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Minireview

Critical metabolic roles of β-cell M_3 muscarinic acetylcholine receptors

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ABSTRACT

Muscarinic acetylcholine (ACh) receptors (mAChRs; M_1–M_5) regulate the activity of an extraordinarily large number of important physiological processes. We and others previously demonstrated that pancreatic β-cells are endowed with M_3 mAChRs which are linked to G proteins of the G_q family. The activation of these receptors by ACh or other muscarinic agonists leads to the augmentation of glucose-induced insulin release via multiple mechanisms. Interestingly, in humans, ACh acting on human β-cell mAChRs is released from adjacent α-cells which express both choline acetyltransferase (ChAT) and the vesicular acetylcholine transporter (vAChT), indicative of the presence of a non-neuronal cholinergic system in human pancreatic islets. In order to shed light on the physiological roles of β-cell M_3 receptors, we recently generated and analyzed various mutant mouse models. Specifically, we carried out studies with mice which overexpressed M_3 receptors or mutant M_3 receptors in pancreatic β-cells or which selectively lacked M_3 receptors or M_3-receptor-associated proteins in pancreatic β-cells. Our findings indicate that β-cell M_3 receptors play a key role in maintaining proper insulin release and whole body glucose homeostasis and that strategies aimed at enhancing signaling through β-cell M_3 receptors may prove useful to improve β-cell function for the treatment of type 2 diabetes (T2D).

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Introduction

The proper control of insulin release from pancreatic β-cells is critical for maintaining proper blood glucose homeostasis. Insulin release is regulated by glucose and other nutrients and by many additional factors including various neurotransmitters and hormones. Like most other cell types, pancreatic β-cells express many different G protein-coupled receptors (GPCRs) which can activate different classes of heterotrimeric G proteins (Ahrén, 2009). The different G proteins are linked to distinct signaling pathways or networks which have multiple effects on β-cell function including the regulation of insulin release. For this reason, GPCRs have emerged as attractive targets for the treatment of type 2 diabetes (T2D; Ahrén, 2009). T2D represents a major threat to human health in the 21st century, primarily fueled by changes in lifestyle and diet. In T2D, pancreatic β-cells are unable to release sufficient amounts of insulin in order to overcome peripheral insulin resistance, resulting in disturbed blood glucose homeostasis. T2D can cause many severe vascular and neurological complications and accounts for a significant portion of all US health care expenditures.

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Although various antidiabetic drugs are in current clinical use, these agents are often associated with severe side effects and/or exhibit limited clinical efficacy. Thus, there is an urgent need to develop novel therapeutic strategies aimed at improving β-cell function for therapeutic purposes.

A large body of work has demonstrated that pancreatic β-cells express muscarinic acetylcholine (ACh) receptors (mAChRs) that are linked to G proteins of the G_q family (Ahrén, 2000; Gilon and Henquin, 2001; Sassmann et al., 2010). Ligand activation of these receptors facilitates glucose-induced insulin release via multiple mechanisms (see below). Several years ago, we (Duttaroy et al., 2004) and others (Zawalich et al., 2004) showed, by using islets prepared from M_3 mAChR receptor knockout (KO) mice, that the M_3 receptor subtype is responsible for mediating the stimulatory effect of ACh on insulin release.

The physiological effects mediated by β-cell M_3 receptors depend on multiple intracellular signaling pathways many of which require G_q-dependent increases in intracellular calcium levels and activation of various PKC isoforms (Ahrén, 2000; Gilon and Henquin, 2001; Sassmann et al., 2010). Gilon and Henquin (2001) summarized studies indicating that the insulinotropic effect of ACh primarily results from a rise in intracellular calcium levels ($[Ca^{2+}]_i$), together with a PKC-mediated increase in the efficiency of Ca^{2+} on exocytosis. Muscarinic stimulation of β-cells also triggers the activation of protein kinase D1 (PKD1; Sumara et al., 2009; Kong et al., 2010), a serine/threonine protein kinase that lies downstream of diacylglycerol (generated by the activation of PLC-β) and PKC signaling pathways. Two recent studies demonstrated that activation of PKD1 is required for mAChR-mediated stimulation of insulin release (Sumara et al., 2009; Kong et al., 2010). Moreover, Sumara et al. (2009) showed that the mitogen-activated protein kinase (MAPK) p38δ catalyzes an inhibitory phosphorylation of PKD1, thereby attenuating stimulated insulin secretion. A recent study using a knock-in mouse strain expressing a phosphorylation-deficient mutant M_3 mAChR suggests that arrestin-dependent signaling pathways contribute to the muscarinic stimulation of PKD1 and insulin release (Kong et al., 2010).

