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Cross-talk between insulin signaling and GPCRs

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Summary

Diabetes is a major risk factor for the development of heart failure. One of the hallmarks of diabetes is insulin resistance associated with hyperinsulinemia. The literature shows that insulin and adrenergic signaling is intimately linked to each other; however, whether and how insulin may modulate cardiac adrenergic signaling and cardiac function remains unknown. Notably, recent studies have revealed that insulin receptor and β_2 adrenergic receptor (β_2AR) forms a membrane complex in animal hearts, bringing together the direct contact between two receptor signaling systems, and forming an integrated and dynamic network. Moreover, insulin can drive cardiac adrenergic desensitization via PKA and GRK phosphorylation of the β_2AR, which compromises adrenergic regulation of cardiac contractile function. In this review, we will explore the current state of knowledge linking insulin and GPCR signaling, especially βAR signaling in the heart, with emphasis on molecular insights regarding its role in diabetic cardiomyopathy (DCM).

Introduction

Diabetes is a major risk factor for development of heart failure (HF) [1]. The Framingham study shows that independent of age, obesity, or dyslipidemia, diabetes alone is linked with a two-fold increased risk of HF in men and a five-fold increased risk in women [2]. There is evidence that diabetes itself is an independent significant predictor of poor outcomes in HF [3, 4]. The association between diabetes and cardiac dysfunction in humans is also supported by data from animal models, which demonstrate distinct diabetes-induced cardiac structural, metabolic, and functional changes, as well as changes in vascular reactivity [5, 6]. The impact of generalized type 2 diabetes mellitus (T2DM) metabolic disorders (including insulin resistance, reduced glucose clearance and increased circulating fatty acids) on the cardiovascular system is complex. A common feature of T2DM is hyperinsulinemia, which results from a chronically insulin-resistant state [7]. Insulin controls a wide variety of biological processes by acting on its receptor tyrosine kinase (RTK). Receptor activation initiates a cascade of phosphorylation events leading to the activation of enzymes that control many aspects of metabolism and growth [8]. In the heart, insulin regulates various physiological and pathophysiological functions, including myocardial energy metabolism, contractility, protein expression, growth and hypertrophy, and ion transport [9, 10]. However,

the impact of insulin on the development of DM-associated cardiomyopathy is not yet well defined.

Cardiovascular homeostasis is regulated by a diverse array of hormones and neurotransmitters, many of which exert their physiological effects through activation of G protein-coupled receptors (GPCRs). Activation of GPCRs initiates cascades of signaling pathways, which modulate critical functional parameters such as heart rate, contractility, vascular tone, and blood volume, and promotes cardiac hypertrophy by phosphorylation of transcriptional factors and changes in gene expression. Thus, abnormal GPCR signaling is a common feature of many chronic cardiovascular pathologies such as hypertension [11], HF [12, 13], and cardiomyopathy [14], making GPCRs an important target for cardiac drug therapy.

GPCRs and RTKs are transmembrane receptors that initiate two major intracellular signaling cascades in response to a diverse array of ligands. Recent studies have shown several different styles of crosstalk between GPCR- and RTK-initiated pathways, with GPCRs or components of GPCR-induced pathways being either upstream or downstream of RTKs [15–18]. GPCRs that are reported to be regulated by RTKs include angiotensin [19], endothelin [16], thrombin [17], α_1-adrenergic [20] and β_2-adrenergic receptors [15, 18]. RTKs use several components of GPCR signaling, such as β-arrestin, G protein-receptor kinases (GRKs), and regulator of G protein signaling, causing integration of the signaling pathways [21]. For example, insulin has been shown to induce tissue RAS activation directly [22] and to cause increases in the AT1 receptor expression in cultured vascular cells [23]. Earlier studies revealed that insulin counter-regulates the action of β-adrenergic catecholamine stimulation, at a point proximal to β-adrenergic receptors (βARs) [24]. Yu et al reports that insulin attenuates the contractile response to βAR stimulation and suppresses isoproterenol (ISO)-induced cardiac dysfunction and cell injury in myocardial ischemia/reperfusion (I/R) [25]. Phosphatidylinositol 3-kinase (PI3K), a downstream kinase in the insulin signaling pathway, inhibits βAR stimulation–induced contractile responses in isolated cardiomyocytes [26] and confines and negates the concurrent β_2AR/Gs-mediated protein kinase A (PKA) signaling [27]. Experimentally-induced diabetes increases α_1-adrenergic sensitivity of rat atria possibly due to an increased receptor affinity, but these changes can be reversed with insulin treatment [28]. Insulin also induces concentration-dependent increases in the phosphorylation state of the α_{1B}-ARs in Rat-1 cells. The cells stimulated by insulin show loss of function and increased α_{1B}-AR phosphorylation and internalization, suggesting that GPCRs themselves may act as substrates for the insulin receptor (InsR) [29]. These studies indicate that the mechanism(s) by which insulin affects GPCR signaling remain poorly understood. Understanding the molecular details of insulin regulation of GPCR signaling pathways in the cardiovascular system has become particularly important to understand the mechanism of diabetic cardiomyopathy. In this review, we will explore the current state of knowledge linking insulin and GPCRs signaling, especially βAR signaling in the heart, with emphasis on molecular insights regarding its role in DCM (See Table 1 for summary).

