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β-Adrenergic Receptor and Insulin Resistance in the Heart

Supachoke Mangmool[1,2], Tananat Denkaew[1], Warisara Parichatikanond[1] and Hitoshi Kurose[3,*]

[1]Department of Pharmacology, [2]Center of Excellence for Innovation in Drug Design and Discovery, Faculty of Pharmacy, Mahidol University, Bangkok 10400, Thailand, [3]Department of Pharmacology and Toxicology, Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka 812-8582, Japan

Abstract

Insulin resistance is characterized by the reduced ability of insulin to stimulate tissue uptake and disposal of glucose including cardiac muscle. These conditions accelerate the progression of heart failure and increase cardiovascular morbidity and mortality in patients with cardiovascular diseases. It is noteworthy that some conditions of insulin resistance are characterized by up-regulation of the sympathetic nervous system, resulting in enhanced stimulation of β-adrenergic receptor (βAR). Overstimulation of βARs leads to the development of heart failure and is associated with the pathogenesis of insulin resistance in the heart. However, pathological consequences of the cross-talk between the βAR and the insulin sensitivity and the mechanism by which βAR overstimulation promotes insulin resistance remain unclear. This review article examines the hypothesis that βARs overstimulation leads to induction of insulin resistance in the heart.

Key Words: β-adrenergic receptor, β-blockers, G protein-coupled receptor kinase, Heart diseases, Insulin resistance, Protein kinase A

ROLE OF G PROTEIN-COUPLED RECEPTORS ON INSULIN RESISTANCE

G protein-coupled receptors (GPCRs) are a conserved family of seven transmembrane receptors (Pierce *et al*., 2002) and are one of the largest receptor classes for drug targeting. β-Adrenergic receptors (βARs) belong to the GPCR family that activates intracellular Gαs protein after binding of catecholamines (Salazar *et al*., 2007). In the heart, acute stimulation of βARs physiologically augments cardiac contraction, whereas chronic stimulation of βARs promotes adverse cardiac remodeling, leading to cardiomyocyte apoptosis, myocardial hypertrophy, and heart failure (Salazar *et al*., 2007). In addition, sustained and overstimulation of myocardial βARs trigger a state of insulin resistance in the heart (Mangmool *et al*., 2016). Insulin resistance is associated with impairments in cardiac function and has been observed in patients with heart failure and dilated cardiomyopathy (Ginsberg, 2000; Shah and Shannon, 2003). Interestingly these conditions are characterized by the dysregulation of the sympathetic nervous system, which results in the up-regulation of βAR. However, a pathological consequence of the relationship between βAR, insulin sensitivity, and the exact mechanisms by which βAR overstimulation leads to impaired insulin activity have not fully described in the heart.

INSULIN RECEPTOR AND ITS SIGNALING

Insulin is a potent anabolic hormone that is synthesized and secreted from the β-cells of the islets of Langerhans in the pancreas, and then circulated throughout the blood stream. Insulin which consists of two polypeptide chains joined by disulfide bonds, play a vital role in cell growth and development, and maintenance of glucose homeostasis (White and Kahn, 1994; De Meyts, 2004). The insulin receptor (IR) belongs to the receptor tyrosine kinase family with intrinsic tyrosine kinase activity.

IR is a heterotetrameric receptor composed of two extracellular α subunits and two membrane-spanning β subunits that are disulfide linked into an $\alpha_2\beta_2$ configuration (Fig. 1) (Becker and Roth, 1990; Lee and Pilch, 1994; Bevan, 2001). The α subunits span the extracellular portion that contains the ligand-binding domain of the receptor, while the β subunits span extracellular, transmembrane, and intracellular domains. The intracellular domain of the β subunits, which expresses in-

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***Corresponding Author**
E-mail: hikurose@phar.kyushu-u.ac.jp
Tel: +81-92-642-6884, Fax: +81-92-642-6884

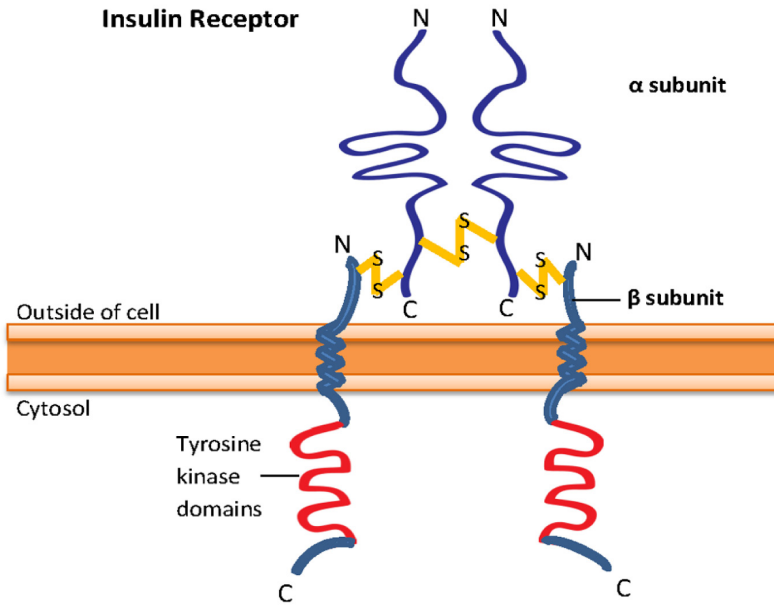

Fig. 1. Structure of insulin receptor. Insulin receptor is a heterotetrameric receptor that contains two α subunits, which is extracellular and has the ligand-binding domain, and two β subunits, which consist of extracellular, transmembrane and intracellular domains. The tyrosine kinase domain of the receptor is present at the intracellular β subunits.

trinsic tyrosine kinase activity, is involved in signal transduction (Ullrich *et al*., 1985; Becker and Roth, 1990; De Meyts, 2004).

Binding of insulin to the α subunits of the receptor activates the intracellular tyrosine kinase activity of the β subunits, resulting in transphosphorylation of one β subunit by the other on specific tyrosine residues, and then autophosphorylation at specific sites in the intracellular tail of the receptor which is followed by tyrosine phosphorylation of cytoplasmic substrate proteins such as Src-homology-2-containing (Shc) proteins and insulin receptor substrate (IRS) proteins (Becker and Roth, 1990; Lee and Pilch, 1994; Bevan, 2001; Taniguchi *et al*., 2006). These substrate proteins interact with their effectors that amplify and extend the insulin signaling cascades (Fig. 2). For example, the phosphorylated Shc induces mitogenesis (Sasaoka and Kobayashi, 2000). Phosphorylation of IRS promotes the interaction of IRS with many signaling proteins including growth factor receptor-bound protein-2 (Grb-2) (Myers *et al*., 1994), Src homology 2 (SH2) domain-containing protein tyrosine phosphatase (SHP-2) (Hayashi *et al*., 2004), and phosphatidylinositol-3-kinase (PI3K) (Sarbassov and Peterson, 1998) to produce many biological responses. The binding of IRS proteins to Grb-2 leads to the activation of the extracellular regulated kinase-1 and -2 (ERK1/2) which promotes mitogenesis, cell growth, and differentiation.

