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Diacylglycerol Kinase Inhibition and Vascular Function

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Abstract

Diacylglycerol kinases (DGKs), a family of lipid kinases, convert diacylglycerol (DG) to phosphatidic acid (PA). Acting as a second messenger, DG activates protein kinase C (PKC). PA, a signaling lipid, regulates diverse functions involved in physiological responses. Since DGK modulates two lipid second messengers, DG and PA, regulation of DGK could induce related cellular responses. Currently, there are 10 mammalian isoforms of DGK that are categorized into five groups based on their structural features. These diverse isoforms of DGK are considered to activate distinct cellular functions according to extracellular stimuli. Each DGK isoform is thought to play various roles inside the cell, depending on its subcellular localization (nuclear, ER, Golgi complex or cytoplasm). In vascular smooth muscle, vasoconstrictors such as angiotensin II, endothelin-1 and norepinephrine stimulate contraction by increasing inositol trisphosphate (IP₃), calcium, DG and PKC activity. Inhibition of DGK could increase DG availability and decrease PA levels, as well as alter intracellular responses, including calcium-mediated and PKC-mediated vascular contraction. The purpose of this review is to demonstrate a role of DGK in vascular function. Selective inhibition of DGK isoforms may represent a novel therapeutic approach in vascular dysfunction.

Keywords

Diacylglycerol kinase; diacylglycerol; phosphatidic acid; protein kinase C; vasoconstrictor; vascular function

Introduction

Intracellular signaling mechanisms play an important role in vascular function by modulating specific cellular processes (contractile proteins, ion transport, etc.) in response to the extracellular stimuli. These stimuli affect vascular reactivity and can participate in vascular diseases. For example, vasoconstrictors, such as angiotensin II (Ang II), endothelin-1 (ET-1), norepinephrine (NE) and serotonin bind to and activate their respective G protein-coupled receptors to stimulate phospholipase C (PLC) or phospholipase D (PLD). PLC hydrolyzes phosphatidylinositol bisphosphate (PIP₂) to release diacylglycerol (DG) and inositol trisphosphate (IP₃). IP₃ acts directly on the sarcoplasmic reticulum to release intracellular calcium (Ca²⁺) and Ca²⁺ and DG activate protein kinase C (PKC). PKC has also been shown to be activated by phosphatidic acid (PA) [1-3]. Since PKC phosphorylates target proteins essential to vascular smooth muscle contraction, PKC is a major signaling protein that contributes to the regulation of vascular function. PLD, which is also stimulated by G protein-coupled receptor agonists, metabolizes phosphatidylcholine into PA and choline. PA can be converted into DG by lipid phosphate phosphohydrolases (LPPs) [4] or into lyso-PA by phospholipase A (PLA).

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Diacylglycerol kinase (DGK) phosphorylates DG to produce PA. Since DG and PA function as lipid messengers to induce vascular cellular responses [5], DGK is thought to be a principal enzyme regulating vascular cell signaling events. Many studies have shown the action of DGK in different cellular responses and recent reviews have discussed the various functions of DGK in cell signaling [5-8]. In the present review, we will focus on the role of DGK in vascular function.

The Diacylglycerol Kinase Family: Structure and Regulation

There are currently 10 known mammalian isoforms of DGKs, which are classified into five groups based on their structural features. Five types of DGKs have a common catalytic domain and C1 domains (Zn²⁺-finger motifs, cysteine-rich). The catalytic domain comprises the entire C-terminal region and is regulated by phospholipids [9-12]. C1 domains have similar sequences for the DG binding domain of PKC [13,14]. Moreover, this domain is proposed to bind to phosphoinositide 3 kinase (PI3K) and Rho [15,16]. Type I DGKs (α , β and γ isoforms) contain EF-hand motifs where Ca²⁺ can bind [17], indicating that type I DGK can be activated by receptor-induced increases in intracellular Ca²⁺. Type II DGKs (δ , η and κ) include PH (pleckstrin homology) domain at the N-terminus and type III (ϵ), thus far, has been shown to contain no regulatory domain. Type IV DGKs, comprised of ζ and ι isoforms, contain ankyrin repeats and a PDZ (postsynaptic density protein-95/Disc large/zona occludens-1) domain at the C-terminus, myristoylated alanine-rich C-kinase substrate (MARCKS, which can be phosphorylated by PKC [18]) and a nuclear localization signal (NLS) [19]. Type V DGK, DGK θ , contains a PR (proline- and glycine-rich) domain, a PH domain and a Ras-association domain [20].