Insulin regulates βAR signaling

Biochemically, InsRs and βARs induce heterologous signal transduction pathways leading to divergent and sometimes opposing cellular processes. InsRs, which belong to RTK family, phosphorylate insulin receptor substrates (IRS1/2) leading to two major signaling branches, the Grb2-depdendent mitogen-activated protein kinase (MAPK) activation and PI3K-dependent Akt activation. The IRS1-induced and PI3K-mediated Akt activity promotes GLUT4 translocation in cardiac and skeletal muscle cells, therefore increasing glucose uptake and oxidation. The insulin-induced Akt activity promotes cardiac hypertrophy [30, 31]. Ligand binding to βARs, which are prototypical members of the GPCR superfamily, induces cAMP-dependent PKA activation and phosphorylation of various substrates such as phospholamban (PLB) leading to increased activities of SERCA2 and RyR2 [32–34]. Enhanced SERCA and RyR2 activities increase myocyte contractility, stroke volume, and cardiac output [34]. Studies in adipocytes and liver cells have suggested that insulin and adrenergic stimulation act reciprocally to blunt each other's signaling [35]. We have recently published evidence showing that insulin impairs myocardial contractility via a mechanism of inducing a Gi-biased β_2-adrenergic signal that inhibits cAMP generation and cardiac contractility induced by βAR stimulation [36] (Figure 1). Since downstream effector pathways for both InsR and GPCRs share common points/nodes that are essential to regulation of cell proliferation, differentiation and metabolism, the counter-regulation of insulin on βARs could happen at multiple levels of each pathway/cascade involving distinct molecules (e.g. receptors, effectors, adaptors, and scaffolds). Notably, recent studies reveal that InsR and β_2AR forms a membrane complex in animal hearts, bringing together the direct contact between two receptor signaling systems, forming an integrated and dynamic network [36].

1. βAR expression and density

In cardiac tissues, there are three pharmacologically distinct subtypes of βARs. β_1 and β_2AR stimulate adenylyl cyclase activity via Gs protein to produce cAMP, which binds to and activates PKA. This leads to phosphorylation of a number of targets including phospholamban (PLB) [37], troponin-I (TnI) [38], and L-type Ca^{2+} channel [39], all being responsible for cardiac contractile function. In healthy hearts and cardiomyocytes, β_1ARs are responsible for catecholamine-induced increase in inotropy and lusitropy, while β_2AR-specific ligands do not affect inotropy or lusitropy [32, 33, 36, 40, 41]. In the same light, β_1AR-specific agonists promote phosphorylation of L-type Ca^{2+} channel [39], PLB [37], ryanodine receptor 2 [42], and TnI [38] while β_2AR-specific agonists only promote phosphorylation of L-type Ca^{2+} channel [43, 44] in healthy cardiomyocytes. Fluorescent resonant energy transfer (FRET) analysis of cAMP and PKA activity levels show that β_1AR-specific stimulation produces robust signals, while β_2AR-specific stimulation only produces small localized signals [36, 45–47]. Unlike β_1 and β_2AR, β_3AR decreases the contractile force in human ventricular muscle through activation of Gi protein and NO synthase signaling pathways [48, 49]. The activation of β_3AR signaling involving both cAMP- and NO-dependent pathways may be a mechanism of preventing the overstimulation of heart muscle by catecholamine.

While β_1AR is the predominant adrenergic receptor isoform for increasing excitation-contraction coupling in healthy hearts, a diabetic state can lead to drastic changes in adrenergic signaling. In hearts from STZ-induced type 1 diabetes mellitus (T1DM) rats, one of the typical features observed is the decreased inotropic and chronotropic responses to βAR stimulation [50–52]. Accordingly, the mRNA and protein levels of β_1AR in the hearts of STZ-induced diabetic rats and their function in promoting heart rate and contractility are decreased; and these alterations progress along with duration of DM [53, 54]. The reduction in myocardial β_1AR numbers and mRNA is prevented by insulin therapy [54–56]. Interestingly, in the same diabetic model, while the β_2AR mRNA level in the heart is increased, the density of β_2AR protein however is decreased [54]. This could be due to an increased rate of β_2AR degradation and/or decreased posttranslational modifications that prevent the maturation of the receptor along the secretory pathway [54]. Meanwhile, the mRNA and protein level of cardiac β_3AR in 14-week STZ DM rats are twice of that in controls [54]. The estimated ratio of β_1, β_2 and β_3AR proteins is approximately 62: 30: 8 and 40: 36: 23 in the heart of control and diabetic rats, respectively; and a two-week insulin treatment of diabetic animals restored the ratio to 57: 33: 10 [54]. These observations support a notion that the abnormality of cardiac response to catecholamines, especially in the case of T1DM, is due to the decrease in β_1AR densities. However, controversial results have been observed in experimental T2DM. In T2DM rats, the responsiveness of the myocardium to βAR stimulation is decreased; however unlike in the T1DM rats, the number of βARs in T2DM rat hearts does not change significantly from the controls [52, 57]. The results indicate that alterations in the contractile parameters and β-adrenergic responses in T2DM cardiomyocytes differ from those observed in T1DM cardiomyocytes. Thus, whether altered cardiac βAR expression is truly responsible for the decreased functional response in diabetic heart is not certain, and the underlying mechanism of insulin-induced restoration of βAR expression in T1DM needs to be further validated. An important question raised in these studies is: what is the contribution of insulin signaling *per se* on βAR expression and the potential contribution of these changes in the hearts of T1DM and T2DM? What is the role(s) of the newly discovered InsR-β_2AR complex in the alterations of βARs in the hearts?

Meanwhile, it is well known that βAR internalizes upon stimulation [58]; and βARs can be redistributed between the plasma membrane and the intracellular compartments [59]. The study in T1DM rats indicates that only the cell surface βAR number decreases [59], which is closely associated with the diabetic state and is reversed by short-term insulin treatment [59]. Thus, the insulin-regulated βAR redistribution may play differential roles in the progress of cardiomyopathy in T1DM and T2DM. The complexities of βAR redistribution regulated by insulin in DCM will be further discussed in the section on Receptor Trafficking.

2. Insulin-induced βAR phosphorylation

Agonist-induced phosphorylation of GPCRs at multiple sites by different kinases is a complex process which results in various signal outcomes [60]. In the case of βARs, studies have shown that both PKA [61] and GRK2 [62] mediate receptor phosphorylation, but they vary in terms of mechanisms of activation, phosphorylation sites, and kinetics [63].

2.1 Insulin-induced PKA phosphorylation of β_2AR switches G-protein coupling

Stimulation of both β_1 and β_2ARs leads to increase G_s-mediated adenylyl cyclase activity and a subsequent increase in intracellular cAMP [64, 65]. The cAMP-dependent-activation of PKA phosphorylates both agonist-occupied and agonist-free receptors at sites within the third intracellular loops and C-terminal tails, which decreases the affinity of the receptor for G_s. For β_2AR, these phosphorylation events increase affinity for G_i [66]. The switch of G protein binding affinity leads to inhibition of adenylyl cyclase and increased MAPK activation including the extracellular-regulated kinases p44 and p42 (ERK1/2), therefore causing β_2AR to have a different signaling profile from β_1AR [66–68].