Interestingly, Rodriguez-Diaz et al. (2011) recently showed that the cholinergic innervation of human islets is sparse and that the α-cells of human islets provide paracrine cholinergic input to adjacent β-cells. Human α-cells were found to express both choline acetyltransferase (ChAT) and the vesicular acetylcholine transporter (vAChT), two key markers of cholinergic cells. Rodriguez-Diaz et al. (2011) also demonstrated that ACh released from α-cells sensitized β-cells in response to increasing glucose concentrations. However, in contrast to human α-cells, mouse α-cells do not seem to contain ACh (Rodriguez-Diaz et al., 2011). In this case, β-cell mAChRs are predicted to be activated by ACh released from parasympathetic nerve endings (Rodriguez-Diaz et al., 2011). However, independent of the source of ACh (non-neuronal versus neuronal), both human and mouse β-cells express mAChRs that can promote insulin release in an ACh-dependent fashion.

During the past few years, we have generated and analyzed various mutant mouse models in order to shed light on the physiological roles of β-cell M_3 receptors. These studies involved the generation of both β-cell-specific transgenic mice as well as mutant mice lacking M_3 receptors or M_3-receptor-associated proteins selectively in pancreatic β-cells. In the following chapter, we will review the key results that have emerged from these studies. These findings may pave the way for the generation of novel classes of drugs useful for the treatment of T2D.

β-Cell M_3 mAChRs are critical for maintaining proper blood glucose homeostasis

Studies with mutant mice lacking M_3 mAChRs in pancreatic β-cells

To examine the relevance of β-cell M_3 mAChRs in maintaining normal blood glucose levels in vivo, we generated mutant mice lacking M_3 receptors selectively in pancreatic β-cells (β-M_3-KO mice; Gautam et al., 2006). Consistent with studies using islets prepared from conventional M_3 receptor KO mice, muscarinic agonist-induced augmentation of glucose-dependent insulin release was greatly reduced in islets prepared from β-M_3-KO mice (Gautam et al., 2006).

In vivo studies demonstrated that β-M_3-KO mice exhibited significantly impaired glucose tolerance after oral or intraperitoneal (i.p.) administration of glucose (Gautam et al., 2006; Fig. 1). In agreement with this phenotype, β-M_3-KO mice released less insulin into the bloodstream than their control littermates following glucose administration (Gautam et al., 2006; Fig. 1). These findings clearly indicated that β-cell M_3 receptors play an important role in maintaining normal blood glucose levels in vivo.

Studies with transgenic mice overexpressing M_3 mAChRs in pancreatic β-cells

We also generated and analyzed a transgenic mouse line that overexpressed M_3 receptors selectively in their pancreatic β-cells (β-M_3-Tg mice; Gautam et al., 2006). These mutant mice exhibited a metabolic phenotype that was opposite to that observed with β-M_3-KO mice (Fig. 1). In vitro insulin release studies demonstrated that muscarinic agonist-induced stimulation of glucose-dependent insulin secretion was significantly greater in islets obtained from β-M_3-Tg mice, as compared to islets from WT littermates (Gautam et al., 2006). The β-M_3-Tg mice also showed several pronounced in vivo phenotypes including significantly reduced blood glucose levels and greatly improved glucose tolerance. Serum insulin measurements strongly suggested that these phenotypes were most likely a consequence of enhanced glucose-induced insulin release in vivo (Gautam et al., 2006). We also found that the β-M_3-Tg mice were protected against the detrimental metabolic effects caused by the chronic consumption of an energy-rich, high-fat diet, such as hyperglycemia and glucose intolerance (Gautam et al., 2006). In contrast to their WT littermates which displayed hyperglycemia and glucose intolerance, β-M_3-Tg mice showed largely normal blood glucose levels and glucose tolerance after chronic consumption of a high-fat diet (Gautam et al., 2006).

