Role of nuclear receptors in the modulation of insulin secretion in lipid-induced insulin resistance

Mary C. Sugden¹ and Mark J. Holness
Centre for Diabetes and Metabolic Medicine, Institute of Cell and Molecular Science, Barts and the London School of Medicine and Dentistry, Blizard Building, 4 Newark Street, London E1 2AT, U.K.

Abstract

In healthy individuals, a hyperbolic relationship exists between whole-body insulin-sensitivity and insulin secretion. Thus, for any difference in insulin-sensitivity, a reciprocal proportionate change occurs in insulin secretion. Such a feedback loop is evident in healthy individuals ingesting diets high in saturated fat and in late pregnancy where, despite lipid-induced insulin resistance, glucose tolerance is maintained through augmented GSIS (glucose-stimulated insulin secretion). NRs (nuclear receptors) are members of a superfamily of ligand-regulated and orphan transcription factors. On activation by a cognate ligand, many ligand-activated NRs recruit the RXR (retinoid X receptor) for heterodimer formation. Such NRs include the PPARs (peroxisome-proliferator-activated receptors), which are involved in lipid sensing and liporegulation. PPARs exert important lipid-lowering effects in vivo, thereby opposing the development of lipid-induced insulin resistance by relieving the inhibition of insulin-stimulated glucose disposal by muscle and lowering the necessity for augmented GSIS to counter lipid-induced insulin resistance. Long-chain fatty acids are proposed as natural PPAR ligands and some specific endogenous pathways of lipid metabolism are believed to generate PPAR agonists. Other NRs, e.g. the LXR (liver X receptor), which senses expansion of the metabolically active pool of cholesterol, and the FXR (farnesoid X receptor; NR1H4), which, like the LXR, is involved in sterol metabolism, also modulate systemic levels and insulin-sensitivity. In this review, we discuss how these NRs impact insulin secretion via effects on the insulin-sensitivity–insulin secretion feedback loop and, in some cases, via direct effects on the islet itself. In addition, we discuss interactions between these nutrient/metabolite-responsive NRs and NRs that are central to the action of metabolically important hormones, including (i) the glucocorticoid receptor, critical for maintaining glucose homeostasis in stress, inflammation and during fasting, and (ii) the thyroid hormone receptors, vital for maintenance of oxidative functions. We present data indicating that the RXR occupies a key role in directly modulating islet function and that its heterodimerization with at least two of its partners modulates GSIS.

Introduction

The pancreatic β-cell contains a range of NRs (nuclear receptors), many implicated in regulation of insulin secretion. In health, a hyperbolic relationship exists between whole-body insulin-sensitivity and insulin secretion, such that altered insulin-sensitivity elicits a reciprocal proportionate change in insulin secretion. This feedback is also evident in healthy individuals ingesting high-saturated-fat diets and in late pregnancy where, despite lipid-induced insulin resistance, glucose tolerance is maintained through augmented GSIS (glucose-stimulated insulin secretion). In this review, we discuss how selected NRs impact insulin secretion via effects on insulin-sensitivity–insulin secretion feedback and/or direct effects on the islet.

NRs: an overview

PPARs (peroxisome-proliferator-activated receptors) (α, γ and δ) are lipid sensing and liporegulatory. PPARγ-1 is detected in pancreatic β-cells [1,2]. The splice variant PPARγ-2 is expressed primarily in adipose tissue. PPARα and PPARδ are expressed in a wide range of tissues, including pancreatic islets [2–4]. PPARγ is activated by eicosanoids, prostanoids [PG (prostaglandin) A1, PGA2 and PGD2] and TZDs (thiazolidinediones). PPARα ligands include unsaturated FAs (fatty acids), HETEs (hydroxyeicosatetraenoic acids) and fibrates [5]. On activation, PPARs recruit the RXR (retinoid X receptor). By lowering circulating lipids, PPARs oppose lipid-induced insulin resistance [6,7], reducing the necessity for augmented GSIS. LXRα (liver X receptors) (α and β) also heterodimerize with RXR. LXRα is abundant in liver, adipose
tissue, intestine, kidney and spleen; LXRβ is ubiquitously expressed. Natural ligands of LXR include oxysterols, and LXRαs operate as cholesterol sensors, protecting from cholesterol overload by increasing the expression of genes involved in removing cholesterol and other lipids from cells.

