FEBS Letters 585 (2011) 269–274

FEBS Letters

journal homepage: www.FEBSLetters.org

Review

Protein kinase C isoforms: Mediators of reactive lipid metabolites in the development of insulin resistance

Sophie Turban [d], Eric Hajduch [a,b,c,*]

[a] Centre de Recherche des Cordeliers, INSERM, U872, Paris F-75006, France
[b] Université Pierre et Marie Curie, Paris VI, UMR S872, Paris F-75006, France
[c] Université Paris Descartes, UMR S872, Paris F-75006, France
[d] Molecular Metabolism Group, Centre for Cardiovascular Science, The Queen's Medical Research Institute, University of Edinburgh, Edinburgh EH16 4TJ, United Kingdom

ARTICLE INFO

Article history:
Received 12 November 2010
Revised 7 December 2010
Accepted 14 December 2010
Available online 19 December 2010

Edited by Robert Barouki

Keywords:
Insulin
Ceramide
DAG
Protein kinase B
Akt
Obesity

ABSTRACT

The role of protein kinase C (PKCs) isoforms in the regulation of glucose metabolism by insulin is complex, partly due to the large PKC family consisting of three sub-groups: conventional, novel and atypical. Activation of some conventional and novel PKCs in response to increased levels of diacylglycerol (DAG) have been shown to counteract insulin signalling. However, roles of atypical PKCs (aPKCs) remain poorly understood. aPKCs act as molecular switches by promoting or suppressing signalling pathways, in response to insulin or ceramides respectively. Understanding how DAG- and ceramide-activated PKCs impair insulin signalling would help to develop treatments to fight insulin resistance.

1. Introduction

Insulin is a hormone essential for growth, energy storage and maintenance of glucose homeostasis. Defects in its secretion and action are critical for the development of metabolic diseases such as diabetes, obesity, hypertension, atherosclerosis and cardiovascular disease [1]. It is known for some time now that elevated circulating free fatty acid (FFA) levels observed during obesity are involved in the development of insulin resistance in peripheral tissues such as white adipose tissue, skeletal muscle, liver, pancreas and heart [2]. In fact, forcing non-adipose cells to store fatty acids beyond their capacities promotes insulin resistance by inducing ectopic fat deposition [2]. First, and like in adipocytes, cells are protected from the deleterious effects of lipids by storing them as triglycerides in small lipid droplets [3]. However, quickly, and in opposite to what is observed in adipocytes, the storage capacity of non-adipose cells is limited, and overload generates lipotoxicity by promoting accumulation of intracellular signalling molecules that are able to inhibit insulin action [4]. Among these fatty acid-derived lipids, diacylglycerol (DAG) and ceramides are the most active to negatively regulate intermediates of the insulin signalling pathway, and consequently to inhibit glucose metabolism [5]. Interestingly, down-regulation of insulin signalling by both DAG and ceramides is mediated through concomitant activation of members of the protein kinase C (PKC) family of serine/threonine kinases. This review summarises the ongoing progress about the involvement of these kinases in the development of insulin resistance in insulin-sensitive tissues.

2. PKC family

PKCs belong to the AGC (cAMP-dependent protein kinase/PKG/PKC) protein kinases family that play important roles in many intracellular signalling events, cell growth and differentiation [6]. The PKC family consists of three different groups: conventional (α, β1, β2 and γ), novel (δ, ε, η and θ), and atypical (ζ and λ/τ) (Fig. 1). Conventional and novel PKC isoforms are lipid-sensitive enzymes and are usually activated by growth factors through stimulation of phospholipase C (PLC) which hydrolyzes phosphatidylinositol-4,5-bisphosphate (PIP2) to generate DAG and inositol triphosphate (IP3). On the other end, atypical PKC (aPKC) are mainly activated by protein–protein interactions and phosphorylations by 3-phosphoinositide-dependent protein kinase 1 (PDK1). Conventional PKC (cPKC) isoforms contain two regulatory domains

* Corresponding author at: Centre de Recherche des Cordeliers, INSERM, U872, Paris F-75006, France. Fax: +33 1 40 51 85 86.
E-mail address: eric.hajduch@crc.jussieu.fr (E. Hajduch).

0014-5793/$36.00
doi:10.1016/j.febslet.2010.12.022

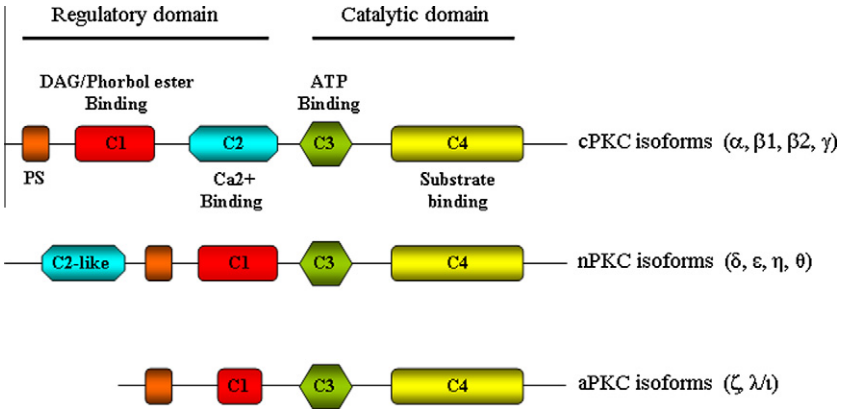

Fig. 1. Structure of PKC isoforms. PKC isoforms are divided into three different groups that contain conserved regions (C1–C4). cPKCs are activated by Ca^{2+} which binds to the C2 region, and by DAG and phorbol esters that bind to the C1 region. nPKCs lack the Ca^{2+} binding site in their C2 region and are solely activated by DAG and phorbol esters through binding to the C1 region. aPKCs, however, completely lack the C2 region and possess a modified C1 region for phosphatidylinositol-3,4,5-triphosphates (PIP3) and ceramides binding. All PKCs possess an auto-inhibitory pseudosubstrate sequence (PS) which is attached to the catalytic domain, therefore blocking access of substrates in the basal state of the kinase, until activators induce conformational changes and promote substrate accessibility. C3 and C4 are highly conserved regions which bind ATP and protein substrates, respectively.