Characterization of a mouse model expressing a constitutively active mutant M_3 receptor in pancreatic β-cells

The five mAChR subtypes share a high degree of sequence homology within the transmembrane receptor core which contains the binding pocket for conventional muscarinic ligands. For this reason, attempts to develop small molecule ligands endowed with a high degree of mAChR subtype-selectivity have remained largely unsuccessful. As is the case for other mAChRs subtypes, compounds that can selectively activate the M_3 receptor are not available at present.

In an attempt to mimic the effects of a drug that can chronically activate β-cell M_3 mAChRs, we generated a mouse line that expressed a modified version of the M_3 receptor selectively in pancreatic β-cells (β-M3-Q490L Tg mice; Gautam et al., 2010). This mutant M_3 receptor

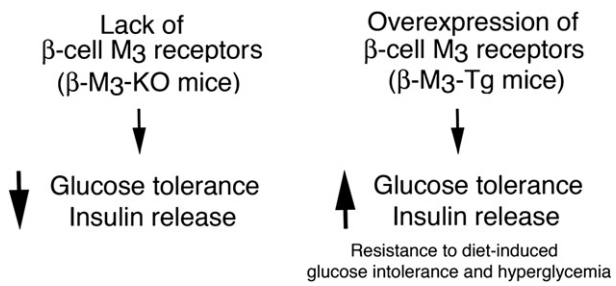

Fig. 1. Scheme summarizing the key phenotypes displayed by β-M_3-KO and β-M_3-Tg mice. See text for details (Gautam et al., 2006).

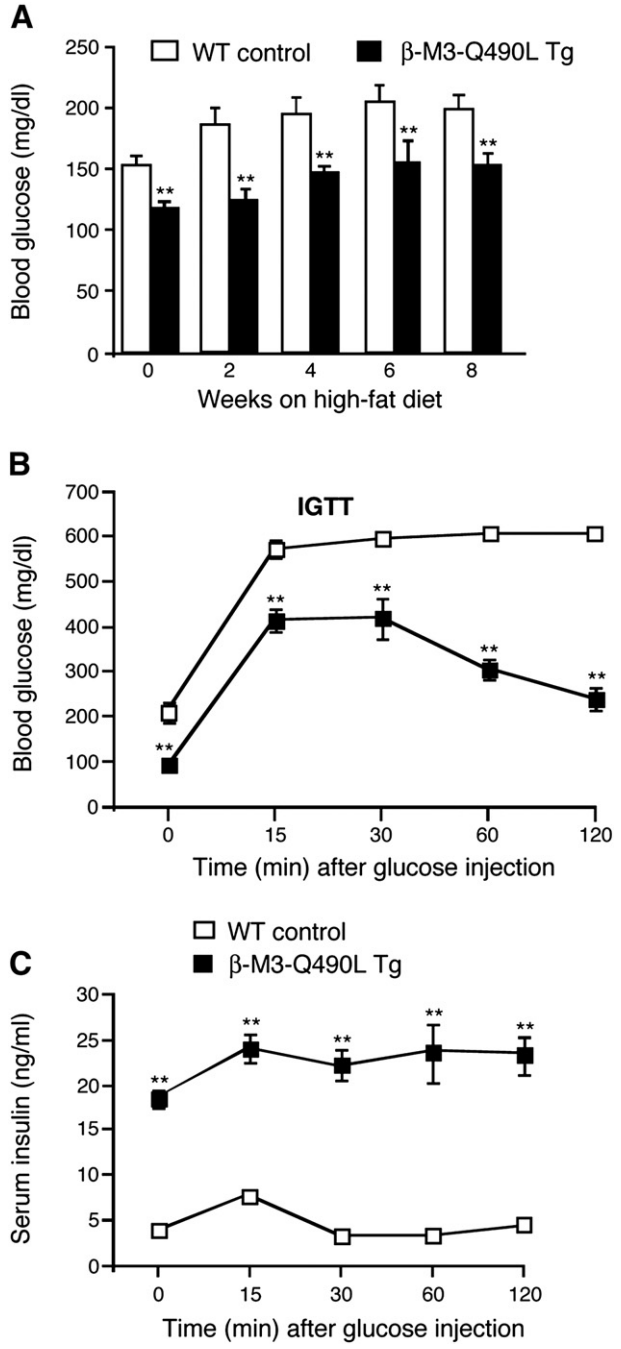

Fig. 2. β-M3-Q490L Tg mice are protected against the detriment metabolic effects associated with the consumption of a high-fat diet. All studies were carried out with male mice maintained on a high-fat diet ($n=6$ or 7 per group). (A) Blood glucose levels of freely fed β-M3-Q490L Tg mice and WT littermates. (B) I.p. glucose tolerance test (IGTT). Blood glucose levels were measured at the indicated time points following i.p. administration of glucose (2 mg/g body weight). (C) Serum insulin levels following i.p. administration of glucose (2 mg/g body weight). Data are expressed as means ± SEM. $^{**}P<0.01$, as compared to the corresponding WT value. Data were taken from Gautam et al. (2010).

