G_o2 G protein mediates galanin inhibitory effects on insulin release from pancreatic β cells

Guanghua Tang[a,1], Ying Wang[a], Sangeun Park[a], Neil S. Bajpayee[a], Diana Vi[a], Yoshiko Nagaoka[a], Lutz Birnbaumer[b,2], and Meisheng Jiang[a,2]

[a]Department of Molecular and Medical Pharmacology, David Geffen School of Medicine, University of California, Los Angeles, CA 90095; and [b]Laboratory of Neurobiology, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709

Contributed by Lutz Birnbaumer, January 5, 2012 (sent for review December 27, 2011)

The neuropeptide galanin regulates numerous physiological activities in the body, including feeding and metabolism, learning and memory, nociception and spinal reflexes, and anxiety and related behaviors. Modulation of blood glucose levels by suppressing insulin release was the first reported activity for galanin. This inhibition was mediated by one or more pertussis toxin-sensitive G proteins of the $G_{i/o}$ subfamily. However, the molecular identities of the specific G protein(s) and intracellular effectors have not been fully revealed. Recently, we demonstrated that mice lacking G_o2, but not other members of the $G_{i/o}$ protein family, secrete more insulin than controls upon glucose challenge, indicating that G_o2 is a major transducer for the inhibitory regulation of insulin secretion. In this study, we investigated galanin signaling mechanisms in β cells using cell biological and electrophysiological approaches. We found that islets lacking G_o2, but not other $G_{i/o}$ proteins, lose the inhibitory effect of galanin on insulin release. Potentiation of ATP-sensitive potassium (K_{ATP}) and inhibition of calcium currents by galanin were disrupted by anti-$G_o2\alpha$ antibodies. Galanin actions on K_{ATP} and calcium currents were completely lost in $G_o2^{-/-}$ β cells. Furthermore, the hyperglycemic effect of galanin is also blunted in $G_o2^{-/-}$ mice. Our results demonstrate that G_o2 mediates the inhibition of insulin release by galanin by regulating both K_{ATP} and Ca^{2+} channels in mice. Our findings provide insight into galanin's action in glucose homeostasis. The results may also be relevant to the understanding of galanin signaling in other biological systems, especially the central nervous system.

glycemia | GTP-binding protein-coupled receptor | heterotrimeric | signal transduction | Alzheimer's disease

Galanin, a 29- to 30-residue neuropeptide hormone initially discovered in porcine intestine (1), is distributed throughout the central and peripheral nervous systems and the intestinal neuroendocrine system of many mammalian species (2, 3). The first 15 N-terminal amino acids, which retain the biological activity of the full-length peptide hormone, are highly conserved, underscoring a physiological importance across species. Galanin coexpresses and colocalizes with many neurotransmitters (4) and functions as an inhibitory modulator.

The biological effects of galanin are mediated by galanin receptors. Three types of galanin receptors (GalR1, GalR2, and GalR3) have been identified by molecular cloning and characterized pharmacologically in various species (5–8). All three subtypes of galanin receptors are members of the GTP-binding protein-coupled receptor (GPCR) superfamily. The three galanin receptors exhibit overlapping but distinctive patterns of expression in the central nervous system and periphery. The distinct distribution patterns of receptors support the notion that each receptor mediates some unique physiological function in the body.

Physiological Effects of Galanin. The biological activity of galanin has been studied intensively in the central and peripheral nervous systems, as well as in the pituitary and the endocrine system. Galanin signaling is involved in feeding and metabolism, learning and memory, nociception and spinal reflexes, anxiety, neuron regeneration, and the pathogenesis of Alzheimer's disease (9–12). The precise role of galanin signaling in these states has not been elucidated. The first reported biological activity of galanin was its effect on plasma glucose levels in dogs and rats (1). The pancreas is highly innervated, and galanin localizes to autonomic nerve terminals in the endocrine pancreas (3) as well as to nerve cell bodies in the celiac ganglion (13). Infusion of galanin into animals results in a significant increase of blood glucose levels by inhibition of insulin secretion from pancreatic β cells (1, 14). Infusion of galanin directly into animals through the pancreatic artery at a concentration that is similar to that released from stimulated pancreatic nerve termini is sufficient to inhibit insulin secretion (15). Conversely, a galanin antagonist can block galanin-mediated inhibition of insulin release from islets (16). In galanin-deficient animals, the inhibition of insulin secretion induced by the chemical activation of sympathetic nerves was observed to be impaired (17). Interestingly, genetically obese *ob/ob* mice have severely decreased pancreatic content of galanin of less than 10% of levels found in control animals (18). In addition, the number of galanin immunoreactive cells is dramatically reduced in diabetic animals (19). Reduced islet innervation has been associated with impaired insulin secretion in type II diabetic hamsters (20). These results suggest that pancreatic galaninergic nerve dysfunction may contribute to the development of type II diabetes, which is an increasing worldwide public health problem.

Galanin-Receptor Signal Transduction. The signaling mechanisms of GalR1 and GalR3, which are coupled to their effectors by pertussis toxin (PTX)-sensitive $G_{i/o}$ G proteins, have been pharmacologically studied. Stimulation of receptors expressed in transfected cell lines or oocytes can inhibit forskolin-stimulated cAMP production, or activate G protein-regulated inwardly rectifying K^+ channels in a PTX-sensitive manner (5, 7, 21, 22). Multiple subclasses of G proteins may be involved in GalR2 signaling (6, 22–24). GalR2 can activate phospholipase C and protein kinase C via $G_{q/11}$ and activate MAPK and/or inhibit forskolin-stimulated cAMP production through PTX-sensitive $G_{i/o}$ G proteins. Like the galanin receptors, all five nonsensory PTX-sensitive $G_{i/o}$ members are expressed in neurons and endocrine tissues. The signal transduction mechanisms mediating galanin's physiological effects have not been studied in native tissue or primary cells. Our previous studies demonstrated that all nonsensory members of the PTX-sensitive G_i/G_o protein family, namely G_i1, G_i2, G_i3, G_o1, and G_o2, are expressed in pancreatic

Author contributions: G.T., Y.W., and M.J. designed research; G.T., Y.W., S.P., N.S.B., D.V., Y.N., and M.J. performed research; G.T., Y.W., S.P., N.S.B., D.V., Y.N., and M.J. analyzed data; and L.B. and M.J. wrote the paper.

The authors declare no conflict of interest.

