

Protein Kinase C β (PKC β): Normal Functions and Diseases

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PKC β I and PKC β II are DAG- and Ca²⁺-dependent conventional or classical isoforms of protein kinase C. Generated by alternative splicing from a single gene, they differ at their C-terminal 50 (β I) or 52 (β II) residues. They are expressed as major PKC isoforms in a variety of tissues, and thus the functions ascribed to “PKC” based on early studies using phorbol esters and PKC inhibitors could be attributed to them. As tools to probe into isoform-specific functions have recently become available, our understanding of the normal functions of these isoforms has dramatically increased. This minireview will focus mainly on two areas of signal transduction where the roles of PKC β I and PKC β II are relatively well-characterized: immunoreceptor and insulin receptor systems. Their involvement in disorders due to perturbations in these signaling systems, i.e., immunodeficiencies and diabetes, is also reviewed. Finally, patterns of PKC action in these and other biologic systems are discussed.

Key words: BCR, diabetes, Fc ϵ RI, insulin, PKC β .

A single gene locus (*PKC β*) encodes two proteins, PKC β I and PKC β II, which are generated by alternative splicing of the C-terminal exons (1). Thus, the difference between these two isoforms resides in the C-terminal V5 domains, which still exhibit a moderate homology (45%) at their amino acid sequences (Fig. 1). Both PKC β I and PKC β II are classified as conventional or classical isoforms whose optimal activity requires diacylglycerol (DAG) and Ca²⁺. They are expressed as major PKC isoforms in a variety of tissues and therefore the functions associated with “PKC” based on early experiments using PKC-activating phorbol esters and general PKC inhibitors could be attributed to them. Thus, PKC β I and β II might function in various signal transducing pathways for proliferation, differentiation, metabolism, and more cell-type-specific functions. Since Nishizuka wrote his landmark review a decade ago after the cloning of most of the currently known PKC isoforms (2), the PKC community has accumulated data on specific functions of PKC β I and β II, using isoform-specific cDNAs (wild-type [wt]), constitutively active, and dominant negative (DN), antisense oligonucleotides, PKC β -specific inhibitors, transgenic mice, and gene knockout mice. This review will focus on recent developments in two areas that illustrate the specific roles of these PKC isoforms and the principles in their signaling networks with regard to other molecules. Readers are referred to other reviews in this series as well as those

by Newton and Mochly-Rosen for activation mechanisms and adaptor or scaffold proteins (3–5).

Immunoreceptor signaling and immunodeficiency

Stimulation of antigen or Fc receptors on immune cells such as the B cell receptor (BCR) on B lymphocytes and the high-affinity IgE receptor (Fc ϵ RI) on mast cells triggers a concerted activation of Src, Syk, and Tec family protein-tyrosine kinases (PTKs). These receptor-proximal PTKs in turn activate several signaling pathways including phosphatidylinositol 3-kinase (PI3K), phospholipase C (PLC)- γ , and Ca²⁺. Products of PLC activity, i.e., DAG and inositol 1,4,5-trisphosphate (IP3), together with consequent increases in Ca²⁺, can activate conventional and novel isoforms of PKC. As can be seen from the fact that phorbol ester plus Ca²⁺ ionophore can circumvent the requirement for receptor stimulation to activate the immune cells, PKC and Ca²⁺ play critical roles in immune cell activation. Indeed, studies on *PKC β* gene knockout mice showed that PKC β is critically important in B cell development and activation (6). These mice had fewer splenic B cells than normal, drastically reduced B-1 lymphocytes and low levels of serum IgM and IgG3, and mounted defective immune responses to thymus-independent type II antigen and reduced primary responses to T cell-dependent antigen. B cell proliferative responses to BCR and lipopolysaccharide stimulation were also reduced. In contrast, T cell development and proliferation in response to CD3 stimulation were normal in *PKC β* $-/-$ mice, although PKC β was expressed in T cells. The B cell phenotype of these mice was similar to those of *btk* knockout mice and X-linked immunodeficient (*xid*) mice with a missense (R28C) mutation in Btk (7), suggesting a signaling link between PKC β and Btk. Indeed, Btk physically interacts with various isoforms of PKC *in vitro* through the interaction between the PH domain of Btk and the C1 domain of PKC (8, 9) and co-immunoprecipitates with PKC β I from mast cell lysates

