Cardiac myocyte contractility is initiated by cell membrane depolarization elicited by action potentials, resulting in a small Ca\(^{2+}\) influx through the voltage-dependent L-type Ca\(^{2+}\) channel (LTCC). To study the effect of \(\alpha_q\) on the cardiac LTCC, we utilized two transgenic mouse lines that selectively express inducible \(\alpha_q\)-estrogen receptor hormone-binding domain fusion proteins (\(\alpha_q\)Q209L-hbER or \(\alpha_q\)Q209L-AAA-hbER) in cardiac myocytes. Both of these proteins inhibit phosphatidylinositol (PI) 3-kinase (PI3K) signaling, but \(\alpha_q\)Q209L-AAA-hbER cannot activate the canonical \(\alpha_q\) effector phospholipase C\(\beta\) (PLC\(\beta\)). L-type Ca\(^{2+}\) current \((I_{\text{Ca,L}})\) density measured by whole-cell patch clamping was reduced by more than 50% in myocytes from both \(\alpha_q\) animals as compared with wild-type cells, suggesting that inhibition of the LTCC by \(\alpha_q\) does not require PLC\(\beta\). To investigate the role of PI3K in this inhibitory effect, \(I_{\text{Ca,L}}\) was measured in the presence of various phosphoinositides infused through the patch pipette. Infusion of PI 3,4,5-trisphosphate (PI(3,4,5)P\(_3\)) into wild-type myocytes did not affect \(I_{\text{Ca,L}}\), but it fully restored \(I_{\text{Ca,L}}\) density in both \(\alpha_q\) transgenic myocytes to wild-type levels. By contrast, PI 4,5-bisphosphate (PI(4,5)P\(_2\)) or PI 3,5-bisphosphate had no effect. Infusion with \(p110^\alpha\)PI3K variants produced by alternative splicing). The class IB catalytic subunit is thought to be activated by both G protein-coupled and tyrosine kinases, and \(p110^\gamma\) is activated in response to stimulation of receptor tyrosine kinases, and \(p110^\gamma\) is activated in response to stimulation of G protein-coupled receptors. \(p110^\gamma\) is thought to be activated by both G protein-coupled and tyrosine kinase receptors (7).

Stimulation of G protein-coupled receptors leads to activation of the heterotrimeric G proteins that consist of \(\alpha\), \(\beta\), and \(\gamma\) subunits. \(\alpha\) and \(\beta\gamma\) then signal independently to downstream effectors. While the \(p110^\gamma\) and \(p110^\beta\) PI3Ks are activated by G\(\beta\gamma\) subunits (7, 8), the role of \(\alpha\) subunits in regulating PI3K is less clear. Recently, we reported that \(\alpha_q\) coprecipitates with and inhibits the lipid kinase activity of the \(p110^\alpha/p85^\alpha\) PI3K complex (9). Using purified recombinant proteins, we demonstrated that \(\alpha_q\) binds directly to the enzyme to inhibit its activity. We also found that the \(\alpha_q\)Q209L mutant, which signals constitutively to its effectors, inhibits \(p110^\alpha\) but not \(p110^\beta\) (9). Furthermore, we found that \(\alpha_q\) can inhibit PI3K without activating its canonical effector phospholipase C\(\beta\) (PLC\(\beta\)), as shown by the use of a mutant (\(\alpha_q\)Q209L-AAA) that cannot activate PLC\(\beta\) (9–11).

Using transgenic mice that selectively express an inducible \(\alpha_q\)Q209L protein in cardiac myocytes, we have demonstrated that activation of \(\alpha_q\) leads to inhibition of the cardiac \(I_{\text{Ca,L}}\). A second line of transgenic mice expressing an inducible \(\alpha_q\)Q209L-AAA protein showed a similar inhibition of the \(I_{\text{Ca,L}}\), suggesting a role for PI3K but not PLC\(\beta\) in this response. In this study, we used whole-cell patch clamping to further investigate the role of PI3K in mediating \(\alpha_q\) inhibition of the \(I_{\text{Ca,L}}\) in myocytes isolated from these transgenic animals. We found that PIP\(_3\) and some PI3K isoforms can reverse the inhibitory effect of \(\alpha_q\) on the cardiac LTCC.