designated C1 and C2 domains. The C1 domain binds DAG and phorbol esters such as phorbol 12-myristate 13-acetate (PMA), while C2 domain binds anionic phospholipids in a calcium-dependent manner (Fig. 1) [6]. Novel PKC (nPKC) isoforms possess a similar C1 domain but lack calcium binding sites in their C2 domain, meaning that they are activated by DAG and phorbol esters independently of calcium (Fig. 1). aPKC isoforms contain a C1 domain that binds phosphatidylinositol-3,4,5-triphosphates (PIP3) and ceramides, but not DAG or phorbol esters and completely lack the calcium-sensitive C2 domain (Fig. 1) [6].

More and more evidences in the literature highlight the crucial role of key PKCs in regulating insulin signalling and especially their participation to mechanisms that induce insulin resistance.

3. PKCs, important actors in the regulation of insulin action

3.1. Positive implication of PKCs in the insulin signalling pathway

In adipocytes and muscle cells insulin signal transduction is initiated by the activation of the insulin receptor, a tyrosine kinase that phosphorylates intracellular substrates, in particular the family of insulin receptor substrates (IRS 1–4 proteins) on several tyrosine residues [7,8]. Numerous proteins can dock on activated IRSs, and among them phosphoinositide-3-kinase (PI3K) and downstream effectors such as serine/threonine kinases protein kinase B (PKB, also known as Akt) and aPKC ζλ which play a crucial role in glucose homeostasis [9,10]. This is the generation of phosphatidylinositol-3,4,5-triphosphate (PIP3) by PI3K that recruits both aPKCs and PKB/Akt to the plasma membrane. Once recruited, aPKCs are phosphorylated by PDK1 on their Thr410/403 sites [8]. Meanwhile, PIP3 binds to the pleckstrin homology (PH) domain of PKB/Akt, inducing a conformational change in the kinase that exposes two regulatory sites, Thr308 and Ser473 (for PKBα/Akt1). Phosphorylation of Thr308 is then mediated by 3-phosphoinositide-dependent protein kinase 1 (PDK1) and Ser473 by mammalian target of Rapamycin(mTOR)–Rapamycin-insensitive companion of mTOR (Rictor) complex [8]. Activated PKB/Akt phosphorylates the Rab-GAP AS160 protein on several sites [11], allowing signal transmission via the activation of Rab proteins (Rab8A, 10 and 14) and the release of Glut4 vesicles to the plasma membrane [11] (Fig. 2). Simultaneously, in skeletal muscle insulin-activated PKB/Akt promotes glycogen synthesis after phosphorylation, and thus inhibition of glycogen synthase kinase 3 (GSK3) [9]. In liver cells, insulin signalling pathway shares great similarities with the one described in adipose or muscle cells, where insulin-activated PKB/Akt promotes glycogen synthesis and inhibits gluconeogenesis (Fig. 2) [5].

Direct implication of aPKCs in regulating Glut4 vesicles translocation under insulin action in adipocytes and muscle cells was shown by overexpressing dominant negative mutants of both PKCζ and PKCλ [12]. Moreover, by using a slightly different approach with PKCζ inhibitors and PKCζ-siRNA, PKCζ has been shown to be involved in the stimulatory effect of insulin on PKB/Akt and subsequent GLUT4 translocation through its interaction with actin cytoskeleton by inducing actin remodelling in L6 muscle cells [13]. This suggests that aPKCs not only participate to insulin signalling cascade but also to insulin-stimulated GLUT4 vesicle trafficking. However, the involvement of aPKCs in the insulin-dependent regulation of glucose transport was recently questioned. A study using PKCλ-depleted L6 muscle cells demonstrated that PKCλ is likely to be dispensable with respect to insulin-stimulated glucose uptake [14]. Indeed PKCλ-depleted muscle cells were more sensitive to insulin than wild-type control cells, suggesting that instead of mediating insulin signalling actions, PKCλ was more likely to restrain them [14]. Those data are in discordance with recent report on the role of PKCλ in vivo in a transgenic mice model lacking PKCλ in their skeletal muscle cells. These mice developed insulin resistance, presented reduced glucose tolerance and dyslipidemia, all common features of the metabolic syndrome [15]. Although those data contribute to the understanding of the effect of aPKCs activation in insulin signalling, a complete dissection of the mechanisms by which aPKCs interact with other components of the signal remains to be realised.

3.2. Other isoforms of PKCs in the insulin signalling pathway

Other cPKC and nPKC isoforms were also found to positively modulate insulin signalling. Indeed, splicing of PKCβ pre-mRNA into PKCβ2 by insulin for remodelling cytoskeleton seems necessary to allow the relocation of GLUT4 vesicles at the plasma membrane in response to insulin in muscle cells [16]. In addition PKCδ was also shown to activate insulin-stimulated glucose transport in muscle cells by interacting and helping internalization of the insulin receptor into internal membranes after stimulation by the hormone.