harbors a single amino acid substitution (Q490L) at the cytoplasmic end of transmembrane domain 6. Studies with different experimental systems have demonstrated that this point mutation allows the M_3 receptor to activate G_q-type G proteins even in the absence of activating ligands (Schmidt et al., 2003). Such constitutively active mutant receptors have proven very useful in studying many different aspects of GPCR function.

Studies with perfused islets prepared from β-M3-Q490L Tg mice and their WT littermates confirmed that the Q490L mutant M_3 receptor showed constitutive activity in mouse β-cells (Gautam et al., 2010). In the presence of a stimulatory concentration of glucose (15 mM), islets expressing the Q490L mutant receptor released considerably more insulin than WT control islets. Importantly, this difference in glucose-induced insulin release was no longer observed following incubation of mutant islets with atropine, an inverse muscarinic agonist that is able to suppress the activity of constitutively active mutant mAChRs. These data clearly support the concept that the Q490L mutant M_3 receptor can signal in an agonist-independent fashion in mouse β-cells.

Studies with β-M3-Q490L Tg mice revealed several striking in vivo metabolic phenotypes (Gautam et al., 2010). Despite unchanged peripheral insulin sensitivity, the β-M3-Q490L Tg mice showed significantly elevated serum insulin levels. Moreover, the β-M3-Q490L Tg mice exhibited pronounced decreases in blood glucose levels (under both fed and fasting conditions) and greatly improved glucose tolerance, most likely due to enhanced insulin release.

To exclude the possibility that the changes in blood glucose and insulin levels displayed by the β-M3-Q490L Tg mice were caused by secondary changes in cell metabolism triggered by the chronic activation of G_q-type G proteins, we injected β-M3-Q490L Tg mice and their WT littermates with an acute dose of the inverse agonist, atropine. While atropine had little or no effect on blood glucose and insulin levels in WT mice, it led to increased blood glucose and decreased serum insulin levels in β-M3-Q490L Tg mice. As a result, atropine treatment abolished the differences in blood glucose levels and serum insulin levels observed with non-injected β-M3-Q490L Tg and WT mice (Gautam et al., 2010). These findings clearly indicated that the Q490L mutant M_3 receptors are also constitutively active in vivo and that this activity is responsible for the in vivo metabolic phenotypes exhibited by the β-M3-Q490L Tg mice.

As already mentioned above, β-M3-Q490L Tg mice displayed reduced blood glucose levels even under fasting conditions. This observation is in agreement with previous findings that persistent muscarinic stimulation of β-cells may sensitize β-cells to the stimulatory effects of glucose (see,

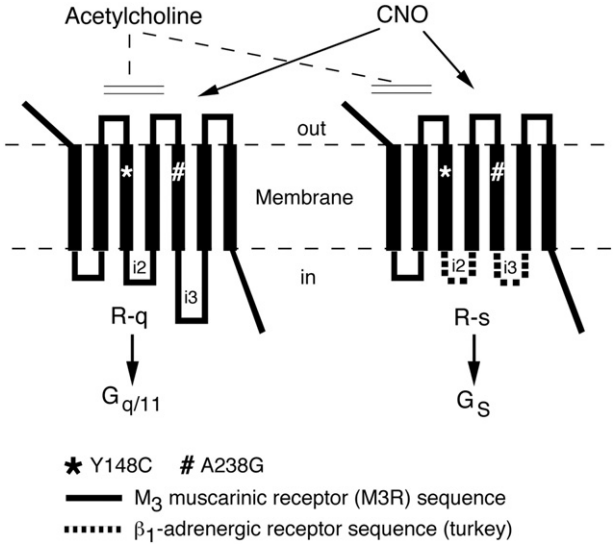

Fig. 3. Examples of ACh-insensitive mutant M_3 mAChRs that can be activated by clozapine-N-oxide (CNO). Both the R-q and R-s mutant receptors contain the Y148C and A238G point mutations (rat M_3 receptor sequence). These two point mutations prevent binding of the neurotransmitter ACh (Armbruster et al., 2007; Guettier et al., 2009). However, both R-q and R-s can be activated by CNO with high potency and efficacy (Armbruster et al., 2007; Guettier et al., 2009). CNO is a pharmacologically inert metabolite of clozapine. The two mutant receptors differ in their G protein-coupling properties (R-q: G_q-type G proteins; R-s: G_s; Guettier et al., 2009). Figure was taken from Guettier et al. (2009).

for example, Zawalich and Zawalich, 1996) or other nutrients such as fatty acids or amino acids.