Further details and methods are provided in the full text of the manuscript.
**Experimental Procedures**

**Materials**—Tamoxifen and 4-hydroxytamoxifen (4-HT) were from Sigma. Recombinant p110β/p85α, p110γ, and PTEN were from Upstate Biotechnology, Inc. (Lake Placid, NY). PI(4,5)P2, di-C8, PI(3,5)P2 di-C8, PI(3,4,5)P3 di-C8 and glutathione S-transferase fused to the pleckstrin homology domain of Grp1 (PH-Grp1) were from Echelon Biosciences, Inc. (Salt Lake City, UT). Akt1/2 antibody was from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Phospho-Erk1/2 antibody was from Cell Signaling Technology, Inc. (Beverly, MA). Recombinant p110α/p85α purified from baculovirus-infected Sf9 cells was described previously (9).

**GαqQ209L-hbER Transgenic Mice**—C57BL/6 transgenic mice expressing either GαqQ209L-hbER or GαqQ209L-AA-hbER in cardiac myocytes under the control of the α myosin heavy chain promoter were described previously. Starting at 8 weeks of age, GαqQ209L-hbER animals were injected intraperitoneally with 1 mg of tamoxifen daily for 14 days, and GαqQ209L-AA-hbER mice were injected for 28 days. Matching wild-type (WT) littermates that were injected with tamoxifen for an equivalent number of days serve as controls; the WT data shown are matched control. The number of cells examined in each group is indicated in parentheses.

**Ventricular Myocyte Isolation**—Myocytes were isolated from tamoxifen-treated WT, GαqQ209L-hbER (QL), and GαqQ209L-AA-hbER (QL-AA) mice, and average peak I_{Ca,L} densities were measured by whole-cell patch clamping. The membrane was held at −50 mV and depolarized for 300 ms to +10 mV. Internal solution with or without phosphoinositides (1 μM) was infused through the patch pipette. ** signifies a statistically significant increase in peak I_{Ca,L} density induced by PI(3,4,5)P3 as compared with the matched control. The number of cells examined in each group is indicated in parentheses.

This commonly used approach assumes a specific capacitance of 1 μF/cm², which could vary slightly depending on physiologic conditions. This variation is expected to be extremely small and well below the detection limit of this technique.

**Phosphoinositides, PI3K Isozymes, PH-Grp1, and PTEN** were diluted 100–500-fold in the internal solution and infused through the patch pipette. PTEN buffer contained 50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 81 mM imidazole, 1 mM EDTA, 5 mM glutathione, 2 mM dithiothreitol, 267 mM sucrose, and 10% glycerol.

**Akt Kinase Assays and Western Blots**—Frozen mouse organs were homogenized with a PRO250 (Pro Scientific, Inc., Monroe, CT) in ice-cold lysis buffer (50 mM HEPES, 1% Triton X-100, 50 mM NaCl, 5 mM EDTA, 50 mM NaF, 10 mM sodium pyrophosphate, 1 mM sodium orthovanadate, 0.5 mM phenylmethylsulfonyl fluoride, and 10 μg/ml each of aprotinin and leupeptin, pH 7.5). Homogenates were centrifuged at 15,000 × g for 30 min at 4 °C. Protein concentrations of the supernatants were determined using the Bradford assay (Bio-Rad). Akt activity was assayed in immunoprecipitates starting with 0.5 mg of tissue lysate protein following a method described earlier (12). Western blot signals were visualized and quantitated using the Odyssey Infrared Imaging System with version 1.2 software (LI-COR Biosciences, Lincoln, NE). IRDye800-conjugated second antibodies were from Rockland Immunochemicals (Gilbertsville, PA).