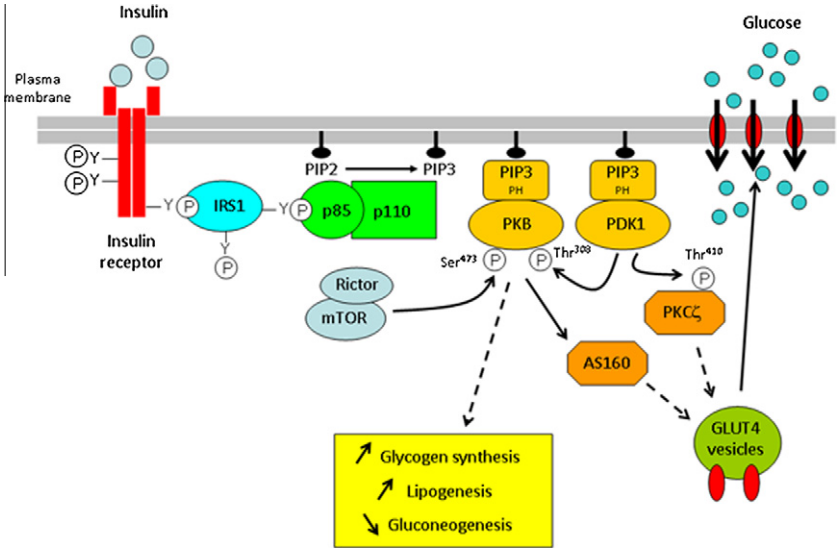

Fig. 2. Insulin signalling pathway. Insulin activates the insulin receptor kinase, leading to the activation of IRSs, PI3-kinase and PKB/Akt. Activated PKB/Akt promotes GLUT4 translocation to the plasma membrane and glucose uptake in adipocytes and skeletal muscle, as well as glycogen synthesis in skeletal muscle and liver. In liver, insulin promotes lipogenesis and inhibits gluconeogenesis.

Overall, very little is known on the role of cPKCs and nPKC in the regulation of insulin signalling and looking at the literature, more members of PKC family might be involved at different levels of regulation.

4. Implication of PKCs as mediators of insulin resistance

Pan et al. were between the first to demonstrate that intramuscular lipid accumulation was associated with insulin resistance [17], followed by Boden et al. showing that infusing lipids intramuscularly to patients was also triggering insulin resistance [18]. Although these data indicated the existence of a relationship between lipid accumulation and reduced insulin signalling pathway, metabolic events connecting fatty acid oversupply to insulin resistance were still not resolved completely at that time. The time frame by which insulin resistance developed (2–4 h after an acute elevation in plasma free fatty acids) indicated an indirect effect of fatty acids on insulin signalling [18], and indeed, an accumulation of reactive lipid intermediates such as DAG and ceramides have been observed in skeletal muscle of insulin-resistant animals [19,20], as well as in human obese subjects with type 2 diabetes [21]. Both lipid intermediates have been demonstrated to play important roles in the establishment of insulin resistance in insulin-sensitive tissues, and their negative action on the insulin signalling pathway is often mediated by several members of the PKC family.

4.1. Diacylglycerol-activated PKCs

DAG, generated by breakdown of phospholipids or through de novo synthesis via the esterification of excess long-chain fatty acyl-CoA into glycerol-3-phosphate, are precursors to triglycerides, but are also thought to act as important second messengers in the regulation of insulin signalling. Interestingly, several studies have shown significant increase in DAG content in skeletal muscle of high-fat fed rats [22], insulin-resistant humans [23], diabetic GK rats [24], as well as in liver of obese homozygous $Lep^{db/db}$ mice [25], and obese Zucker rats [26]. However, to our knowledge there is little evidence for an elevation of DAG contents leading to insulin resistance in adipocytes. Elevated levels of DAG in different tissues lead to the activation of several PKC isoforms such as PKCα, PKCδ, PKCθ, PKCε and PKCβ1 [27]. However, only few of them have been consistently involved in the negative action of DAG on insulin signalling. In rodents or human muscle cells, elevated DAG contents are mainly associated with an increased activation of both PKCθ and PKCε, and related to inhibition of insulin action in these cells [27,28]. The involvement of PKCθ in the development of insulin resistance was confirmed by using PKCθ knockout mice. In these mice, the lack of PKCθ protected muscle cells against insulin resistance induced by lipid infusion [29]. In liver, the specific role of PKCε in mediating DAG harmful actions has been shown by using PKCε antisense oligonucleotides in rats fed with a high fat diet. These rats accumulated DAG in the liver and knocking down PKCε expression specifically in this tissue prevented rats from fat-induced hepatic insulin resistance [27]. PKCε has also been shown to be activated in liver of patients with type 2 diabetes [30] and high-fat fed rats [31] suggesting once again its participation in the development of insulin resistant obesity.