Recently, our group reported that in cardiomyocytes, insulin, like catecholamine, promotes PKA-mediated phosphorylation of β_2AR at serine 261/262 sites. The phosphorylation of β_2AR by PKA switches the receptor coupling from G_s to G_i protein, leading to an insulin-induced inhibitory effect on β_2AR signal cascade [36] (Figure 1). Mechanistically, how insulin promotes PKA activity remains unclear. One possibility is that InsR undergoes autophosphorylation and activates IRS1/2 for subsequent activation of RAS/RAF/MEK/ERK1/2 signaling cascade [69]. ERK can phosphorylate a consensus motif RXSP within the catalytic domains of PDE4B, PDE4C, and PDE4D [70]. ERK phosphorylation of the long form PDE4D3 reduces cAMP-hydrolytic activity (50% inhibition), which in turn leads to increased levels of cAMP for PKA activation. In cardiomyocytes, only the long PDE4D isoforms bind to β_2AR [71], indicating a potential role of these long isoforms in insulin-induced PKA phosphorylation of β_2AR in hearts.

2.2 Insulin-induced GRK2 phosphorylation of β_2AR promotes β-arrestin recruitment and receptor internalization

While PKA phosphorylation of β_2AR is sensitive to low levels of agonist stimulation, at a high occupancy of agonist, β_2AR is also phosphorylated by GRK at distinct sites [72–74]. Recent studies suggest that GRK2 is emerging as a physiologically negative modulator of insulin signaling in skeletal muscle and adipose tissue, as hemizygous GRK2 mice are protected from insulin resistance in multiple model systems [75]. Increased GRK2 abundance is observed in human hearts with metabolic syndrome and in different murine models of insulin resistance [76]. GRK2 can even be used as a biomarker that is consistently upregulated in human HF and animal models of HF. Genetically inhibiting GRK2 leads to cardiac protection in mice [77]. Higher levels of GRK2 can impair βAR-mediated inotropic reserve; its inhibition or molecular reduction improves pump function in animal models including a preclinical pig model of HF [78]. A recent study indicates that insulin can promote formation of IRS-GRK2 complexes in animal hearts [79]. However, the molecular basis underlying the deleterious effects of GRK2 in diabetic cardiomyopathy is not fully understood.

Previous study shows that GRK2-mediated phosphorylation of the β_2AR is necessary for subsequent internalization in cardiomyocytes [80], which contributes to compartmentalization of the G_s-stimulated cAMP signal, and selectively affects cardiac contractile [81]. In a recent study, we demonstrate that GRK2 may represent a molecular link between the InsR and βAR signaling for regulation of cardiac function (Figure 2).

Insulin impairs cAMP generation induced by isoproterenol in myocytes expressing wild-type β_2AR, but not in the cells expressing mutant β_2AR that lacks the GRK phosphorylation sites. Pharmacological inhibition of GRK2 abolishes the insulin-induced phosphorylation of β_2ARs at GRK sites and the insulin-induced impairment of β-adrenergic stimulation of cAMP signaling and myocyte contractility [36]. GRK-phosphorylation also triggers the binding of arrestins to the activated receptors, which impairs G protein coupling to GPCRs such as β_2AR in a process known as desensitization [82]. In contrast, phosphorylation of the β_2AR at PKA/protein kinase C (PKC) sites alone *in vitro* is not sufficient to promote arrestin recruitment [83]. Thus, signaling consequences of these phosphorylation events have overlapping features (e.g. they both result in receptor desensitization), as well as unique properties (e.g. phosphorylation by GRK, but not PKA, mediates arrestin recruitment and receptor internalization) [63, 84, 85].

Mechanistically, insulin promotes GRK phosphorylation of β_2AR via two possible pathways. Insulin stimulation leads to activation of G_i protein [86], a process releasing Gβγ subunits, which is required for GRK activation by recruiting cytosolic GRKs to the plasma membrane near the β_2ARs. Alternatively, GRK2 binds to IRS1 via its C-tail [79]. Thus, the activated InsR recruits IRS1-GRK2 to the complex of InsR and β_2AR; and then GRK2 can phosphorylate the substrates onsite. Deletion of IRS2 disrupts the complex of InsR and GRK2, which attenuates insulin-induced β_2AR phosphorylation at the GRK sites and the counter-regulation effects of insulin on βAR signaling in mouse embryonic fibroblasts (MEFs) [41]. These studies are consistent with the observations showing that knocking down IRS1/2 abolishes insulin-induced phosphorylation of β_2AR at both PKA sites and GRK sites [36].

2.3 Insulin-induced src and Akt phosphorylation of β_2AR

Although a majority of GPCR phosphorylation happens in serine/threonine-rich regions, it also happens on tyrosine residues [87–89]. Studies show that tyrosine 350/354 and 364 in the cytoplasmic region of β_2AR are primary targets of tyrosine kinases including InsR and insulin-like receptor [15]. Insulin stimulation increases β_2AR phosphorylation on tyrosine [24], which augments the action of insulin through promoting internalization of the β_2AR [18, 89, 90]. Likewise, stimulation with insulin-like growth factor-1 (IGF-1), a hormone with similar molecular structure to insulin, promotes phosphorylation of β_2AR *in vivo* predominantly on tyrosine 132/141 sites. IGF-1 also blocks βAR agonist action [18]. Meanwhile, stimulation of InsR promotes PI3K-mediated activation of Akt/PKB, which is able to directly phosphorylate β_2AR at serine 345/346 on the C-terminal tail [91]. This Akt-mediated phosphorylation is required for the inhibitory effect of insulin on β-adrenergic stimulated cAMP accumulation in CHO clones expressing wild type β_2AR; expressing either double mutation in β_2AR (S345A/S346A) or the dominant-negative version of Akt (K179A/T308A/S473A) abolishes insulin-induced inhibition of the cAMP response [91]. This ability of Akt/PKB to phosphorylate β_2AR is also obligate on insulin-induced tyrosine 350 phosphorylation in the receptor [91]. The phosphorylated tyrosine 350 creates an SH2 (src homology domain 2)-binding motif to promote the association of c-Src, Grb2 and dynamin [87, 89, 92]. The consequent binding of the adaptor molecule Grb2 to the phosphorylated β_2AR leads to disruption of receptor-G-protein coupling [93]. Therefore, the

