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Diacylglycerol Kinase Puts the Brakes on Airway Smooth Muscle Contraction

Diacylglycerol (DAG) is an important lipid second messenger (1) that activates isoforms of PKC and classically has procontractile properties in airway smooth muscle (ASM) (2). Upon agonist stimulation of GPCRs (G protein–coupled receptors) with endogenous ligands such as histamine or acetylcholine, the Gq protein activates PLC (phospholipase C), which hydrolyzes phosphatidylinositol 4,5-bisphosphate to DAG and inositol triphosphate (IP_3). IP_3 binds to IP_3 receptors on the sarcoplasmic reticulum and leads to the release of calcium into the cytoplasm, where it activates CaM (calmodulin) and MLCK (myosin light chain kinase), which phosphorylates MLC (myosin light chain), leading to contraction (Figure 1). DAG activates certain isoforms of PKC, which—among many functions—also activate a phosphorylation-dependent inhibitor protein, CPI-17 (17 kD PKC-potentiated inhibitory protein of type 1 protein phosphatase), that inhibits MLCP (myosin light chain phosphatase). Inactivated MLCP is unable to dephosphorylate MLC, thus also favoring contraction. Given its importance in lipid biosynthesis and as a signaling molecule, DAG is tightly regulated by DGKs (DAG kinases) (3), which phosphorylate DAG and convert it to phosphatidic acid (PA). DGKs consist of 10 different isoforms composing five classes that are present in many organisms and cell types (4). Although first discovered in the late 1950s (5), DGKs have not been studied extensively in ASM (6). Interestingly, in this issue of the *Journal* (pp. 658–671), Sharma and colleagues show that the inhibition of DGK and the subsequent altered ratio of DAG:PA is able to inhibit PLC, leading to eventual ASM relaxation and thus revealing a potential novel target for the development of bronchodilating therapies for asthma (7).

Prior studies from the same group showed that DGKζ knockout mice had decreased airway hyperresponsiveness (8) with an unclear mechanism of action, which prompted the studies described in the current issue of the *Journal*. Here, Sharma and colleagues show that inhibition of DGK with R59022—a small molecule DGKI (DGK inhibitor)—leads to relaxation of methacholine- and histamine-induced contraction of airways in human precision cut lung slices. In cell-based studies, pretreatment with a DGKI or siRNA knockdown of DGK isoforms α or ζ leads to a decrease of phospho-MLC, an important component of the contractile machinery. Moreover, inhibition of DGK (pharmacologically or genetically) leads to attenuation of Gq ligand-induced intracellular calcium increases, suggesting inhibition of the Gq–PLC–IP_3–Ca^{2+} pathway. Not surprisingly, the calcium sensitization pathway is also affected—agonist-induced RhoA activity and phosphorylation of MYPT1 T696 (a regulatory subunit of MLCP) are reduced in cells that are pretreated with DGKI, both of which are expected to reduce ASM contraction. To further characterize where the interruption of the calcium surge takes place with DGK inhibition, Sharma and colleagues elegantly show that genetic or pharmacologic inhibition of DGK led to reduced amounts of agonist-induced inositol phosphate, pointing to PLC as a possible site of action because inositol phosphate is a downstream product of PLC activity. They also show that the ratios of DAG:PA are enhanced with DGK inhibition. DGK inhibition, and the subsequent increase in the DAG:PA ratio, also directly reduced agonist-induced PLC activity. Furthermore, the negative feedback effect of DAG on PLC was directly measured by the exogenous addition of a cell-permeable DAG analog, which was found to decrease PLC activity.

This comprehensive series of cell signaling studies employing complementary pharmacologic and genetic inhibition approaches by these investigators strongly supports the inhibitory role of DAG on PLC, which they elegantly explored in cell-based experiments and supported with functional studies in *ex vivo* human lower airways. However, these findings challenge existing dogma with respect to the current knowledge of DAG action (2, 9, 10). The inhibition of DGK would presumably allow for increased concentrations of DAG, which would activate certain PKC isoforms and lead to phosphorylation of CPI-17 and inhibition of MLCP, thus favoring continued muscle contraction. Surprisingly, basal amounts of DAG or histamine-induced elevations in DAG were not further augmented by DGK inhibition. However, DGK inhibition did reduce the amount of histamine-induced PA. These findings support the authors' conclusion that the phospholipid stoichiometry, specifically the DAG:PA ratio, may be the dominant signaling determinant rather than absolute DAG levels. These intriguing and novel findings require a reconsideration of the role of DAG in cell signaling in general, and in particular in the contraction–relaxation pathways in ASM (Figure 1). There are still many questions that remain unanswered. For example, it is unclear what makes this negative feedback pathway (and muscle relaxation) dominant over the DAG-driven procontractile pathway. Is it solely based on the DAG:PA ratio, or are there additional regulatory elements that favor one pathway over the other?

