the modulation of ventricular cardiomyocyte contractility by PI3K$\gamma$ depends on cAMP,2,4 we examined the effects of the cAMP/protein kinase (PKA) antagonist adenosine-3',5' cyclic phosphorothioate (Rp-cAMP; 1×10$^{-4}$ mol/L) on SAN myocytes. Eight minutes of treatment with Rp-cAMP had no effect ($P=0.69$) on spontaneous AP frequency in PI3K$^{+/+}$ SAN myocytes but reduced ($P<0.05$) firing frequency from 170±6 to 140±4 bpm and the DD slope from 48.9±3.5 to 22.2±1.2 mV/sec in PI3K$^{-/-}$ SAN myocytes, without affecting the maximum diastolic potential (Figure 2 and supplemental Table I). Firing frequencies were identical between the groups after Rp-cAMP.

To explore the ionic basis for the changes in AP-firing frequency, we measured $I_{Ca,L}$ and $I_f$ because these currents alter the spontaneous firing rate of SAN myocytes in a cAMP-dependent manner by influencing the DD slope. Figure 3 shows representative $I_{Ca,L}$ recordings, originating from Cav1.2 and Cav1.3 channels, measured with voltage-clamp protocols designed to minimize T-type Ca$^{2+}$ currents.21 These recordings were done in the presence of tetrodotoxin (3×10$^{-5}$ mol/L) or QX-314 (3×10$^{-2}$ mol/L) to block voltage-gated Na$^+$ channels. $I_{Ca,L}$ current densities were larger ($P<0.05$), and the peak of the current–voltage relationship (I-V) curve was shifted toward negative potentials in PI3K$^{-/-}$ versus PI3K$^{+/+}$ SAN myocytes. Note that these I-V curves peak at more negative membrane potentials than ventricular myocytes because of the functional expression of the CaV1.3 channel isoform.21 To better quantify the differences in $I_{Ca,L}$ between the groups, steady-state conductance analysis was performed, which revealed that the maximum conductance ($G_{max}$) was elevated ($P<0.05$) in PI3K$^{-/-}$ (93.5±7.5 pS/pF) compared with PI3K$^{+/+}$ (72.7±7.9 pS/pF) SAN myocytes (Figure 3C). Furthermore, the voltage required for 50% channel activation ($V_{1/2}$) was shifted ($P<0.05$) leftward from −30.6±1.7 mV for PI3K$^{+/+}$ SAN myocytes to −38.5±2.0 mV in PI3K$^{-/-}$ SAN myocytes (Figure 3C). Despite clear differences in channel activation, no differences ($P=0.89$) in the time constants for $I_{Ca,L}$ inactivation (ie, $\tau_{inact}$ and $\tau_{slow}$) were observed between genotypes (supplemental Figure I). These changes in channel activation are expected to increase depo-
larizing currents leading to increased DD slopes and spontaneous firing frequencies.

Because the increase in \( G_{\text{max}} \) and the shift in \( V_{1/2} \) for \( I_{\text{Ca,L}} \) in \( \text{PI3K}_{\gamma^{-/+}} \) SAN myocytes are reminiscent of changes observed following activation of cAMP/PKA in the myocardium, we examined the effects of the cAMP/PKA antagonist Rp-cAMP (1×10^{-4} \text{ mol/L}) on \( I_{\text{Ca,L}} \) measured at −10 mV, a voltage at which the conductance is maximal. As summarized in Figure 3D and 3E, Rp-cAMP had no effect (\( P=0.30 \)) on \( I_{\text{Ca,L}} \) in \( \text{PI3K}_{\gamma^{-/+}} \) SAN myocytes but reduced (\( P<0.05 \)) peak \( I_{\text{Ca,L}} \) in \( \text{PI3K}_{\gamma^{-/-}} \) to levels not different from \( \text{PI3K}_{\gamma^{-/+}} \) myocytes in the presence or absence of Rp-cAMP. Similarly, Rp-cAMP had no effect (\( P=0.12 \)) on \( G_{\text{max}} \) or \( V_{1/2} \) of activation for \( I_{\text{Ca,L}} \) in \( \text{PI3K}_{\gamma^{-/+}} \) SAN myocytes but reduced (\( P<0.05 \)) \( G_{\text{max}} \) and shifted \( V_{1/2} \) in \( \text{PI3K}_{\gamma^{-/-}} \) SAN myocytes to values that were not different (\( P=0.48 \)) from \( \text{PI3K}_{\gamma^{-/-}} \) (supplemental Figure II).

