Diacylglycerol Kinase in the Regulation of Insulin Secretion

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Diacylglycerol (DAG) is a lipid signal messenger and plays a physiological role in β-cells. Since defective glucose homeostasis increases de novo DAG synthesis, DAG may also contribute to β-cell dysfunction in type 2 diabetes. Although the primary function of DAG is to activate protein kinase C (PKC), the role of PKC in insulin secretion is controversial: PKC has been reported to act as both a positive and negative regulator of insulin secretion. In addition to the PKC pathway, DAG has also been shown to mediate other pathways such as the Munc-13-dependent pathway in β-cells. The intracellular levels of DAG are strictly regulated by diacylglycerol kinase (DGK); however, the role of DGK in β-cells and their involvement in β-cell failure in type 2 diabetes remain to be fully elucidated. We have recently reported the roles of type I DGK, DGKα and γ, in insulin secretion from β-cells. DGKα and γ were activated by glucose or high K⁺ stimulation in β-cells, and the inhibition of the DGKs by a type I DGK inhibitor or by knockdown with small interfering RNA (siRNA) decreased insulin secretion. Thus, DGKα and γ are suggested to be activated in response to elevated [Ca²⁺], in β-cells and to act as positive regulators of insulin secretion. In this article, we review the current understanding of the roles of DAG and DGK in β-cell function and their involvement in the development of β-cell dysfunction in type 2 diabetes.

**Key words** diacylglycerol kinase; diacylglycerol; pancreatic β-cell; insulin secretion; diabetes

1. INTRODUCTION

Diacylglycerol (DAG) is one of the most important lipid signal messengers, the level of which is regulated by diacylglycerol kinase (DGK), diacylglycerol lipase, or diacylglycerol acyltransferase (DGAT). Among these, DGK, which phosphorylates DAG to phosphatidic acid (PA), is the dominant acyltransferase (DGAT). Among these, DGK, which phosphorylates DAG to phosphatidic acid (PA), is the dominant enzyme responsible for DAG metabolism. DGK has been reported to have various physiological effects by modulating the balance between DAG and PA in various tissues; however, the information is still limited. We have recently investigated the roles of DGKα and γ in insulin secretion from pancreatic β-cells and suggested that DGKα and γ act as positive regulators of insulin secretion. Depression of DGKα and γ, which causes the accumulation of DAG and a decrease in PA, led to the reduction of insulin secretion. In type 2 diabetes, a chronic metabolic disorder, glucose and lipid metabolism is disrupted. The disruption of DAG metabolism is thus likely to result in the development of insulin resistance and/or insulin secretory dysfunction. In skeletal muscles, down-regulation of DGKδ occurs in type 2 diabetic patients and this DGKδ deficiency induces insulin resistance. In this article, we review the current understanding of the roles of DAG and DGK in pancreatic β-cells and discuss their pathophysiological roles in β-cells.

2. INSULIN SECRETION REGULATED BY DAG

Glucose-stimulated insulin secretion (GSIS) from pancreatic β-cells is well known to involve the ATP-sensitive K⁺ (KATP) channel-dependent pathway. Entry of Ca²⁺ through L-type voltage-dependent Ca²⁺ channels, which are mediated by plasma membrane depolarization resulting from the closure of KATP channels, is a trigger signal for insulin secretion. Insulin vesicle exocytosis can also be modulated by agents activating the DAG/protein kinase C (PKC) pathway. It is well recognized that DAG generated by the activation of phospholipase C (PLC), following stimulation of muscarinic receptors or with high concentrations of glucose, is required for the normal functioning of β-cells, and that the primary function of DAG is to activate PKC. However, the role of PKC in insulin secretion remains controversial. Several studies have shown that conventional PKC isoforms potentiate insulin secretion, whereas others have suggested that these isoforms are not involved. Moreover, the effects of novel PKC isoforms on insulin secretion are also controversial. Hence, the role of PKC in β-cell function is likely complicated. These variable reports might be partly explained by species differences between rats and mice.