phosphorylation of β_2AR at tyrosine 350 effectively precludes the β_2AR from coupling to G_s protein and further activation of adenylyl cyclase for cAMP generation. Mutation of tyrosine 350/354 to phenylalanine abolishes the ability of insulin to counter-regulate β-agonist stimulation of cAMP accumulation [90]. In addition, through a phosphorylated tyrosine-mediated binding to Grb2, the β_2AR acts as a scaffold to promote signal pathways such as MAPK in the absence of its own ligand [94]. The binding to dynamin can also promote β_2AR endocytosis [89, 95]. Currently, the studies on tyrosine phosphorylation are mostly done in DDT1-MF2 smooth muscle cells, human epidermoid carcinoma cells (A431) and Chinese hamster ovary (CHO) cells. The information of tyrosine phosphorylation of β_2AR in cardiac tissues remains unclear and awaits further determination.

3. Insulin-induced βAR trafficking and internalization

Receptor phosphorylation and internalization are prominent features of agonist-induced desensitization of GRCRs. There are two types of desensitization based on the nature and types of kinases involved: homologous desensitization and heterologous desensitization. Chronic use of βAR agonists provokes homologous desensitization that limits the therapeutic use of the agonists [96]. Meanwhile, GPCRs can be subjected to heterologous desensitization when a different membrane receptor is stimulated. This heterologous stimulation can be mediated by another GPCR, an RTK, or a cytokine receptor. Indeed, insulin has been shown to induce desensitization and sequestration of βAR in a way similar to βAR agonists [96]. Earlier data show that *in vitro* incubation with insulin results in a reduction of the sensitivity of cardiac tissues to isoproterenol without altering total βAR number [97]. In rat adipocytes, insulin induces an acute reduction in the ligand binding capacity of βARs, due to a rapid and dose-dependent translocation of βARs to the interior of the cell [98]. Chronic insulin treatment also promotes β-arrestin-1 degradation, which is associated with decreased βAR agonist-mediated MAP kinase signaling [99]. Consistently, injection of insulin to healthy male subjects induces a reduction in β-adrenergic sensitivity [100], which supports a hypothesis that reduced β-adrenergic sensitivity is an important pathophysiological mechanism in hypoglycemia in T1DM [100].

Mechanistically, Grb2 contains one SH2 domain and two SRC homology 3 (SH3) domains. After insulin stimulation, Grb2 can bind directly to β_2AR via SH2-binding domain, and connects the receptor to p85 regulatory subunit of PI3K and dynamin via the SH3 domains. Both PI3K and dynamin are well documented in β_2AR internalization [101–104]. On the other side, activated InsR promotes phosphorylation of tyrosine 895 on IRS1, which also creates a high affinity and specific binding site for the SH2 domain of GRB2 and p85 of PI3K. Insulin also stimulates Src association with β_2AR/AKAP250/PKA/PKC complex [105]. These observations are in line with βAR agonist-induced and clathrin-mediated endocytosis, which requires Src-mediated tyrosine phosphorylation of dynamin [106]. Inhibition of Src blocks the ability of insulin to internalize β_2AR and as well as translocate GLUT4 vesicles [107]. Together, the binding of phosphorylated tyrosine 350 of β_2AR to SH2-domain proteins appears to act in concert with Akt/PKB phosphorylation of serine 345/346 to mediate β_2AR internalization in response to insulin [60, 89, 95].

Moreover, IRS1 interacts with the C-terminus of GRK2 [79]; insulin stimulation induces GRK2 membrane translocation and increases the association of GRK2 and β_2AR in an IRS2-dependent manner [41]. The IRS2-dependent recruitment of GRK2 is necessary to promote β_2AR phosphorylation and internalization [41]. This together with enhanced phosphorylated β_2AR coupling to inhibitory Gi proteins results in inhibition of βAR-activated AC-cAMP-PKA signaling and cardiac contraction [41]. IRS1 and IRS2 share significant homology, and the mechanism involved in the counter-regulation of insulin on βAR signaling in heart by individual IRS proteins remains to be determined.

Considering insulin-induced β_2AR internalization utilizes many components that are widely shared by βAR agonist-induced internalization, such as PI3K, src, dynamin, etc., one would expect that a common pathway is involved in the β_2AR trafficking in response to both stimuli. However, the fact that the Akt/PKB sites of phosphorylation on β_2AR are different from those of the GRK sites [73, 91] suggests that the mechanism of receptor internalization is distinct from that mediated by the GRKs [60]. Accordingly, studies show that compared with βAR agonist-induced receptor internalization and recycling, the insulin-stimulated β_2AR requires a distinct cytoskeletal system, in which the β_2AR sequestration requires an intact actin cytoskeleton, and recycling to the cell surface requires intact microtubules [108].

In comparison to rapid and robust agonist-induced internalization of β_2AR, the internalization of β_1AR is limited [109]. Likewise, β_1AR is shown to be resistant to insulin-induced counter-regulation of function and receptor internalization [110]. Substitution of the C-terminal tail of β_2AR on β_1AR enables the chimeric GPCR to be functionally and spatially regulated by insulin. Specifically, the 15-amino acid motif harboring the SH2-binding motif at Y350 and the Akt phosphorylation site at S345/346 on the C-terminal tail of β_2AR are sufficient to enable β_1AR regulation by insulin [110]. Interestingly, IGF-1 has been shown to functionally antagonize the ability of β_1AR to generate cAMP in response to stimulation by the βAR agonist [111]. The inhibitory effect on β_1AR is accompanied by internalization of β_1AR in response to IGF-1. In cardiomyocytes, the IGF-1 action is blocked by PI3K inhibition or AKT (S412) loss-of-function mutation, suggesting the participation of PI3K/AKT axis in the IGF-1-mediated counter-regulation of β_1AR signal [111]. Overall, the mechanisms by which different subtypes of βARs (β_1AR and β_2AR) are subjected to the counter-regulation induced by distinct types of growth hormones (e.g. Insulin and IGF-1) require further elucidation.

