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The diverse roles of protein kinase C in pancreatic β-cell function

Trevor J. Biden[1], Carsten Schmitz-Peiffer, James G. Burchfield, Ebru Gurisik, James Cantley, Christopher J. Mitchell and Lee Carpenter

Garvan Institute of Medical Research, 384 Victoria Street, Darlinghurst, NSW 2010, Australia

Abstract

Members of the serine/threonine PKC (protein kinase C) family perform diverse functions in multiple cell types. All members of the family are activated in signalling cascades triggered by occupation of cell surface receptors, but the cPKC (conventional PKC) and nPKC (novel PKC) isoforms are also responsive to fatty acid metabolites. PKC isoforms are involved in various aspects of pancreatic β-cell function, including cell proliferation, differentiation and death, as well as regulation of secretion in response to glucose and muscarinic receptor agonists. Recently, the nPKC isoform, PKCε, has also been implicated in the loss of insulin secretory responsiveness that underpins the development of Type 2 diabetes.

Introduction

The PKC (protein kinase C) family of serine/threonine protein kinases comprises a total of ten isoforms, and is subdivided into three groups based on sequence homology and mechanisms of activation [1]. The cPKC (conventional PKC) (PKCα, β and γ) and nPKC (novel PKC) subgroups (PKCδ, ε, η and θ) are both activated by DAG (diacylglycerol), but the cPKCs are additionally sensitive to Ca^{2+}. Both these subgroups are activated downstream of receptors that couple with phospholipase C, phosphoinositide hydrolysis and DAG generation. The aPKC (atypical PKC) isoforms (PKCζ and PKCι/λ) constitute a third group within the PKC family, and are independent of both Ca^{2+} and DAG. Instead, these kinases can be activated in response to receptors that stimulate phosphoinositide 3-kinase.

Receptor-mediated PKC activation in β-cells

Multiple PKC isoforms are expressed in pancreatic islets and β-cell lines, including PKCα, β_{II}, δ, ε, ζ and ι [2–6]. Moreover, pharmacological activation of the cPKCs and nPKCs is sufficient to promote insulin secretion provided a stimulus to raise intracellular Ca^{2+} is also included [7]. The relevance of these findings was quickly established in the context of a role for PKC in mediating secretion in response to agonists acting on receptors coupled with the canonical G_q/phospholipase C signalling pathway [8]. Best studied of these are agonists such as acetylcholine or carbamoylcholine that activate muscarinic cholinergic receptors and thereby stimulate multiple isoforms of PKC [3,4,9]. Inhibitor studies, or protocols to down-regulate PKC, have clearly established at least a partial requirement for PKC activation in the potentiation of insulin secretion due to muscarinic receptor agonists [10–12]. cPKCs are the most strongly implicated isoforms [13]. However, there is also good evidence that PKC activation acts as a feedback inhibitor in the G_q signalling pathway [11,14–17]. It would not be surprising if this effect were mediated by activation of different PKC isoforms to those responsible for the predominant stimulation of secretion, although this has not been investigated extensively. An additional consideration is that muscarinic cholinergic receptors couple with the hydrolysis of both PtdIns and PtdIns(4,5)P_2 [18,19], so it is possible that different PKC isoforms might be activated downstream of these two signalling events, but this has not been demonstrated directly.

PKC and GSIS (glucose-stimulated insulin secretion)

Because glucose promotes phosphoinositide hydrolysis in β-cells, secondary to voltage-gated Ca^{2+} influx and a Ca^{2+}-dependent stimulation of phospholipase C [20], it was envisioned that the DAG/PKC pathway might also be relevant to GSIS. This was strengthened by suggestions that DAG could also be generated via *de novo* synthesis from glucose [21,22]. More recent evidence favours a mechanism whereby alterations in endogenous lipid metabolism lead to increases in PKC activators such as DAG or CoA derivatives of long-chain FAs (fatty acids) [LCACs (long-chain acylcarnitines)] [23–25]. According to this model, glucose leads to increases in malonyl-CoA, which subsequently inhibits β-oxidation via actions on the key regulatory enzyme, CPT1 (carnitine palmitoyltransferase 1). This shifts endogenous FA metabolism towards esterification pathways at the expense of β-oxidation [23]. A similar mechanism is implicated in the potentiation of GSIS by non-esterified FAs [26].

