

# Protein Kinase C Function in Muscle, Liver, and $\beta$ -Cells and Its Therapeutic Implications for Type 2 Diabetes

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**I**ncreased lipid availability is strongly associated with both  $\beta$ -cell dysfunction and insulin resistance, two key facets of type 2 diabetes. Isoforms of the protein kinase C (PKC) family have been viewed as candidates for mediating the effects of fat oversupply because they are lipid-dependent kinases with wide-ranging roles in signal transduction, including the positive and negative modulation of insulin action. Until recently, their involvement was based on correlative studies, but now causative roles for distinct PKC isoforms have also been addressed, in both pancreatic  $\beta$ -cells and insulin-sensitive tissues. Our goal here, therefore, is to review the hitherto disparate fields of PKC function in insulin signaling/resistance on the one hand and in regulating  $\beta$ -cell biology on the other hand. By integrating these two areas, we provide a reappraisal of the current paradigm regarding PKC and type 2 diabetes. In particular, we propose that PKC $\epsilon$  warrants further investigation, not merely as a treatment for insulin resistance as previously supposed, but also as a positive regulator of insulin availability.

## DIACYLGLYCEROL-MEDIATED ACTIVATION OF PROTEIN KINASE C

The protein kinase C (PKC) family comprises 10 isoforms that have been subdivided into three groups (Fig. 1) based on sequence homology and mechanisms of activation (rev. in 1). While differentiated by their sensitivity to  $\text{Ca}^{2+}$ , both the conventional PKCs (cPKC $\alpha$ ,  $\beta$ , and  $\gamma$ ) and novel PKCs (nPKC $\delta$ ,  $\epsilon$ ,  $\eta$ , and  $\theta$ ) are dependent on diacylglycerol (DAG) for full activation. These isoforms are therefore responsive to the stimulation of G protein-coupled receptors or receptor tyrosine kinases, which activate phospholipase C, inducing the hydrolysis of phosphatidylinositol 4,5-bisphosphate at the plasma membrane and the resultant generation of DAG and  $\text{Ca}^{2+}$ . Evidence for the acute elevation of DAG in this fashion by insulin was reported in early studies (2), although the identities of the putative phospholipase(s) and phospholipid substrates involved were never clarified. On the other hand, chronic elevation of DAG through de novo synthesis during periods of lipid oversupply, as in the case of obesity, has been widely correlated with cPKC and nPKC activation, although in this case, DAG is first synthesized in the endoplasmic

reticulum, perhaps resulting in PKC activation at intracellular sites.

As a consequence of the interaction between PKC and membrane-delimited DAG, cPKC and nPKC isoforms generally translocate from a cytosolic to a membrane-associated compartment. PKC isoform translocation, observed by immunoblotting subcellular fractions, is thus commonly used as an indication of activation, particularly because in vitro kinase assays discriminate poorly between isoforms. Longer-term stimulation leads to PKC downregulation by proteolysis, although susceptibility varies between isoforms and depends on cell type.

## PKCs AS INSULIN SIGNAL TRANSDUCERS

The atypical isoforms (aPKC $\zeta$  and aPKC $\iota/\lambda$ ) constitute a third group within the PKC family and are independent of both  $\text{Ca}^{2+}$  and DAG (Fig. 1). (There is confusion in the literature concerning PKC $\lambda$  [3], which is not a distinct isoform but in fact the mouse ortholog of human PKC $\iota$  [4]. In all species, the gene symbol for this isoform is now Prkci.) Instead, these kinases can be activated in response to stimulation of the insulin receptor substrate (IRS)/phosphatidylinositol (PI) 3-kinase pathway, which enables phosphorylation of aPKCs at the "activation loop" near the catalytic site by PI 3-dependent kinase 1 (5).

Atypical PKCs signal in parallel to Akt in muscle and adipose tissue during the stimulation of glucose metabolism, especially via translocation of GLUT4 (6). There appears to be redundancy between aPKC $\zeta$  and aPKC $\iota$  in this respect because one can substitute for the other in overexpression studies. Diminished IRS-1/PI 3-kinase-dependent aPKC activation is observed in muscle and adipose tissue during insulin resistance and type 2 diabetes (6) but remains intact in liver. In this instance, activation occurs predominantly through the IRS-2/PI 3-kinase pathway and is more important for the lipogenic action of insulin, so its continued function may play a role in lipid dysregulation upon hyperinsulinemia in insulin-resistant states (6).

Insulin has also been reported to stimulate the activity of cPKC and nPKC isoforms to promote glucose disposal (7). Putative mechanisms of activation include tyrosine phosphorylation of PKC $\delta$  and alternative splicing of PKC $\beta$  to increase PKC $\beta$ II levels, but these have not been widely substantiated, and the positive effects of these kinases need to be reconciled with the negative regulation of insulin action detailed below.

## PKC AND INSULIN RESISTANCE

An association between PKC activation and insulin resistance in skeletal muscle became apparent from studies linking PKC translocation with defective insulin-stimulated glucose metabolism (8–10). Most often, nPKC isoforms, especially PKC $\theta$  and PKC $\epsilon$ , were implicated