**Data Analysis**—Values are means ± S.E., and Student’s t tests were performed to estimate the significance of the differences between mean values. A value of p < 0.05 was considered significant.

**Results**

**Effects of PI(3,4,5)P3 on I_{Ca,L} in Cardiac Myocytes**—In this study we employed myocytes isolated from two transgenic mouse lines that selectively express silent Gαq proteins in the heart. These proteins, GαqQ209L-hbER and GαqQ209L-AA-hbER, are inactive until they bind to 4-HT. Both of them can inhibit PI3K signaling, but the latter cannot activate PLCβ. Activation of these Gαq proteins in response to injection with tamoxifen, which is converted to 4-HT in animals, causes a large reduction in I_{Ca,L} density in cardiac myocytes from both transgenic animals. This result suggests that inhibition of the cardiac LTCC by Gαq occurs independently of PLCβ and may be due to reduced PI3K signaling. This idea is supported by studies showing that PI3Ks stimulate I_{Ca,L} in isolated portal vein myocytes (2, 3).
As a first test of this hypothesis, we asked if PI(3,4,5)P3, the lipid product of PI3K, reverses the depressed I\textsubscript{Ca,L} in G\textsubscript{qQ209L}-hbER and G\textsubscript{qQ209L-AA}-hbER cells. Myocytes were isolated from tamoxifen-treated WT and transgenic mice, and internal solution with or without 1 μM PI(3,4,5)P3 was infused into the cells through the patch pipette. The peak I\textsubscript{Ca,L} density was measured by whole-cell patch clamping at +10 mV following a single depolarizing step of 300 ms duration from a holding potential of −50 mV. PI(3,4,5)P3 did not have a significant effect on I\textsubscript{Ca,L} density in WT myocytes (Fig. 1). However, PI(3,4,5)P3 significantly increased the I\textsubscript{Ca,L} density by 2.4-fold in G\textsubscript{qQ209L}-hbER myocytes and 2.2-fold in the G\textsubscript{qQ209L-AA}-hbER cells (Fig. 1). The values for PI(3,4,5)P3-treated transgenic myocytes were statistically indistinguishable from those observed in WT myocytes treated with or without PI(3,4,5)P3 (Fig. 1). We performed additional control experiments in G\textsubscript{qQ209L-AA}-hbER myocytes using other phosphoinositides. Infusion with PI(3,5)P2, which is generated from PI 3-phosphate by a PI 5-kinase, had no effect (Fig. 1). Similarly, PI(4,5)P2, which is converted to PI(3,4,5)P3 by PI3K, did not alter the peak I\textsubscript{Ca,L} density (Fig. 1). These results are consistent with the hypothesis that decreased I\textsubscript{Ca,L} density in the transgenic myocytes is due to G\textsubscript{qQ209L}-dependent inhibition of PI3K.

We also constructed current density-voltage (I-V) relationships for WT and transgenic myocytes infused with or without PI(3,4,5)P3. Activation of I\textsubscript{Ca,L} was elicited by depolarizing voltage pulses from −50 mV to +50 mV in 10 mV increments (300 ms duration) from a holding potential of −50 mV. Fig. 2A shows typical recordings of I\textsubscript{Ca,L} activation from WT cells in the absence (top panel) or presence (middle panel) of PI(3,4,5)P3. The peak I-V curves for both conditions are plotted in the bottom panel (Fig. 2A). There was no significant difference in I\textsubscript{Ca,L} density between the two conditions at any of the voltages tested. In contrast, infusion with PI(3,4,5)P3 resulted in a large enhancement of I\textsubscript{Ca,L} activation in both the G\textsubscript{qQ209L}-hbER (Fig. 2B) and G\textsubscript{qQ209L-AA}-hbER (Fig. 2C) myocytes. The I-V relationships (bottom panels of Fig. 2, B and C) show that I\textsubscript{Ca,L} density was increased at nearly all of the voltages tested in both groups of myocytes.