The cAMP-dependent \( I_{\text{Ca,L}} \) elevations in \( \text{PI3K}_{\gamma^{-/-}} \) SAN myocytes contrasts with the lack of effect of \( \text{PI3K}_{\gamma} \) ablation on \( I_{\text{Ca,L}} \) in ventricular myocytes.\(^4\) Because \( I_{\text{Ca,L}} \) is only produced by Cav1.2 channels in ventricular myocytes, but is generated by Cav1.2 and Cav1.3 channels in SAN myocytes, we considered the possibility that \( I_{\text{Ca,L}} \) elevations in \( \text{PI3K}_{\gamma^{-/-}} \) SAN myocytes originate from cAMP-dependent regulation of Cav1.3 channels. To test this possibility, we took advantage of the fact that, like SAN myocytes, working atrial myocytes also express Cav1.2 and Cav1.3 L-type Ca^{2+} channels.\(^{21-23}\) As summarized in Figure 4 (and supplemental Figure I), \( I_{\text{Ca,L}} \) in right atrial myocytes did not differ (\( P=0.85 \)) between \( \text{PI3K}_{\gamma^{-/+}} \) and \( \text{PI3K}_{\gamma^{-/-}} \), suggesting that increased \( I_{\text{Ca,L}} \) in \( \text{PI3K}_{\gamma^{-/-}} \) SAN myocytes is unlikely to result from distinct regulation of Cav1.3 L-type Ca^{2+} channels by \( \text{PI3K}_{\gamma} \).

Next, we recorded \( I_{f} \). Our data illustrate that \( I_{f} \) current density was higher (\( P<0.05 \)) in \( \text{PI3K}_{\gamma^{-/-}} \) SAN myocytes, compared with \( \text{PI3K}_{\gamma^{-/+}} \) myocytes, at membrane potentials less than or equal to −80 mV (Figure 5). These elevated current densities were accompanied by a shift (\( P<0.05 \)) in the \( V_{1/2} \) for channel activation (~−96.4±1.0 mV for \( \text{PI3K}_{\gamma^{-/+}} \) myocytes versus −88.5±0.9 mV for \( \text{PI3K}_{\gamma^{-/-}} \) myocytes) with no change (\( P=0.14 \)) in slope factor (18.5±0.9 for \( \text{PI3K}_{\gamma^{-/-}} \) versus 19.0±0.8 for \( \text{PI3K}_{\gamma^{-/+}} \)). These differences in \( I_{f} \) are anticipated to increase depolarizing currents, leading to a higher DD slopes and firing rates in \( \text{PI3K}_{\gamma^{-/-}} \) myocytes.

To determine whether these \( I_{f} \) differences resulted from altered cAMP/PKA signaling between the groups, the effects of Rp-cAMP (1×10^{-4} \text{ mol/L}) were examined (Figure 6A and 6B). In these studies, the control current levels were measured 30 seconds after rupturing the cell membrane, and the effects of Rp-cAMP were measured after 8 minutes of dialysis (see Materials and Methods). Rp-cAMP increased (\( P<0.05 \)) \( I_{f} \) at −120 mV slightly from −10.2±0.8 to −11.9±0.9 \text{ pA/μF} in \( \text{PI3K}_{\gamma^{-/+}} \) SAN myocytes while reducing (\( P<0.05 \)) \( I_{f} \) from −18.4±0.8 to −15.7±1.2 \text{ pA/μF} in \( \text{PI3K}_{\gamma^{-/-}} \) SAN myocytes. The ability of Rp-cAMP to increase \( I_{f} \) in \( \text{PI3K}_{\gamma^{-/+}} \) SAN myocytes is not unexpected because, whereas Rp-cAMP can block PKA activation, it also directly activates hyperpolarization-activated, cyclic nucleotide-gated (HCN) channels.\(^{24,25}\) Thus, to dissect the contributions of PKA to the enhanced \( I_{f} \) in \( \text{PI3K}_{\gamma^{-/-}} \) myocytes, we sought to use a 14-22-amide protein kinase inhibitor (PKI\(_{14-22}\)) (1×10^{-5} \text{ mol/L}), which is the inhibitor peptide for the PKA catalytic subunit.\(^{26}\) PKI\(_{14-22}\) had no effect on \( I_{f} \) in \( \text{PI3K}_{\gamma^{-/+}} \) SAN myocytes, but caused reductions in \( \text{PI3K}_{\gamma^{-/-}} \) SAN myocytes similar to those seen with Rp-cAMP (Figure 6C and 6D).