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Abbreviations: BCR, B cell receptor; DAG, diacylglycerol; DN, dominant negative; EGF, epidermal growth factor; Egr, Early growth response; Fc ϵ RI, high-affinity IgE receptor; IKK, I κ B kinase; IP3, inositol 1,4,5-trisphosphate; IRS, insulin receptor substrate; LSP1, leukocyte-specific protein-1; PI3K, phosphatidylinositol 3-kinase; PMA, phorbol 12-myristate 13-acetate; PLC, phospholipase C; PTK, protein-tyrosine kinase; Rb, retinoblastoma; VEGF, vascular endothelial growth factor; wt, wild-type; *xid*, X-linked immunodeficient.

(10). PKC β (either β I or β II) can phosphorylate Ser180 in the Tec linker region of Btk and inhibits the membrane translocation and tyrosine phosphorylation at Tyr551 and Tyr223 of Btk (11). Consistent with these observations, Btk phosphorylation was increased in BCR-stimulated PKC β $-/-$ cells (6). On the other hand, Btk in concert with Syk phosphorylates and regulates the activity of PLC- γ 2 (12), and, apparently through this mechanism, Btk positively regulates the membrane translocation and activity of PKC β I, not PKC β II or PKC α , in Fc ϵ RI-stimulated mast cells (10). This Btk/PKC β I pathway is involved in Fc ϵ RI-induced production of IL-2 and TNF- α . PKC β I was also shown to be required for IL-2 secretion from PMA-stimulated T cells (13). Fc ϵ RI stimulation induced a reduced production of IL-6 mRNA and protein in PKC β $-/-$ mast cells (14), which is consistent with an increased accumulation of IL-6 mRNA in PKC β -overexpressing mast cells (15). Since PKC β (and PKC ϵ) can be involved in Fc ϵ RI-induced expression of *c-fos* and *c-jun* mRNAs (16), reduced induction of JunD mRNA may contribute to the reduced IL-6 production in PKC β $-/-$ mast cells. Collectively, not only does Btk

positively regulate PKC β I, but PKC β can negatively regulate Btk activity as a feedback loop inhibitor (Fig. 2). This self-regulatory pathway may contribute to fine-tuning of signal intensity and timing. The importance of this kind of fine-tuning was illustrated in B cell immunodeficiencies because loss-of-function as well as gain-of-function mutations of Btk induce similar immunodeficiencies (7). The phenotypic similarity of PKC β $-/-$ and *btk* $-/-$ (or *xid*) mice may be understood along this line.

An important outcome of immunoreceptor activation is the activation of NF- κ B, which is involved in immune reactions, inflammation, and cell survival. NF- κ B is a heterodimer of p50 and p65 that is sequestered in the cytosol by I κ B, which prevents its nuclear translocation and activity. Upon immune cell activation, I κ B α is phosphorylated by I κ B kinases (IKK α and IKK β) of the IKK complex, which triggers the ubiquitination and subsequent degradation of I κ B through the proteasome pathway. Phosphorylation of I κ B α downstream of BCR triggering is regulated by Btk (17, 18). Defective I κ B α phosphorylation and inefficient Bcl-X $_L$ induction in *btk* $-/-$ or *xid* B cells indicate an important role of Btk-mediated NF- κ B activation in BCR-dependent B cell survival. PKC β was also shown to control NF- κ B activity (19, 20). Similar to *btk* $-/-$ B cells, PKC β $-/-$ B cells are also characterized by poor survival in the absence of IL-4. Consistent with their poor proliferative response, BCR stimulation failed to promote the expression of the anti-apoptotic proteins Bcl-X $_L$ and Bcl-2 in PKC β $-/-$ B cells. In these cells, BCR stimulation also failed to induce the recruitment of the IKK complex to lipid rafts (19) and phosphorylation of IKK α at the critical Ser180 and sustained Ser181 phosphorylation of IKK β (20), which resulted in reduced phosphorylation and more persistent expression of I κ B α , leading to a reduced NF- κ B activity. However, the reduction of NF- κ B activity was only modest (20), which is potentially congruent with the study that indicates that novel PKC isoforms (particularly PKC θ and