4. Insulin activates βAR downstream effectors

4.1 G_i protein

The influence of G_i protein on RTK signaling was first proposed in 1987, based upon the observation that experimentally-induced diabetes leads to the loss of G_i-protein expression in rat liver [86]. Since then, G_{i2} deficiency has been found to increase protein-tyrosine phosphatase activity and attenuate insulin-stimulated tyrosine phosphorylation of IRS *in vivo*. Deletion of $G_{\alpha i2}$ in liver, skeletal muscle and white adipose tissue of transgenic mice has been found to induce insulin resistance [112], whereas tissue-specific expression of a constitutively active mutant of G_{i2} in skeletal muscle, liver and adipose tissues markedly activates translocation of the insulin-sensitive GLUT4 glucose transporter to the cell surface

[113] and enhances glucose-tolerance of transgenic mice [114]. All the evidence implicates $G_{\alpha i2}$ as a positive regulator of insulin action.

Mechanistically, it was demonstrated that $G_{\alpha i2}$ interacts with InsR during insulin signaling [115]. Studies have suggested that this association participates in the regulation of InsR autophosphorylation, yet the mechanisms are controversial. For instance, it has been shown that GTPγs increases the autophosphorylation of partially purified cardiac InsRs [116]. In contrast, an inhibition of RTK activity by GTPγs has been observed in InsR preparations from rat adipocytes [117]. The discrepancy could be due to the conditions used in the experiments. Overall, InsRs can functionally engage G proteins for downstream signaling, similar to GPCRs. In the same vein, a recent finding shows that in cardiomyocytes, insulin promotes β_2AR coupling to G_i; and inhibition of G_i by pertussis toxin (PTX) abolishes the inhibitory effect of insulin on β_2AR and downstream cAMP-PKA signal cascade [36].

4.2 Phosphodiesterase

We have discussed how acute insulin treatment alters the function of the stimulatory pathway of adenylyl cyclases by uncoupling β_2AR from its cognate G protein, which leads to a decrease in cAMP production. Meanwhile, cyclic AMP dependent phosphodiesterases (PDEs) provide additional mechanisms for attenuation of cAMP signal [118]. Interestingly, in rat adipocytes, isoproterenol and insulin increase activity of distinct pools of PDE to modulate subcellular cAMP and PKA activity. With cotreatment with insulin and isoproterenol, there is a rapid, transient, and synergistic activation of particulate cAMP PDE, which temporally correlates with a decrease in PKA activity and reduction in lipolysis [119]. Moreover, among PDE isoforms expressed in 3T3-L1 and primary mouse adipocytes, only PDE3B and PDE4D are upregulated by long-term treatment with insulin in a PI3K-dependent manner. In contrast, long-term treatment with βAR agonist induced down-regulation of PDE3B and up-regulation of PDE4D [120]. In line with the observation in adipocytes, our recent study shows that in rodent hearts, hyperinsulinemia induces upregulation of PDE4D protein expression and activity, which correlates with cardiac dysfunction [121] (Figure 2).

5. Other GPCRs crosstalk with insulin signaling pathway

The cross talk between insulin and angiotensin II (Ang II) signaling pathway has gained great focus due to the significant clinical association between type 2 diabetes and hypertension [122]. The cross talk could happen at different levels. At early steps, Ang II activates JAK and in turn promotes tyrosine phosphorylation of IRS-1 and IRS-2, these events happen in a large signaling complex formed with angiotensin receptor type 1 (AT1R) [123, 124]. However, in contrast to the effect of insulin, Ang II inhibits PI3K activity associated with phosphorylated IRS-1 and IRS2 [123], suggesting that Ang II can block insulin signaling partially via the PI3K pathway; Likewise, Ang II increase serine phosphorylation of the β-subunit of the insulin receptor and IRS-1, which is another mechanism for the inhibition of insulin signaling [124–127]. Thus, multiple serine phosphorylation events are involving in the negative modulation of Ang II on insulin signaling. In the downstream signal events, Ang II could promote the induction of SOCS-3,

which interacts and inhibits insulin signal transduction through the IR and IRS proteins or JAK/STAT signal pathway [128–130]. In addition, activation of AT1R by binding to Ang II promotes activation of ERK1/2 and JNK, which can phosphorylate IRS-1, impairing the transduction of insulin signaling to promote NO production by eNOS [131–133]. In parallel, Ang II also impairs insulin signal transduction through the IRS-PI3K-Akt pathway, which is consistent with the observation of the association of insulin resistance with cardiac hypertrophy [134].

Serotonin and insulin are key regulators of energy hemostasis in the hypothalamus. Under a diabetic situation, an impairment of the serotonergic signaling in the hypothalamus is observed. Moreover, hypothalamic impairment of the serotonin induced-PI3K/Akt pathway can modify downstream signaling cascades, leading to symptoms of type 2 diabetes [135]. These observations suggest the existence of a cross talk between serotonin and insulin in the central nervous system.

6. Therapeutic Interventions

6.1. Targeting the cross-talk between insulin and βAR signaling in heart

Under physiological conditions, insulin promotes glucose uptake, reduces myocardial O_2 consumption and increases cardiac efficiency [136]. Moreover, insulin directly acts on heart muscle to augment cardiomyocyte contraction, which is mediated principally through the Akt/PKB signal pathway [136]. Several animal studies have shown the protective effects of insulin on the heart [137]. A recent study shows that insulin decreased necrosis in cardiomyocytes during ischemia through an Akt/NFκB pathway [138]. The beneficial effect of insulin in the post-ischemic functional improvement in responding to βAR agonist is likely due to the increased cell survival and subsequent more functional cardiomyocytes available within the I/R hearts [25]. However, such “acute” protection by insulin in I/R hearts cannot explain the absence of a benefit with hyperinsulinemia in the development of HF in DM independent of myocardial ischemia [139, 140]. Moreover, during chronic HF induced by pressure overload and myocardial infarction, cardiac tissues display a feature of insulin resistance [10]. Thus, many of the interactions between chronic insulin resistance and HF might differ from those of the beneficial effect in “acute” I/R in hearts.