[1]Present address: Cardiovascular and Metabolism Research Unit, Lakehead University, Thunder Bay, Ontario P7A 7T1, Canada.

[2]To whom correspondence may be addressed. E-mail: birnbau1@niehs.nih.gov or jm@ucla.edu.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1200100109/-/DCSupplemental.

islets, and that only G_o2, and not $G_i1–3$ or G_o1, G protein-deficient mice show an "improved" glucose tolerance test and enhanced insulin release from pancreatic β cells (25). This suggests that G_o2 is the major transducer mediating inhibitory effects on insulin release that prevent oversecretion. In this study, using G_i/G_o α-subunit gene knockout animals established in our laboratory (26, 27), we demonstrate that the G_o2 G protein mediates galanin's inhibitory effect on insulin release. We also identify potentiation of ATP-sensitive potassium (K_{ATP}) currents and inhibition of Ca^{2+} channels as possible molecular mechanisms mediating galanin-GalR-G_o2 signaling.

Results and Discussion

Inhibition of Insulin Release by Galanin Is Lost in $G_o2\alpha^{-/-}$ Islets. In the present study, we addressed the possible molecular mechanisms by which galanin inhibits insulin release. Galanin has been shown to elevate blood glucose levels by inhibiting insulin release. PTX blocks the inhibitory effects of galanin, suggesting the involvement of G_i/G_o proteins in the process. PTX ADP ribosylates a cysteine at the carboxyl termini (−4 position) of the α subunits of $G_{i/o}$ G proteins and collectively blocks all G_i/G_o signaling. Therefore, PTX assays cannot delineate through which specific G protein(s) galanin signals. To investigate the mechanism and to screen the responsible G_i/G_o protein(s) for mediating the inhibition of insulin release by galanin, we established a perifusion assay of isolated islets from G_i/G_o-deficient animals aimed at identifying which G_i/G_o protein(s) mediates galanin's inhibitory effects. A pool of 30–50 islets isolated shortly after a recovery period were placed onto a nylon membrane in a perifusion chamber. After 60 min of perifusion with 1.8 mM glucose in Krebs Ringer bicarbonate (KRB) solution to establish a stable rate of insulin secretion, the glucose concentration in the buffer was elevated to 16.2 mM and islets were continually perifused for 120 min at this high-glucose concentration. The perifusion solution was then switched back to 1.8 mM (low) glucose for 30 min, followed by perifusion with KCl (30 mM) for an additional 30 min. During the high-glucose (16.2 mM) perifusion period, galanin (100 nM) was included transiently (from minute 60 to 90) in the buffer. Fig. 1 shows insulin secretion profiles of islets isolated from WT and knockout mice lacking $G_i2\alpha$, $G_i1/G_i3\alpha$, $G_o1\alpha$, and $G_o2\alpha$. High glucose (16.2 mM) induced insulin release from pancreatic islets effectively from all of the genotypes. When the lower-glucose (1.8 mM) buffer was applied, insulin release from islets declined to basal levels. KCl (30 mM), which directly depolarizes the β-cell membrane, triggered insulin release from islets. Galanin (perifused together with high glucose) suppressed insulin secretion in islets from WT, $G_i1\alpha^{-/-}$ $G_i3\alpha^{-/-}$ double KO, $G_i2\alpha^{-/-}$, and $G_o1\alpha^{-/-}$ mice. Upon removal of galanin from the buffer, insulin secretion resumed at the elevated rates. In contrast, the inhibitory effect of galanin on insulin release was absent in islets from $G_o2\alpha^{-/-}$ mice. The results indicate that G_o2 mediates the inhibitory effect of galanin on insulin release in pancreatic β cells. The levels of insulin released under basal, glucose-stimulated, and K^+-induced conditions were comparable for islets from WT, $G_i2\alpha^{-/-}$, $G_i1\alpha^{-/-}$ $G_i3\alpha^{-/-}$ double knockout, and $G_o1\alpha^{-/-}$ mice, but were significantly higher for islets from $G_o2\alpha^{-/-}$ ($P <$ 0.01). This suggests that, in addition to mediating galanin inhibition of insulin release, G_o2 modulates the sensitivity of islets to glucose and maybe directly or indirectly regulates secretory machinery.

K_{ATP} Channels in $G_o2\alpha^{-/-}$ Islets Show Normal Glucose Sensitivity. It is well-established that glucose and other nutrients promote the depolarization of the β-cell membrane and Ca^{2+} influx through voltage-dependent channels. This constitutes the principal stimulus for insulin exocytosis (28). A glucose transporter (Glut-2) in the β cell facilitates entry of glucose into the cell (29). The enzyme glucokinase phosphorylates glucose to glucose-6-phosphate.