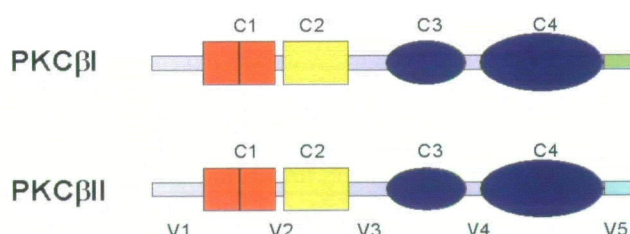


Fig. 1. **Domain structure of PKC β I and PKC β II.** The DAG-binding C1 domain, the acidic phospholipid/Ca²⁺-binding C2 domain and the catalytic domain (C3-V4-C4) are highlighted. The V5 region is different between PKC β I (50 residues) and PKC β II (52 residues), but key autophosphorylation sites (Thr641 and Ser660 in PKC β II) in this region are conserved.

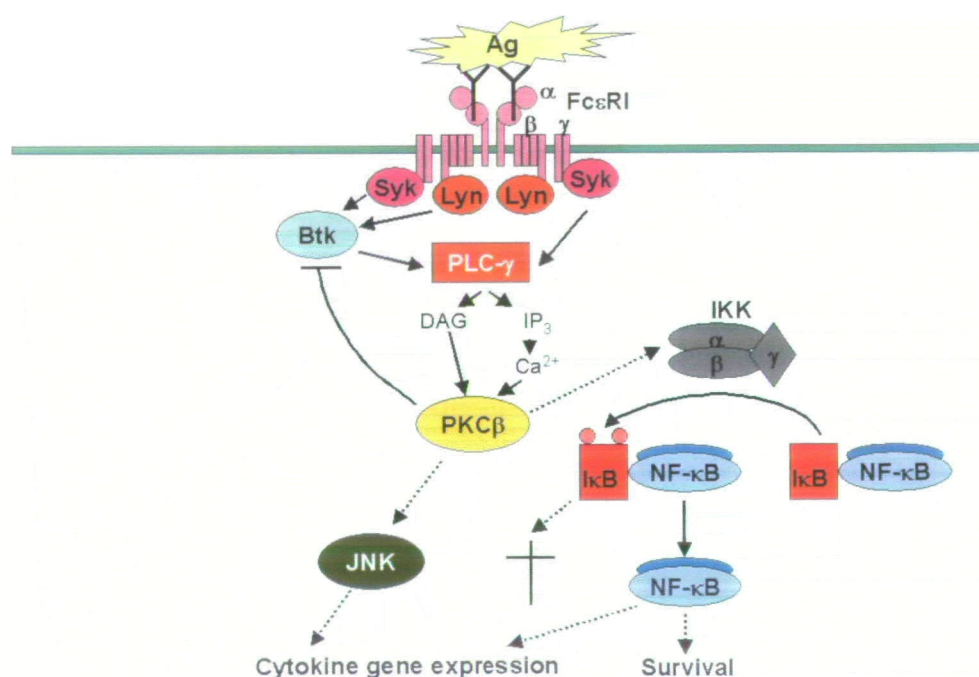


Fig. 2. **PKC β in immunoreceptor signaling.** Btk phosphorylates and activates PLC- γ in concert with Syk upon immunoreceptor stimulation. Second messengers generated by PLC, *i.e.*, DAG and IP₃ (subsequent Ca²⁺ increase), activate various PKC isoforms including the β I isoform. Activated PKC β I can phosphorylate Ser180 of Btk and inhibit the activity of the latter enzyme. PKC β can also regulate transcription of several cytokine and survival genes such as *bcl-X_L* through JNK and IKK activities. The simplified signaling pathways of the Fc ϵ RI system are depicted. BCR signaling also uses similar signaling networks.