The cardiac performance in cardiomyocyte-selective InsR knockout (CIRKO) is mildly impaired, but absence of InsR signaling in the cardiomyocytes leads to worsening catecholamine-mediated myocardial injury [30, 141]. On the other side, despite the resistance in insulin-induced Akt activity, the excessive basal cardiac insulin signaling is detrimental, and can exacerbate systolic dysfunction induced by pressure overload in mice [142]. Moreover, recent clinical therapy of diabetic medications (e.g. glyburide) in HF patients show adverse effects [143, 144], suggesting that aggressive metabolic controls with insulin secretagogues may exacerbate cardiac symptoms by driving InsR-mediated desensitization of adrenergic signal. In this notion, insulin can drive desensitization of β_2AR in the heart independent of sympathetic drive, offering an explanation on insensitivity of diabetic patients to β-blocker therapy.

Together, these studies indicate that acute insulin exposure inhibits β-adrenergic signaling by a mechanism that differs from the chronic insulin exposure in HF. The cross talk between insulin and βAR signaling in diabetic cardiomyopathy is complex and multifactorial. It is necessary to take consideration of both HF stages and insulin resistance in treatment on individual patients with comorbidity of diabetes and HF.

6.2 Other GPCRs as therapeutic targets in diabetes and diabetic complications

The pharmaceutical therapeutic options for diabetes have traditionally targeted insulin receptor (insulin), kinases or ion channels (sulfonylureas and metformin). However, recently several new therapies for diabetes that target GPCRs have been identified. These include Glucagon-like peptide 1 (GLP-1) based agonists and the related DPP-IV inhibitors, D2 dopamine receptor agonist, serotonin agonists, and more recently, GPR40 agonists and selective endothelin-A receptor antagonists.

The most well developed therapies targeting GPCRs are GLP-1 agonists and the related DPP4 inhibitors. GLP-1 is a peptide hormone that is produced in the intestine after a meal. GLP-1 stimulates insulin secretion, inhibits glucagon secretion via activation of GLP-1 receptor, a member of GPCR family [145, 146]. However, the half-life of GLP-1 is very short primarily due to its degradation by the enzyme dipeptidyl peptidase IV (DPP-IV). Thus, efforts to extend the half-life of GLP-1 agonists include replacement of mimetic form of GLP-1 such as Exendin-4 and modification of natural GLP-1 structure, which aim for more resistant to DPP-IV degradation [147–150]. On the other side, by prolonging endogenous GLP-1 concentrations and increasing fasting levels of GLP-1, DPP-IV inhibitors have also been used as adjuvant treatment for diabetes [151–153].

Meanwhile, free fatty acids have also been reported to stimulate insulin secretion in a glucose-dependent manner by activation of GPR40 (free fatty acid receptor 1 or FFAR1) [154–156]. Thus, several GPR40 agonists are being developed such as TAK-875, which was shown both in rodents and humans to enhance insulin secretion and promote glucose control [155, 157].

Endothelin-1 (ET-1) is a peptide with vasoconstrictor activity, and ET-1 is implicated in the pathogenesis of hypertension, diabetes mellitus, and cardiovascular diseases [158, 159]. Plasma ET-1 levels have been shown to be elevated in animal diabetic models and the activity of ET-A receptors is enhanced in diabetic patients [160]. Thus, the therapeutic intervention designed to suppress the ET system may be effective in preventing the development of diabetic complications. Indeed, ET blockade is effective in improving cardiac dysfunction in the diabetic rats [161] and ameliorate experimental diabetic nephropathy [162–164].

The central nervous system (CNS) is of importance to regulate whole body energy homeostasis. Endogenous dopaminergic and serotonergic rhythms in the CNS are reported to be involved in the transition from insulin-sensitive to insulin-resistant state. Within the ventromedial hypothalamus (VMH), serotonin level and activity are increased during insulin-resistant state and decrease to normal with return to insulin-sensitive state in animals that undergo seasonal changes in metabolism. Conversely, dopamine levels are low during

an insulin-resistant state and increase to normal following return of the insulin-sensitive state. The D2 dopamine receptor agonist, bromocriptine, was approved for the treatment of type 2 diabetes recently. [165] Bromocriptine augments low hypothalamic dopamine levels and inhibits excessive sympathetic tone within the CNS, resulting in a reduction in post meal plasma glucose levels, due to enhanced suppression of hepatic glucose production [165]. Meanwhile, a peripheral serotonin inhibitor (LP-533401) has already been patented for treating diabetes and obesity [166], suggesting that in peripheral tissues, suppressing serotonin signaling might represent a new target for treating diabetes by improving insulin resistance [167, 168].

7. Conclusion

With diabetes and HF being common comorbid conditions, it is highly important to develop treatments for diabetic-related HF. Currently, we have learned that activation of the InsR pathway can lead to a desensitization of the cardiac adrenergic signaling pathway, which contributes to cardiac pathophysiology associated with diabetes. While InsR and β_2AR form a direct membrane receptor complex, additional important nodes where signaling crosstalk takes place between the adrenergic and insulin pathways are the G_i and GRK2 proteins. A better understanding the crosstalk between these two cascades will allow us to develop more effective treatment strategies for patients with comorbidity of diabetes and cardiovascular complications.