To further characterize the action of PI(3,4,5)P3 in transgenic myocytes, we investigated its time-dependent effect on I\textsubscript{Ca,L} activation in single cells. In this protocol, the myocyte was infused with or without PI(3,4,5)P3 and repeatedly depolarized with voltage steps to +10 mV from a holding potential of −50 mV (300 ms duration). The I\textsubscript{Ca,L} densities were normalized to the value obtained from the first voltage step.
**Goq Inhibition of the L-type Ca2+ Channel**

**FIGURE 3. Time-dependent effect of PI(3,4,5)P3 on Ica,L activation.** Myocytes were isolated from tamoxifen-treated GoqQ209L-hbER (QL) and GoqQ209L-AA-hbER (QL-AA) mice. Cells were patched and infused with internal solution without (Con) or with 1 μM PI(3,4,5)P3. Peak Ica,L densities were measured following repeated (every 10 s) voltage steps from −50 mV to +10 mV (300 ms duration). Values shown are average Ica,L densities normalized to the value of the first Ica,L recording, which is taken immediately after breaking into the whole-cell mode. Left panel, n = 7; middle and right panels, n = 5 for both groups.

(Insert figure here)

following opening of the patch and initiation of whole-cell recording. As shown in the left panel of Fig. 3, the typical "run-down" of Ica,L density was observed in GoqQ209L-hbER myocytes when the patch pipette contained the control internal solution without PI(3,4,5)P3. Within 180 s, the normalized Ica,L density in these cells decreased by about 20%. In contrast, in the presence of PI(3,4,5)P3, we observed a "run-up" of Ica,L density in both the GoqQ209L-hbER (Fig. 3, middle panel) and GoqQ209L-AA-hbER (Fig. 3, right panel) myocytes. Ica,L density started to decrease after reaching a maximum after ~120 s and the rate of decline was similar to that seen in cells infused with the control solution. Interestingly, even the first voltage step elicited a significantly larger Ica,L in cells infused with PI(3,4,5)P3 as compared with the control internal solution.

**Modulation of Ica,L by PI3K Isozymes in GoqQ209L-AA-hbER Myocytes**—We next asked if infusion of purified PI3K proteins into GoqQ209L-AA-hbER myocytes has the same effect as PI(3,4,5)P3 in increasing Ica,L density. Multiple isoforms of PI3K have been identified in the adult heart of different species (6), and we tested three of them: p110α/p85α, p110β/p85α, and p110γ. The effects of these PI3K isoforms on the peak Ica,L density at +10 mV are shown in Fig. 4. In the absence of PI(4,5)P2, none of the three PI3K isoforms had an effect on Ica,L density. However, in the presence of PI(4,5)P2, both p110β/p85α and p110γ induced a significant increase in Ica,L density (2.6- and 2.2-fold, respectively). These increased Ica,L density values were similar to those observed in WT myocytes (see Fig. 1). Interestingly, infusion with p110α/p85α plus PI(4,5)P2 had no effect on Ica,L density (Fig. 4). These results suggest that Ica,L in the GoqQ transgenic myocytes is modulated by specific PI3K isoforms.

Fig. 5 shows typical traces of Ica,L activation in the presence of PI3K isoforms without (A) or with (B) PI(4,5)P2, that were used to construct I-V relationships (Fig. 5, C and D). The I-V curves show that infusion of the PI3K isoforms alone did not change Ica,L density across the entire voltage range examined as compared with the control cells infused with internal solution only (Fig. 5C). In the presence of PI(4,5)P2, both p110β/p85α and p110γ stimulated Ica,L at nearly all the voltages tested (Fig. 5D). The I-V curve for myocytes infused with p110α/p85α plus PI(4,5)P2 is nearly identical to the curve obtained from cells infused with PI(4,5)P2 alone (Fig. 5D).