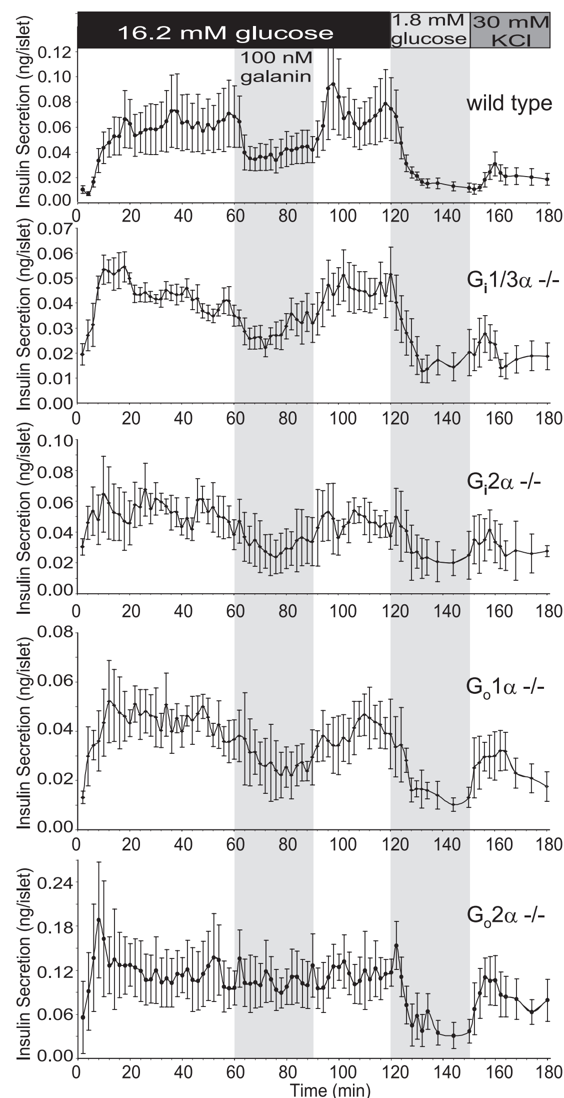

Fig. 1. Lack of $G_o2\alpha$ in the islets impairs the inhibitory effect of galanin on insulin secretion. Insulin release profiles of perifusion assays on islets from $G_o2\alpha^{-/-}$, WT, and other G_i/$G_o\alpha$ knockout mice are shown. Hand-picked islets (30–50) were packed in a perifusion chamber and perifused with low (1.8 mM) and high (16.2 mM) concentrations of glucose solutions and KCl as indicated. Galanin was applied during a 60–90 min period as indicated in the first shaded area in the charts. Insulin contents were measured by ELISA with a mouse insulin standard. Perifusion rate was 0.25 mL/min; fractions were collected every 2 min. Shown are traces of mean values ± SEM of insulin secretion from WT, $G_o2^{-/-}$, $G_o1^{-/-}$, $G_i1/3$ double knockout, and $G_i2^{-/-}$ islets' response to glucose, galanin, and KCl.

Glucokinase appears to function as the fundamental glucose sensor that controls the subsequent β-cell response (30). Glucose is oxidized, leading to a rapid increase of the concentration of intracellular ATP and a decrease of the concentration of ADP. This in turn changes the ATP:ADP ratio in the β cell. ATP is a potent inhibitor of the ATP-sensitive K^+ channel, whereas ADP is a stimulator of the channel (31, 32). Inhibition of K^+ channels by ATP decreases K^+ efflux from the β cell, thus leading to membrane depolarization (33). Depolarization opens voltage-dependent Ca^{2+} channels, causing a rapid increase in the concentration of intracellular Ca^{2+}. This triggers exocytosis of pancreatic β-cell insulin granules (34). As observed, galanin can inhibit insulin secretion from pancreatic β cells in a PTX-sensitive manner.

To investigate whether the G_o2-mediated signaling pathway regulates K_{ATP} and Ca^{2+} channels, we studied channel properties via patch-clamp recording. First, the integrity of K_{ATP} channels was investigated in β cells from $G_o2\alpha$ knockout mice. Specifically, K_{ATP} channels were studied in response to glucose (Fig. S1). Freshly isolated islets were digested briefly with trypsin to generate single β cells. Short-term cultured β cells were used in the studies. Glucose inhibited K_{ATP} currents in β cells from both wild-type and $G_o2\alpha^{-/-}$ (Fig. S1 *A–C*). High glucose (16.2 mM) inhibited K_{ATP} currents in isolated β cells from −58.0 ± 15.6 to −22 ± 6.1 pA/pF for WT ($n = 10$, $P < 0.01$) and −49.8 ± 13.3 to −27.1 ± 5.1 pA/pF for $G_o2\alpha^{-/-}$ ($n = 12$, $P < 0.05$) mice (Fig. S1*D*). Furthermore, glucose deprivation stimulated K_{ATP} currents in both WT (−26.5 ± 5.0 to −62.6 ± 13.5 pA/pF; $n = 9$, $P < 0.01$) and $G_o2\alpha^{-/-}$ mice (−22.26 ± 4.0 to −65.34 ± 14 pA/pF; $n = 6$, $P < 0.01$). This result demonstrates that K_{ATP} channels in β cells isolated from $G_o2\alpha^{-/-}$ mice respond to glucose similarly to WT controls and suggests that lack of $G_o2\alpha$ does not alter the responsiveness of K_{ATP} channels to glucose in β cells.

Galanin Fails to Potentiate K_{ATP} Channels in $G_o2\alpha^{-/-}$ Mice. We next investigated the effects of galanin on the modulation of K_{ATP} channel activity by glucose. K_{ATP} channel currents in the β cells of WT and $G_o2\alpha^{-/-}$ mice were recorded in the absence of galanin, presence of 100 nM galanin, or 50 μM diazoxide. As shown in Fig. 2, the averages of glucose-sensitive K_{ATP} currents in β cells from WT mice were −35.4 ± 6.3 and −58.0 ± 10.7 pA/pF ($n = 12$) before and after application of 100 nM galanin to the bath solution, respectively. The averages of K_{ATP} currents in $G_o2\alpha^{-/-}$ β cells were −46.5 ± 6.7 and −45.3 ± 6.9 pA/pF ($n = 11$) before and after application of 100 nM galanin to the bath solution, respectively (Fig. 2). Diazoxide, a selective K_{ATP} channel opener, was able to further potentiate the channels in both WT and $G_o2\alpha^{-/-}$ β cells in the presence of galanin. Galanin significantly enhanced ($P < 0.01$) K_{ATP} currents in β cells from WT control mice; however, this current augmentation was completely absent in the β cells from $G_o2\alpha^{-/-}$ mice. The results suggest that G_o2 protein mediates the inhibitory effect of galanin on K_{ATP} channels in mouse β cells, and that the potentiation of K_{ATP} by diazoxide is independent of the G_o2-mediated pathway. These data are consistent with the existence of a specific signaling mechanism whereby the inhibitory neuropeptide galanin binds to its receptors and activates K_{ATP} channels through G_o2 to inhibit insulin secretion from β cells.