Interactions occur between nutrient-responsive NRs and endocrine NRs, which include the GR (GC (glucocorticoid) receptor) and TRs (thyroid hormone receptors). As well as affecting glucose homoeostasis, GCs counteract the production of pro-inflammatory cytokines [e.g. IL (interleukin)-6 and TNFα (tumour necrosis factor α)] and stimulate that of anti-inflammatory cytokines. The unliganded GR forms a complex with proteins, including heat-shock proteins and immunophilin chaperones (reviewed in [8]). On ligand binding, the receptor dissociates from the complex, translocates to the nucleus and binds to specific DNA sequences as a homodimer. The anti-inflammatory effects of the GR are exerted through inhibitory interactions with non-NR transcription factors such as NF-κB (nuclear factor κB; reviewed [5]). Many immunoregulatory genes coding for cytokines, such as TNFα and IL-6, contain NF-κB sites in their promoters or regulatory regions.

TRs bind to specific recognition sites on DNA as dimers. TREs (thyroid hormone response elements) recruit either a TR–TR homodimer or an RXR–TR heterodimer [9–12]. In the absence of T3 (tri-iodothyronine), TRs recruit co-repressor proteins [13]: binding of T3 causes release of co-repressors and recruitment of co-activators [14]. RXR ligands have little effect on transcriptional activation by TR–RXR heterodimers [15]. The actions of both the GCs and thyroid hormones on their target tissues are determined by the bioavailability of the hormone (systemic transport) and target tissue-specific hormone-modifying enzymes.

**Mechanisms of islet compensation for insulin resistance**

Increased glucose utilization and oxidation, with increased anaplerosis and pyruvate cycling, all probably contribute to enhanced GSIS in islet compensation for insulin resistance. As well as acutely stimulating GSIS, glucose has a long-term action in enhancing the expression of genes involved in β-cell glucose sensing [Slc2a2, coding for GLUT2 (glucose transporter 2); Gck, coding for Gk (glucokinase)], electrical activity (Abcc8 and Kcnj11, coding for the KATP–channel subunits and sulfonyl receptor 1) and lipogenesis (Fas, FA synthase; Acc1, acetyl-CoA carboxylase; Scd1, SCD1) [31–34]. A feed-forward action of the high level of glucose in insulin resistance [35] may promote islet glucose utilization via enhanced gene expression, resulting in an increased functional capacity for GSIS (Figure 1). Similarly, insulin resistance elevates plasma FA and lipoprotein-TAG concentrations, and so acute beneficial actions of FA to enhance GSIS may contribute to islet compensation (Figure 1).

A GPCR (G-protein-coupled receptor), GPR40 (G-protein-coupled receptor 40)/FFAR1 (free FA receptor 1), is implicated in the acute response of GSIS to exogenous FA [36–38], probably with coupling with Gαq/11 [36]. LPL (lipoprotein lipase), expressed in islets [39], generates monoacylglycerol and FA locally. However, β-cell FA supply is not restricted to exogenous FA and lipoproteins, but includes intracellular TAG. TAG can supply FAs via hydrolysis when exogenous FA supply is low or, as outlined above, TAG can act as a sink for excess incoming FAs for preventing lipid toxicity. Severe depletion of islet TAG or inhibition of lipolysis of islet TAG suppresses GSIS, suggesting that TAG
lipolysis may generate lipid-derived coupling signal(s) optimizing GSIS [40]. GSIS is augmented in ZF (Zucker fatty) rats (that are obese, but not characterized by compromised β-cell function) in conjunction with enhanced TAG/FA cycling (elevated esterification and lipolysis without TAG accumulation), suggesting that TAG/FA cycling as well as pyruvate cycling can enhance β-cell function during islet compensation by generating key signalling lipids [41].

Islet compensation for insulin resistance also involves an increase in β-cell mass, reflecting increased β-cell neogenesis/proliferation relative to apoptosis. The β-cell-specific insulin receptor-knockout mouse shows an age-dependent decrease in β-cell mass, with selective loss of GSIS [42,43]. IRS-1 (insulin receptor substrate-1)-deficient islets and β-cell lines have decreased GSIS, partially restored by re-expression of IRS-1 [43]. IRS-2-null mice show decreased GSIS [42,44,45] and lack of compensatory β-cell hyperplasia despite insulin resistance [45]. Thus the influence of insulin on β-cell mass may be dependent on the insulin signalling status of the islet.