The results above establish that increased spontaneous firing rates of SAN myocytes in \( \text{PI3K}_{\gamma^{-/-}} \) mice are related to elevated cAMP/PKA signaling. To explore cAMP signaling...
further, we investigated the effects of isoproterenol (ISO). As expected, application of ISO (1 × 10⁻⁶ mol/L) increased (P<0.05) AP frequency from 118±9 to 154±6 bpm in PI3Kγ⁻/⁻ myocytes (Figure 7A and 7B), in association with increases (P<0.05) in DD slope, AP overshoot, and AP duration measured at 50% repolarization (APD₅₀) (supplemental Table I). In PI3Kγ⁻/⁻ myocytes, ISO application increased (P<0.05) AP frequency from 183±6 to 207±6 bpm with relatively similar changes in AP profile, as seen in PI3Kγ⁻/⁻ SAN myocytes (Figure 7B and supplemental Table I), establishing that cAMP-dependent signaling is not saturated under baseline conditions in PI3Kγ⁻/⁻ SAN myocytes.

Indeed, both I_Ca,L and I_f were increased (P<0.05) by β-adrenergic receptor stimulation with ISO in PI3Kγ⁻/⁻ and PI3Kγ⁻/⁻ SAN myocytes (Figure 7C through 7F).

SAN pacemaker activity has been shown to be modulated by SR Ca²⁺ loading and release,¹⁴,²⁷,²⁸ which are both regulated by cAMP/PKA and markedly increased in PI3Kγ⁻/⁻ ventricular myocytes.⁴,⁵ Therefore, we tested the effects of the SR Ca²⁺ release blocker ryanodine (1 × 10⁻⁵ mol/L) on spontaneous AP frequency. Representative AP recordings in control conditions and after treatment with ryanodine (minimum 8 minutes; Figure 8) show firing frequency was reduced (P<0.05) by approximately the same percentage in PI3Kγ⁻/⁻.
SAN myocytes (166±11 to 131±8 bpm) as in PI3K\textsuperscript{γ+/-} SAN myocytes (135±5 to 105±6 bpm). Ryanodine also reduced (P<0.05) DD slope and increased (P<0.05) APD\textsubscript{50} (supplemental Table I). Thus, AP-firing frequencies remained elevated (P<0.05) in PI3K\textsuperscript{γ-/-} SAN myocytes after inhibition of SR Ca\textsuperscript{2+} release, suggesting that differences in SR function are not responsible for the higher firing rates in PI3K\textsuperscript{γ-/-} SAN myocytes.

Heart rate is also potently regulated by parasympathetic nervous system-mediated stimulation of M2 muscarinic receptors in the SAN, which activate hyperpolarizing acetylcholine-sensitive K\textsuperscript{+} currents (I\textsubscript{KACH}) via G\textsubscript{i,II} subunits.\textsuperscript{29} Because heart rate (Figure 1) differed between PI3K\textsuperscript{γ+/-} and PI3K\textsuperscript{γ-/-} mice after autonomic blockade, we also examined I\textsubscript{KACH} in SAN myocytes. I\textsubscript{KACH} evoked by carbachol (1×10\textsuperscript{-5} mol/L)\textsuperscript{32} did not differ between PI3K\textsuperscript{γ+/-} and PI3K\textsuperscript{γ-/-} SAN myocytes (supplemental Figure III), indicating that the properties of I\textsubscript{KACH} are not directly affected by PI3K\textsuperscript{γ}.

**Discussion**

Although our studies demonstrate that PI3K\textsuperscript{γ} negatively regulates the spontaneous firing rate of isolated SAN myocytes, heart rate was not different between wild-type and PI3K\textsuperscript{γ-/-} mice under basal conditions. This is not unexpected because heart rate in vivo is regulated by the autonomic nervous system.\textsuperscript{30,31} Indeed, blockade of the sympathetic or parasympathetic arms of the autonomic nervous system unmasked elevated heart rates in PI3K\textsuperscript{γ-/-} mice with the relative heart rate differences after combined propranolol and atropine treatment being similar to the relative differences in spontaneous firing rate observed in isolated SAN myocytes. Interestingly, whereas both propranolol and atropine treatment affected heart rate, sympathetic blockade had a relatively larger effect in agreement with previous mouse studies.\textsuperscript{32-34} Furthermore, the larger heart rate changes with atropine in PI3K\textsuperscript{γ-/-} (compared with PI3K\textsuperscript{γ+/-}) mice suggest that elevated parasympathetic activity is used by PI3K\textsuperscript{γ-/-} mice to suppress higher intrinsic AP rates of their SANs, even though I\textsubscript{KACH} was not directly affected by PI3K\textsuperscript{γ}.

