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Targeting triglyceride/fatty acid cycling in β-cells as a therapy for augmenting glucose-stimulated insulin secretion

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Insulin secretion from pancreatic β-cells is triggered by signals arising from the metabolism of glucose and acting through separate initiation and amplification pathways. Despite decades of investigation, crucial details of this mechanism remain poorly understood, especially those relating to the amplifying pathway(s). Advances in this area are vital if we are to understand why insulin secretion fails in type 2 diabetes and to develop strategies to overcome this failure. Indeed, targeting the amplifying pathway(s) would constitute an attractive therapy for augmenting insulin secretion because it would closely link secretory responsiveness to the prevailing glycaemia. It is therefore noteworthy that the possibility of augmenting the amplification pathway(s) has recently been highlighted by studies investigating a metabolic cycle that links the breakdown of triacylglycerol (TAG), release of fatty acid (FA), and subsequent re-incorporation of that FA into TAG. This work reinvigorates and extends the long-standing idea that partitioning of endogenous lipid metabolism towards esterification products promotes the amplification phase of the secretory response. These conceptual advances, and their possible therapeutic application, will be discussed in the following article.

Key words: amplification pathway, lipolysis, triglyceride, lipotoxicity, protein kinase C

Abbreviations: DAG, diaclyglycerol; FA, fatty acid; GSIS, glucose-stimulated insulin secretion; LCAC, fatty acyl CoA; PKC, protein kinase C; TAG, triaclyglycerol

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The mechanisms underpinning glucose-stimulated insulin secretion (GSIS) from β-cells comprise both initiation and amplification pathways.[1] Both pathways are absolutely dependent on the mitochondrial metabolism of glucose. The initiation mechanisms are understood in some detail: increases in ATP derived from the oxidative phosphorylation of glucose lead to closure of ATP-sensitive K^+ channels; the resultant depolarization of the plasma membrane promotes the gating of voltage-dependent Ca^{2+} channels and influx of Ca^{2+} from the extracellular space; this increase in cytosolic free Ca^{2+} is both necessary and sufficient for initiating signalling pathways that result directly in the activation of exocytosis. In the amplification pathway(s) glucose also generates a metabolic signal that augments the amount of insulin secreted in response to a given rise in cytosolic free Ca^{2+}.[1] Although it is often believed that the initiation and amplification phases correspond temporally with the first and second phases of GSIS respectively, this is not true, since the amplification phase is activated sufficiently rapidly to contribute substantially to first phase secretion.[2]

The amplification pathway(s) is/are very poorly understood at the molecular level, in terms of both the biochemical signal(s) arising from glucose metabolism, and how those signal couple to the mechanics of exocytosis. Although there are several competing theories, each with their own strengths,[1,3] one long-standing hypothesis involves the capacity for glucose-derived metabolites to regulate endogenous lipid metabolism.[4,5] As in many cell-types, basal energy requirements in β-cells are met largely by the oxidation of FAs (β-oxidation) derived in turn from the hydrolysis of neutral lipid stores (largely triglyceride or cholesterol ester). When glucose is present as an alternative fuel, β-oxidation is inhibited and FAs (or their activated CoA derivatives)