Cellular mechanisms by which DAG-activated PKC isoforms down-regulate insulin signalling are better understood. It appears that DAG-activated PKCs target IRS proteins, one of the first events in the insulin signalling pathway (Fig. 3A). Indeed inactivation of IRSs is mediated by phosphorylation on one or several of its 200 serine/threonine residues. On the one hand, PKCθ can act upstream of the stress kinases IκBα kinaseβ (IKKβ) and c-Jun NH2-terminal kinase (JNK) to phosphorylate IRS-1 on serine residues Ser301 [27], Ser302 and Ser307 [32], but PKCθ can also phosphorylate IRS-1 directly on its serine residue Ser1101 [33] and Ser307 [22]. On the other hand, DAG-activated PKCε inhibits IRS-1 by increasing its phosphorylation on Ser636/639 residues [34] (Fig. 3A). In addition, PKCε associates directly with the insulin receptor and thus impairs its kinase activity [35]. More convincing evidences on the implication of IRS serine phosphorylation in the development of insulin resistance was pointed out in mice in which residues Ser302, Ser307, and Ser612 of IRS-1 were mutated to alanine [36]. These mice were partially but significantly protected against fat-induced insulin resistance [36]. However, it seems that the phosphorylation of serine 307 of IRS-1 might be insufficient to impair insulin signalling in skeletal muscle. Surprisingly, knock-in mice in which the serine 307 residue was replaced by an alanine (A/A) residue developed a more profound resistance to insulin compare to their wild-type littermates under diet-induced obesity

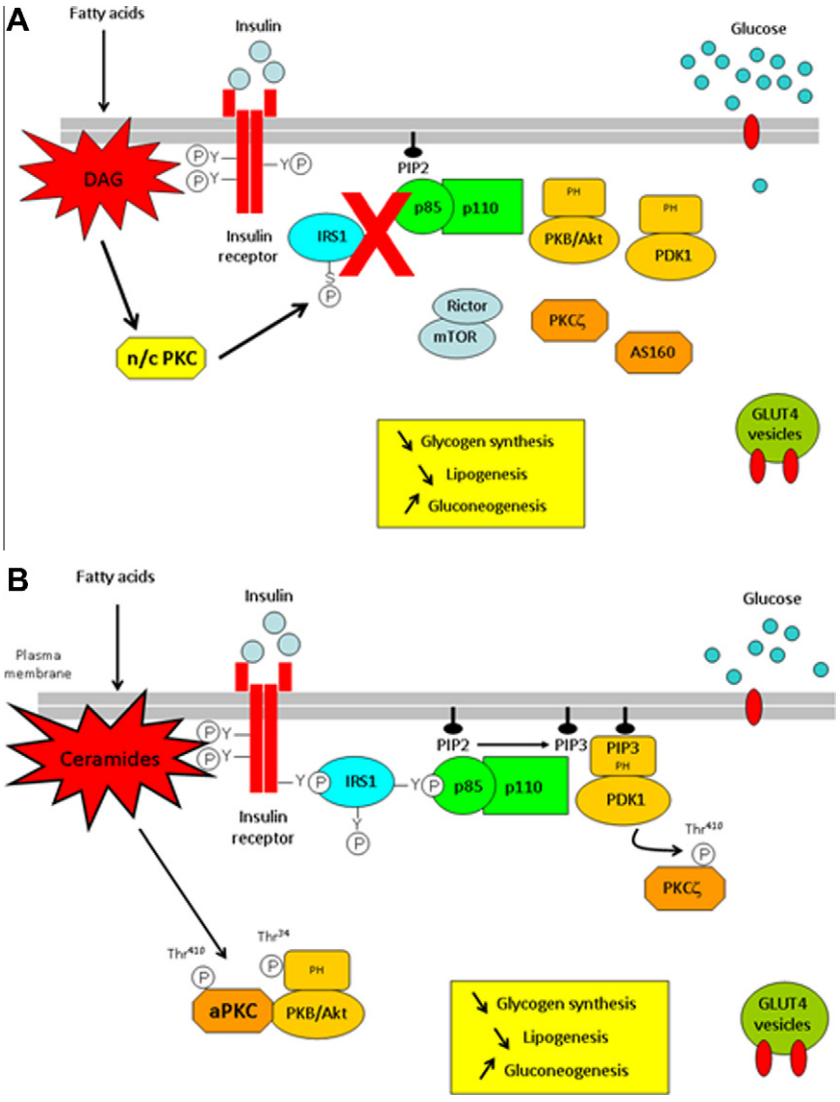

Fig. 3. Implication of PKC isoforms in the development of fatty-acid induced insulin resistance. Insulin resistance result from excess accumulation of fatty acids in ectopic tissues, leading to production of lipid metabolites like DAG and ceramides. (A) High levels of DAG in insulin-sensitive tissues impair insulin signalling at the level of IRSs. DAG is thought to act through both conventional and novel PKCs that induce the phosphorylation of IRSs on serine residues, thus reducing signalling to downstream effectors. (B) Increased levels of ceramides inhibit insulin signalling in insulin sensitive cells through the association between ceramide-activated PKCζ and PKB/Akt. PKCζ-bound PKB/Akt is phosphorylated by PKCζ on its Thr34 residue, therefore preventing PKB/Akt to bind PIP3 and to be recruited to the plasma membrane where it is activated by insulin.

[37]. Hence, this demonstrates that, in vivo, serine 307 residue alone is a positive regulatory site for insulin sensitivity and that more than one serine residue have to be phosphorylated in response to elevated DAG to induce insulin resistance.