Anti-$G_o2\alpha$ Antibodies Suppress Basal K_{ATP} Currents and Abolish the Potentiating Effects of Galanin on K_{ATP} in WT β Cells. To further validate that G_o2 G protein is a critical component in the inhibitory pathway mediating galanin signaling, we used affinity-purified anti-$G_o2\alpha$ antibodies to titrate the endogenous $G_o2\alpha$ protein and tested K_{ATP} current activity in islet β cells. WT β cells dialyzed with anti-$G_o2\alpha$ antibodies showed a reduced basal K_{ATP} channel activity. After rupture of the patch, antibodies diffused into the cells from the holding pipettes, and the K_{ATP} current decreased from −43.7 ± 0.7 to −15.7 ± 0.1 pA/pF ($n = 5$, $P < 0.01$) within 10 min and reached a new steady state. K_{ATP} currents were stable when the holding pipettes contained nonspecific IgG or no antibodies. Galanin (100 nM) was perifused into the cell chamber during the steady phase. As expected, K_{ATP} currents were not enhanced by galanin in β cells dialyzed with anti-$G_o2\alpha$ antibodies. Notably, the antibodies had no effects of the K_{ATP} channel opener diazoxide on β cells (Fig. 3). These findings are in agreement with studies on isolated β cells from $G_o2\alpha^{-/-}$ mice (Fig. 2), and confirm that G_o2 is a critical downstream signal transducer of galanin receptors in β cells. Through modulation of K_{ATP} channel activity, G_o2 regulates insulin secretion. The stimulation of K_{ATP} channels by galanin was abolished by dialyzing anti-$G_o2\alpha$ antibodies into β cells (Fig. 3 *A* and *B*). Thus, antibodies dialyzed into the cytoplasm appear to titrate G_o2 away from their effectors and change the dynamic regulation of effectors. The reduction of the basal K_{ATP} channel activity by anti-$G_o2\alpha$ antibodies suggests that under basal conditions, G_o2 potentiates K_{ATP} channel activity in β cells. The K_{ATP} channels in β cells are involved in maintaining membrane potential, and activation of K_{ATP} channels is required for retaining β cells in the hyperpolarized state to suppress insulin secretion. In addition, the observation of dynamic changes in K_{ATP} activity by dialyzed anti-$G_o2\alpha$ antibodies suggests that the α subunit may be involved directly. Our results from native channel studies are also in agreement with previous findings that demonstrated that the activity of cloned SUR1-Kir6.2 channels (pancreatic form) can be enhanced by a $G_{i/o}\alpha$ protein during inside-out patch recordings in a reconstitution assay (35). In addition, recombinant $G_o\alpha$ protein can potentiate neuronal (brain) K_{ATP} channel activity in inside-out patches (36). Taken together,

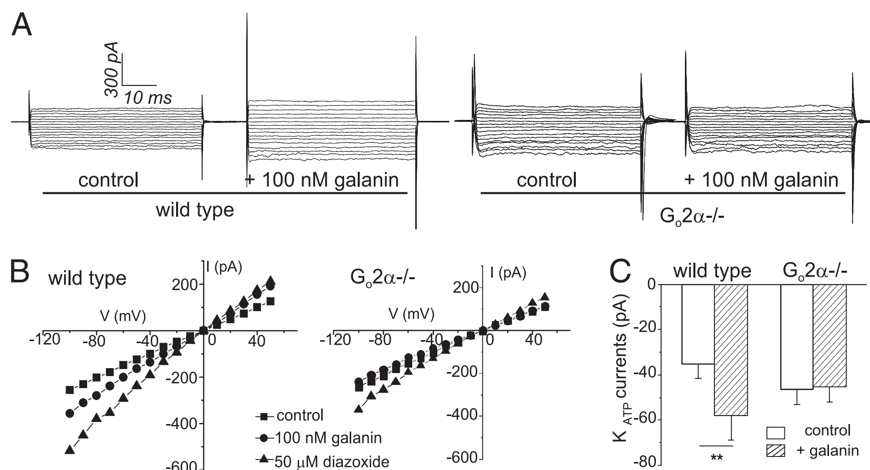

Fig. 2. Galanin-stimulated K_{ATP} channel activity is missing in $G_o2^{-/-}$ β cells. (*A*) Representative K_{ATP} current traces recorded from β cells potentiated by 100 nM galanin. Testing potential (TP) = −100 to +50 mV, holding potential (HP) = −20 mV. (*B*) I–V (current-voltage) relationship curves show the effect of galanin and diazoxide (50 μM) on K_{ATP} current from WT and $G_o2^{-/-}$ β cells. (*C*) Summary of K_{ATP} currents in β cells stimulated by galanin in WT and the diminished galanin stimulation in $G_o2^{-/-}$ in the presence of 10 mM glucose (in normal culture medium-glucose condition) ($n = 11$–12, $^{**}P < 0.01$, TP = −100 mV to +50 mV, HP = −20 mV).

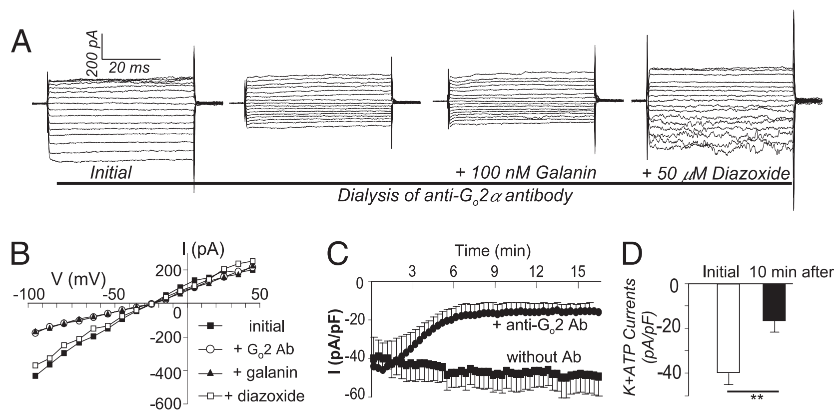

Fig. 3. Anti-G_o2 antibodies reduce basal K_{ATP} currents and abolish galanin-potentiated K_{ATP} currents in WT β cells. (*A*) Representative K_{ATP} current traces in WT β cells dialyzed with anti-$G_o2\alpha$ antibody and perifused with 100 nM galanin or 50 μM diazoxide. TP = −100 to +50 mV, HP = −20 mV. Basal K_{ATP} currents were blocked by the dialyzed anti-G_o2 antibodies, and the anti-G_o2 antibodies abolished galanin-potentiated K_{ATP} currents but not diazoxide-stimulated K_{ATP} currents. (*B*) Corresponding I–V (current-voltage) relationship curves. (*C*) Summary of time course of basal K_{ATP} currents blocked by anti-G_o2 antibodies ($n = 5$, TP = −100 mV, HP = −20 mV). (*D*) Summary of abolishment of galanin-potentiated K_{ATP} currents by anti-G_o2 antibody dialysis. Compare K_{ATP} currents before and 10 min after dialysis of anti-G_o2 antibodies ($n = 5$, $^{**}P < 0.01$, TP = −100 mV, HP = −20 mV).

these results support that the α subunit of G_o also participates in the regulation of K_{ATP} channel activity.