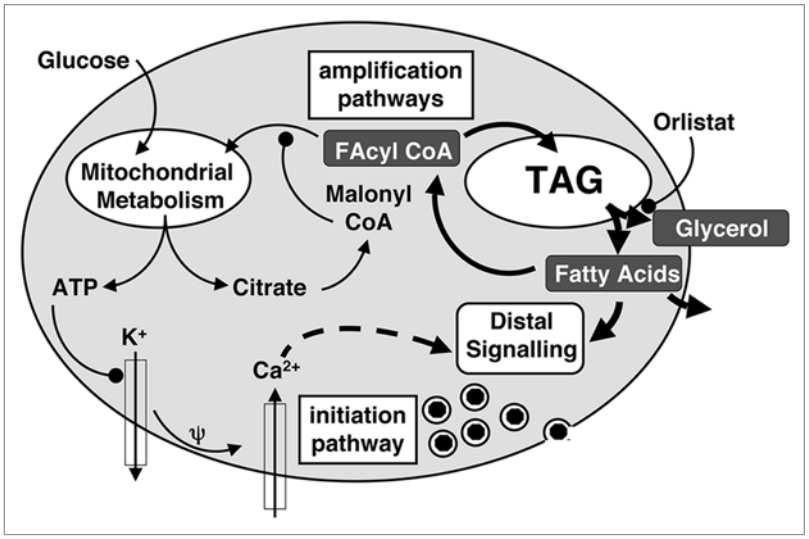

Figure 1. Putative role of a TAG/FA cycle in the amplification pathway of glucose-stimulated insulin secretion. ATP derived from mitochondrial metabolism of glucose leads to depolarization (Ψ) and gating of voltage-dependent Ca^{2+} channels (initiation pathway). Mitochondrial metabolism of glucose via anaplerosis separately leads to cytosolic accumulation of citrate that is converted to malonyl CoA. This metabolite, via inhibition of FA oxidation, partitions FAs towards esterification products, such as TAG. As discussed in the text, liberation of signalling molecules derived from TAG are hypothesised to constitute an amplification signal for glucose-stimulated insulin secretion. Lipases involved in this process can be inhibited by orlistat. Also illustrated is the concept that FAs and TAG pools are being continuously interconverted via a cycle and that this turnover is important for signalling. Alternatively, this might represent a route for detoxification by extrusion of FAs and glycerol, which in β-cells is not re-incorporated into glycerolipids.

are shunted into synthetic pathways. This switch in partitioning is controlled by an increase in anaplerosis whereby the glycolytic endpoint, pyruvate, is converted into intermediates of the tricarboxylic acid cycle (**Fig. 1**). These are then available for export from the mitochondria as citrate, which is metabolized to malonyl CoA in the cytosol. Malonyl CoA inhibits FA transport into the mitochondria, and thus serves as a key regulator of the partitioning of FAs between β-oxidation and esterification (**Fig. 1**). Quantitatively the most important esterification pathway is that of fatty acyl CoA (LCAC) condensation with glycerol 3-phosphate (derived from glycolysis) to from monacylglycerols, diacylglycerols (DAG) and TAG. These compounds, along with cholesterol ester formed by esterification of LCACs with cholesterol, constitute the major stores of cellular neutral lipid.

A weakness of the anaplerosis/lipid partitioning hypothesis remains the absence of a clearly elucidated signalling molecule that is generated by this pathway. Originally DAG was envisioned as fulfilling this role, via its capacity to activate protein kinase C (PKC).[6] On balance, however, PKC does not appear to be a major mediator of GSIS,[7,8] although this does not exclude other signalling functions of DAG.[9] But it is now apparent that the lipid partitioning pathway in β-cells is only one facet of a more complex mechanism. A separate line of enquiry had established that breakdown of neutral lipid stores via lipolysis also contributed to GSIS (**Fig. 1**). This was based on evidence that glucose acutely stimulates lipolysis in β-cells,[10-12] and that GSIS is blocked in rat islets by general lipase inhibitors such as orlistat.[10,12,13] However, conflicting results obtained using different strains of mice deleted in hormone-sensitive lipase led to some confusion in interpretation.[11,14-16] Several recent studies clarify this situation. Firstly, it has been confirmed that when HSL is specifically deleted in β-cells, GSIS is impaired, albeit at a site in distal exocytosis.[17] More importantly for the discussion here, a second publication showed that another enzyme, adipose triglyceride lipase, is the most abundant lipase in rat islets, and that its deletion impairs insulin secretion especially in response to fuel secretagogues.[18] This suggests that hydrolysis of a triglyceride pool leads to the release of an (unidentified) intermediate that serves as an amplification signal.