High-fat diet feeding studies demonstrated that the β-M3-Q490L Tg mice, similar to the β-M_3-Tg mice, were protected against diet-induced hyperglycemia and glucose intolerance, most likely due to enhanced insulin release (Gautam et al., 2010; Fig. 2). This protective effect was observed even in aged β-M3-Q490L Tg mice (>1 year old; D. Gautam and J. Wess, unpublished observations). These findings indicate that the chronic activity of β-cell Q490L M_3 receptors is not associated with a gradual loss of M_3 receptor function due to counter-regulatory cellular events including M_3 receptor desensitization. However, it remains to be seen whether the same holds true for chronic activation of WT β-cell M_3 receptors, either by endogenous ACh or by exogenously administered drugs.

It should be pointed out that the β-M3-Q490L Tg mice, similar to the other transgenic mouse lines reviewed in this chapter, still express endogenous β-cell M_3 mAChRs. Thus, we cannot exclude the possibility that some of the phenotypes that we observed with the various β-cell-specific transgenic mouse strains were modulated, at least to some extent, by the activity of the endogenous WT M_3 mAChR population.

Taken together, studies with β-cell-specific M_3 receptor mutant mice strongly support the concept that β-cell M_3 mAChRs or components of downstream signaling pathways represent attractive targets for stimulating β-cell function for therapeutic purposes.

A novel chemical-genetic approach to study M_3 receptor/G_q-mediated regulation of β-cell function in vivo

Recently, mutant mAChRs have been described that are no longer able to bind the endogenous ligand, ACh, but can be activated by a compound called clozapine-N-oxide (CNO; Armbruster et al., 2007; Guettier et al., 2009). CNO is a pharmacologically inert metabolite of the antipsychotic drug, clozapine. A characteristic feature of these CNO-sensitive mutant mAChRs is that they contain two point mutations in the transmembrane receptor core (corresponding to Y148C and A238G in the rat M_3 receptor; Fig. 3). Armbruster et al. (2007) introduced the term DREADD (designer receptor exclusively activated by designer drugs) to describe this new class of mutant mAChRs. However, these receptors have also been referred to as RASSLs (receptors activated solely by synthetic ligands), a term first coined by Conklin and colleagues (2008). Importantly, expression of these receptors in a cell-type specific fashion in transgenic mice allows the activation of distinct G protein signaling pathways in a drug- and cell type-specific fashion in vivo. Fig. 3 shows the structures of two M_3 receptor-based DREADDs (RASSLs) that are endowed with different G protein-coupling properties (Guettier et al., 2009).