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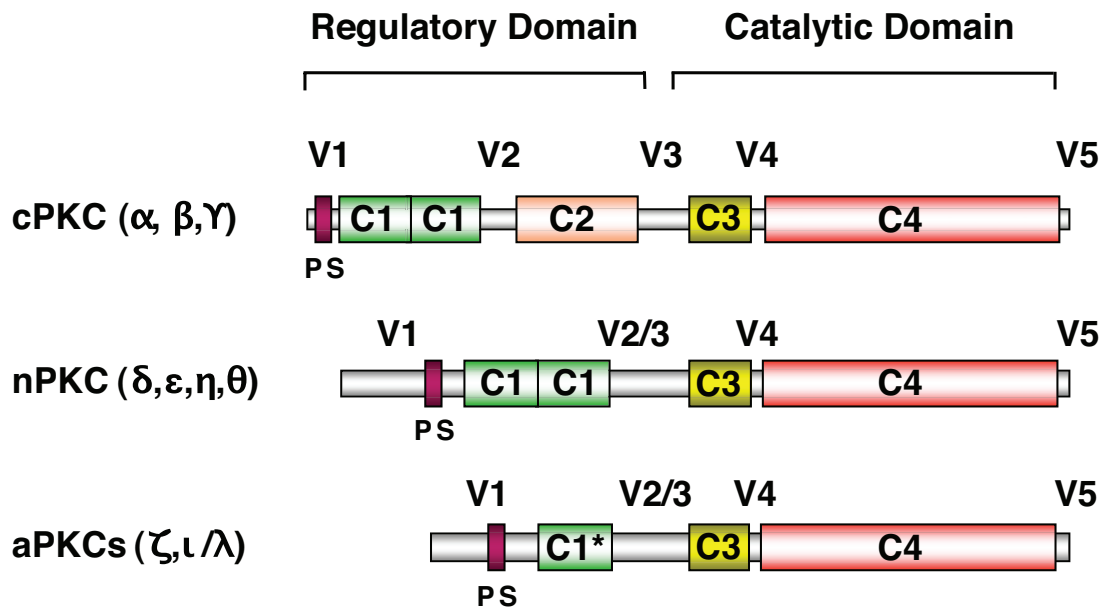
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**FIG. 1.** The PKC family of lipid-activated protein kinases. PKC isoforms contain constant regions (C1–4) and variable regions (V1–5) and can be divided into three subgroups. cPKCs are activated in the presence of calcium, which binds to the C2 domain, and DAG, which binds to the C1 domains. nPKCs lack C2 domains and are  $\text{Ca}^{2+}$ -independent but still require DAG for full activation. aPKCs possess only one nonfunctional C1 domain (C1\*) and no C2 domain and are both  $\text{Ca}^{2+}$ - and DAG-independent. The C3 regions (ATP-binding) and C4 regions (protein substrate binding) are highly conserved between isoforms. In each case, the pseudosubstrate (PS) sequences, found in the V1 variable region, interfere with the catalytic domains to inhibit substrate phosphorylation until conformational changes induced by activators allow full activation.

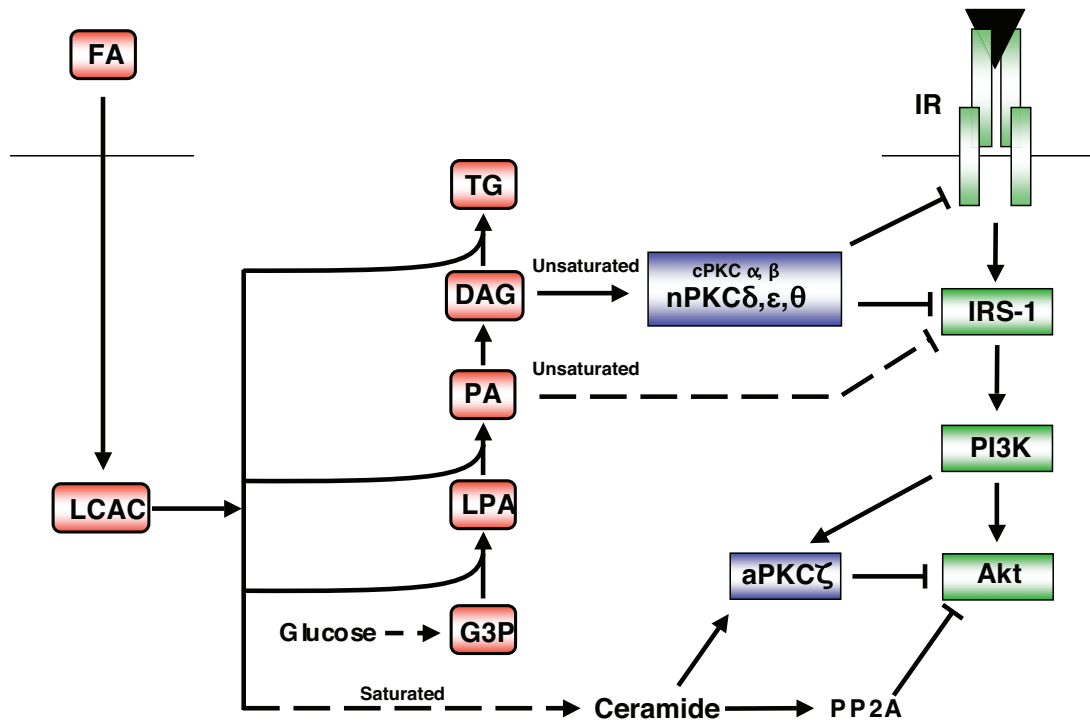
together with an elevation of lipid intermediates such as DAG. Thus, skeletal muscle from rats fed a high-fat diet for 3 weeks exhibited an increase in the translocation of PKC $\theta$  and PKC $\epsilon$  in conjunction with elevated lipid content and diminished glucose disposal (11). PKC redistribution was reversed upon treatment with the insulin sensitizer rosiglitazone (12). Similar alterations in nPKC isoforms were also observed in genetic models of obesity and diabetes (13,14). In more acute models of insulin resistance, PKC $\theta$  translocation was also observed after 5-h lipid infusion (15), whereas 1- or 4-day infusion of glucose, which increased muscle lipid content, promoted activation of PKC $\epsilon$  (16).