Inhibitory Effect of Galanin on Ca^{2+} Currents Is Missing in Pancreatic β Cells from $G_o2\alpha^{-/-}$ Mice. The L-type Ca^{2+} channel is a major route for elevating intracellular Ca^{2+} concentration, which in turn triggers insulin exocytosis. Modulation of Ca^{2+} channel activity would effectively regulate intracellular Ca^{2+} concentrations and insulin release in β cells. It has been shown that mouse β cells express L-type Ca^{2+} channels (both $Ca_v1.2$ and $Ca_v1.3$) (37, 38). To address whether the L-type Ca^{2+} channel is another intracellular effector in the galanin G_o2-mediated pathway, we investigated galanin's effect on the regulation of Ca^{2+} currents. First, we verified the nature of the recorded Ca^{2+} currents in β cells using a Ca^{2+} channel potentiator and blocker. As shown in Fig. 4*A*, Bay K-8644 (0.2 μM) potentiated current, whereas nifedipine (1 μM) inhibited current, confirming the existence of classical L-type Ca^{2+} channels in β cells. The low-voltage T-type Ca^{2+} channels were not detectable in our mouse pancreatic β cells. We next tested the effect of galanin on regulating Ca^{2+} currents in β cells. Galanin caused a reduction in Ca^{2+} flow into β cells by inhibiting Ca^{2+} currents. Galanin (100 nM) significantly reduced Ca^{2+} current in β cells isolated from WT mice, with a decrease of 55% of peak currents from −108.93 ± 25.68 to −60.26 ± 17.96 pA/pF ($n = 8, P < 0.01$) (Fig. 4 *B* and *C*). When the cells were dialyzed with anti-$G_o2\alpha$ antibodies, the average peak currents were −85.13 ± 21.58 and −72.07 ± 26.41 pA/pF ($n = 8$) before and after galanin (100 nM) application, respectively. No significant difference was observed before and after application of galanin in the presence of anti-$G_o2\alpha$ antibodies. This demonstrates that anti-$G_o2\alpha$ antibodies can block the inhibitory effect of galanin on Ca^{2+} currents. These observations suggest that in addition to modulating K_{ATP} channel activities, G_o2 protein could also mediate effects of galanin by regulating the activity of the pancreatic L-type Ca^{2+} channel. To confirm this hypothesis, we performed patch-clamp recordings of Ca^{2+} channel activity of β cells isolated from $G_o2\alpha^{-/-}$ animals. In $G_o2\alpha^{-/-}$ β cells, average peak currents before and after application of galanin were −51.87 ± 11.9 and −38.54 ± 15.6 pA/pF ($n = 6$), respectively. There was also no statistically significant difference before and after application of galanin. These results show that the lack of $G_o2\alpha$ in β cells by gene knockout or titration of $G_o2\alpha$ by antibodies leads to the loss of galanin's inhibitory effect on Ca^{2+} channels. It is noteworthy that there is a statistically significant difference ($P <$ 0.05) in the Ca^{2+} currents in WT and $G_o2\alpha^{-/-}$ β cells. The cause for this reduction of Ca^{2+} channel activity in $G_o2\alpha$ knockout is currently unknown. Taken together, our results suggest that G_o2 mediates the potentiation of K_{ATP} channels and inhibition of Ca^{2+}

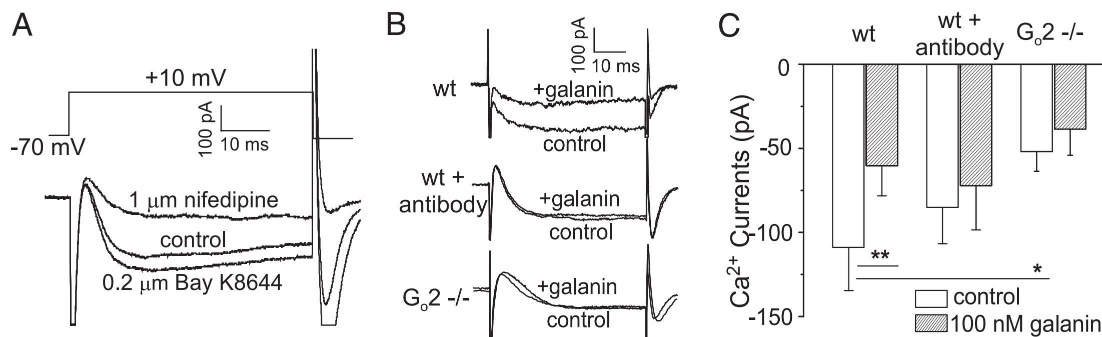

Fig. 4. The inhibition of Ca^{2+} current by galanin is missing in $G_o2\alpha^{-/-}$ β cells. (*A*) Current traces of whole-cell Ca^{2+} currents recorded from WT β cells under a control condition or the presence of 1 μM nifedipine or 0.2 μM Bay K-8644. TP = 0 mV, HP = −70 mV. (*B*) Representative Ca^{2+} current traces before and after 100 nM galanin application to WT β cells without or with dialyzed anti-G_o2 antibodies and to $G_o2\alpha^{-/-}$ β cells (TP = 0 mV, HP = −70 mV). (*C*) Summary of galanin effects on Ca^{2+} currents in β cells from WT without or with dialyzed anti-$G_o2\alpha$ antibodies and from $G_o2\alpha^{-/-}$. The results suggest that G_o2 mediates the inhibition of Ca^{2+} currents by galanin (TP = 0 mV, HP = −70 mV, $^*P < 0.05$, $^{**}P < 0.01$, $n = 5–8$). Values are means ± SEM.

PHYSIOLOGY

channels induced by galanin. Hence, G_o2 is a major transducer for galanin receptor signaling in the pancreatic β cell.