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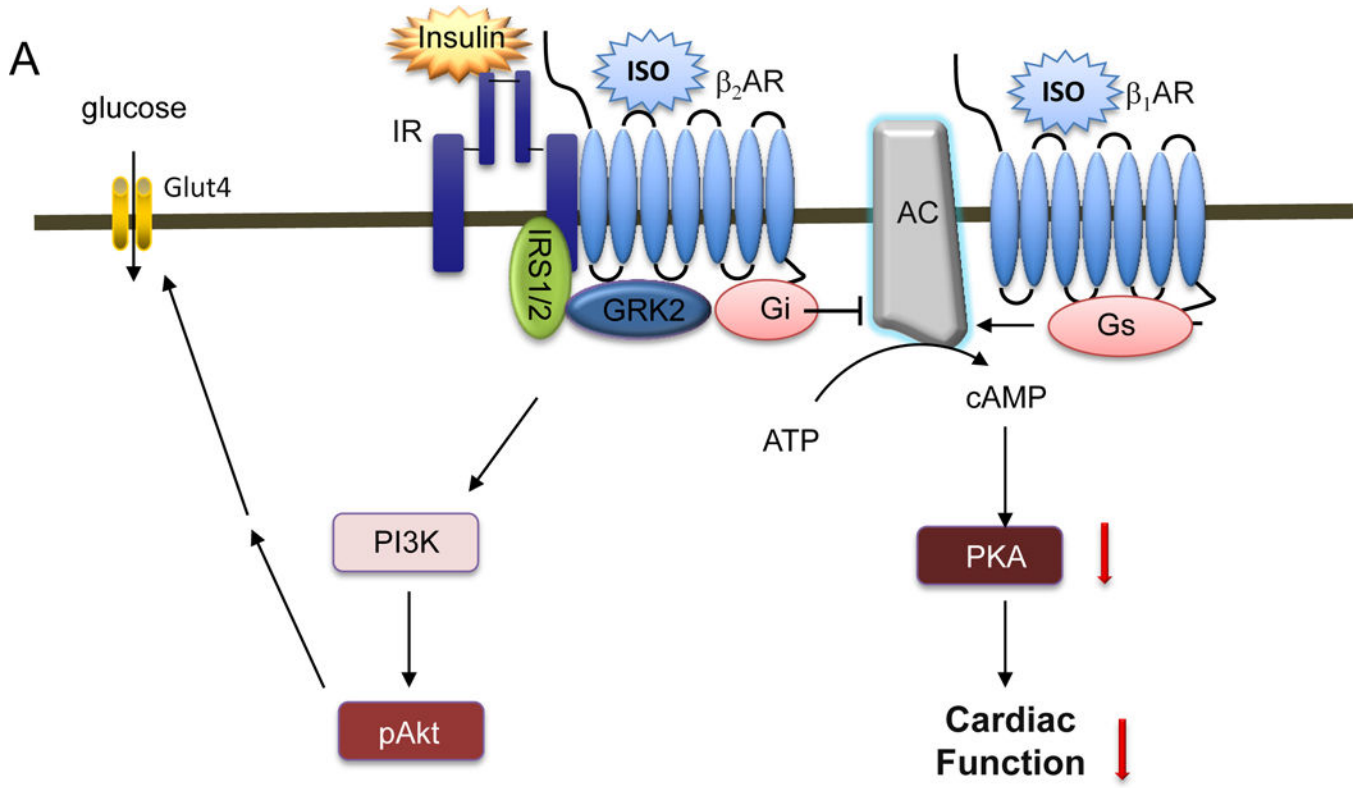

Figure 1.
Acute insulin signaling impairs cardiac adrenergic stimulation via enhanced βAR/G_i coupling

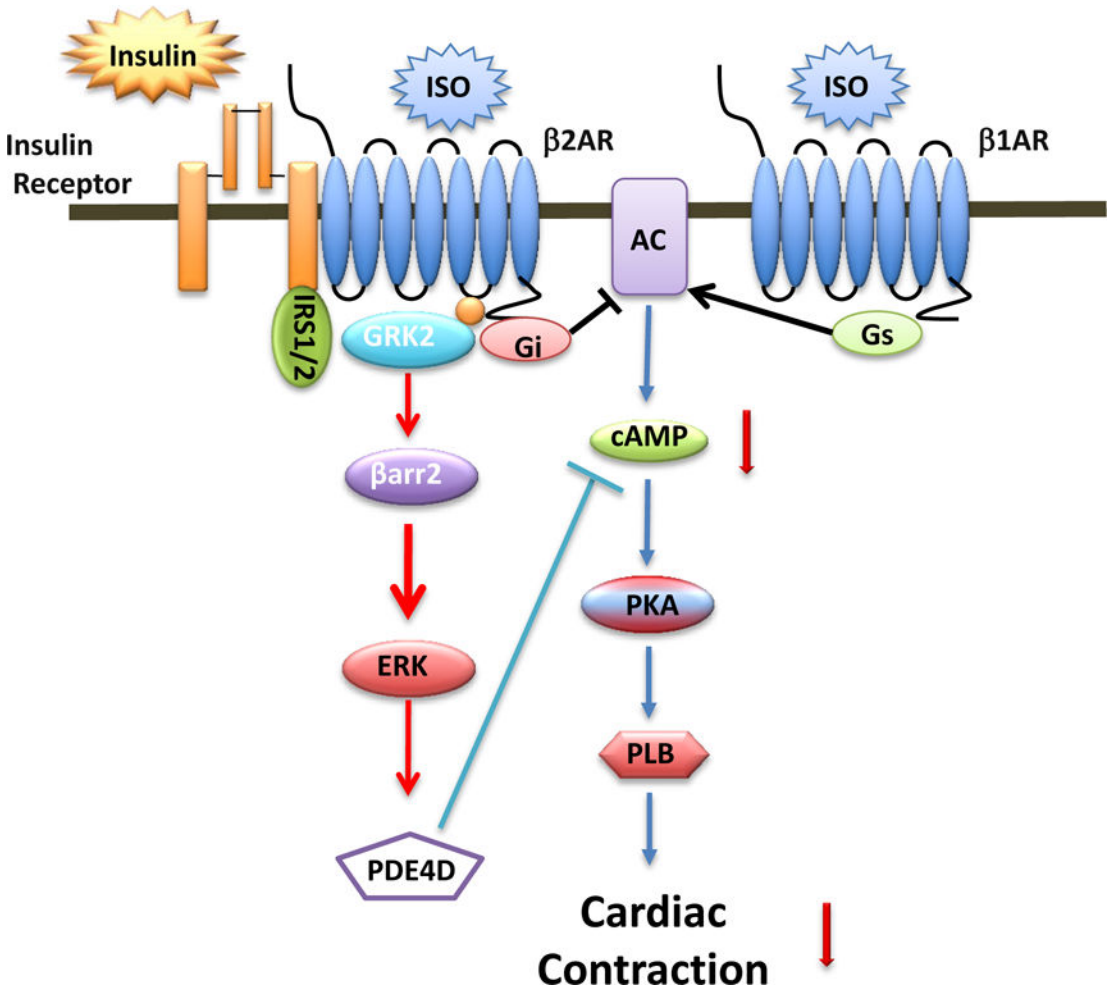

Figure 2. Chronic insulin stimulation promotes β2AR-dependent expression of PDE4D

Briefly, our data indicates insulin receptor transactivates beta2AR through recruiting GRK2 by IRS1/2,triggers Gi coupled signaling, meanwhile, GRK2 recruits betaarrestin2, activates ERK, promotes PDE4D expression. Both pathways decrease cAMP level and myocytes contractile function, leading to heart dysfunction. So GRK2 is the key link between IR and b2AR signaling. Will inhibition of GRK2 helps to preserve heart function in HFD feeding mice?