PKC $\epsilon$  has been the isoform most often implicated in the generation of insulin resistance in liver. This was initially demonstrated using liver biopsies from obese subjects with type 2 diabetes (17). In addition, short-term (3-day) fat feeding of rats, which induces hepatic steatosis, led to translocation of PKC $\epsilon$  in concert with a diminished ability of insulin to reduce endogenous glucose production (18). Other isoforms have been implicated to a lesser extent. Alterations in the cellular localization of PKC $\alpha$  and PKC $\zeta$ , in addition to PKC $\epsilon$ , were observed in diabetic liver (17). PKC $\delta$  translocation has also been observed in muscle of high-fat fed rats (11), as well as after lipid infusion in both liver (19) and muscle where PKC $\beta$  was also activated (20). These studies support the hypothesis that activation of one or more PKC isoforms through increased lipid availability, especially PKC $\theta$  and PKC $\epsilon$ , can interfere with insulin-stimulated glucose disposal. It is not clear why nPKC isoforms are more often implicated in these studies when cPKCs are also sensitive to elevations in DAG. This may be related to the additional sensitivity of cPKCs to  $\text{Ca}^{2+}$ , which may not become elevated upon fat oversupply.

#### MECHANISMS OF PKC-INDUCED INSULIN RESISTANCE

PKC has been reported to inhibit several components of the insulin signaling cascade, as well as downstream metabolic enzymes such as glycogen synthase (rev. in 10). These studies were often based on in vitro phosphorylation or overexpression of PKC and potential substrates in cultured cells and require careful interpretation. In vivo, PKC has also been suggested to act indirectly, such as by upregulation of inflammatory pathways (21). Nevertheless, most studies have addressed the simpler hypothesis that PKC directly phosphorylates serine residues of the insulin receptor substrates, especially IRS-1 (Fig. 2). This results in diminished tyrosine phosphorylation of IRS-1, reduced downstream signaling through the PI 3-kinase/Akt pathway to glucose metabolism, and, ultimately, enhanced IRS-1 degradation (22,23). Several PKC isoforms, especially PKC $\delta$ , have been shown to phosphorylate IRS-1 directly, at least in vitro or in intact cells (Table 1). In addition, PKC may act upstream of other ser/thr kinases (24–26). Thus, PKC activation may enhance the ability of kinases, such as Jun NH<sub>2</sub>-terminal kinase (JNK) and inhibitor of  $\kappa$ B kinase (IKK)- $\beta$ , to phosphorylate IRS-1 at Ser-307, a key regulatory site located close to the domain that interacts with the insulin receptor (27). PKC may also act upstream of p42/44 MAPK to promote Ser-612 phosphorylation, which more specifically modulates PI 3-kinase activation (28,29).

The insulin receptor itself, as well as IRS-1, may be a site of negative regulation by PKC (Fig. 2), particularly PKC $\epsilon$ . While direct phosphorylation by PKC does not appear to modulate tyrosine kinase activity (41), an interaction of PKC $\epsilon$  with the receptor in liver may affect its ability to phosphorylate its substrates (42). Alternatively, such an association may affect insulin receptor trafficking in hepatocytes (43) and hence both the clearance of insulin by the liver (44) and degradation of the receptor itself (14).



**FIG. 2.** Lipid oversupply leads to the generation of distinct intracellular mediators of insulin resistance. Fatty acids entering the cell are activated by the formation of LCAC. Saturated fatty acid favors ceramide accumulation due to the requirement for palmitate during de novo synthesis, which in turn leads to the inhibition of Akt, in part due to aPKCζ action. In contrast, DAG species derived from unsaturated fatty acids favor nPKC activation and inhibition at the level of the insulin receptor (IR), or IRS-1. In addition, another unsaturated fatty acid-derived species, dilinoleoyl-phosphatidic acid (PA), can reduce IRS-1 tyrosine phosphorylation in a PKC-independent manner (49). G3P, glycerol 3-phosphate; LPA, lysophosphatidic acid; TG, triglyceride. See text for further details.

**THE RELATIONSHIP BETWEEN DAG ACCUMULATION, PKC ACTIVATION, AND INSULIN RESISTANCE**

The studies described above have helped to establish the hypothesis that aberrant PKC activation plays a role in the development of insulin resistance. It is generally assumed that elevations in DAG, secondary to intracellular lipid accumulation, provide the essential link in this scheme. But intracellular DAG could also be increased by insulin (45) or from de novo synthesis from glucose (46). Thus, PKCs may contribute to the secondary insulin resistance caused by the hyperinsulinemia and/or hyperglycemia occurring in the glucose-intolerant state. A further corollary of these and similar studies is that different species of fatty acids induce insulin resistance via different mechanisms (rev. in 8). PKC activation is relatively specific to

DAG species composed of unsaturated fatty acid side chains (47). Thus, we have demonstrated that the elevation in DAG observed in muscle cells treated with the saturated fatty acid palmitate does not greatly stimulate PKC translocation, while the relatively minor DAG changes caused by unsaturated fatty acids can do so (48,49). Because both saturated and unsaturated fatty acids cause insulin resistance, this supports the contention that saturated fatty acids do not act principally via the DAG/PKC pathway, but through other mechanisms including ceramide-dependent signaling (50–53). (Ceramide is a potential activator of aPKCζ [Fig. 2], and a cross-talk mechanism involving sequestration of Akt by PKCζ in caveolae has also been proposed to contribute to the disruption of insulin signaling caused by saturated fatty acids [52].)

**TABLE 1**  
PKC-mediated IRS-1 phosphorylation

	Action	Effect	Reference
PKCα	IRS-1 Ser/Thr phosphorylation	Inhibition of IR kinase activity	30
	IRS-1 Ser24 phosphorylation	Reduced IRS-1 phosphatidylinositol-4,5-bisphosphate binding	31
PKCδ	IRS-1 Ser/Thr phosphorylation	Inhibition of IR kinase activity	30
	IRS-1 Ser307,323,574 phosphorylation	Reduced IRS-1 Tyr phosphorylation	32
	IRS-1 Ser24 phosphorylation	Reduced IRS-1 phosphatidylinositol-4,5-bisphosphate binding	33
	IRS-1 Ser318 phosphorylation	Early positive but longer-term negative regulation of IRS-1 signaling	34
	IRS-1 Ser357 phosphorylation	Reduced IRS-1 Tyr phosphorylation	35
PKCθ	IRS-1 Ser/Thr phosphorylation	Inhibition of IR kinase activity	30
	IRS-1 Ser1101 phosphorylation	Reduced IRS-1 Tyr phosphorylation	36
PKCζ	IRS-1 Ser/Thr phosphorylation	Reduced IRS-1 Tyr phosphorylation	37,38
	IRS-1 Ser318 phosphorylation	Reduced IRS-1 Tyr phosphorylation	39,40

IRS-1 phosphorylation sites are listed as they appear in individual references. Ser302, 307, and 318 in mouse IRS-1 are Ser307, 312, and 323 in human IRS-1. IR, insulin receptor.