Key words: glucose-stimulated insulin secretion, insulin secretion, lipotoxicity, pancreatic β-cell, protein kinase C (PKC), Type 2 diabetes.

Abbreviations used: DAG, diacylglycerol; FA, fatty acid; GLP-1, glucagon-like peptide 1; GSIS, glucose-stimulated insulin secretion; IGF-1, insulin-like growth factor-1; PKC, protein kinase C; aPKC, atypical PKC; cPKC, conventional PKC; nPKC, novel PKC.

[1]To whom correspondence should be addressed (email t.biden@garvan.org.au).

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However, because whole islets are not readily amenable to overexpression strategies, and because of the lack of truly isoform-specific inhibitors, the role of PKC activation in GSIS has remained controversial. Nevertheless, glucose does appear to activate classical PKC isoforms (particularly PKCα) in a Ca^{2+}-dependent manner, at least as demonstrated by their translocation to the plasma membrane [27–29]. Glucose also alters the subcellular localization of PKCε [28,30], although the ultimate destination varies between studies [31,32]. Translocation is not necessarily the same thing as functional activation, however, and demonstrations of glucose-stimulated phosphorylation of PKC substrates in β-cells have been inconsistent [6,11,33–35]. This might be explained by activation of protein phosphatases by glucose [8], which would tend to counter the functional consequences of PKC stimulation. Thus phosphorylation of classical PKC substrates in islets was shown to be modest and transient, unless phosphatase inhibitors were also employed during glucose stimulation [6].

Investigations of whether PKC activation is required for GSIS were initially based on the use of relatively non-specific inhibitors or down-regulation of PKC activity by chronic exposure to phorbol esters. These produced variable results [11,33,36,37], which might be partially explained by species differences between rats and mice. The use of more specific inhibitors, capable of selectively targeting cPKCs, resulted in only modest alterations in GSIS, but clearly inhibited secretion in response to phorbol esters under the same conditions [6,13,38]. Consistent with these findings, the use of recombinant adenoviruses to overexpress wild-type or kinase-dead (dominant-negative) PKCα constructs did not alter either first- or second-phase GSIS in perifused rat islets [6]. A similar approach in another laboratory resulted in a slight diminution of second-phase GSIS with PKCα kinase-dead virus, but this was based on batch incubation of islets [39]. Collectively these studies suggest that activation of cPKCs plays, at most, a minor role in GSIS.

Knockout mouse models have been used more recently to address roles of nPKCs and aPKCs in β-cell function. Deletion of aPKCι/λ resulted in decreased GSIS [40], but this was related to a role in general β-cell differentiation rather than stimulus–secretion coupling itself (see below). A partial requirement for PKCδ in GSIS has also been postulated from knockout mouse studies [41], although activation of this isoform by glucose could not be demonstrated in this or several earlier reports [29,32]. Moreover, a role in GSIS was not supported when kinase-dead PKCδ was overexpressed in isolated rat islets using adenovirus [6]. Therefore a role in secretion for this isoform appears less well established than its generally pro-apoptotic function in pancreatic β-cells [42–44].

Glucose tolerance or insulin excursions during whole body glucose tolerance tests were not impaired in PKCε-knockout mice, nor was GSIS altered when measured *ex vivo* [45]. Similarly, a cell-permeant, peptide inhibitor of PKCε did not inhibit the secretory response to glucose using isolated islets [45]. In contrast, a cell-impermeant form of this peptide did inhibit GSIS, possibly as a result of manipulations for permeabilizing the islets to facilitate peptide entry [28]. In other studies, overexpression of a variety of PKCε constructs in β-cells pointed to roles for this PKC isoform in regulating secretion in response to glibenclamide [31], or inositol hexakisphosphate [46], but GSIS was not specifically examined. Moreover, secretion was not assayed directly in these studies, but extrapolated from changes in membrane capacitance.