Loss of $G_o2\alpha$ Blunts the Hyperglycemic Effect of Galanin in Vivo. To confirm our findings that G_o2 mediates galanin receptor signaling in pancreatic β cells and galanin's inhibitory effect on insulin secretion, we tested the effect of galanin administration to $G_o2\alpha^{-/-}$ and WT mice on their blood glucose levels. Galanin can effectively suppress insulin release from β cells; therefore, administration of galanin into the bloodstream will suppress insulin levels, and in turn increase blood glucose levels. Galanin (2 μg/kg) was delivered via the retroorbital vein in overnight-fasted mice. Blood glucose levels were monitored before and after administration of galanin. As shown in Fig. 5, galanin elevates blood glucose levels rapidly, reaching a maximum after 10 min in WT mice and declining slowly thereafter. In WT mice, blood glucose levels were raised from a basal level of 74.5 ± 3.4 mg/dL to 130.1 ± 7.4 mg/dL 10 min postinjection and 121.8 ± 5.7 mg/dL 20 min postinjection. These correspond to a 77.8 and 64.5% increase after administration of galanin, respectively. In contrast, galanin only elevated glucose levels slightly over basal levels in $G_o2\alpha$-null mice. The basal blood glucose level of G_o2-null mice was 60.4 ± 1.7 mg/dL. The glucose levels were raised moderately after galanin injection to 85.5 ± 3.1 mg/dL 10 min postinjection and 81.8 ± 3.7 mg/dL 20 min postinjection, corresponding to a 45.7 and 36.9% increase over basal, respectively. The results demonstrate that galanin can effectively elevate blood glucose levels in WT mice, and that this effect is significantly reduced ($P < 0.01$) in mice lacking $G_o2\alpha$. This suggests that G_o2 mediates a critical signaling pathway for galanin in the modulation of glucose homeostasis in the body. We did not observe a complete loss of galanin's hyperglycemic effect in $G_o2\alpha$-null mice, suggesting that galanin may also use other, G_o2-independent mechanisms to augment glucose levels in β cells. In fact, galanin has been shown to enhance plasma glucagon levels secreted from pancreatic α cells. During the systemic infusion of galanin, pancreatic glucagon output rapidly doubled in dogs and mice (3, 39). Therefore, galanin-induced hyperglycemia is the additive action of lowering basal plasma insulin levels, increasing basal plasma glucagon levels, and possibly other mechanisms. Increasing plasma glucagon by galanin is believed to be mediated by $G_{11/q}$ proteins, but not by $G_{i/o}$ proteins. Therefore, elevation of blood glucose levels by the action of glucagon should not be affected in $G_o2\alpha^{-/-}$ mice. Physiologically, the body responds to hypoglycemia by increasing glucose production and decreasing glucose sequestration (by reducing insulin levels) simultaneously.

The distribution of galanin suggests that it is a neuropeptide. Its activity is exerted locally, rather than systemically as a humoral agent (40). Pancreatic islets are highly innervated, and activation of pancreatic nerves is sufficient to influence islet function (15). G_o was originally identified as the "other" PTX-sensitive G protein in the brain and is highly expressed in the CNS and endocrine cells (41). Logically, antagonizing the G_s pathway by suppressing adenylyl cyclase (AC) activity might be a mechanism for galanin receptor-G_o protein-mediated signaling in cells. Galanin can inhibit AC activity through G_i3 (42) or G_i2/G_i3 proteins in the RINm5F cell line (43). However, galanin can still suppress insulin release from insulin-secreting cells in the presence of cAMP in the media (44). Using static islet incubation assays, we also confirmed that the suppression of insulin release by galanin is not dependent on the AC-cAMP pathway. Islets were incubated with or without galanin in the presence of 0.1 mM dibutyryl-cAMP and 16.2 mM glucose, and released insulin was measured. Galanin can inhibit insulin release from wild-type islets even in the presence of cAMP, as observed previously (45). This observation suggests that inhibiting AC and reducing cAMP levels are unlikely to be a mode for suppressing insulin release by galanin. In fact, G_o is at best a poor AC inhibitor in vitro compared with G_i proteins (46). In addition, cellular cAMP levels are not elevated in $G_o^{-/-}$ islets (47). Taken together, reducing cAMP levels by inhibiting AC activity in β cells does not appear to constitute a major route for the inhibitory effect of galanin on insulin release.

Our findings can be summarized as follows: (*i*) The inhibitory effects of galanin on insulin release are lost in β cells from $G_o2\alpha^{-/-}$ mice, but are intact in the other $G_{i/o}$ knockouts; (*ii*) the potentiation by galanin of K_{ATP} currents is missing in $G_o2\alpha^{-/-}$ β cells; (*iii*) the inhibition of β-cell L-type Ca^{2+} currents by galanin is lost in $G_o2^{-/-}$ β cells; (*iv*) antibodies specific against $G_o2\alpha$ abolish galanin-mediated potentiation of K_{ATP} and inhibition of Ca^{2+} currents in WT β cells; and (*v*) in vivo, the galanin-induced hyperglycemic effect is blunted in mice lacking $G_o2\alpha$.

Galanin inhibits Ca^{2+}-induced insulin release from permeabilized RINm5F cells in a PTX-sensitive manner (48), suggesting that direct inhibition of exocytosis is one mechanism of action for galanin. Constitutively active α subunits of $G_{i/o}$ protein can inhibit the exocytosis process, suggesting α subunits can directly influence the process (49). In addition, enhanced potassium-induced insulin release in $G_o2^{-/-}$ islets shown in Fig. 1 and our previous study (25) supports the notion that G_o2 may act on the insulin exocytosis process directly. Interestingly, Gβγ can also regulate the exocytosis process. Gβγ can bind SNAP-25 directly, as demonstrated in in vitro assays (50). The C terminus of SNAP-25 may be a target of Gβγ for presynaptic inhibition (51). Recently, it has been reported that norepinephrine can block the exocytosis process via the Gβγ subunit, and that this process is mediated specifically by $G\alpha_i1/2$ (52). Through study of G-protein knockout mice, we and others have observed that the expression levels of Gβγ correlate well with α-subunit levels in cells; Gβγ are down-regulated in α-subunit knockout and heterozygous mice (53). This suggests that signaling through Gα and Gβγ is well-coordinated in the cells. The mechanism for G-protein inhibition of the exocytosis process remains to be further elucidated.