Agonists of some GPCRs activate RTKs (receptor tyrosine kinases) in the absence of RTK ligand. Such a transactivation contributes to the growth-promoting activities of many GPCR ligands via the ERK (extracellular-signal-regulated kinase) signalling cascade (reviewed in [46]). The ERK pathway is also involved in pancreatic β-cell growth [47]. RTKs exert some of their effects by engagement of GPCR signalling molecules, including heterotrimeric G-proteins. Insulin and IGF-1 (insulin-like growth factor-1) receptors associate with Gαi and Gβγ proteins to activate the ERK pathway and mitogenesis [48,49]. Given that FA and GLP-1 act via GPCRs, we hypothesize that FA signalling via GPR40/FFAR1 and/or GLP-1 signalling via the GLP1R interact with insulin or IGF-1 to enhance β-cell mass in insulin-resistant states as part of the insulin action–secretion feedback in islet compensation.
PPARγ, insulin resistance and islet compensation for insulin resistance

PPARγ-2 promotes adipogenesis and adipocyte differentiation, modifies adipokine and cytokine production and regulates genes involved in lipid uptake and sequestration. In insulin resistance, impairment of these functions increases exposure of other tissues to liquids (reviewed in [50]). Many characteristics of β-cell dysfunction in diabetes are reproduced on prolonged exposure of islets to high FA concentrations. Thus the combination of increased FA flux and excessive TAG accumulation may lead to failure of GSIS [51,52]. Islet TAG content is increased in PPARγ−/− mice in which adipose-tissue TAG accumulation is reduced, with associated deterioration of GSIS [53]. Increased FA flux from adipose tissue also increases intracellular lipids in liver and skeletal muscle, with associated development of insulin resistance, introducing the requirement for compensatory insulin hypersecretion [35].

Adipose mass and adipocyte size increase during early pregnancy and decline during late pregnancy [54] (reviewed in [55,56]). The pattern of change in adipose mass is depot-specific, with a decline in subcutaneous fat mass late in pregnancy [54]. PPARγ agonism redistributes fat towards the subcutaneous depot away from the viscera [57]. In rats, adipocyte LPL activity (which reflects adipocyte PPARγ signalling [58]) increases early in pregnancy, and decreases in late pregnancy [59,60], suggesting that PPARγ signalling is important in adipocyte adaptations to pregnancy and, by implication, adipocyte lipid buffering, whole-body insulin-sensitivity and the requirement for islet compensation. Altered PPARγ signalling interacts with prolactin and PL (placental lactogen): the latter promote adipocyte TAG breakdown [61]. Decreased PPARγ signalling and increased PRL signalling in adipose tissue as pregnancy advances are predicted to redirect lipid to non-adipose tissue and, as predicted from increased lipid delivery, maternal insulin resistance develops as pregnancy proceeds [62].

In obesity, adipocyte expression and secretion of pro-inflammatory adipokines are increased [63,64]. We propose that exposure to pro-inflammatory cytokines due to increased cytokine production from adipose tissue could impair the β-cell response to lipid-induced insulin resistance, either in response to obesity/high-fat diet or pregnancy. Cross-talk may also exist between insulin-resistant liver and/or skeletal muscle and the pancreatic islet via increased pro-inflammatory cytokine production. High-fat diets and obesity activate NF-κB in liver, and increase hepatic production of IL-6, IL-1β and TNFα [65]. Similarly, muscle-specific PGC-1α (PPARγ co-activator-1α)-knockout mice, although retaining muscle insulin-sensitivity, show glucose intolerance and impaired insulin secretion in conjunction with increased expression of pro-inflammatory genes in muscle and elevated circulating levels of IL-6 [66].

PPARα and islet function

PPPARα opposes PPARγ by activating the expression of genes involved in FA uptake and oxidation. GSIS, both in vivo and ex vivo, decreases during fasting [67–69]. A key role for PPARα in mediating islet adaptations to starvation has been demonstrated using PPARα-null mice, which develop hyperinsulinaemic hypoglycaemia on fasting [70,71]. Islets from fasted PPARα-null mice show increased GSIS and decreased FA oxidation [71], whereas PPARα over-expression in β-cell lines increases FA oxidation and inhibits GSIS [71,72]. PPARα activation, through augmenting FA oxidation, may therefore lower GSIS by increasing the removal (via oxidation) of a lipid-derived molecule that facilitates or augments GSIS. However, as acute FA addition amplifies GSIS to the same extent with WT (wild-type) and PPARα-null islets; PPARα signalling is not required for acute stimulatory effect of FA on GSIS [77].

