Lipases in the pancreatic β-cell: implications for insulin secretion

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Abstract

Lipids have been implicated in β-cell stimulus–secretion coupling. In such a role, lipases in β-cells would be required to generate lipid coupling factors. We have shown previously that glucose stimulates lipolysis in rodent islets. In addition, lipolysis and diacylglycerol lipase activity in islets are abolished by orlistat, an irreversible lipase inhibitor with a broad specificity for substrates. Moreover, orlistat dose-dependently inhibits glucose- and forskolin-stimulated insulin secretion, while leaving glucose oxidation and the rise in the ATP/ADP ratio intact. In an effort to identify β-cell lipase(s), we found that HSL (hormone-sensitive lipase), the rate-limiting enzyme for acylglycerol hydrolysis in adipocytes, is expressed in rodent β-cells. To resolve the role of this lipase, we have created global and β-cell-specific knockout mice. Although our line of global HSL-knockout mice is moderately glucose-intolerant owing to reduced peripheral insulin sensitivity and exhibits normal islet metabolism and insulin secretion, other HSL-knockout lines have displayed impaired insulin secretion under certain conditions. In contrast, β-cell-specific HSL-knockout mice, which are less prone to genetic redundancy, are hyperglycaemic, presumably caused by a perturbation of first-phase insulin secretion. Thus studies by us and others demonstrate that lipases, such as HSL, play a regulatory role in β-cell stimulus–secretion coupling.

Introduction

The metabolic perturbations in Type 2 diabetes are not restricted to the metabolism of glucose, but also involve lipid abnormalities. Lipids are critical in the development of late diabetic complications. In addition, lipids may play a pathogenetic role in how metabolic perturbations in Type 2 diabetes evolve. Lipotoxicity implies that lipids are harmful to the insulin-secreting β-cells and impair insulin sensitivity [1]. Increased levels of circulating lipids, i.e. NEFAs [non-esterified ('free') fatty acids] and triacylglycerols, are thought to perturb cellular function upon accumulation in non-adipose cells, e.g. β-cells, liver and skeletal muscle.

Lipids are also required for normal function of pancreatic β-cells. Islets depleted of triacylglycerols are unresponsive to glucose [2,3]; this phenotype is reversed by adding exogenous NEFAs. Furthermore, glucose-responsiveness in fasted rats both in vivo and in vitro is dependent on NEFAs [4], and similar findings have been made in humans [5]. Thus a lipid-derived factor may play a critical role in insulin secretion. Production of such a factor requires the action of a lipase which mobilizes a potential lipid coupling factor from more complex lipids in the cells.

The link between glucose and lipid metabolism in the β-cell has been proposed to rely on a glucose-stimulated rise in malonyl-CoA levels [6,7]. This metabolite is the building block in fatty acid synthesis, and will block the transport of long-chain acyl-CoA into the mitochondrion, via inhibition of CPT-1 (carnitine palmitoyltransferase 1) [8]. Consequently, levels of long-chain acyl-CoA would rise in the cytoplasm and serve as a coupling factor in GSIS (glucose-stimulated insulin secretion). Generation of long-chain

Key words: coupling signal, fatty acid, islet, lipolysis, orlistat, triacylglycerol

Abbreviations used: A1G1, adipocyte triacylglycerol lipase; C1L1, carboxyl ester lipase gene; GP1, glucagon-like peptide 1; GSI, glucose-stimulated insulin secretion; HSL, hormone-sensitive lipase; KATP-channel, ATP-sensitive K+ channel; α-KIC, α-ketoisocaproic acid; KO, knockout; MODY, maturity-onset diabetes in the young; NEFA, non-esterified ('free') fatty acids; RBP2, rat insulin promoter 2; VNTR, variable number of tandem repeats.

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acetyl-CoAs in the β-cell would also require the action of one or more lipases for release from intracellularly stored lipids. Indeed, islets do contain triacylglycerols, an accumulation of which may increase during the pathogenesis of diabetes [9].