The specific implication of the DAG-PKC pathway in the development of insulin resistance in skeletal muscle has been confirmed recently by artificially increasing DAG contents in muscle cells [24]. Chibalin et al. [24] showed that the inhibition of DGKδ (diacylglycerol kinase δ), enzyme phosphorylating DAG to produce phosphatidic acid in vitro in rat muscle strips, induced an elevation of DAG contents which is followed by the activation of novel/conventional PKCs and increased phosphorylation of IRS-1 on both Ser307 and Ser302 residues. Serine phosphorylation of IRS-1 prevented insulin to induce IRS-1 tyrosine phosphorylation, PI3-kinase activity and PKB/Akt phosphorylation, leading to an inhibition of insulin-induced glucose uptake in these cells [24]. Moreover, this study showed that both the expression and the activity of DGKδ were reduced in skeletal muscle of type 2 diabetic patients, as well as in diabetic animals [24]. Thus, this important finding further emphasises the role of DAG and PKCs as important mediators in the regulation of hyperglycaemia-induced impairment of peripheral insulin sensitivity.

4.2. aPKCs mediate ceramide action

Ceramides are derivatives of sphingomyelin and are generated either by sphingomyelinase, via de novo synthesis using palmitate as a precursor, or by the salvage pathway that uses sphingosine through reacylation to produce ceramides [38]. Ceramides are relatively minor components of cell membranes but are known to be mediators of very important cellular processes such as apoptosis, proliferation and differentiation [5]. Like DAG, ceramides accumulate in skeletal muscle of insulin-resistant animals [19,39], lipid infused humans [40], and in type 2 diabetic patients [21]. Several in vivo and in vitro studies have demonstrated that accumulation of ceramides inhibited insulin signalling [5]. Reciprocally, artificially blocking de novo ceramide biosynthesis by pharmacological inhibition of serine palmitoyl transferase (SPT), first enzyme in the ceramide biosynthesis pathway, prevented the onset of insulin resistance in cells and in animals [5].

A consensus now exists that PKB/Akt is the primary target of ceramides. Indeed, defects in activation of this kinase induced by ceramides have been observed in several cell types, such as white and brown adipocytes, skeletal and smooth muscles, mammary cells and nerve cells [5]. In some cells, ceramides acts on PKB/Akt through the direct activation of phosphatases such as the protein phosphatase-2A (PP2A) [5], a cytosolic serine/threonine phosphatase responsible for dephosphorylating PKB/Akt. Treatment of C2C12 muscle cells, PC12 nerve cells and brown adipocytes with the PP2A inhibitor okadaic acid (OKA) [41] prevented negative effects of ceramides on PKB/Akt [5]. However, in L6 muscle cells and white adipose tissue, we and others have shown that ceramides inhibited insulin-stimulated glucose transport through a mechanism that does not involve a phosphatase [42,43]. Ceramides have been shown for a long time to activate aPKCs [44], and recently, a specific protein fragment of PKCζ has been demonstrated to bind ceramides [45]. We demonstrated that ceramide-activated PKCζ interacted and phosphorylated the PH domain of PKB/Akt on a Thr34/Ser34 residue (Thr34 in PKBα/Akt1 and PKBβ/Akt2, and Ser34 in PKBγ/Akt3), thus preventing PKB/Akt to be recruited to the plasma membrane where the kinase is normally activated in response to insulin (Fig. 3B) [39,44]. Inhibition of PKB/Akt activity after phosphorylation of its Thr34/Ser34 residue by PKCζ in response to ceramides has been confirmed by Fox et al. in rat aorta vascular smooth muscle cells (A7r5) [46]. They overexpressed a mutated PKBγ/Akt3 construct in which the Ser34 residue was replaced by a glutamate to mimic phosphorylation. This mutant reproduced the ceramide repressive action on PDGF- stimulated PKBγ/Akt3 activity. Inversely replacement of Ser34 residue by an alanine to prevent PKCζ phosphorylation completely impaired ceramide inhibition of PKBγ/Akt3 [46]. Altogether, these results demonstrated the importance of PKCζ in mediating ceramide-induced insulin resistance (Fig. 3B).

Recently, we and others have shown that ceramides induced the recruitment of both PKCζ and PKB/Akt in specialized domains of the plasma membrane called caveolin-enriched microdomains (CEM) [46,47]. These microdomains also called lipid rafts are believed to form platforms for conducting a variety of cellular functions [48]. Most of cellular ceramides are concentrated in these domains, typically enriched with cholesterol, sphingolipides and caveolins [46]. Caveolins are the major coat constituent of a class of sub-microdomains present in these CEM, called caveolae. In addition to their structural role, caveolins can segregate signal initiation events by concentrating and organizing signalling molecules through their specific caveolin scaffolding domain [47]. Interestingly, caveolins were shown to interact with PKCζ via their scaffolding domain, explaining why this kinase is well represented in caveolae [49]. Because these caveolae are particularly abundant in adipocytes, endothelial cells and muscle cells [50], we hypothesised that ceramides could repress insulin signalling through segregation of both PKB/PKCζ in these sub-microdomains. We found in adipocytes as well as in L6 muscle cells that elevated contents of ceramides induce the recruitment of PKB/PKCζ complex in CEM where PKB/Akt is held in a repressed state, and unable to support the hormonal activation of glucose transport [47]. However, in cells with low caveolin expression, in other words basically devoid of these domains, the PKCζ pathway is inactive and ceramides inhibit PKB/Akt mainly via PP2A [41]. Therefore the compartmentalization of the plasma membrane into sub-microdomains is a crucial determinant for the pathway used by ceramides to inhibit PKB/Akt [41].