Table 1

Summary of insulin receptor and adrenergic receptor signaling

Models	Pathophysiological Context	Treatment	Biological Function	Key Factor	Unknown and Questions	ref
Cardiomyocytes, Rat-1 cell, Human fat cell, isolated perfusion heart		Insulin,	βAR ligand binding capacity↓ βAR internalization↑ β_1AR-induced cAMP and cardiac contractility ↓ α1B-ARs phosphorylation↑ desensitization↑	β_2AR, PI3K,PKC	The different effects insulin on adrenergic receptor signaling in physiological condition, T1DM and T2DM; the bidirectional role of insulin in diabetes related HF and its underlying mechanisms.	[29,36,98]
Adult rat cardiomyocytes		PI3K inhibitor	β_1AR-induced L-type Ca^{2+} currents↑ intracellular Ca^{2+} transients↑ myocyte contractility↑ β_2AR/Gs-mediated PKA signaling and contractile and relaxant response↑	betaARK1, Gi, Gβγ,		[26, 27]
CIRKO mouse			cardiac performance mildly impaired	IR		[30]
CIRKO mouse		βAR agonist	βAR-mediated contractility and relaxation↑ myocardial injury↑ interstitial and sub-endocardial fibrosis ↑	capillary density		[141]
CIRKO mouse	TAC		cardiac insulin signaling↓ systolic dysfunction↓ cardiac ischemia↓ hypertrophy↓	IR, IRS		[128]
Left atria and papillary muscles	T1DM	insulin	βAR-induced cardiac tissues sensitivity↓			[97]
mouse	T1DM with TAC	STZ	hyperglycemia↓ pressure overload induced myocardia ischemia↑ cardiomyocyte death↑ HF ↑	IR, IRS		[128]
Atria, papillary muscles, ventricular tissue	T1DM alloxan diabetes		βAR density↓, βAR stimulation sensitivity↓, βAR induced inotropic responses↓	βAR affinity, α_1-AR affinity βAR redistribution		[28, 50, 51, 52, 55, 56, 59]
Mouse cardiomyocytes		insulin	βAR induced cAMP↓ cardiomyocyte contractility↓	βAR phosphorylation at S355, 356	What is the effect of insulin- and IGF-1-induced βAR phosphorylation at different sites in heart?	[36]
CHO-cell, A431 cells		Insulin IGF-1	β_1AR function ↓ β_2AR function ↓ internalization↑	Phosphorylation at Y350 and S345, 346 of β2AR		[91, 110, 111,]
DDT1 MF-2 smooth muscle cells		insulin	β_2AR phosphorylation on tyrosyl residue↑ β_2AR phosphorylation on threonyl residue↓ βAR activated AC and cAMP↓ β_2AR internalization↑	β_2AR phosphorylation at Tyr-350, 354		[24, 89, 90]
Myoblast and adipocytes	TNF-α infusion, aging, and HFD	altering GRK2 level	negative regulator of insulin signaling		Whether insulin directly increases GRK2 expression in diabetes?	[75]

Models	Pathophysiological Context	Treatment	Biological Function	Key Factor	Unknown and Questions	ref
Mouse cardiomyocytes, heart, or whole body	GRK2 deletion,		insulin-resistant ↓ obese phenotype↓ cardiac insulin sensitivity↑ and mild heart hypertrophy with preserved systolic function	IRS-1 phosphorylation	And whether GRK2 could be a target in the treatment of diabetes related heart disease?	[76, 77, 79]
MEF		IRS2 deletion	insulin-induced β_2AR phosphorylation↓ β_2AR internalization↓ decreased βAR signaling↑	InsR/ GRK2 complex disruption		[41]
Cardiomyocytes, Human and rat fat cells		insulin	insulin decreased β-mediated cAMP and contractility↑ IR and Gi association ↑	β_2AR-Gi coupling IR-Gi complex	The nature of protein-protein interaction among IR, βAR, and different G proteins and its effect in heart.	[36, 115,]
Rat cardiomyocytes		GTP?s (G protein activation)	IR autophosphorylation ↑	IR-Gi complex		[116,117]
3T3-L1 and primary mouse adipocytes		insulin (long term)	PDE3B↑ PDE4D↑ βAR increased PDE↑PKA↓ lipolysis↓	PI3K	Which PDE subtype is involved in the diabetes related heart disease, and the interactions among insulin, βAR and PDE in the balance of cAMP-PKA signaling in heart.	[119, 120]
Mouse heart, cardiomyocytes	T2DM		PDE4D↑ cardiomyocyte contractility↓	β_2AR, β-arrestin2, ERK		[121]
Cardiomyocytes, rat heart,	I/R	insulin	infarct size↓ cell survival↑ β_1AR induced contractile response↓ cardiac dysfunction↓ cell injury↓	Akt and NF?B.	The difference of interactions between chronic insulin resistance and HF and the beneficial effect in “acute” I/R in hearts. How to use insulin in different type diabetes combined with heart diseases? Whether insulin reduced β-adrenergic sensitivity is involved in hypoglycemia unawareness in T1DM	[25, 124]
Middle-aged and elderly men	insulin resistance		CHF↑ left ventricular systolic dysfunction ↑			[125, 126]
Human	T2DM	glipizide, glyburide, glimepiride	risk of overall mortality↑			[129]
Healthy male subjects		insulin	βAR sensitivity↓			[100]