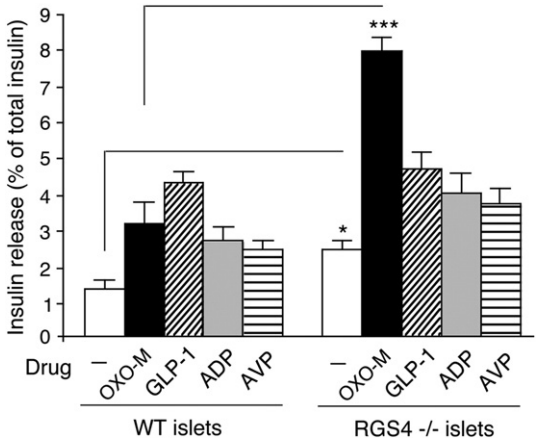

Fig. 4. RGS4 deficiency results in the selective enhancement of M_3 receptor-mediated increases in insulin secretion. Insulin release experiments were carried out with isolated pancreatic islets prepared from WT and RGS4-deficient mice, as described by Ruiz de Azua et al. (2010). Islets were incubated in the presence of a stimulatory concentration of glucose (16.7 mM) in the absence of ligands or in the presence of oxotremorine-M (OXO-M, 0.5 μM), glucagon-like peptide 1 (GLP-1, 10 nM), ADP (100 μM), or arginine vasopressin (AVP, 100 nM). These ligands are known to promote insulin secretion via activation of specific β-cell GPCRs. OXO-M stimulates the release of insulin from pancreatic β-cells via activation of M_3 mAChRs (Duttaroy et al., 2004; Zawalich et al., 2004). Data are expressed as means ± SEM of three independent experiments, each carried out in triplicate. *$P<0.05$, ***$P<0.001$, as compared to the corresponding WT value. Data were taken from Ruiz de Azua et al. (2010).

We recently characterized a transgenic mouse line that expressed a CNO-sensitive mutant rat M_3 mAChR (containing the Y148C and A238G point mutations) selectively in pancreatic β-cells (Guettier et al., 2009). In vitro studies with cultured cells had demonstrated that this mutant receptor (R-q), when treated with CNO, selectively activated G proteins of the G_q family (Armbruster et al., 2007; Guettier et al., 2009; Fig. 3). The resulting mutant mice (referred to as β-R-q Tg mice below) showed a series of interesting metabolic phenotypes that are summarized in the following paragraphs.

CNO treatment of β-R-q mice resulted in dose-dependent reductions in blood glucose levels, most probably due to CNO-induced increases in plasma insulin concentrations (Guettier et al., 2009). Thus, the use of this system makes it possible to modulate the extent of G_q signaling in pancreatic β-cells in vivo by simply varying the administered CNO dose. When treated with CNO, β-R-q Tg mice also displayed a pronounced increase in first-phase insulin release, followed by a more prominent long-lasting second phase of insulin secretion, as compared to WT littermates. Considerable evidence suggests that first-phase insulin release is critical for post-prandial glucose homeostasis and impairments in this early insulin response may be indicative of β-cell dysfunction in the early stages of T2D (Del Prato et al., 2002). Our finding that activation of β-cell G_q signaling greatly promoted first-phase insulin release is therefore of considerable clinical relevance.

We also demonstrated that CNO-dependent activation of β-cell G_q signaling in β-R-q Tg mice resulted in a dramatic improvement in glucose tolerance (Guettier et al., 2009). This effect was observed with mice maintained on regular mouse chow or a high-fat diet. We found that glucose treatment of β-R-q Tg mice resulted in greatly enhanced increases in plasma insulin levels, as compared to WT littermates. This observation indicated that activation of β-cell G_q signaling leads to improved glucose homeostasis by stimulating the release of insulin from pancreatic β-cells.

Strikingly, chronic treatment of β-R-q Tg mice with CNO resulted in a significant increase in β-cell mass, associated with an increase in mean islet size and β-cell hypertrophy (Guettier et al., 2009). This finding convincingly demonstrated that persistent activation of β-cell G_q signaling affects signaling pathways that exert a stimulatory effect on β-cell mass. To obtain clues regarding the molecular mechanisms underlying these changes in islet morphology, we studied the expression of a series of genes important for β-cell function and growth. For these studies, cDNA was generated from total islet RNA prepared from β-R-q Tg mice and WT littermates treated with CNO for 4 weeks (1 mg/kg i.p. per day). Real-time qRT-PCR studies demonstrated that the expression of several genes critical for β-cell function and growth was significantly increased in samples obtained from the CNO-treated β-R-q Tg mice. For example, we found that preproinsulin (*Ins2*) and proprotein convertase 1 and 2 transcript levels were elevated in this group of mice, suggesting that chronic activation of β-cell G_q signaling promotes insulin synthesis (S. Jain and J. Wess, unpublished results). However, CNO treatment of the β-R-q Tg mice also selectively increased the expression of several other genes important for β-cell function, such as GLUT2, pyruvate carboxylase, and IRS2 (S. Jain and J. Wess, unpublished results). Previous studies have shown that IRS-2 plays a key role in maintaining normal β-cell function and β-cell mass (White, 2006). On the basis of these findings, we are currently studying whether IRS-2 acts as a signaling molecule that links activation of β-cell G_q signaling to increased β-cell mass. Clearly, more detailed studies are

needed to delineate the molecular pathways regulating β-cell mass in response to enhanced G_q signaling. Moreover, the use of β-R-q Tg mice will allow us to investigate whether drug (CNO)-dependent activation of G_q signaling in pancreatic β-cells has beneficial effects on whole body glucose homeostasis in various mouse models of diabetes.