This work, however, also supports the concept advanced by Nolan and Prentki that the cycling of lipid intermediates between storage and breakdown pools is important, and not just the steady state levels of those intermediates (**Fig. 1**).[19,20] Cycling confers several advantages over a linear pathway, albeit at the expense of ATP consumption.[21] Firstly, cycles are highly sensitive to regulation at both input and outputs. Secondly, a cycle might serve to disperse or restrict metabolites to cellular locations differing from those associated with a linear pathway. Finally, this particular cycle might serve as a detoxification mechanism as well as, or instead of, a signalling function.[20,21] This follows from the fact that the glycerol arising from lipase activity is unable to be re-esterified in β-cells but is exported from the cell (**Fig. 1**). Thus during hyperglycaemia excess glucose might be metabolized via glycolysis and exported as glycerol. This might form an elegant adaptation for de-toxifying glucose without compromising the crucial glucose-sensing function of the β-cell. Similarly excess FA uptake during lipotoxicity might also be balanced by re-export of FAs via action of the TAG/FA cycle.[20,21]

Defective GSIS is one of the key features of β-cell failure in type 2 diabetes, and hence the maintenance of secretory function is a key therapeutic goal. There is now growing evidence that targeting the TAG/FA cycle might be beneficial in this regard. Firstly, it was shown that enhanced flux through this cycle was associated with the compensatory enhancement of GSIS that is responsible for the maintenance of glucose tolerance in Zucker fatty rats, a rodent model of obesity without progression to diabetes.[22] When diabetes was induced in these animals using partial pancreatectomy the TAG/FA cycle was subtly perturbed as witnessed by an accumulation of monoacylglycerol and DAG, but not TAG.[23] Moreover, at least two pharmacological agents capable of modulating GSIS have now been shown to do so in a manner displaying parallel alterations in TAG/FA cycling. Thus pioglitazone inhibited secretion via non-genomic effects that correlated with a diminished lipolysis.[24] Conversely,

an agonist of nuclear liver X receptors augmented both basal and GSIS in a manner inhibited by orlistat and associated with enhanced turnover of TAG pools.[25]

Our own studies on the role of PKCε deletion have also highlighted a role for TAG/FA cycling.[26,27] Functional ablation of PKCε dramatically improves whole body GSIS in dietary and genetic models of glucose intolerance and type 2 diabetes.[26] These and ex vivo studies using isolated islets confirmed that the restoration of GSIS required both loss of PKCε and chronic lipid exposure, and was associated most obviously with an augmentation of the amplification pathway(s).[26,27] The underlying mechanism involved a shift in FA partitioning toward esterification. In particular there was an enhanced turnover of TAG pools, resulting in an increase in glucose-stimulated lipolysis that was absolutely required for the enhancement of GSIS under these conditions.[27] In contrast, the glucose response in normal mouse islets (that is wild-type for PKCε and not pre-treated with FAs) was not inhibited by orlistat.[26] This differs from the situation using rat islets where lipolysis seems necessary for full GSIS, even in the absence of lipid pretreatment.[10,12,13] Interestingly, the second phase of GSIS is much more pronounced in rat than mouse (or human) islets.[2,28] We therefore propose that this amplification pathway in mice (and potentially humans) ia limited by activation of PKCε, but this might be less apparent in rats where, indeed, inhibition of PKCε does not further augment (and actually appears to inhibit) GSIS.[29,30]

Although some aspects of this topic remain conjectural there is sufficient evidence on the one hand that TAG/FA cycling is important for β-cell function, and on the other hand that it represents an ideal therapeutic target for intervening in β-cell dysfunction and therefore warrants further investigation. In particular, some of the apparent differences in the roles of TAG lipolysis in rat versus mouse islets need to be explored further and the situation in humans clarified. We still need to identify the signalling molecule released by lipolysis, both for a better understanding of the downstream pathways, and to help resolve whether the total TAG pool is a precursor for this molecule, or a pool restricted by either cellular localization or FA subspecies. The contribution of different lipase enzymes to TAG breakdown under both basal and stimulated conditions requires quantification, especially if a signalling sub-pool is identified. It will also be of interest to identify the molecular targets for PKCε and genes regulated by LXR that contribute to these processes. In turn these experiments might provide valuable tools with which to address the crucial question of whether TAG/FA cycling represents a signalling or detoxification pathway.

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