We also examined the time-dependent effect of PI3K isoforms plus PI(4,5)P2 on Ica,L in GoqQ209L-AA-hbER myocytes subjected to repeated depolarizing voltage steps. Infusion of p110α/p85α plus PI(4,5)P2 did not prevent the typical run-down (Fig. 6, left panel). In contrast, a run-up of Ica,L followed by a slow decrease was observed when either p110β/p85α or p110γ plus PI(4,5)P2 were infused into the cells (Fig. 6, middle and right panels).

**Reduction of Ica,L by Decreasing Endogenous PI(3,4,5)P3 in WT Myocytes**—Since the reduction of Ica,L density in GoqQ transgenic myocytes can be reversed by infusion exogenous PI(3,4,5)P3, or some PI3Ks, we hypothesized that depletion of endogenous PI(3,4,5)P3 in WT myocytes would lower Ica,L density. Two approaches were used to test this hypothesis. In the first approach, we infused myocytes isolated from WT mice (not treated with tamoxifen) with purified PH-Grp1 protein to sequester intracellular PI(3,4,5)P3. PH-Grp1 has been shown to bind specifically to PI(3,4,5)P3 (13) and has been used to block PI3K signaling (4). In the presence of 20 nM PH-Grp1, the peak Ica,L density measured after a 300 ms pulse at +10 mV from a holding potential of −50 mV was 6.6 ± 0.6 pA/pF (n = 7) as compared with 9.6 ± 0.4 pA/pF (n = 7) for cells infused with the control solution. This 31% decrease is statistically significant (t test, p < 0.01). Shown in Fig. 7A (top two panels) are representative current traces that were used to construct the I-V relationships. The I-V curves show that PH-Grp1 reduced Ica,L density at nearly all of the voltages tested (Fig. 7A, bottom panel).
In the second approach, we infused myocytes from untreated WT mice with the lipid phosphatase PTEN, which specifically dephosphorylates the D3 position on the inositol ring of PI(3,4,5)P3 to form PI(4,5)P2. When 20 nM purified PTEN protein was infused through the patch pipette, the peak $I_{Ca,L}$ density at $-100$ mV measured after a 300 ms pulse from a holding potential of $+50$ mV was reduced by 28% to $6.5 \pm 0.4$ pA/pF ($n = 20$) versus $9.0 \pm 0.6$ pA/pF ($n = 5$) for control cells infused with an equivalent volume of PTEN buffer diluted into internal solution. The difference between the two conditions is statistically significant ($t$ test, $p < 0.01$). The top two panels in Fig. 7B show sample current traces that were used to construct the $I$-$V$ relationships for these two conditions (Fig. 7B, bottom panel). The $I_{Ca,L}$ densities were reduced at nearly all of the voltages tested when PTEN was infused into the cells as compared with the control solution. Taken together, these results

**FIGURE 5.** $I$-$V$ relationships for myocytes infused with PI3K isozymes. Myocytes were isolated from tamoxifen-treated $G_{\alpha_q}$Q209L-AA-hbER mice, and $I_{Ca,L}$ was elicited using the protocol described in the legend of Fig. 2. A and B, representative recordings of $I_{Ca,L}$ activation in cells infused with 20 nM of PI3K in the absence (A) or presence (B) of 1 $\mu$M PI(4,5)P2. Mean $I$-$V$ relationships in the absence (C) or presence (D) of 1 $\mu$M PI(4,5)P2. Con, $n = 5$; p110$\alpha$/p85$\alpha$, $n = 5$; p110$\beta$/p85$\alpha$, $n = 4$; and p110$\gamma$, $n = 4$.