Galanin has been shown to be an important neuropeptide that regulates glucose homeostasis in the body (17). Our study demonstrates that galanin inhibition of insulin release is mediated mainly by G_o2, not other G_i/G_o proteins in pancreatic β cells. Other G_i or G_o proteins cannot compensate for the loss of G_o2 in mediating galanin's modulation of insulin secretion. This indicates that galanin receptors preferentially couple to effectors by G_o2 protein in β cells in vivo. At the molecular level, loss of G_o2 protein in β cells results in impairment of galanin's modulation of K_{ATP} and Ca^{2+} channels. This suggests in the insulin secretion pathway that galanin achieves its inhibitory effects on insulin release by at least at three different action sites: by potentiating K_{ATP} channels, inhibiting L-type Ca^{2+} currents, and regulating the exocytosis process. Inhibitory pep-

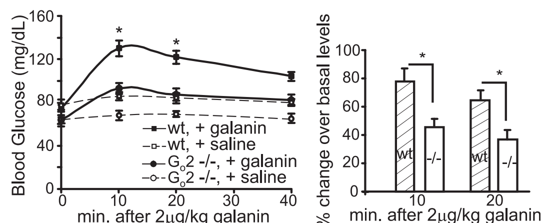

Fig. 5. Effects of galanin on blood glucose levels in G_o2 knockout mice. Galanin at 2 μg/kg bodyweight was given at time 0. Blood glucose levels were monitored. (*Left*) Actual glucose levels. (*Right*) Percentage changes over the basal. WT: $n = 9$, 74.5 ± 3.4 (basal at 0 min), 130.1 ± 7.4 (10 min, increased 77.8% ± 14.3), 121.8 ± 5.7 (20 min, increased 64.5% ± 8.1); $G_o2\alpha^{-/-}$: $n = 18$, 60.4 ± 1.7 (basal at 0 min), 85.5 ± 3.1 (10 min, increased 45.7% ± 5.7), 81.8 ± 3.7 (20 min, increased 36.9% ± 6.7). For the control no-galanin injection experiment, $n = 6$ and 8 for WT and $G_o2\alpha^{-/-}$ mice, respectively. *$P < 0.01$; statistical significance was determined using the Student's *t* test from unpaired data or analysis of variance.

tide hormones play a critical role in maintaining euglycemia by preventing oversecretion of insulin into the bloodstream. Oversecretion of insulin could result in hypoglycemia that may impair the normal biological function of the endocrine and nervous systems. Furthermore, prolonged oversecretion of insulin may desensitize peripheral tissues and lead to the development of insulin resistance characteristic of type II diabetes.

Materials and Methods

All procedures were designed and performed in accordance with the generally accepted ethical standards for animal experimentation and approved by the Chancellor's Animal Research Committee of the University of California, Los Angeles. Detailed methods for islets and β-cell isolations, islet perifusion, electrophysiological recordings, and in vivo tests for galanin effects on glucose levels are described in *SI Materials and Methods*. Statistical analyses were done using paired and unpaired Student's *t* tests and analyses of variance in conjunction with Newman–Keuls tests where appropriate. Group differences at the level of $P < 0.05$ were considered statistically significant.

ACKNOWLEDGMENTS. This work is supported by National Institutes of Health Grants DK069771 (to M.J.) and DK19318 (to L.B.) and in part by Intramural Research Program of the NIH Project Z01-ES101643 (to L.B.).

