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_{2}, but not other members of the G_{i/o} protein family, secrete more insulin than controls upon glucose challenge, indicating that G_{2} 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_{2}, 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_{2}αx antibodies. Galanin actions on K_{ATP} and calcium currents were completely lost in G_{2}−/− β cells. Furthermore, the hyperglycemic effect of galanin is also blunted in G_{2}−/− mice. Our results demonstrate that G_{2} 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.

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 PTX-sensitive 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/o} protein family, namely G_{1}, G_{2}, G_{3}, G_{6}, and G_{2}, are expressed in pancreatic 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). Inhibition 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.
islets, and that only G\(_2\)α, and not G\(_{1\pi-3}\) or G\(_{3\pi-1}\) protein-deficient mice show an “improved” glucose tolerance test and enhanced insulin release from pancreatic \(\beta\) cells (25). This suggests that G\(_2\)α is the major transducer mediating inhibitory effects on insulin release that prevent oversecretion. In this study, using \(G\_2\alpha, G\_3\beta\)-subunit gene knockout animals established in our laboratory (26, 27), we demonstrate that the G\(_2\)α 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\(_2\)α signaling.

**Results and Discussion**

**Inhibition of Insulin Release by Galanin Is Lost in G\(_2\alpha^{-/-}\) 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 \(G\_2\alpha/G\_3\beta\) proteins in the process. PTX ADP ribosylates a cysteine at the carboxyl termini (–4 position) of the \(\alpha\) subunits of G\(_{1\pi-3}\) G proteins and collectively blocks all \(G\_2\alpha/G\_3\beta\) signaling. Therefore, PTX assays cannot delineate through which specific G protein(s) galanin signals. To investigate the mechanism and to screen the responsible \(G\_2\alpha/G\_3\beta\) protein(s) for mediating the inhibition of insulin release by galanin, we established a perfusion assay of isolated islets from \(G\_2\alpha\)–deficient animals aimed at identifying which \(G\_2\alpha/G\_3\beta\) 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 perfusion chamber. After 60 min of perfusion 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 perfused for 120 min at this high-glucose concentration. The perfusion solution was then switched back to 1.8 mM (low) glucose for 30 min, followed by perfusion with KCl (30 mM) for an additional 30 min. During the high-glucose (16.2 mM) perfusion 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 \(G\_2\alpha, G\_1\alpha/G\_3\beta, G\_1\alpha\), and \(G\_2\alpha\). 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 \(\beta\)-cell membrane, triggered insulin release from islets. Galanin (perfused together with high glucose) suppressed insulin secretion in islets from WT, \(G\_1\alpha^{-/-} G\_3\beta^{-/-}\) double KO, \(G\_2\alpha^{-/-}\), and \(G\_1\alpha^{-/-}\) 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 \(G\_2\alpha^{-/-}\) mice. The results indicate that \(G\_2\alpha\) mediates the inhibitory effect of galanin on insulin release in pancreatic \(\beta\) cells. The levels of insulin released under basal, glucose-stimulated, and K\(^{+}\)-induced conditions were comparable for islets from WT, \(G\_2\alpha^{-/-}\), \(G\_1\alpha^{-/-} G\_3\beta^{-/-}\) double knockout, and \(G\_1\alpha^{-/-}\) mice, but were significantly higher for islets from \(G\_2\alpha^{-/-}\) (\(P<0.01\)). This suggests that, in addition to mediating galanin inhibition of insulin release, \(G\_2\alpha\) modulates the sensitivity of islets to glucose and maybe directly or indirectly regulates secretory machinery.