The integral link between specific fatty acids, DAG, and PKC activation has been confirmed in studies addressing the effects of DAG kinase (DGK) isoforms, which lower intracellular DAG levels and reverse the activation of PKC, but these have also highlighted the complexity of DAG signaling (49,54,55). On the one hand, a reduction in DGK $\delta$  activity potentially contributes to hyperglycemia-induced activation of PKC $\delta$  and the resultant inhibition of insulin signaling in skeletal muscle from subjects with type 2 diabetes (55). On the other hand, overexpression of DGK $\epsilon$  in muscle cells, while reducing the activation of several PKCs as expected because of chronic exposure to unsaturated fatty acid, in fact compounds defects in insulin signaling (49). Such discrepancies are perhaps explained by the different substrate specificities, regulation, and cellular localization of the DGK isoforms (56) involved. In addition, and taken together with the failure of dominant-negative nPKC mutants to overcome insulin resistance in cultured myotubes (48), it can be argued that the diminished insulin action arising from lipid oversupply is not always simply explained by the accompanying DAG accumulation and PKC activation. Indeed, other lipid intermediates such as dilinoleoyl-phosphatidic acid (49) have been recently implicated as mediating insulin resistance induced by unsaturated fatty acids via pathways independent of PKC. This makes it essential to define precisely the situations where PKC does play a role and to quantify its contribution using *in vivo* models.

#### RECENT FINDINGS FROM PKC TRANSGENIC AND KNOCKOUT MICE

The importance of the correlation between the translocation of various PKC isoforms and insulin resistance has now been addressed in a more causative fashion, mostly using PKC knockout mice. Deletion studies of conventional PKC isoforms have argued against these enzymes acting to significantly reduce insulin action *in vivo* (57,58), although it now appears that PKC $\beta$  can impinge on this indirectly by reducing lipid oxidation in adipose tissue and thereby enhancing fat accumulation (59).

PKC $\theta$  has been extensively investigated, because it is relatively highly expressed in skeletal muscle compared with other tissues, suggesting it as a prime candidate for regulating glucose homeostasis. A complex picture has emerged. First, PKC $\theta$  knockout mice are protected against the short-term insulin resistance and defective insulin signaling in skeletal muscle generated by acute lipid infusion (60). In contrast, longer-term studies revealed that these animals exhibit an increased susceptibility to obesity and diet-induced insulin resistance (61). Likewise, a dominant-negative approach to block PKC $\theta$  by overexpressing a kinase-inactive mutant in skeletal muscle also resulted in obesity, glucose intolerance, and hyperinsulinemia (62). It therefore appears that while PKC $\theta$  can acutely inhibit insulin signal transduction, it plays other important roles that protect against obesity. Pertinently, activation of PKC $\theta$  has usually been inferred from an increased membrane-to-cytosolic ratio, but this is often the consequence of the reduction in cytosolic protein rather than an increase in membrane association (11,12,63). These features could equally be interpreted as an overall downregulation of PKC $\theta$  and diminished functional capacity, consistent with it playing a positive role in insulin sensitivity in the longer term.

Other studies addressing causation have indicated mul-

iple roles for hepatic PKC $\epsilon$  in the modulation of glucose homeostasis. Antisense oligonucleotides against PKC $\epsilon$  were able to reverse a defect in insulin receptor kinase activity and the suppression of hepatic glucose production observed in rats fed for 3 days on a high-fat diet based on safflower oil (rich in the unsaturated fatty acid linoleate) (42). This extends the correlation between PKC $\epsilon$  translocation and liver insulin resistance observed under short-term conditions (18). In contrast, no improvement in hepatic insulin signaling was observed in mice globally deleted in PKC $\epsilon$  and maintained on a long-term diet rich in saturated fatty acids and sucrose, which induces obesity and profound insulin resistance (44). Surprisingly, however, glucose tolerance was completely restored in the PKC $\epsilon$  knockout mice. This was explained by a greater availability of insulin, which compensated for, rather than reversed, insulin resistance. Two phenotypes in the PKC $\epsilon$  null mice contributed to this compensation: an enhancement in glucose-stimulated insulin secretion by pancreatic  $\beta$ -cells (see below) and a reduction in hepatic insulin clearance (44). Importantly, there was no increase in hepatic triglyceride or fatty acid content in PKC $\epsilon$  knockout mice, indicating that the transiently enhanced insulin levels in response to a glucose challenge did not promote steatosis.

The contrasting interpretations of the role of PKC $\epsilon$  in hepatic insulin action arising from these studies might be explained by differences in both the type and the duration of the fat diets used (42,44). First, the use of a diet rich in unsaturated fat is more likely to activate PKC (48), while a saturated fat diet may act predominantly through the elevation of ceramide (50,51,53). Second, it is possible that PKC $\epsilon$  contributes significantly to hepatic insulin resistance in an acute fashion but that other PKC $\epsilon$ -independent mechanisms become prevalent over time, such that PKC $\epsilon$  deletion is no longer sufficient to protect insulin signaling. Finally, compensation for PKC $\epsilon$  deletion by other PKC isoforms, such as PKC $\delta$ , might occur in liver and muscle, but not in  $\beta$ -cells. Under these conditions, enhanced insulin secretion (see below) might overcome the ongoing loss of insulin sensitivity because of the high-fat diet.