1. Tatemoto K, Rökaeus A, Jörnvall H, McDonald TJ, Mutt V (1983) Galanin—A novel biologically active peptide from porcine intestine. *FEBS Lett* 164(1):124–128.
2. Skofitsch G, Jacobowitz DM (1985) Immunohistochemical mapping of galanin-like neurons in the rat central nervous system. *Peptides* 6:509–546.
3. Dunning BE, et al. (1986) Galanin: A novel pancreatic neuropeptide. *Am J Physiol* 251 (1 Pt 1):E127–E133.
4. Melander T, et al. (1986) Coexistence of galanin-like immunoreactivity with catecholamines, 5-hydroxytryptamine, GABA and neuropeptides in the rat CNS. *J Neurosci* 6:3640–3654.
5. Habert-Ortoli E, Amiranoff B, Loquet I, Laburthe M, Mayaux JF (1994) Molecular cloning of a functional human galanin receptor. *Proc Natl Acad Sci USA* 91:9780–9783.
6. Smith KE, et al. (1997) Expression cloning of a rat hypothalamic galanin receptor coupled to phosphoinositide turnover. *J Biol Chem* 272:24612–24616.
7. Smith KE, et al. (1998) Cloned human and rat galanin GALR3 receptors. Pharmacology and activation of G-protein inwardly rectifying K^+ channels. *J Biol Chem* 273: 23321–23326.
8. Wang S, He C, Hashemi T, Bayne M (1997) Cloning and expressional characterization of a novel galanin receptor. Identification of different pharmacophores within galanin for the three galanin receptor subtypes. *J Biol Chem* 272:31949–31952.
9. Counts SE, Perez SE, Ginsberg SD, De Lacalle S, Mufson EJ (2003) Galanin in Alzheimer disease. *Mol Interv* 3(3):137–156.
10. Steiner RA, et al. (2001) Galanin transgenic mice display cognitive and neurochemical deficits characteristic of Alzheimer's disease. *Proc Natl Acad Sci USA* 98:4184–4189.
11. Wrenn CC, et al. (2004) Learning and memory performance in mice lacking the GAL-R1 subtype of galanin receptor. *Eur J Neurosci* 19:1384–1396.
12. Zorrilla EP, Brennan M, Sabino V, Lu X, Bartfai T (2007) Galanin type 1 receptor knockout mice show altered responses to high-fat diet and glucose challenge. *Physiol Behav* 91:479–485.
13. Ahrén B, Lindskog S (1992) Galanin and the regulation of islet hormone secretion. *Int J Pancreatol* 11(3):147–160.
14. McDonald TJ, et al. (1985) Galanin inhibits insulin secretion and induces hyperglycemia in dogs. *Diabetes* 34(2):192–196.
15. Dunning BE, Taborsky GJ, Jr. (1989) Galanin release during pancreatic nerve stimulation is sufficient to influence islet function. *Am J Physiol* 256(1 Pt 1):E191–E198.
16. Gregersen S, et al. (1993) Blockade of galanin-induced inhibition of insulin secretion from isolated mouse islets by the non-methionine containing antagonist M35. *Eur J Pharmacol* 232(1):35–39.
17. Ahrén B, Pacini G, Wynick D, Wierup N, Sundler F (2004) Loss-of-function mutation of the galanin gene is associated with perturbed islet function in mice. *Endocrinology* 145:3190–3196.
18. Dunning BE, Ahrén B (1992) Reduced pancreatic content of the inhibitory neurotransmitter galanin in genetically obese, hyperinsulinemic mice. *Pancreas* 7:233–239.
19. Adeghate E, Ponery AS (2001) Large reduction in the number of galanin-immunoreactive cells in pancreatic islets of diabetic rats. *J Neuroendocrinol* 13:706–710.
20. Kohnert KD, et al. (1999) Islet neuronal abnormalities associated with impaired insulin secretion in type 2 diabetes in the Chinese hamster. *Regul Pept* 82(1–3):71–79.
21. Parker EM, et al. (1995) Cloning and characterization of the rat GALR1 galanin receptor from Rin14B insulinoma cells. *Brain Res Mol Brain Res* 34(2):179–189.
22. Kolakowski LF, Jr., et al. (1998) Molecular characterization and expression of cloned human galanin receptors GALR2 and GALR3. *J Neurochem* 71:2239–2251.
23. Wang S, Hashemi T, He C, Strader C, Bayne M (1997) Molecular cloning and pharmacological characterization of a new galanin receptor subtype. *Mol Pharmacol* 52: 337–343.
24. Fathi Z, et al. (1998) Molecular characterization, pharmacological properties and chromosomal localization of the human GALR2 galanin receptor. *Brain Res Mol Brain Res* 58(1-2):156–169.
25. Wang Y, et al. (2011) Augmented glucose-induced insulin release in mice lacking G(o2), but not G(o1) or G(i) proteins. *Proc Natl Acad Sci USA* 108:1693–1698.
26. Jiang M, et al. (1998) Multiple neurological abnormalities in mice deficient in the G protein Go. *Proc Natl Acad Sci USA* 95:3269–3274.
27. Rudolph U, et al. (1995) Ulcerative colitis and adenocarcinoma of the colon in G α i2-deficient mice. *Nat Genet* 10(2):143–150.
28. Ashcroft FM, Gribble FM (1999) ATP-sensitive K^+ channels and insulin secretion: Their role in health and disease. *Diabetologia* 42:903–919.
29. Guillam MT, et al. (1997) Early diabetes and abnormal postnatal pancreatic islet development in mice lacking Glut-2. *Nat Genet* 17:327–330.
30. Matschinsky FM (1996) Banting Lecture 1995. A lesson in metabolic regulation inspired by the glucokinase glucose sensor paradigm. *Diabetes* 45:223–241.
31. Aguilar-Bryan L, et al. (1998) Toward understanding the assembly and structure of K_{ATP} channels. *Physiol Rev* 78:227–245.
32. Babenko AP, Gonzalez G, Bryan J (1999) Two regions of sulfonylurea receptor specify the spontaneous bursting and ATP inhibition of K_{ATP} channel isoforms. *J Biol Chem* 274:11587–11592.
33. Ashcroft FM, Rorsman P (1990) ATP-sensitive K^+ channels: A link between B-cell metabolism and insulin secretion. *Biochem Soc Trans* 18(1):109–111.
34. Ashcroft FM, et al. (1994) Stimulus-secretion coupling in pancreatic β cells. *J Cell Biochem* 55(Suppl):54–65.
35. Sánchez JA, Gonoi T, Inagaki N, Katada T, Seino S (1998) Modulation of reconstituted ATP-sensitive K(+)-channels by GTP-binding proteins in a mammalian cell line. *J Physiol* 507:315–324.
36. VanDongen AM, et al. (1988) Newly identified brain potassium channels gated by the guanine nucleotide binding protein Go. *Science* 242:1433–1437.
37. Namkung Y, et al. (2001) Requirement for the L-type Ca(2+) channel α(1D) subunit in postnatal pancreatic β cell generation. *J Clin Invest* 108:1015–1022.
38. Schulla V, et al. (2003) Impaired insulin secretion and glucose tolerance in β cell-selective Ca(v)1.2 Ca^{2+} channel null mice. *EMBO J* 22:3844–3854.
39. Lindskog S, Ahrén B (1987) Galanin: Effects on basal and stimulated insulin and glucagon secretion in the mouse. *Acta Physiol Scand* 129:305–309.
40. Dupré J (1988) Galanin: A selective inhibitor of insulin secretion? *Pancreas* 3(2): 119–121.
41. Jiang M, Bajpayee NS (2009) Molecular mechanisms of Go signaling. *Neurosignals* 17 (1):23–41.
42. de Mazancourt P, Goldsmith PK, Weinstein LS (1994) Inhibition of adenylate cyclase activity by galanin in rat insulinoma cells is mediated by the G-protein Gi3. *Biochem J* 303:369–375.
43. McDermott AM, Sharp GW (1995) Gi2 and Gi3 proteins mediate the inhibition of adenylyl cyclase by galanin in the RINm5F cell. *Diabetes* 44:453–459.
44. Sharp GW, et al. (1989) Galanin can inhibit insulin release by a mechanism other than membrane hyperpolarization or inhibition of adenylate cyclase. *J Biol Chem* 264: 7302–7309.
45. Drews G, Debuyser A, Henquin JC (1994) Significance of membrane repolarization and cyclic AMP changes in mouse pancreatic B-cells for the inhibition of insulin release by galanin. *Mol Cell Endocrinol* 105(1):97–102.
46. Taussig R, Iñiguez-Lluhi JA, Gilman AG (1993) Inhibition of adenylyl cyclase by Gi α. *Science* 261:218–221.
47. Zhao A, et al. (2010) Gαo represses insulin secretion by reducing vesicular docking in pancreatic β-cells. *Diabetes* 59:2522–2529.
48. Ullrich S, Wollheim CB (1989) Galanin inhibits insulin secretion by direct interference with exocytosis. *FEBS Lett* 247:401–404.
49. Lang J, et al. (1995) Direct control of exocytosis by receptor-mediated activation of the heterotrimeric GTPases Gi and G(o) or by the expression of their active G α subunits. *EMBO J* 14:3635–3644.
50. Blackmer T, et al. (2001) G protein βγ subunit-mediated presynaptic inhibition: Regulation of exocytotic fusion downstream of Ca^{2+} entry. *Science* 292:293–297.
51. Gerachshenko T, et al. (2005) Gβγ acts at the C terminus of SNAP-25 to mediate presynaptic inhibition. *Nat Neurosci* 8:597–605.
52. Zhao Y, Fang Q, Straub SG, Lindau M, Sharp GW (2010) Noradrenaline inhibits exocytosis via the G protein βγ subunit and refilling of the readily releasable granule pool via the α(i1/2) subunit. *J Physiol* 588:3485–3498.
53. Valenzuela D, et al. (1997) G α(o) is necessary for muscarinic regulation of Ca^{2+} channels in mouse heart. *Proc Natl Acad Sci USA* 94:1727–1732.