In addition to closure of KATP channels (ATP-sensitive K+–channels), it has now been widely recognized that nutrients also stimulate insulin secretion via KATP-channel independent or amplifying pathways [10]. KATP-independent pathways have been suggested to be primarily responsible for the second phase of GSIS. Lipids constitute one class of nutrients which has been suggested to be responsible for KATP-independent insulin secretion [7]. This review describes potential lipases and processes which may account for the production of lipid-derived coupling signals in the β-cell.

Lipolysis and insulin secretion

Lipolysis, i.e. the hydrolysis of acyl groups from the glycerol backbone in triacylglycerols, is one potential process whereby lipases are thought to regulate insulin secretion. To explore this possibility, we used orlistat [11], known previously as tetrahydrolipstatin. This lipophilic drug is a wide-spectrum inhibitor of lipases, binding irreversibly to the catalytic site of the enzymes [12]. Orlistat is widely used in the treatment of obesity [13], owing to its inhibition of intestinal lipases. At high concentrations in vitro, orlistat will traverse biological membranes [14]. Orlistat has previously been shown to inhibit insulin secretion stimulated by cAMP and GLP-1 (glucagon-like peptide 1) [15,16].

Lipolytic activity in β-cells was initially suggested by studies in Corkey’s group [15]: acidification and efflux of fatty acids in HIT-T15 cells in response to glucose and the incretin hormone GLP-1 were observed. We found that glucose promotes glycerol release, an index of lipolysis, from primary rat β-cells [11], and glucose also stimulates glycerol release from INS-1 cells, correlating with insulin release [17]; similar findings were made in islets from mice [17,18]. Lipolysis, as well as diacylglycerol lipase activity, are abolished by orlistat [11], and the lipase inhibitor dose-dependently inhibits insulin secretion stimulated by glucose and cAMP. Moreover, using a perfusion system for rat islets, we found that orlistat significantly inhibits the second, but not the first, phase of insulin secretion [11]. This suggests that a putative lipid-derived factor predominantly acts during the second and KATP-independent phase of insulin secretion. In agreement with this notion, we observed no effect of orlistat on the glucose-induced rise in the ATP/ADP ratio, a prerequisite for GSIS triggered by the KATP-dependent pathway. However, when palmitate is added to the islets incubated with 16.7 mM glucose and orlistat, the insulin secretory response is recovered, suggesting that palmitate substitutes for a lipid-derived intracellular signal (Figure 1). In agreement, 3,5-dimethyloxazolidone, another anti-lipolytic agent, also inhibits insulin secretion [19]. Finally, a lipase inhibitor selective for HSL (hormone-sensitive lipase) inhibits insulin secretion from rat islets [20].

Figure 1 | Insulin secretion in rat islets in the presence of orlistat and palmitate

Islets were incubated in the presence and absence of 200 μM orlistat (orli) at the indicated glucose (Glc) concentrations for 1 h. There was a significant reduction in insulin secretion in the presence of orlistat, which was recovered when 1 mM palmitate (PA) was added. Data taken from [11].

Lipases in pancreatic β-cells

Hormone-sensitive lipase

It has long been recognized that β-cells store triacylglycerols and utilize lipids as fuel when ambient glucose is low [21,22]. However, the identity of β-cell lipases remained unconfirmed until we were able to demonstrate that both HSL mRNA and protein are expressed in various preparations of rodent β-cells [23]. The molecular form of HSL in β-cells is slightly larger than the predominant form in adipocytes (89 compared with 84 kDa). This difference is accounted for an additional N-terminal 43-amino acid peptide [24]. In islets, HSL is predominantly expressed in β-cells [23] (Figure 2), but it also occurs in the other cell types. It was estimated that HSL is responsible for ~25% of diacylglycerol lipase activity [23,25]. It has also been shown that chronic exposure to high glucose increases expression and activity of HSL in INS-1 cells and rat islets, and, in parallel, stimulates lipolysis [25]. Moreover, prolonged high-fat feeding of C57BL/6J mice results in decreased HSL expression in islets [26]. Interestingly, this is paralleled by triacylglycerol accumulation and perturbed insulin secretion both in vivo and in vitro.