In particular, insulin action is primarily dependent on the activation of PI3K. IRS interacts with PI3K, leading to activate the PI3K activity (Backer *et al*., 1992). The activated PI3K then generates phosphatidylinositol 3,4,5-trisphosphate (PIP_3) from the substrate phosphatidylinositol 4,5-bisphosphate (PIP_2) on the plasma membrane, which regulates the activity of several protein effectors, including Akt (known as protein kinase B, PKB) and atypical isoforms of protein kinase C (aPKC) (Lee and Pilch, 1994; Bevan, 2001; Taniguchi *et al*., 2006) that control many aspects of cellular insulin action, including protein synthesis, glycogen synthesis, and glucose transporter (GLUT) translocation from intracellular to the cell surface and ultimately triggers glucose uptake into the cells (Czech and Corvera, 1999). Activation of Akt leads to an increased glucose transport and persistent translocation of GLUT subtype 4 (GLUT4) to the plasma membrane in adipocytes (Kohn *et al*., 1996, 1998). In addition, stimulation of PKCζ or PKCλ induces GLUT4 translocation, whereas inhibition of PKCλ suppresses GLUT4 translocation (Kitamura *et al*., 1998; Kotani *et al*., 1998). GLUT4 is widely expressed in various tissues such as skeletal muscle, cardiac muscle and adipose tissue that constitute important sites of glucose utilization after food intake (Zhao and Keating, 2007). In the basal condition, most GLUT4 is localized in the intracellular space. Upon stimulation of IRs, GLUT4 is rapidly translocated to the cell surface, where it facilitates inward transport of glucose from the circulation into the cells (Watson and Pessin, 2001). Glucose that enters the cell is rapidly phosphorylated by hexokinase to generate glucose-6-phosphate (G-6-P), and is subsequently used for metabolism or stored in the cell as glycogen or triglyceride (TG). Hexokinase II is found in association with GLUT4 in skeletal and cardiac muscle and in adipose tissue (Postic *et al*., 1993).

ACTIONS OF INSULIN

Downstream effects of insulin are initiated through the binding of insulin to the extracellular β subunits of its receptor and transmission of the signal across the plasma membrane that activates several insulin signaling pathway. Although the details linking between the second messengers to the metabolic effects of insulin are still under investigation, the metabolic effects of insulin action are well understood. IR is widely ex-

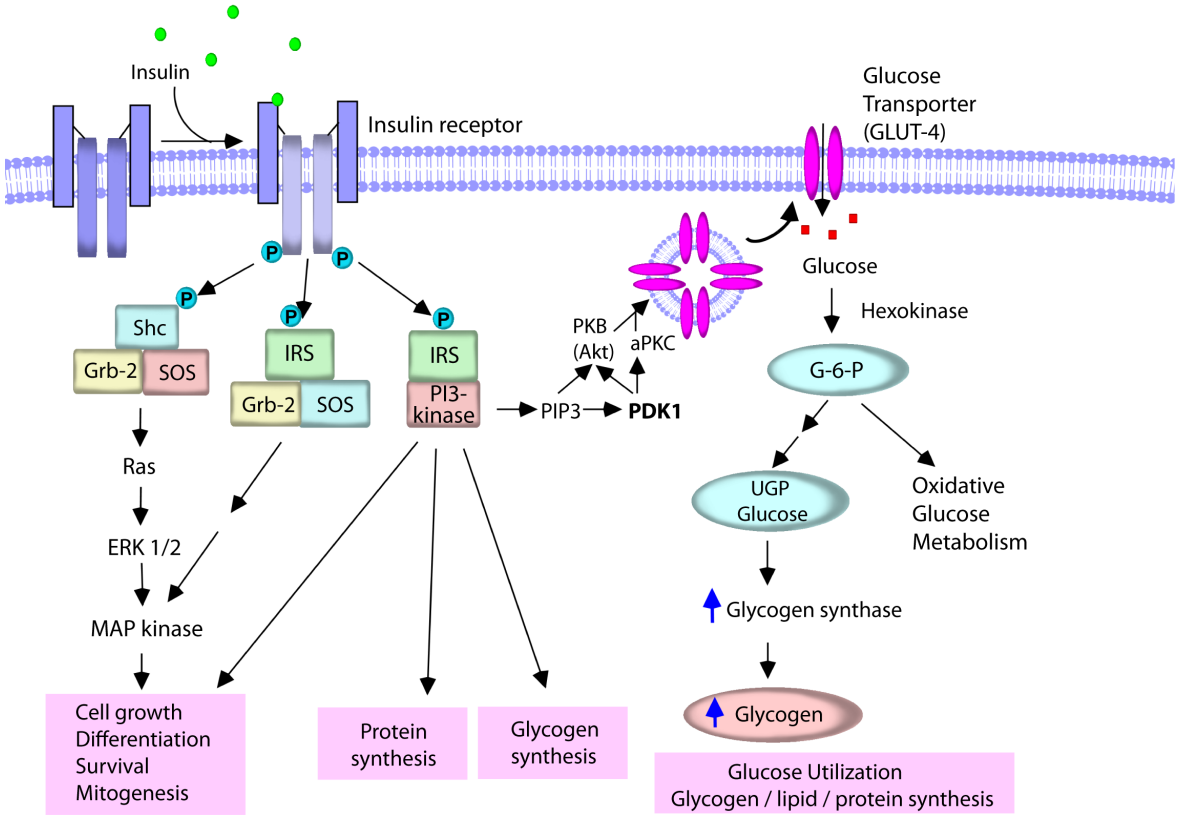

Fig. 2. Insulin signaling pathway. Upon binding of insulin to its receptor, a cascade of intracellular events in the cells is initiated. The activated insulin receptor phosphorylates and activates substrate proteins such as Shc and IRS. Phosphorylation of Shc promotes the formation of Shc/Grb-2/SOS complex which stimulates MAP kinase pathway, resulting in mitogenesis, cell growth, and differentiation. Phosphorylated IRS proteins interact with many other signaling proteins including Grb-2 and PI3K, and change cellular function. PI3K catalyzes the formation of PIP_3 which, in turn, activates Akt and aPKC, and controls many aspects of insulin action, including protein synthesis, glycogen synthesis, and glucose transport via the translocation of GLUT4 to the plasma membrane. Glucose that enters the cells is rapidly phosphorylated by hexokinase enzymes to generate glucose-6-phosphate (G-6-P), and is subsequently utilized for metabolism and/or stored in the cells as glycogen or TG.

pressed on many cell types, implicating the broad array of biological responses to insulin (Fig. 3). The tissues that are considered important for control of glucose homeostasis are liver, skeletal muscle and adipose tissues. In addition, the brain, pancreatic islets, and cardiac muscle are also main targets for insulin action.