The experiments by Sharma and colleagues strongly suggest that DGK might be a promising target for the development of asthma drugs. But before we can fully exploit this novel therapeutic approach, more studies are needed in animal models of asthma to determine how these effects translate into desired physiologic outcomes *in vivo*. Such studies might address the effect on airway constriction in asthmatic mice pretreated with DGKI/R59022, the route of inhibitor delivery, or the ability to design inhibitors that are DGK isotype specific. The authors have enhanced our mechanistic understanding of how targeting DGK favors relaxation of Gq-stimulated ASM contraction. What is clear is that DAG and its regulation have a more complex role in ASM contraction–relaxation than previously realized. Sharma and colleagues have elucidated novel signaling mechanisms

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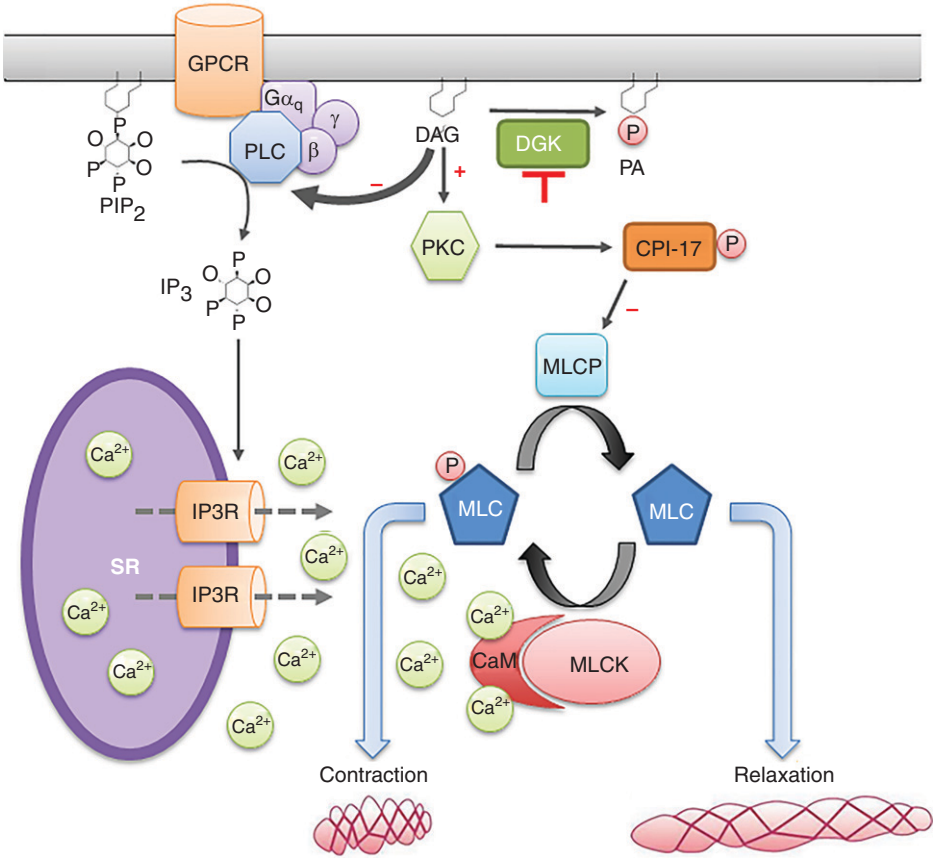

Figure 1. Negative feedback inhibition of PLC (phospholipase C) by elevated diacylglycerol (DAG) levels from DGK (DAG kinase) inhibition favors airway smooth muscle relaxation. Cell surface receptors responding to contractile mediators (e.g., histamine or acetylcholine) couple to heterotrimeric GPCR (G protein–coupled receptor) that activates PLC via the Gq protein. PLC then cleaves a phosphodiester linkage in phosphatidylinositol 4,5-bisphosphonate (PIP_2) to liberate inositol triphosphate (IP_3) and DAG. IP_3 stimulates release of Ca^{2+} from the sarcoplasmic reticulum (SR) that induces Ca^{2+} oscillations, which, in turn, mediate contraction by multiple mechanisms, including the stimulation of MLCK (myosin light chain kinase), favoring MLC (myosin light chain) phosphorylation and contraction. DAG activates PKC isoforms that phosphorylate CPI-17 (17 kD PKC-potentiated inhibitory protein of type 1 protein phosphatase), which inhibits the phosphatase activity of MLCP (myosin light chain phosphatase), favoring the phosphorylated state of MLC and contraction. DAG signaling is regulated by DGK, which phosphorylates DAG into phosphatidic acid (PA). Inhibition of DGK leads to elevated DAG:PA ratios, which inhibits the Gq–PLC–IP_3–Ca^{2+} pathway, thereby leading to muscle relaxation. This appears to be the dominant functional pathway over the DAG–PKC–pCPI–17 procontractile pathway. CaM = calmodulin; IP3R = IP_3 receptor; P = phosphate.

in ASM, leading to a need for additional studies to understand the complex role of messenger lipids in these events. ■

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