These data highlight that redundant pathways exist within cells to mediate negative actions of ceramides on insulin sensitivity. This clearly indicates that targeting a single pathway (either PKCζ or PP2A) would not be efficient strategies to fight ceramide-induced insulin resistance. Instead, finding a way to prevent ceramide accumulation in insulin-sensitive tissues would be a better approach as possible therapeutic intervention.

5. Conclusion and future direction

Recent studies have highlighted PKCs as a family of multifunctional enzymes that play crucial roles in the transduction of many cellular signals by phosphorylating various targets. In the specific context of glucose metabolism, some are operating either as mediators (PKCζλ, β2, γ) or inhibitors (PKCζλ, α, δ, θ, ε, β1) of the insulin signal, depending on their activating stimuli. Regarding the concerning picture of worldwide increasing incidence of insulin resistant obesity, highlighting PKC isoforms as regulators of lipid-induced insulin resistance have been a real breakthrough into the understanding in the mechanisms leading to this disease. Indeed, it has now become clear that PKCθ in muscle, and PKCε in liver relay DAG-induced insulin resistance in these tissues. Similarly, implication of PKCζλ in mediating the deleterious effects of ceramides in muscle cells and adipocytes has now been well established.

However, most of the studies have been carried out in these tissues, and future research should be extended to other tissues such as pancreas and heart, also critical organs targeted during diabetes and cardiovascular diseases. Therefore, understanding how DAG- and ceramide-activated PKCs impair insulin signalling in ectopic tissues will help to design new drugs to treat insulin resistance and other obesity-linked disorders.

References

[1] Sesti, G. (2006) Pathophysiology of insulin resistance. Best. Pract. Res. Clin. Endocrinol. Metab. 20, 665–679.
[2] Boden, G. (2003) Effects of free fatty acids (FFA) on glucose metabolism: significance for insulin resistance and type 2 diabetes. Exp. Clin. Endocrinol. Diabetes 111, 121–124.
[3] Listenberger, L.L., Han, X., Lewis, S.E., Cases, S., Farese Jr., R.V., Ory, D.S. and Schaffer, J.E. (2003) Triglyceride accumulation protects against fatty acid-induced lipotoxicity. Proc. Natl. Acad. Sci. USA 100, 3077–3082.
[4] Schmitz-Peiffer, C. (2000) Signalling aspects of insulin resistance in skeletal muscle: mechanisms induced by lipid oversupply. Cell. Signal. 12, 583–594.
[5] Holland, W.L. and Summers, S.A. (2008) Sphingolipids, insulin resistance, and metabolic disease: new insights from in vivo manipulation of sphingolipid metabolism. Endocr. Rev. 29, 381–402.
[6] Steinberg, S.F. (2008) Structural basis of protein kinase C isoform function. Physiol. Rev. 88, 1341–1378.
[7] Litherland, G.J., Hajduch, E. and Hundal, H.S. (2001) Intracellular signalling mechanisms regulating glucose transport in insulin-sensitive tissues (review). Mol. Membr. Biol. 18, 195–204.
[8] Pearce, L.R., Komander, D. and Alessi, D.R. (2010) The nuts and bolts of AGC protein kinases. Nat. Rev. Mol. Cell Biol. 11, 9–22.
[9] Hajduch, E., Litherland, G.J. and Hundal, H.S. (2001) Protein kinase B (PKB/Akt) – a key regulator of glucose transport? FEBS Lett. 492, 199–203.
[10] Hajduch, E., Alessi, D.R., Hemmings, B.A. and Hundal, H.S. (1998) Constitutive activation of protein kinase B alpha by membrane targeting promotes glucose and system A amino acid transport, protein synthesis, and inactivation of glycogen synthase kinase 3 in L6 muscle cells. Diabetes 47, 1006–1013.
[11] Sano, H., Kane, S., Sano, E., Miinea, C.P., Asara, J.M., Lane, W.S., Garner, C.W. and Lienhard, G.E. (2003) Insulin-stimulated phosphorylation of a Rab GTPase-activating protein regulates GLUT4 translocation. J. Biol. Chem. 278, 14599–14602.
[12] Farese, R.V. (2002) Function and dysfunction of aPKC isoforms for glucose transport in insulin-sensitive and insulin-resistant states. Am. J. Physiol. Endocrinol. Metab 283, E1–E11.
[13] Liu, L.Z., Cheung, S.C., Lan, L.L., Ho, S.K., Chan, J.C. and Tong, P.C. (2010) The pivotal role of protein kinase C zeta (PKCzeta) in insulin- and AMP-activated protein kinase (AMPK)-mediated glucose uptake in muscle cells. Cell. Signal. 22, 1513–1522.
[14] Stretton, C., Evans, A. and Hundal, H.S. (2010) Cellular depletion of atypical PKC{lambda} is associated with enhanced insulin sensitivity and glucose uptake in L6 rat skeletal muscle cells. Am. J. Physiol. Endocrinol. Metab. 299, E402–E412.
[15] Farese, R.V., Sajan, M.P., Yang, H., Li, P., Mastorides, S., Gower Jr., W.R., Nimal, S., Choi, C.S., Kim, S., Shulman, G.I., Kahn, C.R., Braun, U. and Leitges, M. (2007) Muscle-specific knockout of PKC-lambda impairs glucose transport and induces metabolic and diabetic syndromes. J. Clin. Invest. 117, 2289–2301.
[16] Chappell, D.S., Patel, N.A., Jiang, K., Li, P., Watson, J.E., Byers, D.M. and Cooper, D.R. (2009) Functional involvement of protein kinase C-betaII and its