Overall, it can be concluded that individual PKC isoforms, especially PKC $\theta$  and PKC $\epsilon$ , play multiple roles in the modulation of insulin action, even within one particular tissue such as muscle or liver. In the case of PKC $\theta$ , these roles appear to be discordant, making this enzyme less suitable as a therapeutic target. In contrast, although deletion or reduction of PKC $\epsilon$  has been shown to produce divergent effects, collectively these should all be beneficial for glucose homeostasis. It still remains to be determined, however, whether the key mechanism of action of PKC $\epsilon$  relates to the generation of insulin resistance, as originally envisioned, or whether the newly recognized role in regulating insulin availability predominates.

#### PKC IN B-CELLS

Surprisingly, given the longstanding focus on PKCs in insulin resistance, there have been relatively few studies addressing a corresponding role in  $\beta$ -cell dysfunction. Before describing this work, however, it is necessary to outline what is known about the normal physiological functions of PKC in  $\beta$ -cells, particularly its controversial role in nutrient-stimulated insulin secretion and its regulation of  $\beta$ -cell mass. Other aspects less directly relevant to type 2 diabetes, such as the well-established involvement

of PKC downstream of muscarinic cholinergic receptors, have been reviewed elsewhere and will not be addressed further here (64,65).

#### PKC ISOFORMS AND GLUCOSE-STIMULATED INSULIN SECRETION

It has been known for more than 25 years that activation of PKC, using pharmacological stimulators such as phorbol esters, was sufficient to augment insulin secretion, particularly in conjunction with a stimulus that raised intracellular  $Ca^{2+}$  (66). Because glucose promoted phosphoinositide hydrolysis in  $\beta$ -cells, secondary to voltage-gated  $Ca^{2+}$  influx and a  $Ca^{2+}$ -dependent stimulation of phospholipase C (67), it was envisioned that the DAG/PKC pathway might contribute to glucose-stimulated insulin secretion (GSIS). However, DAG could also be generated via de novo synthesis, either from glucose directly (68,69) or secondarily to a switch in endogenous fatty acid metabolism away from  $\beta$ -oxidation and toward esterification products. The latter phenomenon has been widely documented and arises via glucose-enhanced anaplerosis leading to increases in malonyl CoA, which subsequently inhibits CPT1, a key control point of  $\beta$ -oxidation (70). The further realization that CoA derivatives of long-chain fatty acids (long-chain acyl-CoA [LCAC]) could themselves activate PKCs has underpinned ongoing support for this proposed signaling function of lipid partitioning. Thus, irrespective of whether PKC activation was triggered by DAG generated from phosphoinositide hydrolysis or by LCACs from endogenous metabolism, the net result would be an amplification of the more proximal  $Ca^{2+}$ -dependent signals that initiate GSIS (70,71).

Nevertheless, for technical reasons, it has been difficult to prove or disprove this role of PKC. Rat and mouse islets and (to variable extents)  $\beta$ -cell lines express multiple PKC isoforms, including PKC $\alpha$ ,  $\beta_{II}$ ,  $\delta$ ,  $\epsilon$ ,  $\zeta$ , and  $\iota$  (72–75). Moreover, the framework within which PKCs function might differ between rat and mouse  $\beta$ -cells. Rat islets display a second phase of GSIS, which is both greatly exaggerated compared with mouse and much more sensitive to the (nonspecific) PKC inhibitor staurosporine (76). Human islets behave more similarly to mouse than to rat islets, displaying a modest second phase that is resistant to staurosporine (69,77). Another technical limitation is that although measuring PKC translocation by immunoblotting is more specific than activity assays, this may suffer from redistribution artifacts during sample processing. Subcellular localization by microscopy is potentially compounded by cross-reactivity of antisera (for studies of endogenous protein) or overexpression artifacts (when using transfected reporters). Optimal visualization of the latter often also relies on complex correction of background signals and/or somewhat artificial conditions.

Nevertheless, there is a general consensus that glucose promotes a rapid  $Ca^{2+}$ -dependent translocation of cPKC isoforms (particularly PKC $\alpha$ ) to the plasma membrane (78–80). Glucose also stimulates a redistribution of PKC $\epsilon$ , in this case independently of  $Ca^{2+}$  (79,81), to sites containing insulin granules at either the plasma membrane (82) or near the nucleus (83). Translocation does not necessarily equate to functional activation, however, and demonstrations of glucose-stimulated phosphorylation of PKC substrates in  $\beta$ -cells have been inconsistent or poorly correlated with secretion (75,84–86). We demonstrated that glucose normally induces only a modest and transient

increase in phosphorylation of cPKC substrates in islets, but this is significantly enhanced in the presence of phosphatase inhibitors (75). This implies that a concomitant stimulation of protein phosphatases by glucose (64) might counteract the consequences of PKC activation.

Determining whether PKC activation is necessary for GSIS has also been problematic. Global downregulation of PKC activity by chronic exposure to phorbol esters, or using nonspecific inhibitors, has produced variable results (84–88) possibly explained by species differences. Nevertheless, there is consistent evidence that inhibitors of conventional PKCs completely block insulin secretion in response to phorbol esters, but affect GSIS only modestly at best (75,86,89,90). Overexpression of a kinase-dead PKC $\alpha$  construct in rat islets augmented first-phase, and slightly diminished second-phase, GSIS in batch incubations (91), but this was not observed when assessed directly using islet perfusion (75). Thus, the general conclusion from these studies is that conventional PKCs are not required for a robust first- or second-phase GSIS, although their activation by glucose might exert a minor modulatory influence.