Adipocyte triacylglycerol lipase

In 2004, a novel lipase was identified in adipocytes by three independent groups; the lipase was called adipocyte triacylglycerol lipase (ATGL), desnutrin or iPLA2ζ (Ca2+-independent phospholipase A2 ζ) [27–29]. In the mouse, ATGL is found predominantly in adipose tissue, but the enzyme is also expressed in other tissues, such as cardiac and...
skeletal muscle, and testis [27]. We have found ATGL to be expressed in the mouse islet as well as in clonal β-cells of rat origin [30].

In adipose tissue, ATGL is mainly thought to be responsible for the catalytic breakdown of triacylglycerols to diacylglycerols, working in concert with HSL to maintain lipolysis [27–29]. Convincing evidence has been presented by several groups demonstrating the triacylglycerol substrate-specificity of ATGL: Zimmermann et al. [27] have shown that HepG2 cells transduced with ATGL, using a recombinant adenovirus, and incubated with radiolabelled triolein/diolein substrates display a markedly higher tri- than di-acylglycerol lipase activity. This supports a role for ATGL as the primary enzyme in the initial step of lipolysis. ATGL appears to be regulated by nutritional status, since ATGL in mice is induced by fasting and suppressed by re-feeding [28]. Moreover, up-regulation of ATGL mRNA has been observed in dexamethasone-treated 3T3-L1 pre-adipocytes [28]. Furthermore, ATGL is down-regulated in adipose tissue in ob/ob and db/db mice. This finding supports a role for the enzyme in fat breakdown, and, when down-regulated, lack of ATGL action may contribute to the development of obesity [28]. Clearly, ATGL is an important enzyme in overall energy homeostasis and basal lipolysis.

Because ATGL is expressed in islets and lipolysis has been shown to be a prerequisite for normal insulin secretion [11,17], this novel lipase may serve an important role in insulin secretion, possibly working in concert with other lipases in creating the lipid signals essential for normal insulin secretion. However, these and other possible roles for this enzyme remain to be investigated. In fact, very little is known about lipid droplet formation, lipid storage and lipid mobilization in the pancreatic β-cell.

**Figure 2 | Hormone-sensitive lipase is expressed in mouse islets**

A section from mouse pancreas was immunostained with an antibody against HSL. Most islet cells were immunofluorescent, indicating the presence of HSL in β-cells.

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**HSL-KO mice: a summary of the phenotype**

Four independent lines of HSL-KO mice have been reported [34–37]. Surprisingly, HSL-KO mice are not obese [34], but all lines exhibit male infertility, because of the absence of HSL from testes [34,38,39]. Interestingly, stimulated release of both NEFAs and glycerol from adipocytes is observed both in vivo and in vitro [34–36,40]. This strongly suggests the existence of one or more additional lipases in adipocytes, and led to the discovery of ATGL by Zechner’s group [27]. This enzyme is also present in humans [41], and is primarily responsible for hydrolysis of tri- to di-acylglycerols [27]. Accumulation of diacylglycerols in adipocytes is consistently found in the different HSL-KO lines [36,37], leading to the notion that the primary role of HSL is hydrolysis of diacylglycerols. Neutral cholesteryl ester hydrolase activity is abolished in adipocytes from HSL-KO adipocytes [34,37], suggesting that an important role of HSL is hydrolysis of cholesteryl esters. An important issue is whether ATGL is controlled by hormonal signals. Here, species differences are apparent: in human adipocytes, ATGL accounts for basal lipolysis, while HSL controls stimulated lipolysis [41]. The situation in pancreatic β-cells is still unresolved.

**Glucose homoeostasis in HSL-KO mice**

HSL-KO mice created by Mitchell’s group [35] exhibit retarded glucose elimination due to a virtually lost insulin response.