PKC δ), but not PKC β II, seem to play a critical role in BCR-mediated NF- κ B and JNK activation (21). Importantly, PKC β inhibitors blocked survival of cell lines derived from non-Hodgkin's diffuse large B cell lymphomas (19), suggesting a clinical potential of PKC β inhibitors in treating these B cell tumors. The importance of PKC β I in B cell survival was also shown in a subline of WEHI-231 cells that express C-terminal residues 179–330 of leukocyte-specific protein 1 (LSP1) (22). This LSP1 truncate, termed B-LSP1, inhibited anti-IgM-induced membrane translocation of PKC β I, but not PKC β II or PKC α , and ERK2 activation, and increased anti-IgM-induced apoptosis. Inhibition of ERK2 activation contributes to the increased apoptosis. Although B-LSP1 can directly interact with PKC β I, but not PKC β II or PKC α , it is not clear whether the endogenous LSP1 protein plays the same role as a PKC β I-sequestering protein in BCR-mediated apoptosis. However, the role of PKC β I in survival could be cell-type-specific; no adverse effects of the lack of PKC β were observed on mast cell proliferation and survival (14).

Degranulation is a cardinal feature of Fc ϵ RI-induced mast cell activation that requires both PKC and Ca²⁺ for maximal activity. Early studies implicated PKC β and PKC δ in this function based on the reconstitution of degranulatory activity in permeabilized and PKC-depleted mast cells by incubation with individual recombinant PKC isoforms (23). Indeed, PKC β $-/-$ mast cells exhibited a lower degranulatory activity than wt cells in response to Fc ϵ RI or Ca²⁺ ionophore stimulation (14). However, PKC δ $-/-$ mast cells showed a higher degranulatory activity, particularly when IgE-primed cells were stimulated with high concentrations of antigen (24). PKC ϵ $-/-$ and PKC θ $-/-$ mast cells degranulated indistinguishably from wild-type cells (our unpublished data).

Insulin receptor signaling and diabetes

Like other receptor PTKs such as those for epidermal growth factor (EGF) and platelet-derived growth factor, stimulation of insulin receptor also activates PLC and pro-

duction of DAG, which in turn activates several PKC isoforms. PKCs may activate Ras and the Raf/MEK/ERK pathway, while DAG-responsive PKCs may activate Raf in a Ras-independent manner. In L6 skeletal muscle cells, insulin-induced activation of PKC α , PKC β , ERK1/2, and DNA synthesis was largely dependent on phosphorylation of insulin receptor substrate 1 (IRS-1), not IRS-2. Blocking PKC β (not PKC α) with either antisense oligonucleotide or the PKC β -specific inhibitor LY379196 decreased the insulin-induced ERK activity and DNA synthesis, without affecting EGF- or serum-stimulated mitogenesis. In contrast, the inhibition of Ras largely spared insulin-induced ERK activation and DNA synthesis, whereas it blocked EGF-induced ERK activation and mitogenesis. PKC β blockade did not affect Ras activity but inhibited insulin-induced Raf activation and coprecipitation of Raf with PKC β . Based on these observations, a signaling pathway was proposed for PKC β -mediated regulation of the Raf/MEK/ERK module and mitogenesis (Fig. 3) (25). Interestingly, PKC ζ plays a major role in insulin induction of ERK activity in rat adipocytes (26).

In addition to the positive role of PKC β in insulin receptor signaling, activation of PKC is associated with an inhibition of insulin receptor PTK activity in various cell types. Downregulation of insulin receptor kinase activity contributes to the pathogenesis of cellular insulin resistance in diabetes mellitus. When the human insulin receptor was coexpressed with PKC β I and β II isoforms and stimulated by insulin in the presence of phorbol ester in HEK293 cells, tyrosine autophosphorylation of the insulin receptor was inhibited, while coexpression with the other isoforms did not significantly modify receptor autophosphorylation, suggesting that PKC β I and β II isoforms might be candidates for insulin receptor inhibition (27). However, similar overexpression of PKC isoforms in Chinese hamster ovary cells did not affect insulin-stimulated tyrosine phosphorylation of the receptor or its kinase activity. PKC α , but not β I, γ , or ϵ isoforms, inhibited *in vivo* insulin receptor kinase activity (28). Exposure to high glucose in L6 cells induced the acti-