The actions of insulin are anabolic, and insulin signaling is critical for promoting the uptake, utilization, and storage of the major nutrients (e.g., glucose, lipids, and amino acids), resulting in glycogenesis, lipogenesis, and protein synthesis. Insulin also inhibits the catabolism of these macromolecules. In the liver, insulin increases glucokinase activity, thereby mediating the phosphorylation and trapping of glucose in hepatocytes. The increased glucose uptake into the hepatocytes accelerates the glycogen synthesis, glycolysis, and fatty acid synthesis. Insulin also reduces hepatic glucose output through the reduction of gluconeogenesis and glycogenolysis. Moreover, insulin suppresses the production and secretion of very-low-density lipoprotein (VLDL) in the liver (Chirieac *et al*., 2000). In muscle, insulin increases amino acid uptake, stimulates the ribosomal protein synthesis machinery, and promotes glycogen synthase activity and subsequent glycogen storage. In adipose tissue, insulin promotes the expression of lipoprotein lipase, which hydrolyzes TG from circulating lipoproteins for uptake into fat cells. In addition, insulin inhibits the release of free fatty acids (FFAs) from adipose tissue, increases TG and FFAs synthesis and also trigger glucose uptake into adipose tissue (Eckel *et al*., 2005).

Furthermore, insulin has anti-atherogenic actions in blood vessels (Eckel *et al*., 2005). Insulin enhances production of the vasodilator nitric oxide (NO) (Montagnani *et al*., 2002), and exerts anti-inflammatory and anti-oxidative stress effects (Yu *et al*., 2011). Insulin also inhibits platelet aggregation (Trovati and Anfossi, 1998) and plasminogen activator inhibitor type 1 (PAI-1) activity (Juhan-Vague *et al*., 1996). Furthermore, insulin has been suggested to be a growth factor that induces vascular cell growth and synthesis of some matrix proteins (Feener and King, 1997; McFarlane *et al*., 2001).

INSULIN RESISTANCE

Insulin resistance is presented as an insulin function deficiency whereby normal circulating concentrations of insulin are insufficient to induce signals regulating normal glucose absorption and glucose homeostasis of tissues and organs

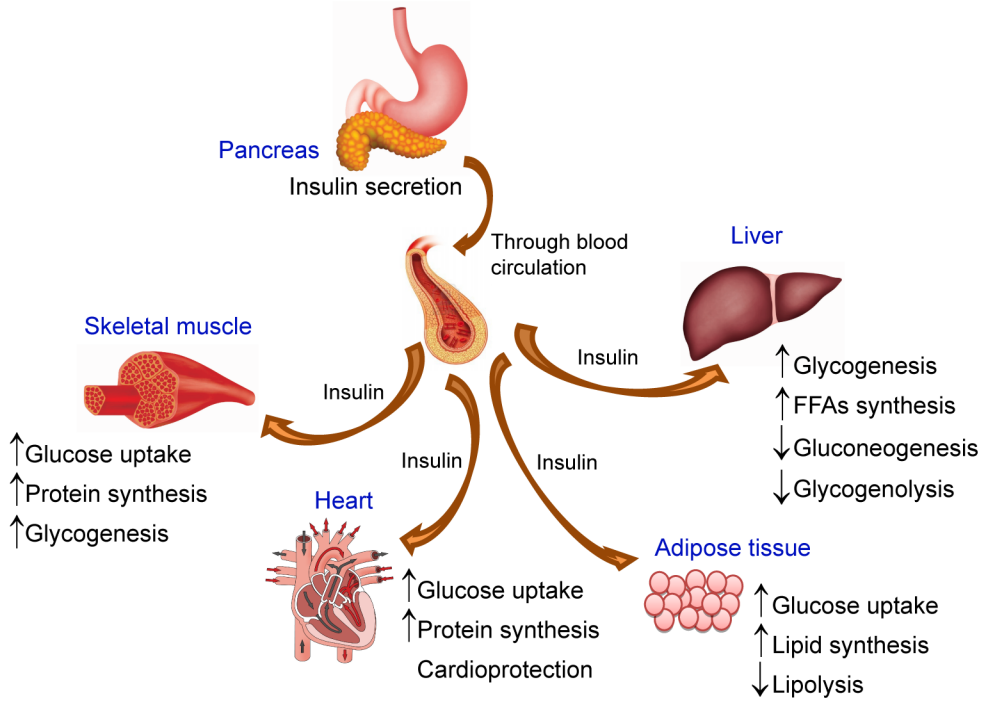

Fig. 3. Insulin actions on many tissues. Insulin is secreted from the pancreas from the β-cells of the islets of Langerhans and circulated throughout the body via the bloodstream. The released insulin acts on insulin receptors located on the plasma membrane of target tissues (i.e., skeletal muscle, liver, adipose tissue and heart). In skeletal muscle, cardiac muscle, and liver, insulin promotes glucose uptake and storage as glycogen or TG. In adipose tissue, insulin promotes conversion of glucose to TG by increasing lipid synthesis.

Table 1. The biological actions of insulin and insulin resistance in many tissues/organs

Tissues/organs	Insulin actions	Insulin resistance
Liver	Increase glycogen synthesis[1,2] Decrease hepatic glucose production[1,2] Inhibit glycogenolysis[1,2] Increase fatty acids synthesis[2,3] Suppress the production of very-low-density lipoprotein (VLDL)[4,5,6]	Decrease glycogen synthesis and glycogen storage[4,5] Increase glucose production[4,5] Increase glycogenolysis[6]
Skeletal muscle	Increase amino acid uptake and protein synthesis[2] Increase glycogenesis[2] Increase glucose uptake by stimulation of GLUT4 translocation[4,7]	Impair insulin-stimulated glucose uptake[8,9] Impair GLUT4 translocation[6,9]
Adipose tissue	Increase TG and fatty acids synthesis[3] Inhibit lipolysis and the release of free fatty acids (FFAs) from adipose tissue[3,4,6] Increase glucose uptake by stimulation of GLUT4 translocation[4,7]	Impair glucose uptake by altering GLUT4 translocation[10,11] Decrease responsiveness of adipose tissue to normal levels of insulin to increase lipid synthesis and inhibit lipolysis[6] Increase intracellular hydrolysis of TG and release of FFAs[12,13]
Cardiac muscle	Increase protein synthesis[4] Increase glucose uptake[14,15] Elicit cardioprotective actions[16,17]	Impair glucose uptake[14,15] Increase FFAs metabolism[18] Impair cardiac functions[19,20,21]

[1]Becker and Roth, 1990; [2]De Meyts, 2004; [3]Boden and Shulman, 2002; [4]Eckel *et al*., 2005; [5]Jellinger, 2007; [6]Sesti, 2006; [7]Czech and Corvera, 1999; [8]Chiasson *et al*., 1981; [9]DeFronzo and Tripathy, 2009; [10]Mulder *et al*., 2005; [11]Kashiwagi *et al*., 1983; [12]Ginsberg, 2000; [13]Lamounier-Zepter *et al*., 2006; [14]Mangmool *et al*., 2016; [15]Morisco *et al*., 2005; [16]Morisco *et al*., 2007; [17]Akikawa *et al*., 2000; [18]How *et al*., 2006; [19]Mazumder *et al*., 2004; [20]McFarlane *et al*., 2001; [21]Boudina *et al*., 2009.