**FIGURE 6.** Time-dependent effect of PI3K isozymes plus PI(4,5)P2 on $I_{Ca,L}$ activation. Myocytes were isolated from tamoxifen-treated $G_{\alpha_q}$Q209L-AA-hbER mice, and $I_{Ca,L}$ densities were measured following repeated depolarization steps and normalized as described in the legend of Fig. 3. Cells were infused with 20 nM PI3K isozymes plus 1 $\mu$M PI(4,5)P2 ($n = 4$ for all three conditions).
**DISCUSSION**

Our initial study showing that $I_{\text{Ca,L}}$ in $\alpha_q\text{Q209L-hbER}$ and $\alpha_q\text{Q209L-aa-hbER}$ transgenic myocytes is similarly depressed as compared with WT myocytes suggested that PLC$\beta$ was not involved in this response. The ability of both of these fusion proteins to inhibit P13K signaling led us to examine this pathway as a possible mediator of $\alpha_q$ inhibition of the LTCC. In this study, we demonstrate that infusion of exogenous P(3,4,5)$\text{P}_3$ into $\alpha_q\text{Q209L-hbER}$ and $\alpha_q\text{Q209L-aa-hbER}$ myocytes completely reverses the inhibition of $I_{\text{Ca,L}}$. $I_{\text{Ca,L}}$ is also fully restored in $\alpha_q\text{Q209L-aa-hbER}$ myocytes infused with certain P13K isozymes in the presence of their phospholipid substrate. Together, these results support the idea that $\alpha_q$ inhibits certain P13Ks to cause a reduction in $I_{\text{Ca,L}}$. Furthermore, since reduction of endogenous P(3,4,5)$\text{P}_3$ levels in WT myocytes depresses $I_{\text{Ca,L}}$ density, it appears that constitutive P13K signaling is required for normal LTCC function.

It is well established that activation of $\alpha_q$ and subsequent activation of cAMP-dependent protein kinase stimulates cardiac LTCC function. On the other hand, activation of pertussis toxin-insensitive G$\alpha$ proteins such as $\alpha_q\epsilon_{11}$ inhibits neuronal L-type (14) and N- and P/Q-type (15) Ca$^{2+}$ channels. G$\beta\gamma$ subunits released from pertussis toxin-sensitive G proteins also inhibit N- and P/Q-type channels through direct protein-protein interactions, but they do not bind to or inhibit the LTCC (15). It is not known how activation of $\alpha_q$ leads to inhibition of neuronal Ca$^{2+}$ channels, but our results here using myocytes suggest that it could be due to inhibition of P13K. Interestingly, a recent study indicates that inhibition of the neuronal LTCC by the M1 muscarinic receptor is mediated by $\alpha_q\epsilon_{11}$ but does not appear to involve PLC$\beta$ (16).

Our studies consistently support the concept that $\alpha_q$ can inhibit...
PI3K independently of PLCβ activation. We initially showed that Goq_q11-coupled α1A adrenergic receptors inhibit growth factor and insulin activation of PI3K (12, 17). Subsequently, we demonstrated that activated Goq directly binds to and inhibits the p110α/p85α PI3K3 (9). By contrast, transfected Goq_q209Δi-hbER did not inhibit p110β immunoprecipitated from cotransfected cells (9). We have also reported that activated Goq does not bind to p110γ, and studies are ongoing in our laboratory to determine whether GTP-bound Goq interacts with other isoforms of PI3K. Results in this study show that p110β/p85α or p110γ, but not p110α/p85α, reversed Goq inhibition of ICa,L (Figs. 4 and 5). One explanation for this result is that activated Goq inhibits only p110α, and the transgenic myocytes express enough activated Goq proteins to neutralize the infused p110α/p85α.

Activation of PI3K potentiates ICa,L in rat portal vein myocytes and rat cerebellar granule neurons (1, 2). In contrast, we found that exogenous PI(3,4,5)P3 had no effect on ICa,L activation in WT mouse cardiac myocytes (Figs. 1 and 2). We have also found that infusion of canine cardiac myocytes with PI(3,4,5)P3 and are maximally active at the level found in these cells. Alternatively, PI3K signaling might be maximally activated in cardiac myocytes, so addition of exogenous lipid second messenger would not have an effect. This possibility seems remote because basal Akt activity in the mouse heart is very low but can be strongly increased by insulin treatment (Fig. 8). We have also measured PI3K activity in p110α, p110β, or p110γ immunoprecipitates from freshly prepared heart lysates and found the activities to be very low (data not shown). We believe that LTCC function or localization at the plasma membrane is near maximal in cardiac myocytes, despite the low PI3K activity. Therefore, increasing PI3K signaling does not further stimulate ICa,L, but inhibition of this signaling pathway by activated Goq_q PH-Grp1, or PTEN can lead to a reduction in ICa,L.