**K\(_{ATP}\) Channels in G\(_2\alpha^{-/-}\) Islets Show Normal Glucose Sensitivity.** It is well-established that glucose and other nutrients promote the depolarization of the \(\beta\)-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 \(\beta\) cell facilitates entry of glucose into the cell (29). The enzyme glucokinase phosphorylates glucose to glucose-6-phosphate. Glucokinase appears to function as the fundamental glucose sensor that controls the subsequent \(\beta\)-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 \(\beta\) 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 \(\beta\) 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 \(\beta\)-cell insulin granules (34). As observed, galanin can inhibit insulin secretion from pancreatic \(\beta\) cells in a PTX-sensitive manner.
To investigate whether the G\(_2\alpha\)-mediated signaling pathway regulates K\(_{\text{ATP}}\) and Ca\(^{2+}\) channels, we studied channel properties via patch-clamp recording. First, the integrity of K\(_{\text{ATP}}\) channels was investigated in \(\beta\) cells from G\(_2\alpha\) knockout mice. Specifically, K\(_{\text{ATP}}\) channels were studied in response to glucose (Fig. S1). Freshly isolated islets were digested briefly with trypsin to generate single \(\beta\) cells. Short-term cultured \(\beta\) cells were used in the studies. Glucose inhibited K\(_{\text{ATP}}\) currents in \(\beta\) cells from both wild-type and G\(_2\alpha\)-mice (Fig. S1A–C). High glucose (16.2 mM) inhibited K\(_{\text{ATP}}\) currents in isolated \(\beta\) 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\(_2\alpha\)-mice \((n=12, P<0.05)\) mice (Fig. S1D). Furthermore, glucose deprivation stimulated K\(_{\text{ATP}}\) currents in both WT (−26.5 ± 5.0 to −62.6 ± 13.5 pA/pF; \(n=9, P<0.01\)) and G\(_2\alpha\)-mice (−22.26 ± 4.0 to −65.34 ± 14 pA/pF; \(n=6, P<0.01\)). This result demonstrates that K\(_{\text{ATP}}\) channels in \(\beta\) cells isolated from G\(_2\alpha\)− mice respond to glucose similarly to WT controls and suggests that lack of G\(_2\alpha\) does not alter the responsiveness of K\(_{\text{ATP}}\) channels to glucose in \(\beta\) cells.

**Galanin Fails to Potentiate K\(_{\text{ATP}}\) Channels in G\(_2\alpha\)-/− Mice.** We next investigated the effects of galanin on the modulation of K\(_{\text{ATP}}\) channel activity by glucose. K\(_{\text{ATP}}\) channel currents in the \(\beta\) cells of WT and G\(_2\alpha\)-/− mice were recorded in the absence of galanin, presence of 100 nM galanin, or 50 \(\mu\)M diazoxide. As shown in Fig. 2, the averages of glucose-sensitive K\(_{\text{ATP}}\) currents in \(\beta\) 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\(_{\text{ATP}}\) currents in G\(_2\alpha\)-/− \(\beta\) 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\(_{\text{ATP}}\) channel opener, was able to further potentiate the channels in both WT and G\(_2\alpha\)-/− \(\beta\) cells in the presence of galanin. Galanin significantly enhanced \((P<0.01)\) K\(_{\text{ATP}}\) currents in \(\beta\) cells from WT control mice; however, this current augmentation was completely absent in the \(\beta\) cells from G\(_2\alpha\)-/− mice. The results suggest that G\(_2\alpha\) protein mediates the inhibitory effect of galanin on K\(_{\text{ATP}}\) channels in mouse \(\beta\) cells, and that the potentiation of K\(_{\text{ATP}}\) by diazoxide is independent of the G\(_2\alpha\)-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\(_{\text{ATP}}\) channels through G\(_2\alpha\) to inhibit insulin secretion from \(\beta\) cells.

**Anti-G\(_2\alpha\) Antibodies Suppress Basal K\(_{\text{ATP}}\) Currents and Abolish the Potentiating Effects of Galanin on K\(_{\text{ATP}}\) in WT \(\beta\) Cells.** To further validate that G\(_2\alpha\) G protein is a critical component in the inhibitory pathway mediating galanin signaling, we used affinity-purified anti-G\(_2\alpha\) antibodies to titrate the endogenous G\(_2\alpha\) protein and tested K\(_{\text{ATP}}\) current activity in isolated \(\beta\) cells. WT \(\beta\) cells dialyzed with anti-G\(_2\alpha\) antibodies showed a reduced basal K\(_{\text{ATP}}\) channel activity. After rupture of the patch, antibodies diffused into the cells from the holding pipettes, and the K\(_{\text{ATP}}\) 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\(_{\text{ATP}}\) currents were stable when the holding pipettes contained nonspecific IgG or no antibodies. Galanin (100 nM) was perfused into the cell chamber during the steady phase. As expected, K\(_{\text{ATP}}\) currents were not enhanced by galanin in \(\beta\) cells dialyzed with anti-G\(_2\alpha\) antibodies. Notably, the antibodies had no effects of the K\(_{\text{ATP}}\) channel opener diazoxide on \(\beta\) cells (Fig. 3). These findings are in agreement with studies on isolated \(\beta\) cells from G\(_2\alpha\)-/− mice (Fig. 2), and confirm that G\(_2\alpha\) is a critical downstream signal transducer of galanin receptors in \(\beta\) cells. Through modulation of K\(_{\text{ATP}}\) channel activity, G\(_2\alpha\) regulates insulin secretion. The stimulation of K\(_{\text{ATP}}\) channels by galanin was abolished by dialyzing anti-G\(_2\alpha\) antibodies into \(\beta\) cells (Fig. 3A and B). Thus, antibodies dialyzed into the cytoplasm appear to titrate G\(_2\alpha\) away from their effectors and change the dynamic regulation of effectors. The reduction of the basal K\(_{\text{ATP}}\) channel activity by anti-G\(_2\alpha\) antibodies suggests that under basal conditions, G\(_2\alpha\) potentiates K\(_{\text{ATP}}\) channel activity in \(\beta\) cells. The K\(_{\text{ATP}}\) channels in \(\beta\) cells are involved in maintaining membrane potential, and activation of K\(_{\text{ATP}}\) channels is required for retaining \(\beta\) cells in the hyperpolarized state to suppress insulin secretion. In addition, the observation of dynamic changes in K\(_{\text{ATP}}\) activity by dialyzed anti-G\(_2\alpha\) antibodies suggests that the \(\alpha\) 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\(_{\text{ATP}}\alpha\) protein during inside-out patch recordings in a reconstitution assay (35). In addition, recombinant G\(_{\text{ATP}}\alpha\) protein can potentiate neuronal (brain) K\(_{\text{ATP}}\) channel activity in inside-out patches (36). Taken together,