Consistent with previous reports showing that PI3K\textsuperscript{γ} is a negative regulator of cAMP in the ventricular myocardi um,\textsuperscript{1-3} we found that the cAMP/PKA blocker Rp-cAMP reduced AP firing frequency in PI3K\textsuperscript{γ-/-} SAN myocytes to levels indistinguishable from PI3K\textsuperscript{γ+/-} SAN myocytes in the absence or presence of Rp-cAMP. The differences in AP firing observed between PI3K\textsuperscript{γ+/-} and PI3K\textsuperscript{γ-/-} SAN myocytes, as well as the changes induced by Rp-cAMP, correlated tightly with changes in the slope of the DD. Under
physiological conditions, several channels whose activities are increased by β-adrenergic receptor-mediated elevations in cAMP/PKA signaling provide depolarizing current during the diastolic period.

\( I_{\text{CaL}} \) is an attractive candidate to explain the differences in spontaneous AP firing between PI3Kγ\(^{+/+}\) and PI3Kγ\(^{-/-}\) SAN myocytes and the response to Rp-cAMP in PI3Kγ\(^{-/-}\) SAN myocytes, for several reasons. First, \( I_{\text{CaL}} \) in SAN myocytes is generated by 2 distinct L-type Ca\(^{2+}\) channel α1 subunits: Ca\(_{\alpha1,2}\) and Ca\(_{\alpha1,3}\). Whereas Ca\(_{\alpha1,2}\) channels contribute primarily to the AP upstroke, Ca\(_{\alpha1,3}\) channels activate at diastolic period. 

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Second, more negative membrane potentials that correspond to the myocytes, for several reasons. First, primarily to the AP upstroke, Cav1.3 channels activate at diastolic period. 

The effects of Rp-cAMP on \( I_{\text{CaL}} \) closely mirrored the effects on spontaneous firing rates. Specifically, the elevation in \( I_{\text{CaL}} \) density that was observed in PI3Kγ\(^{-/-}\) SAN myocytes was eliminated by Rp-cAMP. On the other hand, Rp-cAMP had no effect on \( I_{\text{CaL}} \) in PI3Kγ\(^{+/+}\) SAN myocytes. Thus, our findings suggest that cAMP-dependent regulation of \( I_{\text{CaL}} \) is a major determinant of the firing rate of the mouse SAN, as concluded previously.\(^{19,21}\) Consistent with this suggestion, \( I_{\text{CaL}} \) is a prototype for cAMP/PKA-dependent phosphorylation,\(^{35,36}\) and \( I_{\text{CaL}} \) blockers are routinely used in the effective treatment of atrial tachyarrhythmias.\(^{37}\) It could be hypothesized that the negative shift in \( I_{\text{CaL}} \) activation properties (ie, \( V_{1/2} \)) between genotypes results from an increased role for Ca\(_{1,3}\) channels in PI3Kγ\(^{-/-}\) SAN myocytes. However, this seems unlikely because \( V_{1/2} \) (as well as \( G_{\text{max}} \)) was identical between genotypes after Rp-cAMP addition.

Another potential contributor to the elevated AP-firing rates in PI3Kγ\(^{-/-}\) SAN myocytes is the “pacemaker current” \( I_{f} \), even though the role of \( I_{f} \) in cardiac pacemaking has been challenged.\(^9\) Careful analysis of our AP recordings reveal that the maximum diastolic potential in mouse SAN myocytes is approximately \(-60\) mV for both genotypes. Because our \( I_{f} \) I-V curves revealed that the \( I_{f} \) density was not statistically different between PI3Kγ\(^{+/+}\) and PI3Kγ\(^{-/-}\) SAN myocytes at potentials above \(-80\) mV, it could be concluded that \( I_{f} \) does not contribute to the rate differences between these genotypes. However, our \( I_{f} \) activation curve measurements reveal that voltages of approximately \(-60\) mV are sufficiently positive to activate \( I_{f} \) in both genotypes, consistent with previous studies showing that \( I_{f} \) contributes to AP firing in mouse SAN.\(^{18,38}\) More importantly, because \( V_{1/2} \) is significantly shifted to more positive potentials and because the maximal \( I_{f} \) densities are increased, the degree of \( I_{f} \) activation is greater in PI3Kγ\(^{-/-}\) SAN myocytes at these diastolic voltages, suggesting that \( I_{f} \) differences can contribute to higher firing rates in PI3Kγ\(^{-/-}\) SAN myocytes. It is also conceivable that differences in \( I_{f} \) channel gating attributable to hysteresis, a property demonstrated in HCN channels,\(^{39,40}\) which was not assessed in our studies, could increase the impact of \( I_{f} \) on SAN firing rates in the 2 genotypes.