**CEL (carboxyl ester lipase)**

Recently, a positional cloning approach identified mutations in the VNTR (variable number of tandem repeats) of the CEL gene [31]. These mutations were subsequently shown to cause β-cell dysfunction and early onset diabetes: a novel autosomal dominant form of inherited diabetes and exocrine pancreas dysfunction, reminiscent of MODY (maturity-onset diabetes in the young). Interestingly, CEL is expressed in the exocrine, but not endocrine, pancreas, implying an intimate functional interplay between the two pancreatic tissue types. Normally, the exocrine tissue produces bicarbonate and enzymes, which are secreted into the duodenum and promote digestion of nutrients. Therefore it was thought that the main function of CEL was to hydrolyse cholesteryl esters in the duodenum.

The authors suggest that common variations in the VNTR of the CEL gene may play a role in exocrine pancreas dysfunction as measured by faecal elastase deficiency, which is present in 10–35% of all cases of Type 1 and 2 diabetes [31]. Recently, it was shown that the prevalence of pancreatic exocrine dysfunction and faecal elastase deficiency in MODY3 was similar to that in Type 1 diabetes [31,32]. These observations highlight the importance of assessing whether patients with any type of diabetes also exhibit exocrine pancreatic dysfunction. However, it still remains unresolved how CEL deficiency in acinar cells causes progressive β-cell failure. So far, studies in CEL-KO (knockout) mice have not clarified this issue, since no clear exocrine or endocrine phenotype was reported in this model [33].

**Genetic inactivation of HSL**

Four independent lines of HSL-KO mice have been reported [34–37]. Surprisingly, HSL-KO mice are not obese [34], but all lines exhibit male infertility, because of the absence of HSL from testes [34,38,39]. Interestingly, stimulated release of both NEFAs and glycerol from adipocytes is observed both in vivo and in vitro [34–36,40]. This strongly suggests the existence of one or more additional lipases in adipocytes, and led to the discovery of ATGL by Zechner’s group [27]. This enzyme is also present in humans [41], and is primarily responsible for hydrolysis of tri- to di-acylglycerols [27]. Accumulation of diacylglycerols in adipocytes is consistently found in the different HSL-KO lines [36,37], leading to the notion that the primary role of HSL is hydrolysis of diacylglycerols. Neutral cholesteryl ester hydrolase activity is abolished in adipocytes from HSL-KO adipocytes [34,37], suggesting that an important role of HSL is hydrolysis of cholesteryl esters. An important issue is whether ATGL is controlled by hormonal signals. Here, species differences are apparent: in human adipocytes, ATGL accounts for basal lipolysis, while HSL controls stimulated lipolysis [41]. The situation in pancreatic β-cells is still unresolved.

**Glucose homoeostasis in HSL-KO mice**

HSL-KO mice created by Mitchell’s group [35] exhibit retarded glucose elimination due to a virtually lost insulin response.
However, glucose tolerance in these HSL-KO mice improved with further breeding [18]. The HSL-KO mice created by Zechner also exhibit increased fasting plasma glucose levels, while insulin levels are decreased [43]. In the Ishibashi line [34], fasting plasma glucose levels are either unchanged in female mice or decreased in male mice, whereas insulin levels are unaltered [44].

The HSL-KO mice created in our laboratory are hyperglycaemic and hyperinsulinaemic in the fasted state [37]. They show an exaggerated insulin response in both intravenous and oral glucose tolerance tests [37]. These observations suggested that our HSL-KO line is insulin-resistant, which is supported by an insulin tolerance test. A hyperinsulinaemic–euglycaemic clamp in our HSL-KO line showed an impaired hepatic insulin response. In addition, insulin-stimulated glucose uptake in soleus muscle and lipogenesis in isolated adipocytes are reduced [37]. A hyperinsulinaemic–euglycaemic clamp in the Zechner line of HSL-KO mice confirmed that there is no effect on whole-body glucose uptake [43]. Instead, insulin sensitivity is increased in liver, which could be attributed to decreased hepatic triacylglycerol levels. Increased hepatic insulin sensitivity was later reproduced in the Mitchell HSL-KO line in the fasted state [45], similar to Zechner’s HSL-KO mice [43]. Apparently, insulin sensitivity has been examined under different conditions in the HSL-KO lines.