(Jellinger, 2007). Thus, insulin resistance is a defect in insulin action. Insulin resistance is a key feature of type 2 diabetes and metabolic syndrome, and contributes to abnormalities in many tissues (Table 1). Insulin resistance in muscle and fat is generally marked by a decrease in glucose clearance from the circulation. Insulin resistance in the liver generally refers to a blunted ability of insulin to suppress glucose production, leading to an overproduction of glucose through gluconeogenesis and glycogenolysis, which contribute to hyperglycemia and development of diabetes (Matthaei *et al*., 2000; DeFronzo, 2004). In adipocytes, insulin resistance causes increased rates of lipolysis resulting in excessive breakdown of stored TG into FFAs, which is responsible for hyperlipidemia in the obese state (Boden and Shulman, 2002). In skeletal muscle, insulin resistance causes the reduction in both glucose transport and glycogen synthesis, which leads to inhibit glucose clearance (DeFronzo and Tripathy, 2009). At the cellular level, insulin resistance involves blunted steps in the signaling cascade from the IR tyrosine kinase to translocation of GLUT4, but the molecular mechanisms are incompletely defined.

Insulin resistance is associated with inflammation, oxidative stress, cardiac remodeling, and endothelial dysfunction that results in diminished endothelial NO synthase (eNOS) expression leading to an increase in vascular tone (Scherrer *et al*., 1994; Kuboki *et al*., 2000). Insulin resistance leading to hyperinsulinemia and hyperglycemia is a predictor and/or marker of cardiovascular diseases. Insulin resistance is a hallmark in diabetic patients (Olefsky *et al*., 1973) and is associated with hypertension (Reaven, 1991), obesity (Mingrone *et al*., 1997), heart failure (Swan *et al*., 1994, 1997), and dilated cardiomyopathy (Shah and Shannon, 2003). In non-ischemic heart failure patients, the prevalence of insulin resistance highly contributes to heart failure development (Witteles and Fowler, 2008).

A previous study reported that fatty acids compete with glucose for substrate oxidation in isolated rat heart muscle and rat diaphragm muscle (Randle *et al*., 1963). They speculated that an increase in fat oxidation might be responsible for insulin resistance in obese states. Moreover, lipid accumulation within skeletal muscle and liver inhibits tyrosine phosphorylation of IRS-1, resulting in blockade of IRS-1/PI3K interaction which, in turn, blunts PI3K activity (Savage *et al*., 2005). Therefore, there is an association between insulin resistance found in type2 diabetes and the development of heart disease. It is believed that the relationship between heart disease and diabetes is bidirectional whereby heart disease induces insulin resistance, and vice versa.

INSULIN RESISTANCE CAUSES HEART DISEASE

The relationship between diabetes and heart disease are well established. Diabetic patients who are diagnosed at younger ages are at much greater risk for cardiovascular diseases (Hillier and Pedula, 2003). The incidence rate of congestive heart failure in diabetic patients is much greater than patients without diabetes (Nichols *et al*., 2004), emphasizing the demand of early diagnosis and an intensive treatment of diabetes for preventing the progression of cardiac dysfunction and heart disease. Alström syndrome is a rare autosomal recessively inherited disorder that affects many organs. In patients with Alström syndrome, metabolic disturbances begin in childhood including severe insulin resistance, hyperinsulinemia and type 2 diabetes. These abnormalities result in a potentially fatal dilated cardiomyopathy and congestive heart failure (Marshall *et al*., 2005). Studies using isolated perfused heart preparations and cardiac myocytes have demonstrated insulin resistance in both human and animal models of the diabetic heart (Ohtake *et al*., 1995; Kolter *et al*., 1997). Insulin resistance develops in the hearts of mice as early as after high-fat feeding that involves reduction in glucose uptake and GLUT4 expression in the heart (Park *et al*., 2005). A condition of insulin resistance and dysregulation of glucose metabolism might induce pathological conditions such as systolic/diastolic dysfunction and cardiac remodeling (Park *et al*., 2005). Moreover, cardiac insulin resistance reduces the metabolic efficiency of the heart, which can lead to contractile dysfunction (Belke *et al*., 2000; Mazumder *et al*., 2004). In addition, impaired myocardial insulin signaling promotes oxidative stress and mitochondrial dysfunction in the heart (Boudina *et al*., 2009). Thus, a condition involving insulin resistance and the dysregulation of glucose metabolism might induce other pathological conditions, such as systolic/diastolic dysfunction and cardiac remodeling. Despite the importance of insulin resistance in the diabetic heart, the molecular mechanisms by which insulin resistance develops in the heart are not fully understood.

Heart failure is twice as high in diabetic men while five times as high in diabetic women. Furthermore, the prevalence of heart failure in elderly diabetic patients is high as 39% (Bell, 2003). The cardiomyopathic heart in the setting of insulin resistance is the worst scenario for energy metabolism, as insulin resistance causes down-regulation of genes regulating glucose homeostasis and metabolism that consequently leads to a state of energy starvation, which promotes further deterioration in cardiac functions (Witteles and Fowler, 2008). The reduction of GLUT4 expression in the myocardium can be found in patients who have insulin resistance with concomitant cardiac hypertrophy (Paternostro *et al*., 1999). Furthermore, reduction in GLUT4 and increase in FFAs concentrations have also been found in ischemic patients with mild chronic heart failure (Murray *et al*., 2004). Moreover, insulin resistance often precedes the development of heart disease due to the altered metabolic environment (e.g., altered ATP generation, increased FFA oxidation, and downregulation of genes for glucose oxidation). These abnormalities result in myocardial dysfunction and HF (Heck and Dutka, 2009). However, the pathophysiological consequences of insulin resistance to cause heart disease are incompletely understood.

HEART DISEASE INDUCES INSULIN RESISTANCE

The evidence for insulin resistance causing heart failure is more extensive than the reverse. Patients with coronary artery disease, heart failure, or a history of myocardial infarction are insulin resistant (Paternostro *et al*., 1996). Patients with severe heart failure have been reported to exhibit insulin resistance, and the degree of insulin resistance has been associated with the severity of heart failure (Swan *et al*., 1997). The mechanisms underlying the worsening of insulin resistance in patients with heart failure are not quite understood. The possible mechanisms include sympathetic overactivation, endothelial dysfunction, and increased circulating cytokines such as TNF-α (Coats and Anker, 2000; Heck and Dutka, 2009). The evi-

dences that support the mechanisms for heart disease causing insulin resistance include sympathetic overactivation such as increased catecholamine secretion through βAR. Overstimulation of βAR leads to the inhibition of insulin-stimulated glucose uptake in cardiomyocytes (Mangmool *et al*., 2016). It has been proposed that infarcted rat hearts have reduced rates of insulin-induced glucose uptake and GLUT4 protein levels (Murray *et al*., 2006). In addition, cardiac glucose uptake is defective after myocardial ischemia and after chronic βAR stimulation (Ciccarelli *et al*., 2011). Moreover, decreased GLUT4 expression has been observed in an animal model of cardiac hypertrophy (Paternostro *et al*., 1995). Increased norepinephrine concentration is found in heart failure (Thomas and Marks, 1978). Norepinephrine can cause the elevation of FFA through the stimulation of lipolysis (Paolisso *et al*., 1991). In addition, an increase of plasma FFA levels stimulates cardiac sympathetic activity (Paolisso *et al*., 2000) and adversely affects insulin signaling, thereby reducing glucose utilization by cardiac muscle. In the same way, the detrimental metabolic effects following sympathetic overstimulation extend further inhibition of pancreatic insulin secretion and increased glucose production, both of which worsen hyperglycemia (Nonogaki, 2000). Currently, the interrelationship between insulin resistance and heart disease has been an interest topic. Although many questions remain with regard to the relationship between insulin resistance and heart disease, a number of exciting future research and trial promise to contribute to the amelioration of heart disease prognosis.