A role for PKC $\epsilon$  in GSIS has also been proposed (79,82). In one study, in which rat islets were “skinned” to facilitate entry of a PKC $\epsilon$  inhibitory peptide, a partial requirement for PKC $\epsilon$  in GSIS was demonstrated (79). Using the same peptide, but in a cell-permeant form, we observed no requirement for PKC $\epsilon$  in GSIS from mouse islets (44). Another report concluded that overexpression of PKC $\epsilon$  in  $\beta$ -cells of *ob/ob* mice was sufficient to enhance secretion, although this was not assayed directly, but extrapolated from changes in membrane capacitance during the initial recording (82). Capacitance changes due to glibenclamide (82) or inositol hexakisphosphate (92) were also blocked by kinase-dead and/or antisense constructs, but this approach was not extended to examine a direct requirement of PKC $\epsilon$  in GSIS. In contrast, using PKC $\epsilon$  knockout mice, glucose tolerance and insulin excursions during whole-body glucose tolerance tests were similar to wild-type animals (44). Likewise, no differences in GSIS were observed when control and PKC $\epsilon$  null islets were compared in batch incubations or perfusion studies *ex vivo* (44).

#### PKC AND INSULIN SECRETION DUE TO FATTY ACIDS

There are also indications of PKC involvement in the potentiation of GSIS by fatty acids (71). First, treatment of  $\beta$ -cells with fatty acids has been variously shown to activate PKC (93–96). Second, general PKC inhibitors partially attenuate the enhancement of insulin secretion due to fatty acids (97–99), even in mouse islets where these inhibitors barely affect GSIS (97,98). Isoform specificity is uncertain, since selective inhibitors or overexpression strategies have not been used in this context. Most evidence, however, points to an involvement of non-cPKCs and therefore novel or atypical isoforms (99–101). Early studies emphasized the importance of endogenous lipid metabolism, and especially a switch from  $\beta$ -oxidation to esterification products, in the mechanism of activation (94). This is consistent with demonstrations that the novel PKCs in particular can be directly activated by LCACs (96). Another possibility, yet to be explored fully, is that PKC could be activated downstream of the cell surface GPR40 receptor, which binds a variety of fatty acids and is known to couple to phospholipase C and hence presumably to phosphoinositide hydrolysis (102).

## REGULATION OF B-CELL MASS OR DIFFERENTIATION BY PKC

There is strong evidence to suggest that PKC $\zeta$  positively regulates  $\beta$ -cell proliferation in response to a variety of growth factors, although the signaling partners up- and downstream of PKC $\zeta$  are yet to be determined (103–105). Specificity was confirmed in one study by demonstrating that proliferation was not altered by knockdown of PKC $\iota$  expression (105), consistent with observations that islets from PKC $\iota$  knockout mice are no smaller than those from wild-type animals (106). There is also evidence that PKC $\zeta$  can positively regulate expression of the key  $\beta$ -cell transcription factor Pdx-1 in response to either IGF-1 (104) or glucose (107). This would seem more relevant to the control of differentiation, as opposed to proliferation, and is consistent with a requirement for PKC $\zeta$  in the glucose-dependent expression of K<sup>+</sup> channel subunits required for GSIS (108). A developmental role for PKC $\iota$  has also been strongly supported. PKC $\iota$  knockout mice display defective GSIS *in vivo* and *ex vivo*, but this is explained less by a specific role in stimulus secretion coupling than by a general effect on  $\beta$ -cell differentiation exerted upstream of the HNF3 $\beta$  transcription factor (106). This is not dissimilar to the purported roles of PKC $\zeta$  in the glucose-dependent differentiation program described above (107,108) and so may point to redundant roles for aPKCs in this particular instance.

There is less extensive evidence for involvement of nPKC isoforms in regulating  $\beta$ -cell mass. Fatty acids disrupt signaling downstream of receptors important for  $\beta$ -cell proliferation, and nPKC isoforms have been implicated in this context (96). Islet mass was not altered by PKC $\epsilon$  deletion in mice maintained on a chow diet (44). On a high-fat diet, however, islet cell mass and proliferation were augmented in wild-type animals in partial compensation for the accompanying insulin resistance. These increases in mass and proliferation were not observed in the PKC $\epsilon$  knockout mice (44). This may represent an adaptive response, in that compensation would no longer be required because of the improved glucose tolerance observed under these conditions. Alternatively, PKC $\epsilon$  might play a more active role in  $\beta$ -cell proliferation, which is absent in the knockout mice. Further work is required to resolve these issues.

As in many cell types, PKC $\delta$  exerts a pro-apoptotic role in pancreatic  $\beta$ -cells (95,109,110). This was first shown in cytokine-mediated cell destruction and involved an increase in mRNA stabilization for transcripts of inducible NO synthase (109). A more distal role, secondary to generation of a constitutively active PKC $\delta$  fragment after its cleavage by caspase 3, was also implicated (110). Inhibition of PKC $\delta$  also partially protected against lipopoptosis (95). In this instance, activation appeared to be downstream of G $_q$ , which potentially implicates a receptor such as GPR40. Further investigation of this role of PKC $\delta$  would be of interest. Seemingly at odds with this pro-apoptotic function, other authors have demonstrated a partial requirement in GSIS using islets from PKC $\delta$  knockout mice (111). This contrasted with an earlier study using overexpression of kinase-dead PKC $\delta$  in isolated rat islets using adenovirus (75). Moreover, translocation of PKC $\delta$  has never been observed when tested in response to glucose (80,83,100,111).