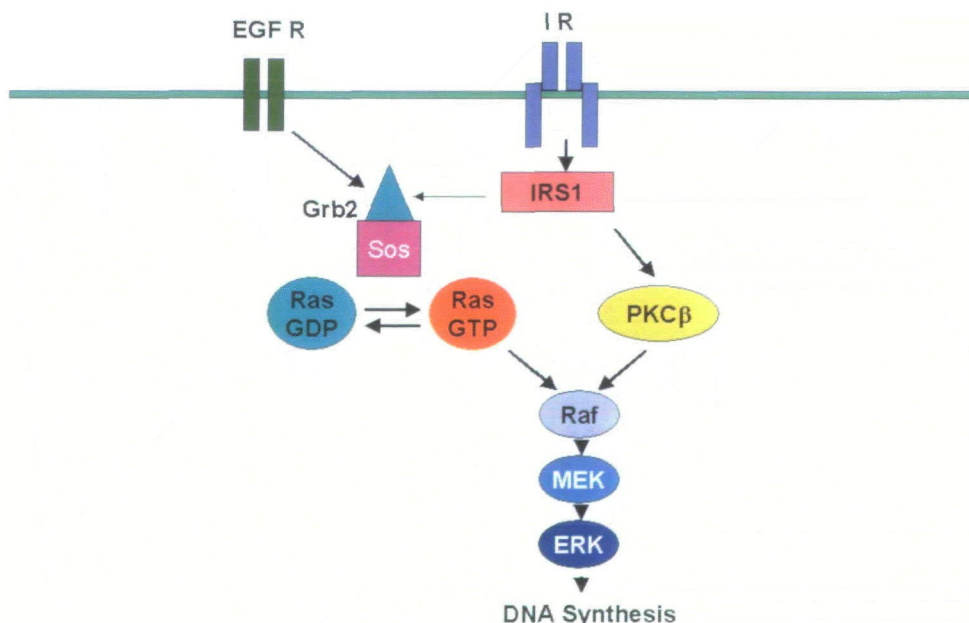


Fig. 3. PKC β in insulin signaling. Insulin-stimulated insulin receptor (IR) phosphorylates IRS1. IRS1 recruits various signaling molecules through SH2-phosphotyrosine interactions. IRS1-binding proteins containing an SH2 domain include Grb2, PI3K, SHP-2, and Nck. Insulin-induced ERK activation in L6 myocytes occurs largely through Raf-1 rather than Ras activation. In contrast, EGF-induced ERK activation is dependent on Grb2/Sos-mediated Ras activation.

vation of insulin receptor kinase activity as well as membrane translocation of glucose transporters, GLUT1 and GLUT4, and glucose uptake. These glucose effects were paralleled by a decrease in receptor-associated PKC activity, which was mostly accounted for by dissociation of PKC α , but not of PKC β or δ , from the receptor. Thus, glucose autoregulation appears to involve PKC α dissociation from the insulin receptor (29). Interesting is the finding that glucose transport is increased in some tissues in PKC β $-/-$ mice and that the increased glucose transport may be partly due to the loss of PKC β I, which negatively modulates insulin-stimulated GLUT4 translocation (30).

Early studies indicated that PKC activity is increased in the retina, aorta, heart, and renal glomeruli of diabetic animals, probably because of an increase in *de novo* synthesis of DAG induced by hyperglycemia. PKC β II was shown to be preferentially increased in membrane fractions in the aorta, heart, and glomeruli of diabetic rats (31). The involvement of PKC β II in the development of retinopathy and nephropathy in diabetes was strongly suggested by experiments using a specific PKC β inhibitor: LY333531 (IC₅₀ of 4.7 and 5.9 nM for β I and β II, respectively) inhibited PKC activity in the retina and glomeruli, and ameliorated the glomerular filtration rate, albumin excretion rate, and retinal circulation in streptozotocin-induced diabetic rats in an oral dose-responsive manner (32). On the other hand, overexpression of PKC β II in the myocardium in transgenic mice induced left ventricular hypertrophy, cardiac myocyte necrosis, multifocal fibrosis and decreased left ventricular performance. The severity of the phenotype was gene dosage-dependent, and this cardiovascular disease was largely prevented or reversed by LY333531 treatment (33).