Four PDHKs (pyruvate dehydrogenase kinases) (PDHKs 1–4) suppress PDC activity by phosphorylation [73,74]. PDHKs 1, 2 and 4 are expressed in islets [3]. Inactivation of PDC by overexpression of PDHK3 does not affect GSIS [75]. Nevertheless, an islet PDHK isoform shift exists on fasting, with selective enhancement of PDHK4 [3]. PPARα activation also selectively increases islet PDHK4 expression [3] (see Figure 2). Thus enhanced PDHK4 expression is a likely component of the GSIS starvation adaptation. PDHK up-regulation restricts entry of pyruvate into the tricarboxylic acid cycle via PDC (Figure 2). Although this impairs ATP generation from glucose, it facilitates fat oxidation by increasing the entry of FA-derived acetyl-CoA into the tricarboxylic acid cycle as citrate by increasing the availability of pyruvate for oxaloacetate formation, regenerating CoA.

The impact of PPARα deficiency on the insulin secretion–action feedback loop in dietary-lipid-induced insulin resistance cannot be assessed in vivo, as PPARα-null mice do not respond to high-fat feeding with the development of insulin resistance [76]. We therefore addressed whether activation of PPARα signalling impacts GSIS when there is islet compensation for insulin resistance in high-saturated-fat-fed rats [78]. Although dietary fat can provide PPARα ligands [79], we observed that islet compensation after dietary saturated fat feeding was not associated with increased islet PDHK4 expression (M.J. Holness and M.C. Sugden, unpublished work; results not shown), suggesting that the compensating islet could be ‘blind’ to PPARα ligands (e.g. because of decreased PPARα expression or, alternatively, that the fate of incoming saturated FAs is such that PPARα ligands are not generated). Polysaturated FAs activate PPARα (e.g. [80,81]) and their effects on gene expression are almost entirely mediated via PPARα [82]. Replacement of long-chain ω-3 FAs for 6–7% of dietary saturated fat prevents the development of insulin resistance [83–85]. We found that 24 h exposure to long-chain ω-3 FAs attenuated GSIS, without enhancing whole-body insulin-sensitivity [86], indicating a direct effect on the islet. Treatment of insulin-resistant high-saturated fat-fed rats with a PPARα ligand for 24 h in vivo also rapidly reversed insulin hypersecretion, mimicking the action of long-chain ω-3 FA enrichment [78]. We conclude that specific FAs variably influence islet function according to the extent that they activate PPARα signalling.
Islets contain TRα1 and TRβ1 [67, 87–89] and thyroid hormone signalling is associated with impaired GSIS, despite increased islet glucose utilization and oxidation [90]. We tested the hypothesis that hyperthyroidism might influence the PPARα-led islet adaptations to dietary fat via competition by TR with PPARα for RXR. Importantly, ex vivo, hyperthyroidism and high-fat feeding together elicited a major impairment of islet function (M.J. Holness and M.C. Sugden, unpublished work; results not shown). Thus, like PPARα activation, TR signalling impairs islet adaptations to dietary saturated fat.

**Pregnancy and PPARα signalling to GSIS**

Substantial alterations in β-cell function occur during mid- to late pregnancy: enhanced GSIS and a lowered glucose-stimulated threshold are associated with increased islet GLUT2 expression and GK activity [91], mediated by prolactin and the PLs. Glucose utilization and oxidation rates exceed those of non-pregnant islets [91], a pattern reminiscent of that induced by chronic exposure to high glucose [34]. Of relevance, the latter suppresses PPARα gene expression and the expression of PPARα-regulated genes [92]. Similarly, hyperglycaemia in vivo following 90% partial pancreatectomy in the rat model suppresses islet PPARα expression levels [93]. We examined the responses of insulin secretion by perifused rat islets to PPARα activation at days 15 and 19 of gestation (term=23 days) [69, 94]. The absence of a response to PPARα activation at day 15 suggested a shift away from a lipo-oxidative metabolic profile. The glucose threshold became increasingly susceptible to suppression by antecedent PPARα activation in vivo as pregnancy proceeded [69, 94], indicating a link with lipid catabolism.

We addressed whether an additional demand for insulin secretion imposed by dietary saturated fat could be...
impaired insulin secretion islet exposure to GCs in vivo pregnancy were modified by PPARα in augmenting islet function by PPARα urated fat during pregnancy were augmented or ameliorated function. We therefore assessed whether effects of dietary sat-

Emerging roles for the LXR in the insulin action–secretion feedback
LXR activation decreases glycaemia in diabetic animal models [99], an action attributed in part to enhanced insulin-sensitivity and in part to altered GSIS. Pharmacological LXR activators, including the non-steroidal agonist T0901317, have been used to investigate the functional effects of LXR activation on GSIS by isolated islets and in vivo. LXRβ activation in pancreatic β-cells increases insulin secretion accompanied by changes in expression of genes crucial for the β-cell phenotype [100–102]. Glucose stimulates LXR transcriptional activity in liver [103] and induces nuclear localization and activation of LXRs in β-cells [104]. After LXR activation by T0901317, insulin secretion and glucose uptake were increased at 8 mM glucose, but decreased at subthreshold glucose.