**Insulin secretion in HSL-KO mice**

Our line of HSL-KO mice responds vigorously to glucose and arginine in vivo [37], whereas the mice created by Mitchell exhibit a perturbation of insulin secretion in vivo [18,42]. In contrast, insulin secretion from perfused islets is similar in both HSL-KO and wild-type mice generated in our laboratory [37,46]. Moreover, in static incubations of islets from our HSL-KO line, insulin secretion in response to glucose under KATP-dependent and -independent conditions, in response to GLP-1, carbacholine, palmitate or α-KIC (α-ketoisocaproic acid), is unchanged [37,46]. Furthermore, the intracellular glucose-induced Ca\(^{2+}\) response is indistinguishable between the two genotypes.

In contrast with our observations, Prentki and co-workers have reported perturbations of insulin secretion in vitro [18,42] in the mice created by Mitchell and colleagues [35]. This phenotype is evident in male mice; in fact, female mice from this line exhibit increased GSIS both in islets isolated from fed and fasted animals, whereas fed mice exhibit a much diminished phenotype [18]. The reasons for these discrepancies are unclear. It is possible that one or more unknown genes on the allele containing the mutated HSL locus has been transferred to the HSL-KO, but not wild-type offspring, and may account for the phenotype. We have backcrossed our mice on to C57BL/6J for ten generations, but this has not led to perturbed insulin secretion, which we also observed initially (H. Mulder, M. Fex, M. Sørhede Winzell, B. Ahren and C. Holm, unpublished work). Moreover, the Mitchell mice were back-crossed for five generations, which should have provided some unifying of the genetic background [18,42]. The genetic background of the two lines is different. This is relevant because it has become increasingly clear that gene targeting in different genetic backgrounds may produce divergent results [47]. Finally, genetic redundancy may play an important role, particularly since the targeting of the HSL locus is present from the gastrula stage. The discovery of ATGL has fuelled this notion [27]. Indeed, we have identified ATGL mRNA in mouse and rat islets, including HSL-KO islets, and clonal β-cells [30].

**β-Cell-specific KO of HSL**

Because the results from the different HSL-KO lines have been contradictory, a clear role for HSL in β-cells has yet to be provided. To circumvent the compensatory mechanisms that may come into play in a global KO of HSL, we have created a conditional KO of HSL in β-cells (β-HSL-KO). This model was created by LoxP-Cre-mediated recombination, crossing a mouse line (SV129/C57BL6J) carrying a mutated HSL allele, in which exons 2–7 were flanked by LoxP sites as described previously [36], with a line (C57BL/6J) expressing the Cre recombinase under control of the RIP2 (rat insulin promoter 2) [48]. An intravenous glucose tolerance test in 12-week-old female mice showed that the β-HSL-KO exhibit elevated levels of plasma glucose, delayed glucose clearance and perturbed insulin secretion in vivo. A possibility existed that our findings in the β-HSL-KO could be explained by glucose intolerance in RIP2-Cre mice [49]. Upon investigation of our line of RIP2-Cre, which has been back-crossed on to a pure C57BL/6J background for 14 generations, we found no disturbances in glucose tolerance [50]. Therefore we feel confident that we will be able to resolve the controversy regarding the role of HSL in pancreatic β-cells, and thereby increase the understanding of lipid coupling signals and lipolytic events regulating insulin secretion from the pancreatic β-cell, using our conditional KO of HSL.

**Conclusion**

We have reviewed the existing evidence that lipid metabolism in pancreatic β-cells plays a role in stimulus–secretion coupling. Control of this metabolism is exerted, in part, by lipases, one of which is HSL. We have demonstrated its expression and activity in β-cells, but also a novel lipase, ATGL, is expressed here. Global KOs of HSL have generated conflicting data on the role of HSL in insulin secretion. A novel conditional β-cell KO of HSL created by us exhibits perturbed insulin secretion. We hope to be able to resolve the role of HSL in β-cells using this experimental model.

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References


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