Studies with β-R-s Tg mice (Fig. 3) demonstrated that activation of β-cell G_s also improves β-cell function and triggers an increase in β-cell mass (Guettier et al., 2009), consistent with published reports (Doyle and Egan, 2007; Baggio and Drucker, 2007; Ahrén, 2009). In general, the β-R-q Tg mice showed more pronounced in vivo phenotypes than the β-R-s Tg mice. However, a direct comparison between the two different mutant mouse strains is complicated by the fact that the R-s designer receptor showed some degree of agonist-independent signaling which may have triggered counter-regulatory responses in the β-R-s Tg mice (Guettier et al., 2009).

To the best of our knowledge, the study by Guettier et al. (2009) provided the first piece of direct evidence that chronic activation of β-cell G_q signaling can enhance β-cell mass. In contrast, previous studies have already established that stimulation of β-cell G_s promotes β-cell proliferation (Doyle and Egan, 2007; Baggio and Drucker, 2007; Ahrén, 2009).

In summary, studies with β-R-q and β-R-s Tg mice strongly suggest that compounds that can promote signaling through β-cell G_q or G_s should prove beneficial in the treatment of T2D. In fact, drugs that stimulate signaling through G_s-linked GLP-1 receptors, which are expressed by β-cells as well as other cell types, have been approved for the treatment of T2D recently (Doyle and Egan, 2007; Baggio and Drucker, 2007; Ahrén, 2009). We would also like to note that the approach described here should be of broad general usefulness to study the in vivo roles of distinct G protein signaling pathways in specific cell types.

RGS4 as a potent negative regulator of β-cell M_3 receptor signaling

As outlined above, the activity of β-cell M_3 mAChRs plays an important role in maintaining proper whole body glucose homeostasis. At present, little is known about the cellular pathways that modulate M_3 receptor function in pancreatic β-cells. However, a better understanding of these processes may potentially reveal novel targets for enhancing β-cell M_3 receptor activity for therapeutic purposes.

In agreement with studies with other GPCRs, agonist stimulation of the M_3 receptor triggers several cellular events aimed at inhibiting or terminating M_3 receptor signaling. Protein kinases, including PKC and different GRKs (GPCR kinases), are known to play central roles in these processes (Luo et al., 2008; Tobin et al., 2008). Moreover, distinct members of the superfamily of regulators of G protein signaling (RGS proteins) act as GTPase-activating proteins (GAPs) to greatly accelerate the rate of Gα-GTP hydrolysis, thus limiting the lifetime of active Gα-GTP G protein subunits. RGS proteins represent a large protein family consisting of more than 30 different members in mammals (Ross and Wilkie, 2000; Hollinger and Hepler, 2002).

Since the role of RGS proteins in regulating GPCR function in β-cells remained unknown, we recently carried out a study to identify RGS proteins that modulate M_3 receptor function in pancreatic β-cells (Ruiz de Azua et al., 2010). We initially used MIN6 mouse insulinoma cells as an in vitro model system. We demonstrated that MIN6 cells almost exclusively express the M_3 receptor subtype and that stimulation of these cells with a muscarinic agonist (oxotremorine-M or short OXO-M) resulted in a pronounced increase in insulin release (Ruiz de Azua et al., 2010). Interestingly, real-time qRT-PCR studies demonstrated that RGS4 mRNA was by far the most abundant RGS transcript that could be detected in MIN6 cells (Ruiz de Azua et al., 2010). RGS4 was also found to be highly expressed in mouse islets. We next showed that siRNA-mediated knockdown of RGS4 expression in MIN6 cells led to pronounced increases in OXO-M-stimulated elevations in $[Ca^{2+}]_i$ and OXO-M-induced insulin secretion (Ruiz de Azua et al., 2010), indicating that RGS4 inhibits M_3 receptor function in this β-cell line.