In liver, LXR activation stimulates the expression of SREBP-1c (sterol-regulatory-element-binding protein-1c). Elevated glucose increases SREBP-1c mRNA expression and total and mature SREBP-1c protein in β-cells [105,106]. Thus effects of LXR activation in the pancreatic β-cell would be predicted to mimic that of SREBP-1c overexpression or exposure to elevated glucose. Forced overexpression of SREBP-1c in β-cells up-regulated Fas and Acc1, effects observed in conjunction with massive islet TAG accumulation and suppressed GSIS [107], but islets lacking SREBP1 failed to induce lipogenic genes in response to high glucose; in addition, SREBP1 appears to be required for the induction by glucose of β-cell genes involved in glucose sensing [107]. Islets lacking SREBP1 also failed to induce SCD gene expression [107]. It is predicted that, in vivo, SCD1 deficiency might predispose islets to lipotoxicity, particularly if FA oxidation capacity is low (e.g. if PPARα signalling is deficient). Within the context of islet compensation for insulin resistance, SCD1 deficiency would be predicted to restrict the formation of TAG and thus the accelerated TAG/FAt cycling that has been shown to augment GSIS. It will be of interest to examine whether islet SCD1 expression is enhanced in mid-

GR–PPARα interactions in pregnancy
Treatment with the synthetic GC, dexamethasone, during the last third of pregnancy has been used to manipulate fetal development such that the offspring is 'programmed' to develop insulin resistance in adulthood (reviewed in [96]). We observed that such a treatment also caused maternal glucose intolerance [69,97]. Effects of dexamethasone in inducing hepatic insulin resistance require signalling via PPARα [98], and so we investigated whether effects of dexamethasone in pregnancy were modified by PPARα activation. Excessive islet exposure to GCs in vivo from day 14 of pregnancy impaired insulin secretion in vivo at day 19 of pregnancy [97], and pregnancy and GC treatment had non-additive effects in augmenting islet function in vivo [69]. Islet perfusions demonstrated no major differences in the characteristics of GSIS by perifused islets at day 19 of pregnancy between the control and dexamethasone-treated pregnant groups [69]. However, we found that antecedent PPARα activation select-

Does PGC-1α link NR signalling to islet function and compensation?
PGC-1α acts as a transcriptional co-activator for PPARs and LXR [109]. The ability of GR to bind to a GRE is modulated by PGC-1α [109,110]. Effects of thyroid hormone resemble those regulated by PGC-1α, and PGC-1α gene and protein expression are strongly regulated by T3 in vivo [111–113]. Given the involvement of all of these NRs in islet compensation, one might speculate that there could be a potential role for PGC-1α. Fasting (24 h) increases PGC-1α gene expression in islets, an effect reversed by refeeding
PGC-1α expression in islets from ob/ob mice and ZDF (Zucker diabetic fatty) rats [115]. PGC-1α overexpression impairs GSIS, suggesting that it can precipitate β-cell dysfunction [115]. Forced expression of PGC-1α in intact islets reduces GLUT2 expression by >50% and GK expression by approx. 75% [115], suggesting that enhanced islet GLUT2 and GK expression during pregnancy could reflect lowered PGC-1α expression.

Effects of chronic hyperlipidaemia and hyperglycaemia on PGC-1α gene expression have been investigated. Rat islets cultured with FA showed a dose-dependent increase in PGC-1α expression, whereas inhibition of PGC-1α (using small interfering RNA) improved the impairment of GSIS induced by chronic exposure to FA [114,116,117], suggesting that enhanced PGC-1α expression participates in chronic hyperlipidaemia-induced β-cell failure. However, exposure to very high (25 mM) glucose (which also impairs insulin secretion) suppressed PGC-1α mRNA expression [114]. With high-glucose and FA in combination, the effect of hyperglycaemia was dominant [114]. Suppression of PGC-1α expression could therefore become maladaptive if the islets are simultaneously exposed to high levels of lipids as occurs during late pregnancy, where there is significant hypertriglyceridaemia.

The existence of high levels of PGC-1α in islets of animal models of diabetes suggests that factors other than glycaemia become dominant for the regulation of islet PGC-1α expression as insulin resistance develops towards diabetes. Clearly, the role of PGC-1α and other NR co-activators and repressors in the physiology/pathophysiology of islet metabolism, particularly in relation to PPARα, needs to be further elucidated.

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