Angiotensin II and cell-matrix adhesion: PKC\(\varepsilon\) is essential

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Angiotensin II (Ang II) is an essential regulator of homeostasis and response to pathological stimuli in the cardiovascular system. In addition to its short- and long-term hemodynamic effects, Ang II plays an important role in cardiovascular remodeling in hypertension, myocardial infarction and chronic heart failure. In recent years, great efforts have been made to identify specific intracellular signaling pathways that are activated in cardiac myocytes and fibroblasts during the process of ventricular remodeling. Despite significant progress, the precise role of individual pathways and their relevance in cardiac myocytes and fibroblasts are only partly understood.

In this issue of *Cardiovascular Research*, Stawowy et al. [1] describe a novel signaling pathway for Ang II-mediated adhesion of cardiac fibroblasts to extracellular matrix. This study convincingly demonstrates that a specific protein kinase C subtype, PKC\(\varepsilon\), is essential for inside-out signaling of \(\beta_1\)-integrin-activated adhesion of cardiac fibroblasts to the extracellular matrix.

Protein kinase C may be regulated by a number of different stimuli. All G protein-coupled receptors that activate \(G_{q/11}\) proteins, e.g., \(\text{AT}_1\) receptors via phospholipase C\(\beta\), which releases diacylglycerol and inositol triphosphate, initiate PKC activation (Fig. 1). Other stimuli that are associated with PKC activation, e.g., ischemia/reperfusion or pressure overload, are less well characterized. According to their physiological behavior, at least 11 PKC isoforms can be distinguished: classical, Ca\(^{2+}\)-dependent kinases (PKC\(\alpha\), \(\beta\), \(\gamma\)), novel, Ca\(^{2+}\)-independent PKCs (PKC\(_{1\alpha}\), \(\theta\), \(\delta\), \(\epsilon\)) and atypical, diacylglycerol-independent enzymes (PKC\(\eta\), \(\mu\), \(\zeta\)) [2].

In the heart, PKC contributes to the induction of the hypertrophic phenotype, and co-activation of Ca\(^{2+}\)-dependent PKC isoforms by phenylephrine is required to shape the hypertrophic signal elicited by \(\alpha_1\)-adrenergic receptors in rat cardiomyocytes [3]. In addition, activation of PKC isoforms has been associated with the cardioprotective effect of early ischemic preconditioning [4]. Both classical and novel PKC subtypes have been implicated as regulators of cell adhesion.

Now, Stawowy et al. [1] provide experimental evidence that the novel PKC isoform PKC\(\varepsilon\) controls Ang II-mediated interaction of cardiac fibroblasts with extracellular matrix. In fibroblasts isolated from rat heart or mouse aorta, Ang II stimulated adhesion of cells to a collagen I matrix within 2 h. Cellular adhesion was abolished by broad-spectrum PKC inhibition and by pharmacological blockade of angiotensin \(\text{AT}_1\) receptors. Stimulation of fibroblasts with Ang II coincided with phosphorylation of PKC\(\varepsilon\). In contrast, phosphorylation of PKC\(\alpha\) or PKC\(\delta\)–two other isoforms expressed in fibroblasts–were not affected by Ang II treatment. In addition, Ang II increased phosphorylation of \(\beta_1\)-integrins at threonine 788, 789 residues.

Two pieces of evidence link the Ang II-induced phosphorylation of \(\beta_1\)-integrin and subsequent adhesion to collagen with the \(\varepsilon\) subtype of PKC: (1) activation of PKC\(\varepsilon\) with a subtype-selective agonist, \(\text{L-}\alpha\)-phosphatidylinositol-3,4,5-trisphosphate (\(\text{L-}\alpha\)-\(\text{PIP}_3\)), induced \(\beta_1\)-integrin phosphorylation and collagen adhesion; (2) “myofibroblasts” were isolated from aortas of wild-type and of gene-targeted mice lacking PKC\(\varepsilon\) (PKC\(\varepsilon\)/C0/C0). While cells derived from wild-type mice showed Ang II-mediated adhesion to collagen, this effect was completely abolished in PKC\(\varepsilon\)/−/− cells. Furthermore, some evidence is provided that PKC\(\varepsilon\) and \(\beta_1\)-integrins associate within cardiac fibroblasts. By
immunofluorescence staining, PKCe and β1-integrins could be detected together in focal contacts and associated with stress fibers, which were induced by Ang II. In immunoprecipitation experiments, PKCe and β1-integrin were co-precipitated after Ang II stimulation, suggesting that both molecules may associate with each other or occur in a signaling complex.

Future studies will reveal whether PKCe and β1-integrin directly interact in fibroblasts or whether additional signaling molecules are necessary. For PKC kinases, translocation to the plasma membrane provides a mechanism to regulate access to substrate and has been taken as the hallmark of activation [5]. In this context, PKCs are known to interact with adapter proteins that are important for their correct targeting. In glioma cells, the association of PKCe with the adapter protein RACK1 ("receptor for activated C-kinase 1") and with β1-integrins has been demonstrated [6]. RACKs bind only activated PKC molecules and thereby provide an optimal strategy to recruit activated PKC isoforms to their intracellular target substrates. Proteomics may help to identify further components of the PKCe, RACK1 and β1-integrin signaling complex. Indeed, other combinations of similar signaling partners may orchestrate specific cellular responses: signaling complexes containing PKCe, β1-integrins, Src and PKB/Akt associate in CWR-R1 cell cultures of prostate cancer cells [7].

One of the major questions that arises relates to the pathophysiological significance of these findings: Is the Ang II-activated signaling cascade engaging PKCe and β1-integrins in cardiac fibroblasts "good or bad" within the context of a remodeling process of the injured heart? There has been a long-standing discussion about the effects of Ang II in cardiac fibroblasts and its relevance for left ventricular remodeling and fibrosis [8–10]. Transgenic and gene-targeted mice may provide a path to settle this debate. Targeted disruption of PKCe in mice did not affect normal cardiac growth, but it abolished the protective effects of PKCe on cardiac infarct volume of ischemic preconditioning [11] or sphingosine-1-phosphate [12]. In gene-targeting models, left ventricular pressure overload caused similar degrees of hypertrophy in wild-type and PKCe-deficient mice [13]. Most interestingly, PKCe−/− hearts displayed enhanced fibrosis, increased collagen deposition and diastolic dysfunction after transverse aortic constriction as compared with wild-type hearts [13]. However, whether this phenotype is causally related to the disruption of the PKCe gene or whether compensatory upregulation of PKCα is the culprit is difficult to distinguish. With the identification of PKCe as a key regulator of Ang II-induced β1-integrin activation, cell-type specific deletion of the PKCe gene in cardiac myocytes will be important to determine the role of Ang II and cardiac fibroblasts in the ventricular remodeling process. Furthermore, compensatory upregulation of other protein kinase isoforms (or additional signaling molecules) may significantly contribute to the phenotype observed in vivo—here, inducible gene-targeting or RNAi may help to identify cause and effect. Until then, angiotensin II-activated PKCe remains a central player in orchestrating cardiac fibroblast adhesion to extracellular matrix via β1-integrins.

References