substrate, myristoylated alanine-rich C-kinase substrate (MARCKS), in insulin-stimulated glucose transport in L6 rat skeletal muscle cells. Diabetologia 52, 901–911.
[17] Pan, D.A., Lillioja, S., Kriketos, A.D., Milner, M.R., Baur, L.A., Bogardus, C., Jenkins, A.B. and Storlien, L.H. (1997) Skeletal muscle triglyceride levels are inversely related to insulin action. Diabetes 46, 983–988.
[18] Boden, G., Lebed, B., Schatz, M., Homko, C. and Lemieux, S. (2001) Effects of acute changes of plasma free fatty acids on intramyocellular fat content and insulin resistance in healthy subjects. Diabetes 50, 1612–1617.
[19] Turinsky, J., O'Sullivan, D.M. and Bayly, B.P. (1990) 1,2-Diacylglycerol and ceramide levels in insulin-resistant tissues of the rat in vivo. J. Biol. Chem. 265, 16880–16885.
[20] Turinsky, J., Bayly, B.P. and O'Sullivan, D.M. (1990) 1,2-Diacylglycerol and ceramide levels in rat skeletal muscle and liver in vivo. Studies with insulin, exercise, muscle denervation, and vasopressin. J. Biol. Chem. 265, 7933–7938.
[21] Haus, J.M., Kashyap, S.R., Kasumov, T., Zhang, R., Kelly, K.R., DeFronzo, R.A. and Kirwan, J.P. (2009) Plasma ceramides are elevated in obese subjects with type 2 diabetes and correlate with the severity of insulin resistance. Diabetes 58, 337–343.
[22] Yu, C., Chen, Y., Cline, G.W., Zhang, D., Zong, H., Wang, Y., Bergeron, R., Kim, J.K., Cushman, S.W., Cooney, G.J., Atcheson, B., White, M.F., Kraegen, E.W. and Shulman, G.I. (2002) Mechanism by which fatty acids inhibit insulin activation of insulin receptor substrate-1 (IRS-1)-associated phosphatidylinositol 3-kinase activity in muscle. J. Biol. Chem. 277, 50230–50236.
[23] Itani, S.I., Ruderman, N.B., Schmieder, F. and Boden, G. (2002) Lipid-induced insulin resistance in human muscle is associated with changes in diacylglycerol, protein kinase C, and IkappaB-alpha. Diabetes 51, 2005–2011.
[24] Chibalin, A.V., Leng, Y., Vieira, E., Krook, A., Bjornholm, M., Long, Y.C., Kotova, O., Zhong, Z., Sakane, F., Steiler, T., Nylen, C., Wang, J., Laakso, M., Topham, M.K., Gilbert, M., Wallberg-Henriksson, H. and Zierath, J.R. (2008) Downregulation of diacylglycerol kinase delta contributes to hyperglycemia-induced insulin resistance. Cell 132, 375–386.
[25] Li, Y.L., Su, X., Stahl, P.D. and Gross, M.L. (2007) Quantification of diacylglycerol molecular species in biological samples by electrospray ionization mass spectrometry after one-step derivatization. Anal. Chem. 79, 1569–1574.
[26] Shin, O.H., da Costa, K.A., Mar, M.H. and Zeisel, S.H. (1997) Hepatic protein kinase C is not activated despite high intracellular 1,2-sn-diacylglycerol in obese Zucker rats. Biochim. Biophys. Acta 1358, 72–78.
[27] Samuel, V.T., Petersen, K.F. and Shulman, G.I. (2010) Lipid-induced insulin resistance: unravelling the mechanism. Lancet 375, 2267–2277.
[28] Idris, I., Gray, S. and Donnelly, R. (2001) Protein kinase C activation: isozyme-specific effects on metabolism and cardiovascular complications in diabetes. Diabetologia 44, 659–673.
[29] Kim, J.K., Fillmore, J.J., Sunshine, M.J., Albrecht, B., Higashimori, T., Kim, D.W., Liu, Z.X., Soos, T.J., Cline, G.W., O'Brien, W.R., Littman, D.R. and Shulman, G.I. (2004) PKC-theta knockout mice are protected from fat-induced insulin resistance. J. Clin. Invest. 114, 823–827.
[30] Considine, R.V., Nyce, M.R., Allen, L.E., Morales, L.M., Triester, S., Serrano, J., Colberg, J., Lanza-Jacoby, S. and Caro, J.F. (1995) Protein kinase C is increased in the liver of humans and rats with non-insulin-dependent diabetes mellitus: an alteration not due to hyperglycemia. J. Clin. Invest. 95, 2938–2944.
[31] Samuel, V.T., Liu, Z.X., Qu, X., Elder, B.D., Bilz, S., Befroy, D., Romanelli, A.J. and Shulman, G.I. (2004) Mechanism of hepatic insulin resistance in non-alcoholic fatty liver disease. J. Biol. Chem. 279, 32345–32353.
[32] Werner, E.D., Lee, J., Hansen, L., Yuan, M. and Shoelson, S.E. (2004) Insulin resistance due to phosphorylation of insulin receptor substrate-1 at serine 302. J. Biol. Chem. 279, 35298–35305.
[33] Li, Y., Soos, T.J., Li, X., Wu, J., Degennaro, M., Sun, X., Littman, D.R., Birnbaum, M.J. and Polakiewicz, R.D. (2004) Protein kinase C theta inhibits insulin signaling by phosphorylating IRS1 at Ser(1101). J. Biol. Chem. 279, 45304–45307.