PROLONGED STIMULATION OF βAR INDUCES CARDIAC INSULIN RESISTANCE

Previous studies have reported that up-regulation of βAR plays an important role in the pathogenesis of insulin resistance in several tissues, especially the heart (Morisco *et al*., 2005, 2007). Insulin resistance might be associated with sustained βAR stimulation and excessive sympathetic nerve activity leading to structural and functional abnormalities in the heart. Acute βAR stimulation physiologically augments cardiac contraction, whereas chronic stimulation of βAR promotes adverse cardiac remodeling leading to cardiac myocyte apoptosis, myocardial hypertrophy and heart failure (Rockman *et al*., 2002). In isolated perfused rat hearts, decreased insulin sensitivity was observed following isoproterenol (ISO) infusion and was accompanied by decreased GLUT4 levels (Heather *et al*., 2009). This study demonstrated that ISO infusion impaired *in vivo* cardiac functions, induced hypertrophy and decreased both fatty acid and glucose metabolism, which are similar to the alterations observed in animal models of myocardial infarction (Heather *et al*., 2009). In addition, sustained and overstimulation of myocardial βAR trigger a state of insulin resistance in cardiomyocytes (Morisco *et al*., 2005) and in heart tissue (Mangmool *et al*., 2016). Insulin resistance can be assessed by uptake of glucose, GLUT expression and translocation, insulin receptor-beta (IR-β) expression, and IRS-1 expression and phosphorylation. In our previous study (Mangmool *et al*., 2016), we reported that sustained βAR stimulation induces cardiac insulin resistance through attenuation of glucose uptake and inhibition of GLUT4 synthesis in the heart.

Stimulation of βARs has a biphasic effect on insulin-induced glucose uptake in cardiomyocytes (Morisco *et al*., 2005). Rapid stimulation of βAR (within minutes) has synergistic effects on insulin-induced glucose uptake. This effect of βAR is mediated by phosphorylation of Akt at threonine 308 (Thr308) through protein kinase A (PKA)/Ca^{2+}-dependent and PI3K-independent pathway, whereas insulin-induced Thr phosphorylation of Akt exclusively depends on PI3K (Morisco *et al*., 2005). On the other hand, chronic stimulation of βAR inhibits both insulin-induced glucose uptake and autophosphorylation of the IRs which is mediated by serine 473 phosphorylation of Akt through PKA/Ca^{2+} and PI3K-dependent pathways (Morisco *et al*., 2005).

Catecholamines (i.e., epinephrine, norepinephrine) including glucagon, cortisol and growth hormone are up-regulated in patients with heart failure and likely play a role in the development of insulin resistance and alteration of glucose homeostasis (Lager, 1991; Nikolaidis *et al*., 2004). Up-regulation of sympathetic nervous system (e.g., elevation of catecholamines) activity not only enhances the development of insulin resistance but also directly contributes to the progression of heart failure. Elevation of catecholamine levels also stimulates lipolysis, increasing circulating FFA levels and exacerbating insulin resistance (Nonogaki, 2000). Treatment with carvedilol (βAR antagonist) could decrease the utilization of FFA with improved myocardial efficiency (Wallhaus *et al*., 2001; Nikolaidis *et al*., 2006).

In addition, insulin-induced glucose uptake might be suppressed by epinephrine, norepinephrine, or isoproterenol (ISO) in adipocytes. This inhibition was characterized by stimulation of several subtypes of βARs (Klein *et al*., 1999). Stimulation of βARs with epinephrine or norepinephrine reduced the amount of plasma membrane GLUT4 by inhibiting insulin-induced GLUT4 translocation in adipocytes. In the same way, stimulation of βAR in brown adipose tissue has the similar effects (Klein *et al*., 1999). The inhibitory effect of catecholamines on insulin-induced glucose uptake has also been observed in rat skeletal muscle (Chiasson *et al*., 1981; Lee *et al*., 1997).

The above results indicate that alterations of glucose homeostasis and insulin resistance are often associated with pathological conditions characterized by excessive activation of sympathetic nervous system and sustained stimulation of βARs. (Deibert and DeFronzo, 1980; Morisco *et al*., 2005; Cipolletta *et al*., 2009). However, the exact molecular mechanism by which βAR mediates cardiac insulin resistance and changes in its signaling have not been fully elucidated.

A few studies have demonstrated that βAR-mediated insulin resistance may be induced through cAMP- and PKA-dependent signaling pathways. For example, cAMP analogs inhibited insulin-stimulated glucose uptake in 3T3-L1 adipocytes and rat adipocytes (Kashiwagi *et al*., 1983; van Putten and Krans, 1985). Moreover, the activation of adenylyl cyclase (AC) by forskolin prevented insulin-stimulated glucose transport in rat adipocytes (Joost and Steinfelder, 1987). In 3T3-L1 adipocytes, treatment with either forskolin or 8-bromo-cAMP resulted in the down-regulation of GLUT4 mRNA (Kaestner *et al*., 1991). Similarly, blockade of AC abolishes the inhibitory effect of ISO on insulin-stimulated glucose uptake and GLUT4 mRNA expression in rat cardiomyocytes (Mangmool *et al*., 2016). Therefore, cAMP appears to be an important mediator of βAR-mediated insulin resistance in the heart. Cellular responses to cAMP might be associated with many cAMP effectors, including PKA and a cAMP-regulated guanine nucleotide exchange factor (Epac). Therefore, the divergent cellular

responses induced by cAMP and their compartmentalization remain to be defined.

After agonist binding, the βAR can couple with the α subunit of heterotrimeric G protein ($G\alpha_s$), which results in activation of AC, followed by elevation of cAMP levels (Pierce *et al*., 2002). There are at least two pathways induced by cAMP, a PKA-dependent and PKA-independent pathway including Epac (Bos, 2006; Oestreich *et al*., 2007). Chronic stimulation of βAR inhibited both insulin-induced glucose uptake and the autophosphorylation of IRs, which is mediated by PKA/Ca^{2+} and PI3K-dependent pathways (Morisco *et al*., 2005). In brown adipocytes, insulin-induced glucose uptake was inhibited by prestimulation of β_3ARs (Klein *et al*., 1999). This effect was mediated via a PKA-dependent signaling pathway. In cardiomyocytes, sustained βAR stimulation-mediated inhibition of insulin-induced glucose uptake and GLUT4 expression was not affected by Epac depletion or inhibition of its activity, whereas blockade of either AC or PKA activity strongly inhibited these effects (Mangmool *et al*., 2016). Thus, the cAMP/PKA-dependent signaling pathway plays a major role in the pathogenesis of insulin resistance that is associated with an overactive sympathetic system in the heart.

Moreover, βAR stimulation impairs insulin signaling mechanism through an Akt-dependent pathway in the heart, demonstrating that Akt crucially contributes to the regulation of insulin sensitivity and plays a key role in βAR stimulated insulin resistance in cardiomyocytes (Morisco *et al*., 2005). There is therefore ample evidence to link insulin resistance to up-regulation of the sympathetic nervous system.