Activation of Goq-coupled receptors might have complex effects on the modulation of the LTCC. G protein-coupled receptors can activate more than one type of Go subunit, and these could modulate LTCC function in diverse ways. Furthermore, some of the released βγ subunits could activate p110γ or p110β. In addition, p110α can complex with different p85 isofoms, and these heterodimers may be differentially affected by Goqq. Interestingly, Macrez and co-workers (18) found that angiotensin II stimulation of the portal vein myocyte LTCC is mediated by Gβγ activation of p110γ. The Gβγ dimer in question appears to be released from Goq11 rather than Goq_q (19). Not all βγ dimers are equivalently in activating PI3K. Gβγ dimers containing β5 are least able to activate p110γ and p110β, while those containing γγ5 are least able to activate p110γ (8, 20). We predict that stimulation of a Goq-coupled receptor will negatively modulate the LTCC if (a) the activated Goq inhibits p110α and (b) its released Gβγ dimer is a weak activator of p110β and p110γ. More studies are needed to determine how cells integrate these competing signals to mount an appropriate Ca2+ channel response.

LTCCs are composed of α1, β, and δ/ε α subunits and, in some forms, an additional γ subunit. There are also multiple isoforms of each subunit. The α1 subunit forms the pore of the channel and the intracellular β subunit regulates cellular localization. Cardiac LTCCs are mostly composed of α1C and β2a isoforms, although other β variants are also present in the heart (21). In a heterologous expression system, PI3K signaling stimulates ICa,L through Akt-mediated phosphorylation of β2a subunits, leading to increased trafficking of the LTCC to the plasma membrane (4). Akt specifically phosphorylates the β2a subunit on a consensus sequence that is not present in β1b, β3, or β4 (4). Inhibition of Akt by Goq11 may reduce trafficking of the cardiac LTCC to the plasma membrane. Additional studies are planned to determine whether infusion of purified activated Akt proteins reverses the inhibition of ICa,L in our transgenic myocytes and if these cells show a decreased amount of LTCC proteins in the plasma membrane. Finally, it would be of interest to explore whether G protein-coupled receptors, Goq_q, and p110α PI3K form a macromolecular signaling complex with the LTCC at the myocyte plasma membrane so that the current through these channels can be specifically regulated.

In conclusion, results from this study indicate that negative modulation of the LTCC by activated Goq11 in cardiac myocytes is mediated by inhibition of PI3K, perhaps specifically by the p110α isoform. Further studies are needed to determine whether L-type and other types of Ca2+ channels present in other excitable cell types are also inhibited by Goq_q through a similar mechanism.

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FIGURE 8. Akt activity in hearts from WT and Goq_q209Δi-hbER mice. Mice were injected with 1 mg of tamoxifen daily for 28 days, fasted overnight, and then injected intraperitoneally with 10 units of insulin/kg of body weight or saline as control. Animals were sacrificed 10 min later and the hearts removed. A, Akt activity was measured in heart lysates after immunoprecipitation with an Akt antibody. The number of mice used per group is shown in parentheses. ** indicates a significant difference between Goq_q209Δi-hbER (QL-AA) + insulin and WT + insulin (t test, p < 0.001). B, Western blot analysis of heart lysate proteins prepared from mice that were injected with insulin. The blot was probed with an Akt antibody (top panel). Signals were quantitated using the Odyssey Infrared Imaging System. WT, 3.86 ± 0.23 arbitrary units; QL-AA, 4.07 ± 0.35 arbitrary units. The difference is not statistically significant (t test). The blot was stripped and reprobed with a phospho-specific Erk1/2 antibody (bottom panel).
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