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**Fig. 2.** Galanin-stimulated K\(_{\text{ATP}}\) channel activity is missing in G\(_2\alpha\)-/− \(\beta\) cells. (A) Representative K\(_{\text{ATP}}\) current traces recorded from \(\beta\) 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 \(\mu\)M) on K\(_{\text{ATP}}\) current from WT and G\(_2\alpha\)-/− \(\beta\) cells. (C) Summary of K\(_{\text{ATP}}\) currents in \(\beta\) cells stimulated by galanin in WT and the diminished galanin stimulation in G\(_2\alpha\)-/− 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).
these results support that the $\alpha$ subunit of $G_{\alpha}$ also participates in the regulation of $K_{ATP}$ channel activity.

**Inhibitory Effect of Galanin on $Ca^{2+}$ Currents Is Missing in Pancreatic $\beta$ Cells from $G_{2\alpha^{-/-}}$ 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 $\beta$ cells. It has been shown that mouse $\beta$ cells express L-type $Ca^{2+}$ channels (both Ca$_{1.2}$ and Ca$_{1.3}$) (37, 38). To address whether the L-type $Ca^{2+}$ channel is another intracellular effector in the galanin $G_{2\alpha}$-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 $\beta$ cells using a $Ca^{2+}$ channel potentiator and blocker. As shown in Fig. 4A, Bay K-8644 (0.2 $\mu$M) potentiated current, whereas nifedipine (1 $\mu$M) inhibited current, confirming the existence of classical L-type $Ca^{2+}$ channels in $\beta$ cells. The low-voltage T-type $Ca^{2+}$ channels were not detectable in our mouse pancreatic $\beta$ cells. We next tested the effect of galanin on regulating $Ca^{2+}$ currents in $\beta$ cells. Galanin caused a reduction in $Ca^{2+}$ flow into $\beta$ cells by inhibiting $Ca^{2+}$ currents. Galanin (100 nM) significantly reduced $Ca^{2+}$ current in $\beta$ cells isolated from WT mice, with a decrease of 55% of peak currents from $-108.93 \pm 25.68$ to $-60.26 \pm 17.96$ pA/pF ($n = 8$, $P < 0.01$) (Fig. 4B and C). When the cells were dialyzed with anti-$G_{2\alpha}$ antibodies, the average peak currents were $-85.13 \pm 21.58$ and $-72.07 \pm 26.41$ 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-$G_{2\alpha}$ antibodies. This demonstrates that anti-$G_{2\alpha}$ antibodies can block the inhibitory effect of galanin on $Ca^{2+}$ currents. These observations suggest that in addition to modulating $K_{ATP}$ channel activities, $G_{2\alpha}$ 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 $\beta$ cells isolated from $G_{2\alpha^{-/-}}$ animals. In $G_{2\alpha^{-/-}}$ $\beta$ cells, average peak currents before and after application of galanin were $-51.87 \pm 11.9$ and $-38.54 \pm 15.6$ 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 $G_{2\alpha}$ in $\beta$ cells by gene knockout or titration of $G_{2\alpha}$ 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 $G_{2\alpha^{-/-}}$ $\beta$ cells. The cause for this reduction of $Ca^{2+}$ channel activity in $G_{2\alpha}$ knockout is currently unknown. Taken together, our results suggest that $G_{2\alpha}$ mediates the potentiation of $K_{ATP}$ channels and inhibition of $Ca^{2+}$...
channels induced by galanin. Hence, \( G_o,2 \) is a major transducer for galanin receptor signaling in the pancreatic \( \beta \) cell.