Although the basic function of DGKs is to metabolize DG, DGKs have been reported to associate with DG-mediated proteins, such as conventional and novel PKC. Some DGKs associate more with certain PKC isoforms, leading to an interactive modulation of both enzymes. For instance, DGK ζ inhibits PKC α activity through attenuating local accumulation of DG and this regulation is impaired by PKC α -mediated DGK ζ phosphorylation [21]. PKC γ and DGK γ are correlated functionally by their direct association and phosphorylation, contributing to subtype-specific activation of DGK γ [22]. DGK θ interacts selectively with and is phosphorylated by PKC ϵ and η and activation of PKC ϵ directly leads to DGK θ translocation to the plasma membrane [23]. Accordingly, DGK may play a pivotal role in vascular signaling due to its direct interaction with PKC.

DGKs Cellular Localization

When we consider basic DGK function and phosphorylation, translocation of DGKs to the plasma membrane is critical for the activation of the membrane-bound DG. Additionally, DGKs can be redistributed by extracellular stimuli. For example, DGK isoforms α and δ are translocated from the cytoplasm to the plasma membrane after glucose exposure. Induction of these DGK isoforms result in a reduction of PKC α activity and activation of insulin receptor signaling [24]. In addition to DG in the plasma membrane, several studies have shown that DG can be generated in various subcellular compartments, such as the nucleus, internal membrane, and cytoskeleton [25-27]. Thus, DGK association with DG is involved in various subcellular localizations.

Kobayashi *et al.* [28] investigated the subcellular localization of DGK isozymes using an epitope-tag expression system in cultured cells. This study revealed that DGK α is expressed in the nucleus and the cytoplasm, DGK β colocalizes with actin filaments, DGK γ with the Golgi complex, DGK ϵ with the ER and DGK ζ with the nucleus. Since DGK ι has a nuclear localization signal (NLS), which characterizes type IV DGK, it may localize in the nucleus

similar to DGK ζ . Translocated DGK α to the nucleus participates in nuclear phospholipid metabolism occurring at the intermediate stage of lymphocyte activation [29]. By binding to actin filaments, DGK β participates in the reorganization of actin stress fibers [28]. In the case of DGK γ , the C1 domain acts as a nuclear transport signal and nuclear DGK γ positively regulates the cell cycle and growth [30]. In neurons, DGK ζ is primarily a nuclear protein, but in some conditions, it can be found in the cytoplasm. Subcellular location depends not only on the cell type but also on the developmental state or growth conditions of the cell [31]. Some DGKs including α , γ , θ , and ζ are known to translocate from the cytosol to the nucleus [32-35] and recent reviews have summarized regulation and roles regarding nuclear DGKs [36,37]. Taken together, these observations suggest that each DGK isoform is known to be diversely located inside the cell and therefore may be responsible for the regulation of DG in the specific subcellular area.

DGKs in Vascular Function

Several physiological stimuli increase DG, which may be regulated by DGK. NE and ET-1 increase IP₃ and DG through stimulation of phosphatidylinositol signaling pathway, initiating vascular contraction, and activate DGKs in caveolae/rafts of rat mesenteric arteries [38]. Additionally, some studies have shown that DGK activity is regulated differentially by vasoconstrictor agents. NE increased membrane-associated DGK activity in rat small arteries, but Ang II did not activate membrane-associated DGK, although Ang II increased cellular DG [39]. NE, but not ET-1 or Ang II, activate and translocate DGK θ through the phosphatidylinositol 3-kinase (PI3K) signaling pathway, especially with protein kinase B, which has been demonstrated as a potential activator of DGK θ in intact small arteries [38,40]. Among DGK isoforms, DGK θ has a Ras-association domain and is known to interact with RhoA, a Ras protein family member. When activated RhoA is specifically bound to DGK θ , RhoA returns to an inactivated state [41]. In vascular signaling, RhoA/Rho-kinase activation contributes to vascular smooth muscle contraction *via* Ca²⁺ sensitization. Thus, the binding of DGK θ to RhoA may be an upstream regulator for vascular cellular responses. Moreover, studies have shown that DGKs interact with a family of small G proteins. RasGRP (Ras guanyl nucleotide-releasing protein) are a family of RasGEF (Ras guanine nucleotide exchange factor) and are attenuated by DGK α [42,43]. DGK ζ inhibits RasGRP 1, 3, and 4, but DGK ι , which is similar in structure (type IV) to DGK ζ , inhibits only RasGRP3 and activates Ras downstream signaling [44]. β 2-Chimaerin, a GTPase-activating protein (GAP) for Rac (RacGAP), is activated by DGK γ [45]. Accordingly, some DGK isozymes along with several small G proteins may regulate downstream cascades of vascular signal transduction (Fig. 1).