SUBTYPE SPECIFICITY OF βARS ON CARDIAC INSULIN RESISTANCE

Insulin resistance is an underlying common feature of heart disease, probably consequent to chronic βAR stimulation (Morisco *et al*., 2005, 2006). Although all β_1-, β_2- and β_3-ARs can couple to the Gs protein and stimulate AC, to generate the second messenger cAMP, there are considerable differences in their ability to activate downstream signaling pathways. For instance, the CaMKII-dependent induction of fetal genes and apoptotic pathways in cardiac myocytes is specifically mediated by β_1ARs, but not β_2ARs (Zhu *et al*., 2003; Sucharov *et al*., 2006). In contrast, stimulation of β_2ARs activates cell survival signals, whereas β_1ARs elicit apoptotic pathways in cardiac myocytes (Communal *et al*., 1999).

The β_2AR is widely expressed in several tissues including the vasculature, liver, skeletal muscle, adipocytes, and cardiac muscle, and therefore participates in cardiac function and body metabolism. Indeed, epidemiological studies have shown an association of β_2AR genetic polymorphism with obesity, diabetes, and hyperlipidemia (Ishiyama-Shigemoto *et al*., 1999; Yamada *et al*., 1999; Iaccarino *et al*., 2005b). βAR (but not αAR) stimulation attenuates insulin-induced glucose uptake by inhibiting GLUT4 translocation to the plasma membrane in 3T3-L1 adipocytes (Mulder *et al*., 2005). This inhibitory effect on GLUT4 translocation is mediated at least by the β_2- and β_3-ARs. The insulin-induced tyrosine phosphorylation of IRs, IRS-1, and IRS-2 was reduced by stimulating β_3ARs in brown adipose tissue (Klein *et al*., 1999). In addition, insulin-induced glucose uptake was completely blocked by stimulating β_3ARs (Klein *et al*., 1999). In HEK-293 cells that stably overexpressed β_2AR, chronic β_2AR stimulation resulted in impaired insulin-induced glucose uptake and IRS-1 phosphorylation (Cipolletta *et al*., 2009). Moreover, overstimulation of β_2ARs inhibited insulin-induced GLUT4 translocation in β_2AR-overexpressing HEK-293 cells and rat cardiomyocytes (Mangmool *et al*., 2016). Thus, β_2AR is considered as the βAR subtype that interferes with insulin-mediated GLUT4 translocation in the heart. Moreover, the reduction of insulin-induced autophosphorylation of IRs in response to chronic βAR stimulation was associated with Thr phosphorylation of the β-subunit of IRs (Morisco *et al*., 2005). In rat cardiomyocytes, ISO-induced phosphorylation of the β-subunit of IRs on Thr residues was mediated by the β_1AR subtype (Morisco *et al*., 2005). In contrast, our recent study showed that overstimulation of β_2AR significantly reduced the ability of insulin to induce glucose uptake and the translocation of GLUT4 in cardiomyocytes and heart tissue (Mangmool *et al*., 2016). That previous study showed that ISO-induced phosphorylation of IR on Thr residues was inhibited by propranolol (a non-selective β-blocker) and betaxolol (a selective β_1AR antagonist), whereas pretreatment with ICI118,551 (a specific β_2AR antagonist) did not affect this response. These results suggest that β_1AR selectively mediates Thr phosphorylation of IR after long term stimulation by ISO in rat neonatal cardiomyocytes (Morisco *et al*., 2005). Nonetheless, the mechanisms by which β_1AR stimulation inhibits the insulin-mediated phosphorylation of IRs, and stimulation of β_2AR suppresses insulin-induced glucose uptake and GLUT4 translocation in cardiomyocytes have not been fully resolved.

Even though β_1AR and β_2AR share 54% sequence identity, the C-terminus (CT) tail of these two subtypes play different roles in receptor endocytosis and signaling pathways. The binding affinity of β-arrestin1 and 2 to the CT tail of the β_1AR is lower than that for the β_2AR (Shiina *et al*., 2000). A chimeric β_2AR containing the C-tail region of β_1AR loses its ability to promote β-arrestin2-mediated ERK nuclear translocation (Kobayashi *et al*., 2005). In addition, β_1ARs and β_2ARs have different PDZ-binding motifs within their CT tail leading to the recruitment of unique regulatory proteins when stimulated by their agonists. For example, the Na^+/H^+ exchanger regulatory factor (NHERF) binds to a DSLL motif in the CT tail of β_2ARs to stimulate Na^+/H^+ exchange (Hall *et al*., 1998), whereas *N*-methyl-D-aspartate receptor binds to an ESKV motif within the CT of β_1ARs and promotes receptor internalization (Hu *et al*., 2000). The CT tail of β_1AR, but not β_2AR, mediates a unique conformation of β-arrestin that allows scaffolding of Epac and CaMKII to form a stable complex, leading to the subsequent cAMP/Epac-mediated activation of CaMKII (Yoo *et al*., 2009; Mangmool *et al*., 2010). It is still unknown how the CT tail of βARs (both β_1- and β_2-AR) regulates βAR-mediated insulin resistance in the heart.

THE ROLE OF GRK ON CARDIAC INSULIN RESISTANCE

βARs belong to the GPCR family. Agonist binding of βAR leads to a dissociation of the heterotrimeric G protein into Gα and Gβγ subunits, both of which activate several effectors (Pierce *et al*., 2002). In parallel, agonist stimulation also triggers the termination of GPCR signals with rapid attenuation of receptor responsiveness, termed as receptor desensitization.

This process is initiated by GRKs that specifically bind to and phosphorylate the agonist-occupied receptors (Benovic *et al*., 1986). GRK-mediated phosphorylation promotes the binding of β-arrestins to the receptors, and β-arrestins sterically inhibit further interactions of the receptors with G proteins (Ferguson *et al*., 1996). The β-arrestin-bound receptors are then internalized from the plasma membrane via clathrin-coated vesicles (Krupnick and Benovic, 1998). Overexpression of GRK enhances the βAR-mediated β-arrestin translocation to the plasma membrane and βAR internalization (Mangmool *et al*., 2006).

Based on sequence and structural homology, the members of the GRK family can be subdivided into the following three groups: rhodopsin kinase subfamily (GRK1 and GRK7); βARK subfamily (GRK2 and GRK3); and GRK4 subfamily (GRK4, GRK5, and GRK6) (Penn *et al*., 2000). GRKs are composed of three distinct domains as follows: (1) a highly conserved centrally located catalytic domain flanked by (2) an amino-terminal domain that includes a region of homology to the regulator of G protein signaling (RGS) protein and (3) a carboxyl-terminal domain of various lengths (Penela *et al*., 2003).