Hyperglycemia is associated with the reduced expression of many islet β -cell-associated genes including the insulin gene. However, *c-myc* expression is induced in diabetic states. Among PKC isoforms (α , β II, δ , ϵ , and ζ) expressed in rat pancreatic islets, wt and DN mutant of PKC β II, but not other isoforms, influenced *c-myc* expression: wt PKC β II increased *c-myc* expression, and *c-myc* induction by high glucose was suppressed by DN PKC β II (34). Further, overexpression of wt PKC β II led to suppression of insulin gene transcription.

Neovascularization is involved in various diseases such as proliferative diabetic retinopathy, tumor growth, and rheumatoid arthritis. Vascular endothelial growth factor (VEGF) plays a central role in the development of neovascularization. Angiogenic response to oxygen-induced retinal ischemia was dramatically increased in transgenic mice overexpressing PKC β II and significantly reduced in PKC β $-/-$ mice (35). The mitogenic action and ERK1/2 activation by VEGF, a potent hypoxia-induced angiogenic factor, was increased in retinal endothelial cells by the overexpression of wt PKC β I or β II isoforms and inhibited by the expression of DN PKC β II. PKC β II was also shown to be physically associated with retinoblastoma (Rb) protein and to phosphorylate the latter at specific serine residues. These observations suggest that Rb phosphorylation by PKC β could lead to an increased transcriptional activity of E2F and eventually to increased VEGF-induced endothelial cell proliferation (35). PKC β activation is also involved in fibrin deposition in hypoxemic vasculature by inducing tissue factor. Hypoxia induces membrane translocation and auto-

phosphorylation of PKC β II, but not α or ϵ isoforms, in U937 monocytic cells (36). Tissue factor expression in an oxygen-deficient environment is driven by the transcription factor Early growth response (Egr)-1. PKC β $-/-$ mice exhibited markedly blunted tissue factor and vascular fibrin deposition responses when exposed to hypoxia. Consistent with the role of Egr-1 in these responses, the mutant mice displayed only a minor elevation of Egr-1 mRNA, protein and activity. PKC β mediates the activation of ERK1/2, which in turn activates the transcription factor Elk-1. Elk-1, in complex with serum response factor, is the likely proximal trigger of Egr-1 transcription (36).

Concluding remarks

PKC β I and β II play crucial roles in numerous cellular functions. Unfortunately, the mechanisms for these functions are still not completely understood. In particular, identification of direct phosphorylation targets and roles of associated proteins are lacking in most cases. However, some features in PKC β action have emerged. First, a single PKC isoform exerts apparently positive as well as negative effects along the same signaling pathway (e.g., PKC β I in BCR and Fc ϵ RI signaling). Second, different isoforms play the same role depending on cell types (e.g., PKC β and novel PKCs in BCR-mediated NF- κ B activation and atypical PKCs in TNF- α -mediated NF- κ B activation, PKC β in myocytes and PKC ζ in adipocytes for insulin induction of ERK activation). Third, different isoforms sometimes play opposing roles in a cellular function [e.g., positive and negative roles of PKC β and PKC δ , respectively, in Fc ϵ RI-induced degranulation, and cardioprotective action of PKC ϵ and cardioprotective action of PKC δ (37)]. These features of PKC action seem to contribute to fine-tuning of signal transduction. However, they could be an obstacle to overcome when inhibitors and activators of PKC isoforms are used as therapeutics. Careful study at preclinical and clinical stages will be able to monitor potential unwanted side-effects. Importantly, several hopeful reports of PKC β inhibitors in preclinical and clinical settings (32, 38) are encouraging the PKC research community and pharmaceutical industry alike.

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