PKC and the regulation of β-cell mass

In addition to the above described role for PKCι/λ in controlling β-cell differentiation [40], the aPKCs are strongly implicated as regulators of β-cell proliferation. PKCζ is essential for full proliferative responses due to growth factors such as GLP-1 (glucagon-like peptide 1) [47], IGF-1 (insulin-like growth factor-1) [48], PTHrP (parathyroid hormone-related protein) and HGF (hepatocyte growth factor) [49]. Moreover, PKCζ can positively regulate expression of the key β-cell transcription factor PDX-1 (pancreatic duodenal homeobox-1) in response to either IGF-1 [48] or glucose [50]. There is also some evidence for involvement of nPKC isoforms in regulating β-cell mass, and activation of these isoforms by FAs could account for the disruptions in growth factor receptor signalling that are caused when β-cells are chronically exposed to lipid *in vitro* [25]. In whole animals the situation is more complex, since islet cell mass and proliferation are augmented by high-fat diets, as part of the compensatory response to the accompanying insulin resistance. These increases in β-cell mass and proliferation were not observed in the PKCε-knockout mice, although there was no difference in islet mass between when wild-type and PKC$\varepsilon^{-/-}$ animals were maintained on a chow diet [45]. This might indicate a requirement for PKCε in the β-cell proliferation that accompanies insulin resistance. But the situation is complicated by an accompanying improvement in whole-body glucose tolerance [45], and so might simply represent an adaptive response whereby proliferation is normalized under conditions where β-cell compensation is no longer required.

PKC in β-cell dysfunction

Type 2 diabetes represents the inability of pancreatic β-cells to compensate, via enhanced insulin secretion, for the insulin resistance that accompanies obesity. This failure to compensate is characterized by both secretory defects, especially in GSIS, and a relative loss of β-cell mass due to enhanced apoptosis. Both these features can be repeated *in vitro* by chronically exposing β-cells to FAs. This raises the possibility of PKC involvement in mediating β-cell dysfunction in response to FAs, in a manner akin to that of the postulated role of PKCs in causing insulin resistance.

The first direct evidence of a potential involvement of PKC in β-cell dysfunction was the observation that overexpression of kinase-dead PKCδ partially protected against lipoapoptosis [44]. More recently, an unexpected role in the development of insulin-secretory defects in models of lipotoxicity was demonstrated using PKCε-knockout mice [45]. As discussed above, deletion of PKCε resulted in a

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normalization of glucose tolerance in fat-fed mice, which was due to an enhancement of insulin availability rather than improved insulin-sensitivity. This was confirmed by comparing GSIS from islets isolated from wild-type and PKCε-knockout animals chronically exposed to elevated FAs in tissue culture. The secretory defects induced under these conditions were prevented by deletion of PKCε, and likewise GSIS was improved in islets of diabetic *db/db* mice when treated *ex vivo* with a PKCε 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 PKCε [45]. Although multiple mechanisms might contribute to this phenotype, the most likely appears to involve an enhancement of the amplification pathway of GSIS. Thus deletion of PKCε restored the partitioning of FA metabolism away from β-oxidation towards esterification products [45]. In wild-type, but not PKC$\varepsilon^{-/-}$ islets, this partitioning is inhibited by chronic lipid exposure.

Inhibition of PKC as a therapy for improving β-cell dysfunction

The recently documented involvement of PKCε in β-cell dysfunction might be of potential relevance to the future treatment of Type 2 diabetes. An inhibitor of PKCε would appear to possess advantages over existing therapies for promoting insulin secretion. First, inhibiting PKCε appears to act very proximally to the site of impaired secretion, which is very different from the mode of action of therapeutics such as sulfonylureas or GLP-1 agonists. These override the secretory defect rather than directly addressing the cause of the impaired secretion. Mechanistically, sulfonylureas or GLP-1 agonists augment intracellular Ca^{2+}, and so could complement administration of a PKCε inhibitor, which is predicted to act by amplifying events downstream of Ca^{2+} signals. Another advantage is that PKCε deletion selectively augmented the first phase of GSIS [45], 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. Both aspects contributed to the enhanced availability of insulin [45]. Taken together, these features suggest that a single therapy, based on inhibition of PKCε, could act at multiple sites, in manners different from, and therefore complimentary to, existing treatments for Type 2 diabetes. However, much more work is needed to determine whether PKC inhibitors will ever fulfil their clinical potential. On the other hand, further study of the role of PKCε in regulating glucose homoeostasis could yield valuable insights into the mechanisms underlying β-cell dysfunction, and further our knowledge of the amplification pathway of GSIS itself.

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