**Loss of \( G_o,2\alpha \) Blunts the Hyperglycemic Effect of Galanin in Vivo.** To confirm our findings that \( G_o,2 \) mediates galanin receptor signaling in pancreatic \( \beta \) cells and galanin’s inhibitory effect on insulin secretion, we tested the effect of galanin administration to \( G_o,2\alpha^{-/-} \) and WT mice on their blood glucose levels. Galanin can effectively suppress insulin release from \( \beta \) cells; therefore, administration of galanin into the bloodstream will suppress insulin levels, and in turn increase blood glucose levels. Galanin (2 \( \mu \)g/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 \( G_o,2\alpha^{-/-} \) null mice. The basal blood glucose level of \( G_o,2\alpha^{-/-} \) 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 glucose levels in WT mice, and that this effect is significantly reduced (\( P < 0.01 \)) in mice lacking \( G_o,2\alpha \). This suggests that \( G_o,2 \) 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 \( G_o,2\alpha^{-/-} \) mice, suggesting that galanin may also use other, \( G_o,2 \)-independent mechanisms to augment glucose levels in \( \beta \) cells. In fact, galanin has been shown to enhance plasma glucagon levels secreted from pancreatic \( \alpha \) 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 \( G_{11/o} \) proteins, but not by \( G_o,2 \) proteins. Therefore, elevation of blood glucose levels by the action of glucagon should not be affected in \( G_o,2\alpha^{-/-} \) 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 hormonal 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 \( G \) pathway by suppressing adenyl cyclase (AC) activity might be a mechanism for galanin receptor-\( G_o \) protein-mediated signaling in cells. Galanin can inhibit AC activity through \( G_o,2 \) or \( G_o,3 \) 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 \( \beta \) cells does not appear to constitute a major role 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 \( \beta \) cells from \( G_o,2\alpha^{-/-} \) mice, but are intact in the other \( G_o,3 \) knockout; (ii) the potentiation by galanin of K\(^{+}\)-channel currents is missing in \( G_o,2\alpha^{-/-} \) \( \beta \) cells; (iii) the inhibition of \( \beta \)-cell L-type Ca\(^{2+}\) currents by galanin is lost in \( G_o,2\alpha^{-/-} \) \( \beta \) cells; (ii) antibodies specific against \( G_o,2 \) abolish galanin-mediated potentiation of K\(^{+}\)-channel activity; (iv) \( G_o,2 \) abolishes galanin-mediated potentiation of K\(^{+}\) currents and inhibition of Ca\(^{2+}\) currents in WT \( \beta \) cells; and (v) in vivo, the galanin-induced hyperglycemic effect is blunted in mice lacking \( G_o,2\alpha \).

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 \( \alpha \) subunits of \( G_o,3 \) protein can inhibit the exocytosis process, suggesting \( \alpha \) subunits can directly influence the process (49). In addition, enhanced potassium-induced insulin release in \( G_o,2\alpha^{-/-} \) islets shown in Fig. 1 and our previous study (25) supports the notion that \( G_o,2 \) may act on the exocytosis process. Therefore, \( G_o,2 \) may be a direct regulator of the exocytosis process. \( G_o,2 \) can bind SNAP-25 directly, as demonstrated in in vitro assays (50). The C terminus of SNAP-25 may be a target of \( G_o,2 \) for presynaptic inhibition (51). Recently, it has been reported that norepinephrine can block the exocytosis process via the \( G_o,2 \) subunit, and that this process is mediated specifically by \( G_o,1/2 \) (52). Through study of G-protein knockout mice, we and others have observed that the expression levels of \( G_o,2 \) correlate well with \( \alpha \) subunit levels in cells; \( G_o,2 \) are down-regulated in \( \alpha \)-subunit knockout and heterozygous mice (53). This suggests that signaling through \( G_o,2 \) and \( G_o,2 \) 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 \( G_o,2 \) and \( G_o,3 \) proteins in pancreatic \( \beta \) cells. Other \( G_o \) or \( G_o,3 \) proteins cannot compensate for the loss of \( G_o,2 \) in mediating galanin’s modulation of insulin secretion. This indicates that galanin receptors preferentially couple to effectors by \( G_o,2 \) protein in \( \beta \) cells in vivo. At the molecular level, loss of \( G_o,2 \) protein in \( \beta \) cells results in impairment of galanin’s modulation of K\(^{+}\) currents 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\(^{+}\) channels, inhibiting L-type Ca\(^{2+}\) currents, and regulating the exocytosis process. Inhibitory pep-
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 j-cell isolation, islet perfusion, 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.

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