GRK expression and activity has been found to be altered in heart disease. It has been shown that GRK2 synthesis and its activity are increased in the failing heart with consequent decrease in β_1AR levels (Ungerer *et al*., 1993). Increased GRK2 expression and activity was found in cardiomyocytes from patients with heart failure (Ungerer *et al*., 1994). Moreover, overexpression of GRK2 reduces the response to βAR stimulation as a result of increased receptor desensitization (Koch *et al*., 1995). Inhibition of GRK2 activity enhanced cardiac contractility and βAR responsiveness (Koch *et al*., 1995; Akhter *et al*., 1998), confirming the essential role of GRK2 in mediating βAR desensitization. Beyond its classical function on receptor desensitization, GRK2 mediates the inhibitory effects of βAR on insulin signaling (Usui *et al*., 2004b; Cipolletta *et al*., 2009), through mechanisms that are still subject to debate. In animal models of diabetes (both Zucker diabetic fatty rats and db/db mice), inhibition of GRK2/3 through synthetic peptides (derived from a kinase-substrate interaction site in GRK2/3) rescues glucose tolerance and enhances insulin sensitivity (Anis *et al*., 2004), suggesting that GRK2 phosphorylates an unknown substrate to induce impaired glucose tolerance. It is interesting to note that GRKs are ubiquitously expressed in mammalian tissues, and their activities and production are increased in conditions characterized by chronic adrenergic activation, such as human congestive heart failure (Iaccarino *et al*., 2005a) and hypertension (Izzo *et al*., 2008). For example, GRK2 is up-regulated through chronic βAR activation (Iaccarino *et al*., 2001). All the above considerations support the hypothesis that GRK2 is involved in the progression of insulin resistance induced by chronic βAR activation.

Endothelin-1 (ET-1) treatment leads to an enhanced IRS-1 degradation, a decreased tyrosine phosphorylation of IRS-1 and a decreased insulin-stimulated glucose transport in 3T3-L1 adipocytes (Ishibashi *et al*., 2001). Insulin-induced GLUT4 translocation was inhibited by pretreatment with ET-1 for 24 h. This inhibitory effect was rescued by microinjection of anti-GRK2 antibody or GRK2 short interfering RNA (Usui *et al*., 2005), suggesting a potential role of GRK2 on insulin resistance. Overexpression of kinase dead GRK2 mutant, but not wild-type GRK2, inhibited ET-1-induced serine 612 phosphorylation of IRS-1 and restored activation of this pathway. Moreover, overexpression of the kinase dead GRK2 mutant suppressed ET-1-induced IRS-1 degradation, whereas wild-type-GRK2 did not, demonstrating a potential role for GRK2 kinase activity in this mechanism. Inhibition of GRK2 could also rescue the ET-1-induced insulin resistance (Usui *et al*., 2005). Taken together, these results suggest that GRK2 mediates ET-1-induced insulin resistance by its kinase activity on IRS-1 serine phosphorylation and degradation.

Treatment with insulin causes tyrosine phosphorylation of IRS-1 and induces glucose uptake in human kidney embryonic (HEK-293) cells (Cipolletta *et al*., 2009). Overexpression of β_2AR increases GRK2 expression that is associated with significant deficit of IRS1 activation and glucose uptake by insulin (Cipolletta *et al*., 2009). Similarly, overexpression of GRK2 prevents insulin-induced tyrosine phosphorylation of IRS1 and insulin-induced glucose uptake. This study also found that GRK2 interacts with IRS1, but not IR (Cipolletta *et al*., 2009). These results provide a regulatory network between insulin signaling and GRK2 in which GRK2 kinase activity mediates βAR-induced insulin resistance and that inhibition of GRK2 activity leads to enhanced insulin sensitivity both in cell and animal models of insulin resistance.

Chronic stimulation of βAR signaling or marked overexpression of β_2ARs causes the development of insulin resistance through an increase in GRK2 expression levels. GRK2 may function as a key negative regulator of insulin responsiveness. In endothelial cells, insulin stimulates a signaling pathway which leads to elevation of NO through the activation of endothelial NO synthase (eNOS) and subsequently promotes vasodilation (Kuboki *et al*., 2000). A recent study showed that the interaction between GRK2 and Akt inhibited the phosphorylation of Akt on Thr 308, resulting in decreased eNOS phosphorylation and a consequent reduction of NO production (Taguchi *et al*., 2012). However, the precise mechanism for GRK2 binding to IR and formation of the complex with IR and/or Akt remains to be elucidated. Recently, Luan *et al*. (2009) reported that insulin stimulates the formation of a new β-arrestin2 signal complex in which β-arrestin2 acts as a scaffold for the translocation of Akt to IR, even though insulin is not a GPCR. In contrast, Taguchi *et al*. (2012) demonstrated that up-regulation of GRK2 and a decrease in β-arrestin2 inhibited insulin-induced stimulation of Akt/eNOS signaling and that GRK2 overactivation may be induced by an increase in PKC activity in aortas from diabetic mice with hyperinsulinemia. In the normal aorta, β-arrestin2 binds to Akt under insulin stimulation. In contrast, insulin causes translocation of GRK2 to the plasma membrane in diabetes, where it binds to Akt and prevents β-arrestin2 from binding to Akt as GRK2 remains bound (Taguchi *et al*., 2012). As mention above, previous studies have described a relationship between insulin resistance and increased GRK2 levels in pathological conditions. GRK2 inhibition can improve glucose uptake and insulin resistance in the heart (Ciccarelli *et al*., 2011). Thus, GRK2 inhibitor (e.g., βARKct; C-terminus of βAR kinase) could be used for the possible treatment of insulin resistance in the heart.

THE ROLE OF β-ARRESTINS ON INSULIN RESISTANCE

β-Arrestins are key regulators of βAR (G protein-coupled receptor) endocytosis and trafficking, and G protein-indepen-

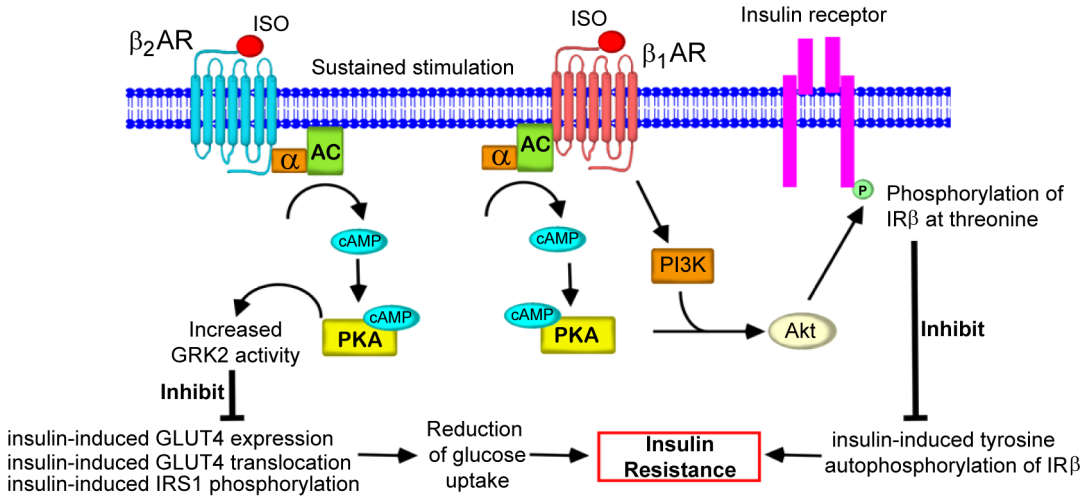

Fig. 4. Schematic diagram representing the signaling pathway for βAR-mediated cardiac insulin resistance. Agonist binding to β_2ARs leads to the G protein-mediated activation of AC and cAMP generation. cAMP directly binds to and activates PKA. In β_2AR signaling, PKA phosphorylates GRK2, which subsequently increases GRK2 activity. This leads to the inhibition of insulin-induced GLUT4 expression and the translocation of GLUT4 to the plasma membrane, thereby interfering with glucose uptake into cells and insulin-induced IRS1 phosphorylation. In β_1AR signaling, PKA and PI3K activate Akt by phosphorylation at Ser 473, which in turn, phosphorylates the β subunit of insulin receptor (IRβ) at Thr residues. Tyrosine phosphorylation of IRβ inhibits insulin-induced tyrosine autophosphorylation of IRβ, leading to impairment of insulin signaling. These conditions lead to insulin resistance in the heart. Treatment with β-blockers antagonizes ISO-mediated insulin resistance in the heart.