Our results show that Rp-cAMP increased \( I_{f} \) in PI3Kγ\(^{-/-}\) SAN myocytes, but slightly increased \( I_{f} \) in wild-type myocytes. Moreover, \( I_{f} \) remained elevated in PI3Kγ\(^{-/-}\) SAN myocytes after Rp-cAMP treatment. These complex results can be understood by recognizing that Rp-cAMP can affect \( I_{f} \) in 2 ways. First, like cAMP and other cAMP analogs, Rp-cAMP can affect \( I_{f} \) channel gating attributable to hysteresis, a property demonstrated in HCN channels,\(^{41,42}\) consistent with the presence of several consensus PKA phosphorylation sites in HCN4 channels, the major HCN homolog in mouse SAN.\(^{38,43}\) Thus, in PI3Kγ\(^{-/-}\) SAN myocytes Rp-cAMP addition is expected to enhance \( I_{f} \) by direct binding to the channels. By contrast, in PI3Kγ\(^{+/+}\) myocytes Rp-cAMP treatment is expected to inhibit PKA, thereby reducing \( I_{f} \) while simultaneously adding to the elevated cAMP pool, which results in elevations of \( I_{f} \) above those seen in wild-type SAN. Consistent with this interpretation, PKA inhibition with PKI14–22 had no affect on \( I_{f} \) in PI3Kγ\(^{+/+}\) SAN myocytes but reduced \( I_{f} \) in PI3Kγ\(^{-/-}\) SAN to levels still exceeding wild-type mice, because of persistent cAMP elevations in PI3Kγ\(^{-/-}\) myocytes. These data suggest that elevated cAMP in PI3Kγ\(^{-/-}\) mice increases \( I_{f} \) both by...
pig phosphodiesterases and A kinase anchoring proteins.45–47 The basis for these differences between myocytes from myocytes but not in working atrial or ventricular myocytes. Therefore, if PI3K dependent modulation of CaV1.3 versus CaV1.2 channels does not observed. These results suggest that selective cAMP-dependent modulation of CaV1.3 versus CaV1.2 channels.

Increased ICaL in PI3Kγ−/− SAN myocytes is clearly distinct from the absence of differences in ICaL in PI3Kγ−/− ventricular myocytes.7 In the current study, we also observed no differences in ICaL density or activation kinetics between wild-type and PI3Kγ−/− right atrial myocytes. Importantly, CaV1.3 channels are functional in working mouse atrial myocytes,23 as well as SAN myocytes.21,22 Therefore, if PI3Kγ were selectively regulating CaV1.3 channels, alterations in ICaL activation kinetics would be expected for the working right atrial myocytes in PI3Kγ−/− mice, which was not observed. These results suggest that selective cAMP-dependent modulation of CaV1.3 versus CaV1.2 channels does not underlie the elevated ICaL in PI3Kγ−/− SAN myocytes. Rather, our data support the conclusion that PI3Kγ is critical for the baseline suppression of cAMP levels in intracellular microdomains containing L-type Ca2+ channels in SAN myocytes but not in working atrial or ventricular myocytes. The basis for these differences between myocytes from different regions is unclear, but tight spatiotemporal regulation of cAMP in microdomains of cells appears to involve macromolecular complexes containing many players, including phosphodiesterases and A kinase anchoring proteins.45–47 It is possible that PI3Kγ is differentially integrated into such macromolecular complexes in a regional-dependent manner in the heart.

Significance
SAN dysfunction is a major burden that progressively increases with age and in disease states. For example, bradycardia associated with sick sinus syndrome account for a large proportion of sudden deaths during heart failure.28 The cause of SAN dysfunction in heart failure is unclear, but heart disease is associated with impaired β-adrenergic/cAMP/PKA signaling, as well as altered function of L-type Ca2+ channels in the SAN.10,48 In the present study, we show that PI3Kγ profoundly modulates SAN function and heart rate and that this modulation is cAMP dependent. Previous studies have shown that PI3Kγ expression is strongly increased in disease39; therefore, it is possible that PI3Kγ may contribute to the onset and maintenance of bradyarrhythmias in heart disease, as well as to SAN dysfunction more generally.

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Disclosures
None.

References