Various stimuli affect vascular responses related to DGK activity and are linked to several diseases. Fatty acids inhibit growth-factor-induced DGK α activation *via* increased PKC activity and amplify platelet derived growth factor (PDGF)-induced DG accumulation in vascular smooth muscle cells [46]. Vascular endothelial growth factor-A (VEGF-A) stimulates DGK α activity in endothelial cells, suggesting that DGK α may participate in the promotion of angiogenesis by proliferative and migratory responses [47]. Since angiogenesis is a complex phenomenon including revascularization, DGK is thought to act diversely in vascular responses. This is further evidenced by peroxisome proliferator-activated receptor (PPAR) γ agonists, which are well known as antihyperglycemic drugs and have anti-inflammatory effects in cardiovascular diseases. Verrier *et al.* [48] showed that PPAR γ agonists upregulate DGK, leading to endothelial cell activation *via* suppression of the DG-induced PKC signaling pathway. Taken together, DGK activity is influenced by various stimuli including vasoconstrictors and could diversely regulate vascular signal transduction.

Effects of DGKs Inhibition

Two DGK inhibitors, R59022 and R59949, have been used to look at the function of DGK in cellular responses. Jiang *et al.* [49] demonstrated that R59949 inhibits Ca^{2+} -activated DGK, especially $\text{DGK}\alpha$, more selectively than R59022. However, this study did not test all DGK isoforms and therefore isoform-specific function regarding these two agents remains unclear. A novel DGK inhibitor, stemphone (cochlioquinone A), isolated from *Drechslera sacchari*, inhibits Ca^{2+} -independent DGK isoforms [50,51]. To date, selectivity and functionality of stemphone-mediated inhibition of DGK are unknown.

Nobe *et al.* [50,52] showed that inhibition of Ca^{2+} -independent DGK decreases vascular contraction. Moreover, these authors found that DGK hyper-reactivity enhances aortic smooth muscle contractility. Since these phenomena are associated with high glucose levels and diabetic models, they may be distinguished from normal conditions. This group used R59022, a DGK inhibitor, and stemphone, a novel DGK inhibitor, in mouse aorta and porcine coronary artery to test vascular reactivity and observed a dose-dependent vascular relaxation induced by these agents. Stemphone suppressed U46619 (thromboxane A_2 analog)-induced endothelial cell (EC) layer permeability [53]. EC dysfunction can be caused by diabetic hyperglycemia or inflammatory conditions and results in vascular dysfunction. These data suggest that stemphone can improve vascular endothelial dysfunction *via* inhibition of DGK.

Our laboratory has investigated two DGK inhibitors, R59022 and R59949, which have different effects in the vasculature. Both inhibitors induced vascular relaxation in aorta contracted with phenylephrine, however inhibition with R59022 resulted in greater relaxation than R59949. As mentioned above, since DGK isoform-specific function of R59022 and R59949 are unknown, differences in vascular reactivity to the two agents should be further evaluated. We also studied R59022 to compare vascular reactivity between aortas from normotensive and hypertensive mice. R59022 causes vascular relaxation in aorta contracted with phenylephrine and this effect was decreased in our hypertensive model as compared with normotensive, suggesting that DGK plays an important role in vascular smooth muscle contraction and has different actions between normotensive and hypertensive models.

Conclusions and Perspectives

DGKs exist as an enzyme family and are comprised of diverse structures. Moreover, each DGK isozyme localizes to different subcellular areas, suggesting that DGKs may play unique roles inside the cell in response to global cellular stimulation. The specific function of each DGK remains unclear based on the lack of isoform-selective DGK inhibitors, however DGKs are known to play an important role in vascular signal transduction, participating with many signaling proteins, such as PKC, RhoA and members of the Ras family. We will enhance our understanding of DGKs in vascular function through research regarding DG, as a substrate, and PA, product of DGK. Since several studies have shown isoform-specific DGK functions, these studies can be applied to vascular function and diseases, such as hypertension, which DGKs may reveal itself as a novel therapeutic target.

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Fig. 1.

Diacylglycerol kinase (DGK) signaling in vascular smooth muscle contraction. Agonist (A) binds to its receptor (R), stimulates PLC, liberating IP₃ from DG (formally PIP₂), or PLD, which produces PA. IP₃ stimulates calcium (Ca²⁺) release from the sarcoplasmic reticulum. Ca²⁺ and DG activate PKC signaling pathway. DGK is activated directly by agonists, or indirectly by PI3K and PKC. DGK stimulates PKC, β2-Chimaerin, and vascular contraction. PLC, phospholipase C; PLD, phospholipase D; IP₃, inositol trisphosphate; DG, diacylglycerol; PIP₂, phosphatidylinositol biphosphate; PA, phosphatidic acid; PKC, protein kinase C; PI3K, phosphatidylinositol 3-kinase; PC, phosphatidylcholine; CaM, calmodulin; MAPK, mitogen-activated protein kinase.