dent signaling. β-Arrestins interact with agonist-occupied and phosphorylated receptors and inhibit further G protein activation (Ferguson *et al*., 1996). Beyond their classical function, β-arrestins also function as scaffolding proteins linking receptors to several effectors such as ERK1/2, JNK, Src and Mdm2, an ubiquitin ligase (Lefkowitz and Shenoy, 2005; Lefkowitz *et al*., 2006). The scaffolding of Ca^{2+}/calmodulin kinase II (CaMKII) and Epac by β-arrestin with subsequent translocation of this multimeric complex to agonist-occupied β_1ARs is an essential mechanism for β_1AR-mediated CaMKII activation (Mangmool *et al*., 2010).

Insulin resistance is a central feature of type 2 diabetes and is caused by a deficiency in IR signaling. Luan *et al*. (2009) reported that the amount of β-arrestin2 was decreased either in a mouse model of type 2 diabetes (db/db) or in liver samples from diabetic patients. Deletion of β-arrestin2 induces insulin resistance, whereas overexpression of β-arrestin2 restores insulin sensitivity (Luan *et al*., 2009). They also found that β-arrestin 2 forms the complex with Akt and Src after insulin stimulation which is consistent with previous studies reporting that β-arrestin interacts with Akt (Beaulieu *et al*., 2005) and Src (Luttrell *et al*., 1999). This association between Src and Akt was reduced in liver samples from β-arrestin 2 KO mice, leading to the disturbance of insulin signaling and development of insulin resistance. In addition, insulin-stimulated phosphorylation of Akt, GSK3β and Foxo1 were reduced in livers of β-arrestin2 KO mice while increased in β-arrestin2 transgenic mice (Luan *et al*., 2009), emphasizing that β-arrrestin2 controls the insulin signaling pathway.

In addition to β-arrestin2, β-arrestin1 also plays a role in insulin signaling and insulin resistance. Overexpression of β-arrestin-1 attenuated insulin-induced IRS-1 degradation, leading to enhanced insulin signaling downstream of IRS-1. Whereas insulin-induced degradation of IRS-1 was increased in cells treated with β-arrestin1 siRNA, stimulation of IR promotes the formation of a complex between IRS-1 and Mdm2, an E3 ubiquitin ligase enzyme, and this complex could be inhibited by β-arrestin1, resulting in a decreased ubiquitin content of IRS-1 (Usui *et al*., 2004a). From these results, they postulated that both β-arrestin1 and IRS-1 competitively bind to Mdm2, which can ubiquitinate IRS-1. Moreover, dephosphorylation of S412 on β-arrestin and the amino terminus of β-arrestin1 are required for this effect of β-arrestin on IRS-1 degradation (Usui *et al*., 2004a). Inhibition of β-arrestin1 leads to enhanced IRS-1 degradation and accentuates cellular insulin resistance.

TREATMENT WITH β-BLOCKERS IMPROVES βAR-MEDIATED CARDIAC INSULIN RESISTANCE

β-Blockers are widely used to treat heart failure, angina and myocardial infarction. They act by blocking the overstimulation of catecholamine to βAR in the heart (Bangalore *et al*., 2007). During heart failure, a large amount of catecholamine is released from synaptic ends and this exerts harmful effects on the heart. Considering that βAR mediates cardiac insulin resistance as shown in previous studies (Morisco *et al*., 2005; Ciccarelli *et al*., 2011; Mangmool *et al*., 2016), β-blockers are expected to exert beneficial effects by suppressing insulin resistance in the heart.

Propranolol and metoprolol were able to abolish ISO inhibition of insulin-induced glucose uptake, GLUT4 expression, and GLUT4 translocation, whereas atenolol had no effect. The differential potency of these β-blockers might be due to differences in their affinity to βAR subtypes. We show that ISO-mediated cardiac insulin resistance involves β_2AR; hence, a β-blocker that has a higher affinity for β_2AR could potentially exert an increased beneficial effect. Propranolol was found to antagonize both the β_1AR and β_2AR subtypes, while atenolol

and metoprolol displayed a selectivity for β_1AR (Hoffmann *et al*., 2004). Previous studies have investigated the K_i values by βAR binding assay and showed that various β-blockers have different Ki values for human β_2AR: propranolol (0.8 nM), metoprolol (2,960 nM), and atenolol (8,140 nM) (Hoffmann *et al*., 2004). Moreover, the offset in activity induced by β-blockers in the heart is related to their lipophilicity (Doggrell and Henderson, 1998). The βAR blocking activity of atenolol, which exhibits low lipophilicity, was offset more quickly than that of propranolol, which exhibits high lipophilicity on contractile responses to ISO (Doggrell and Henderson, 1998). Collectively, propranolol showed a more superior effect than atenolol and metoprolol in suppressing β_2AR-mediated insulin resistance.

Interestingly, in addition to blocking βAR effects, some β-blockers have been shown to evoke signal transduction through the βARs in G protein-independent and β-arrestin-dependent manner (Wisler *et al*., 2007; Nakaya *et al*., 2012). Long-term administration of metoprolol to mice induced cardiac fibrosis through β-arrestin2 and GRK5-dependent pathway (Nakaya *et al*., 2012). Although metoprolol induces cardiac fibrosis, it still has a beneficial effect on heart failure by inhibiting catecholamine overstimulation in heart failure. Thus, it will be worthwhile to determine G protein-independent signal transduction of β-blockers on cardiac insulin resistance. However, some β-blockers have additional effects in addition to βAR blockades, such as intrinsic sympathomimetic activity, and antioxidant and vasodilating properties. Thus, these additional effects of β-blockers require further study for their ability to attenuate βAR-mediated insulin resistance in the heart.

SUMMARY

Based on data from previous studies, we propose that sustained and overstimulation of βARs leads to the development of heart failure and is associated with the pathogenesis of insulin resistance in the heart. Overstimulation of β_2ARs enhances insulin resistance in the heart by inhibiting insulin-induced glucose uptake, GLUT4 synthesis, and translocation of GLUT4 to the plasma membrane via the cAMP/PKA/GRK2-dependent pathway (Fig. 4). As treatment with β-blockers such as propranolol and metoprolol could antagonize the effects of ISO-mediated cardiac insulin resistance in the heart, β-blockers may exert beneficial effects that can improve insulin resistance in the heart. Moreover, inhibition of GRK2 by βARKct gene therapy could be used for the improvement of glucose uptake in the heart.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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