To examine whether RGS4 exerts a similar effect in pancreatic islets, we performed insulin secretion studies using isolated islets prepared from RGS4-deficient mice (RGS4 KO mice; Ruiz de Azua et al., 2010). Consistent with the results that we obtained with cultured MIN6 cells, we found that OXO-M treatment of RGS4-deficient islets led to a significant augmentation of glucose-induced insulin secretion, as compared to islets prepared from WT littermates (Ruiz de Azua et al., 2010; Fig. 4). These findings further supported the concept that RGS4 acts as a potent negative regulator of M_3 receptor function in pancreatic β-cells. In contrast, additional studies with both MIN6 cells and islets prepared from RGS4 KO mice demonstrated that RGS4 deficiency had little or no effect on insulin release stimulated by the activation of other β-cell G_q- or G_s-coupled receptors (Ruiz de Azua et al., 2010; Fig. 4). At present, the molecular basis underlying the selectivity of RGS4 in inhibiting β-cell M_3 receptor signaling remains unclear. However, accumulating evidence indicates that GPCR/RGS signaling complexes contain additional signaling or scaffolding proteins, including spinophilin, 14-3-3 proteins, or Ca^{2+}/calmodulin (Abramow-Newerly et al., 2006). It is therefore tempting to speculate that the observed selectivity of RGS4 in regulating β-cell M_3 receptor activity may depend on the selective interaction of the M_3 receptor with specific components of the RGS4 signaling complex including RGS4 itself.

RGS4 is not only expressed by pancreatic β-cells but is also found in other peripheral tissues or cell types and in different parts of the brain (Bansal et al., 2007; Ruiz de Azua et al., 2010). Thus, in order to be able to examine the effects of RGS4 regulation of β-cell M_3 receptor activity in vivo, we employed Cre/loxP technology to generate mutant mice that selectively lacked RGS4 in pancreatic β-cells (β-RGS4-KO mice; Ruiz de Azua et al., 2010). Under basal conditions, β-RGS4-KO mice did not show any obvious metabolic phenotype. We therefore injected β-RGS4-KO mice and their WT littermates with bethanechol, a peripherally acting muscarinic agonist, and then monitored the resulting changes in blood glucose and insulin levels. Consistent with previous work (Fukudo et al., 1989), we found that bethanechol treatment of WT mice stimulated insulin secretion, resulting in a moderate decrease in blood glucose levels (Ruiz de Azua et al., 2010). These effects were absent in mutant mice selectively lacking M_3 receptors in β-cells (β-M_3-KO mice), strongly suggesting that bethanechol acts on β-cell M_3 receptors to promote insulin release in WT mice (Ruiz de Azua et al., 2010). Interestingly, bethanechol treatment of β-RGS4-KO mice resulted in significantly enhanced increases in insulin secretion and more robust reductions in blood glucose levels, as compared with control littermates (Ruiz de Azua et al., 2010). This observation strongly supports the concept that RGS4 also acts as a potent negative regulator of β-cell M_3 receptor activity in vivo.

As discussed in the past, RGS proteins represent attractive new targets for drug development (Neubig and Siderovski, 2002). Our findings raise the possibility that peripherally acting RGS4 inhibitors may prove useful for the treatment of T2D by enhancing signaling through β-cell M_3 receptors. Clearly, additional experiments are required to further explore the feasibility of this approach.

Concluding remarks

Studies with M_3 mAChR mutant mice strongly suggest that strategies aimed at enhancing signaling through β-cell M_3 receptor may become useful in the treatment of T2D by promoting insulin release and improving β-cell function in general. Since M_3 receptors are selectively coupled to G proteins of the G_q family, the findings summarized in this chapter may also be relevant for other β-cell GPCRs that are linked to this class of G proteins, such as the fatty acid receptor GPR40 or different peptide GPCRs (Ahrén, 2009). Moreover,

our findings suggest that proteins that regulate the activity of β-cell M_3 receptors may also represent potential novel drug targets.

Recently, considerable progress has been made in developing drugs that selectively stimulate M_1 or M_4 receptors via targeting allosteric receptor sites (Conn et al., 2009). Given these advances in the development of mAChR subtype-selective agents, together with the recent availability of a high-resolution X-structure of the M_3 receptor (Kruse et al., 2012), it should also be possible to obtain allosteric agonists selective for the M_3 receptor. Obviously, such agents will also stimulate non-β-cell M_3 receptors (e.g. M_3 receptors expressed by smooth muscle or glandular tissues). However, since β-cell M_3 receptors appear very efficiently coupled to downstream signaling pathways, the possibility exists that administration of low doses of an M_3 receptor agonist could improve glucose homeostasis without causing major side effects.

Conflict of interest statement

The authors declared no conflicts of interest.

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