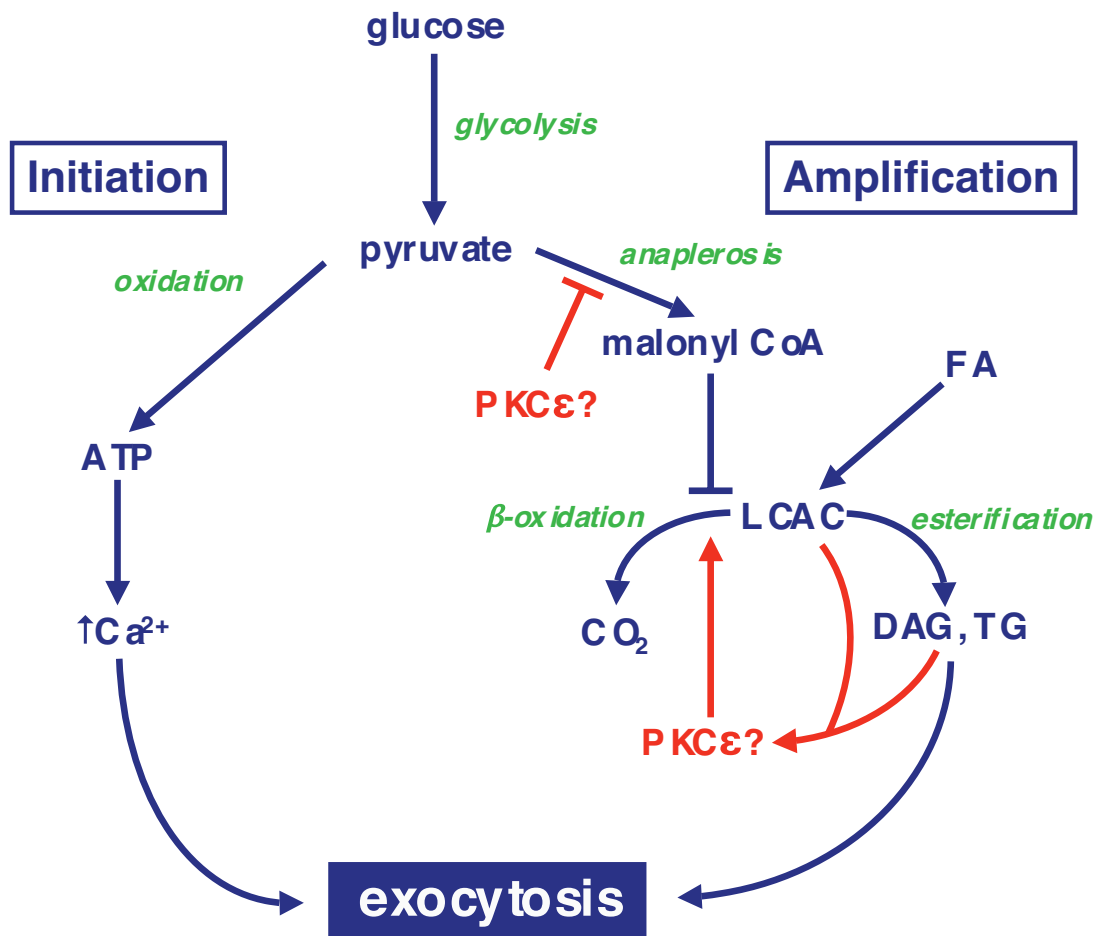
## PKC IN SECRETORY DYSFUNCTION OF B-CELLS

We have recently established an unexpected role for PKC $\epsilon$  in the development of  $\beta$ -cell lipotoxicity (44). As discussed above, deletion of PKC $\epsilon$  resulted in a normalization of glucose tolerance in fat-fed mice because of an enhancement of insulin availability rather than improved insulin sensitivity. This was confirmed by comparing GSIS from wild-type or PKC $\epsilon$  null islets chronically exposed to elevated fatty acids *ex vivo*. The secretory defects induced under these conditions were prevented by deletion of PKC $\epsilon$ , and likewise GSIS was improved in islets of diabetic *db/db* mice when treated *ex vivo* with a PKC $\epsilon$  inhibitory peptide. In all cases, *in vivo* and *ex vivo*, the enhancement of insulin secretion was dependent on a diabetic milieu or prior lipid exposure; unimpaired GSIS was not altered by functional inhibition of PKC $\epsilon$  (44). This suggests that activation of PKC $\epsilon$  is either intimately involved in the actual process whereby secretory defects are induced by chronically elevated fatty acid or lipid overload or that it acts proximally to that process.

PKC $\epsilon$  most probably affects secretion via multiple mechanisms. Deletion of this isoform resulted in slight (~25%) increases in both insulin content and insulin mRNA, suggesting that it might play a minor role in regulating insulin gene expression (44). But the modesty of these increases, and their lack of dependence on prior lipid exposure, suggests they do not make a major contribution to the reversal of defective secretion. Instead, our findings implicated the amplification pathway of GSIS, based on observations of lipid partitioning in  $\beta$ -cells during acute exposure to glucose (44). Normally this is associated with a switch from  $\beta$ -oxidation toward esterification pathways, but chronic pretreatment with fatty acids disrupts this switch by upregulating  $\beta$ -oxidation (70). Deletion of PKC $\epsilon$  helps to restore the appropriate balance between esterification and oxidation, and this may contribute to the normalization of insulin secretion (Fig. 3). At present, however, the role of lipid partitioning in regulating GSIS remains somewhat controversial. It is unclear whether this phenomenon is itself causal, activating signaling cascades that augment secretion, or whether it is merely a readout of potentially more important events occurring upstream in anaplerotic pathways (Fig. 3). It is possible that further defining the role of PKC $\epsilon$  might actually help resolve some of these basic unsolved questions in stimulus-secretion coupling in  $\beta$ -cells.

## PKC AS A THERAPEUTIC TARGET FOR TYPE 2 DIABETES

Much of the work described above is based on the premise that inhibition of PKCs could be of benefit in the treatment of type 2 diabetes. Within that framework, we will now attempt to summarize our view of the current state of the field (Fig. 4). Most speculation over the last decade has centered on PKC antagonists as potential insulin sensitizers. This is based on a body of work demonstrating that PKCs possess the capacity to disrupt insulin signaling and that they are activated in muscle and liver during insulin resistance. A causal relationship, however, between PKC activation and insulin resistance has been difficult to substantiate. In particular, the early promise of PKC $\theta$  as potential mediator of muscle insulin resistance has been clouded by an additional role in protecting against obesity (61,62). In liver, inhibition of PKC $\epsilon$  might improve insulin sensitivity in short-term models (42); this was not the case