[34] Mack, E., Ziv, E., Reuveni, H., Kalman, R., Niv, M.Y., Jorns, A., Lenzen, S. and Shafrir, E. (2008) Prevention of insulin resistance and beta-cell loss by abrogating PKCepsilon-induced serine phosphorylation of muscle IRS-1 in *Psammomys obesus*. Diabetes Metab. Res. Rev. 24, 577–584.
[35] Ikeda, Y., Olsen, G.S., Ziv, E., Hansen, L.L., Busch, A.K., Hansen, B.F., Shafrir, E. and Mosthaf-Seedorf, L. (2001) Cellular mechanism of nutritionally induced insulin resistance in *Psammomys obesus*: overexpression of protein kinase Cepsilon in skeletal muscle precedes the onset of hyperinsulinemia and hyperglycemia. Diabetes 50, 584–592.
[36] Morino, K., Neschen, S., Bilz, S., Sono, S., Tsirigotis, D., Reznick, R.M., Moore, I., Nagai, Y., Samuel, V., Sebastian, D., White, M., Philbrick, W. and Shulman, G.I. (2008) Muscle-specific IRS-1 Ser->Ala transgenic mice are protected from fat-induced insulin resistance in skeletal muscle. Diabetes 57, 2644–2651.
[37] Copps, K.D., Hancer, N.J., Opare-Ado, L., Qiu, W., Walsh, C. and White, M.F. (2010) Irs1 serine 307 promotes insulin sensitivity in mice. Cell Metab. 11, 84–92.
[38] Summers, S.A. (2010) Sphingolipids and insulin resistance: the five Ws. Curr. Opin. Lipidol. 21, 128–135.
[39] Powell, D.J., Turban, S., Gray, A., Hajduch, E. and Hundal, H.S. (2004) Intracellular ceramide synthesis and protein kinase Czeta activation play an essential role in palmitate-induced insulin resistance in rat L6 skeletal muscle cells. Biochem. J. 382, 619–629.
[40] Straczkowski, M., Kowalska, I., Nikolajuk, A., Dzienis-Straczkowska, S., Kinalska, I., Baranowski, M., Zendzian-Piotrowska, M., Brzezinska, Z. and Gorski, J. (2004) Relationship between insulin sensitivity and sphingomyelin signaling pathway in human skeletal muscle. Diabetes 53, 1215–1221.
[41] Blouin, C.M., Prado, C., Takane, K.K., Lasnier, F., Garcia-Ocana, A., Ferre, P., Dugail, I. and Hajduch, E. (2010) Plasma membrane subdomain compartmentalization contributes to distinct mechanisms of ceramide action on insulin signaling. Diabetes 59, 600–610.
[42] Summers, S.A., Garza, L.A., Zhou, H. and Birnbaum, M.J. (1998) Regulation of insulin-stimulated glucose transporter GLUT4 translocation and Akt kinase activity by ceramide. Mol. Cell. Biol. 18, 5457–5464.
[43] Hajduch, E., Balendran, A., Batty, I.H., Litherland, G.J., Blair, A.S., Downes, C.P. and Hundal, H.S. (2001) Ceramide impairs the insulin-dependent membrane recruitment of protein kinase B leading to a loss in downstream signalling in L6 skeletal muscle cells. Diabetologia 44, 173–183.
[44] Powell, D.J., Hajduch, E., Kular, G. and Hundal, H.S. (2003) Ceramide disables 3-phosphoinositide binding to the Pleckstrin homology domain of protein kinase B (PKB)/Akt by a PKCzeta-dependent mechanism. Mol. Cell. Biol. 23, 7794–7808.
[45] Wang, G., Krishnamurthy, K., Umapathy, N.S., Verin, A.D. and Bieberich, E. (2009) The carboxyl-terminal domain of atypical protein kinase Czeta binds to ceramide and regulates junction formation in epithelial cells. J. Biol. Chem. 284, 14469–14475.
[46] Fox, T.E., Houck, K.L., O'Neill, S.M., Nagarajan, M., Stover, T.C., Pomianowski, P.T., Unal, O., Yun, J.K., Naides, S.J. and Kester, M. (2007) Ceramide recruits and activates protein kinase C zeta (PKC zeta) within structured membrane microdomains. J. Biol. Chem. 282, 12450–12457.
[47] Hajduch, E., Turban, S., Le, L.X., Le Lay, S., Lipina, C., Dimopoulos, N., Dugail, I. and Hundal, H.S. (2008) Targeting of PKCzeta and PKB to caveolin-enriched microdomains represents a crucial step underpinning the disruption in PKB-directed signalling by ceramide. Biochem. J. 410, 369–379.
[48] Simons, K. and Ikonen, E. (1997) Functional rafts in cell membranes. Nature 387, 569–572.
[49] Oka, N., Yamamoto, M., Schwencke, C., Kawabe, J., Ebina, T., Ohno, S., Couet, J., Lisanti, M.P. and Ishikawa, Y. (1997) Caveolin interaction with protein kinase C. Isoenzyme-dependent regulation of kinase activity by the caveolin scaffolding domain peptide. J. Biol. Chem. 272, 33416–33421.
[50] Blouin, C.M., Le Lay, S., Lasnier, F., Dugail, I. and Hajduch, E. (2008) Regulated association of caveolins to lipid droplets during differentiation of 3T3-L1 adipocytes. Biochem. Biophys. Res. Commun. 376, 331–335.