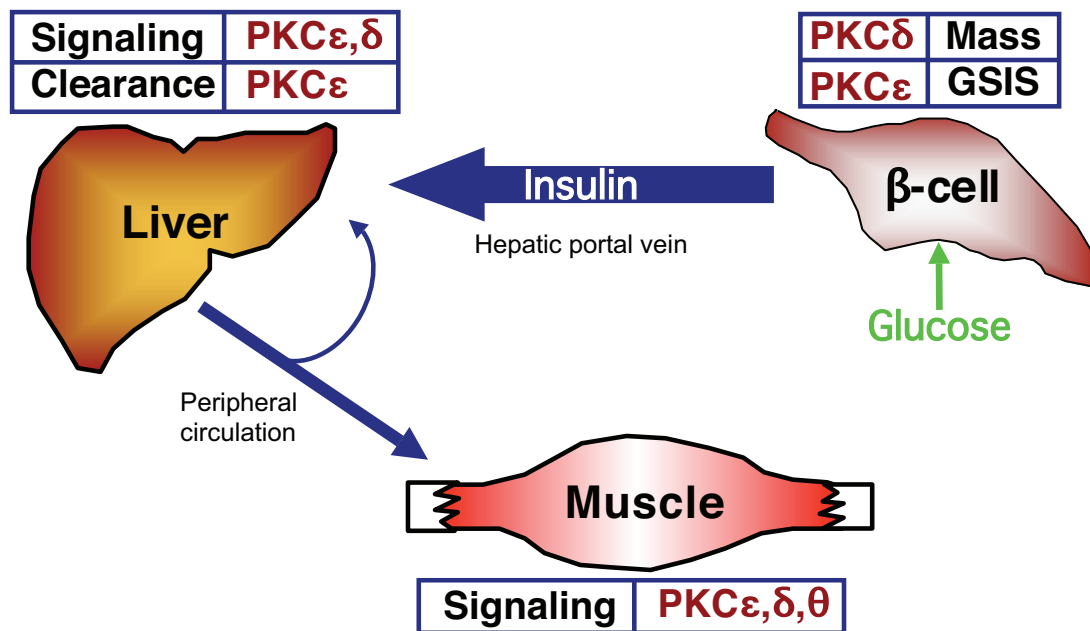
**FIG. 3.** Putative site of action of PKCε in the amplification pathway of GSIS. Pyruvate, derived from glycolysis, undergoes two metabolic fates in mitochondria that together regulate GSIS. In the initiation pathway, it is subjected to oxidative phosphorylation to generate ATP, which leads to closure of ATP-dependent K<sup>+</sup> channels, depolarization, and the gating of Ca<sup>2+</sup> influx. In the amplification pathway, pyruvate augments Krebs cycle intermediates (anaplerosis), some of which can be exported to the cytosol to generate malonyl-CoA. This results in an inhibition of the β-oxidation of LCACs derived from exogenous fatty acids or mobilization of endogenous lipid stores. This favors the formation of esterification products di- or triacylglycerol (DAG or TG, respectively). PKCε, potentially activated by LCACs or DAG, appears to promote oxidation of lipid fuels at the expense of esterification pathways that are implicated in the amplification pathway. Whether PKCε acts directly at this site, or upstream at a step in anaplerosis, remains to be determined. For further explanation, see the text and Ref. 44.

in longer-term dietary regimens that are arguably more representative of obesity-induced insulin resistance (44). Further work is required to resolve these issues, particularly because a compensatory activation of other PKC isoforms may have masked the effects of PKCε deletion on insulin sensitivity in the knockout model. A possible candidate in that regard is PKCδ, which has been implicated in studies of IRS phosphorylation (30,32–35), although its role *in vivo* has not been assessed directly.

Regardless of the ultimate involvement or not of PKCs in insulin resistance, the rationale for targeting PKCs in the treatment of type 2 diabetes has been extended by our recent demonstration of an unexpected role for PKCε in regulating insulin availability (Fig. 4). Indeed, targeting PKCε is likely to have several advantages over existing therapies that enhance insulin secretion. Most importantly, PKCε inhibition appears to act very close to the actual cause of impaired secretion, or at least specifically addresses its consequences. To our knowledge, no other strategy for promoting insulin secretion shows this benefit. Existing therapies, such as sulfonylureas or GLP-1 agonists, bypass the secretory defect rather than addressing it directly. Another advantage is that PKCε deletion selectively augmented the first phase of GSIS, which is

crucial for regulating glucose tolerance and which is lost early in the development of type 2 diabetes. Finally, the effects of inhibiting PKCε in β-cells were complemented by a reduction in hepatic insulin clearance (44). Both aspects contributed to the enhanced availability of insulin. Taken together, these features suggest that a single therapy, based on inhibition of PKCε, could act at multiple sites and in manners different from, and therefore complimentary to, existing treatments for type 2 diabetes.

Obviously, it is difficult to develop truly selective inhibitors of protein kinases and, indeed, it remains to be seen whether other potential consequences of PKCε inhibition might preclude its adoption as a therapy. On the other hand, off-target effects might actually prove to be beneficial if they included, for example, inhibition of PKCδ, which might help preserve β-cell mass and/or overcome insulin resistance (Fig. 4). Regardless of the ultimate clinical utility of PKC inhibitors, however, it seems that there is much useful information to be obtained in the short term by the further study of the roles of PKC in regulating glucose homeostasis. This is a particular need for mechanistic insight into how PKCε controls insulin uptake in hepatocytes and restores GSIS in diabetic β-cells.



**FIG. 4.** Potential sites at which individual PKC isoforms might be beneficially targeted for the treatment of type 2 diabetes. Inhibition of PKC $\epsilon$  is predicted to improve insulin availability by restoring defective GSIS and by diminishing hepatic insulin clearance. This may also improve insulin sensitivity in liver and muscle. Targeting PKC $\delta$  may be of benefit in maintaining  $\beta$ -cell mass and in treating insulin resistance in muscle and liver. See text for details.

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