In addition to the DAG/PKC pathway, DAG has also been reported to mediate other pathways in β-cells, e.g., Ras guanine nucleotide-releasing protein (Ras-GRP), protein kinase D, and transient receptor potential (TRP) channels. In addition, DAG regulates insulin secretion by modulating the pathway dependent on Munc-13, which is a synaptic protein that regulates vesicle release independently of PKC. Munc-13-1 mice show impairment of GSIS both in vivo and in isolated islets. Thus, Munc-13 may act as an effector molecule in β-cell lipid signaling.

DAG is metabolized through three major pathways: phos-
phorylation by DGK to produce phosphatidic acid, hydrolysis of fatty acyl chain by diacylglycerol lipase to generate a monoacylglycerol and a free fatty acid, and formation of triacylglycerol by diacylglycerol acyltransferase (DGAT). Since the expression of DGK, diacylglycerol lipase, and DGAT is detected in isolated islets, DAG level and DAG signaling in β-cells might be strictly and intricately regulated by these enzymes. In most cases, however, the conversion to PA catalyzed by DGK seems to be the primary pathway for DAG metabolism in β-cells.1,2)

3. FUNCTION AND REGULATION OF DGK

DGK is a lipid kinase that catalyzes phosphorylation of DAG to PA, a lipid second messenger, and is a terminator of DAG-mediated signaling. Ten mammalian DGK isoforms (α, β, γ, δ, ε, ζ, η, θ, ι, and κ) have been identified so far, and are divided into five groups based on their structure (Fig. 1). All of the mammalian DGK isoforms have two common structural features: a catalytic domain and two cysteine-rich regions (except for DGKθ, which has three) similar to C1A and C1B motifs of PKC. Other structural properties are as follows: Type I DGK isoforms, DGKα, β, and γ, have a pair of Ca2+-binding EF-hand motifs, making these isoforms more active in the presence of Ca2+.17) Type II DGK isoforms, DGKδ, η, and κ, have a pleckstrin homology (PH) domain at the N-terminus, which is considered to be involved in interactions with lipids. Type III DGK isoform, DGKε, has only a C1 domain. Type IV DGK isoforms, DGKζ and ι, contain a region homologous to the phosphorylation site of the PKC substrate MARCKS (myristoylated alanin-rich C-kinase substrate). Type V DGK isoform, DGKθ, has a PH domain overlapping with a Ras associating domain. Each DGK isoform specifically expresses and localizes in various tissues and possesses isoform-specific functions. Recent studies have revealed that DGKα is highly expressed in T lymphocytes and acts during T-cell activation and proliferation.18) DGKβ is abundantly expressed in the brain, especially in the caudate putamen, hippocampus, and cerebral cortex, and regulates spine formation and branching in the maintenance of neural networks.19) DGKγ is highly expressed in the cerebellum and has functional roles in the development of neurons.20) DGKη is also reported to regulate macrophage differentiation21) and mast cell degranulation.22) DGKδ is suggested to contribute to insulin resistance in the progression of type 2 diabetes.4) DGKε is the only DGK isoform that shows substrate specificity for DAG with an arachidonoyl acyl chain at the sn-2 position, and is likely to be involved in the occurrence of seizures.23) Finally, DGKζ localizes to the nucleus in various cell types and is considered to regulate nuclear DAG signaling.24,25) Thus, DGK isoforms are distinctively expressed in each tissue and play crucial roles in various cellular functions as lipid signaling molecules regulating the balance between DAG and PA.

4. ROLES OF DGKS IN INSULIN SECRETION

We have recently reported the roles of type I DGK isoforms in insulin secretion from pancreatic β-cells5) (Fig. 2). DGKα and γ are highly expressed in the cytoplasm of mouse β-cells and are activated by [Ca2+]i elevation in the pancreatic β-cell line MIN6. Treatment with the type I DGK inhibitor R59949 and double knockdown of DGKα and DGKγ by small interfering RNA (siRNA) led to a significant decrease in insulin secretion induced by glucose or high K+ stimulation. These findings suggest that DGKα and γ are activated in response to elevated [Ca2+]i, in β-cells and act as positive regulators of insulin secretion. R59949 also led to an apparent decrease in [Ca2+]i, elevation induced by glucose or high K+, accompanied by an increase in intracellular DAG level in MIN6 cells. Presumably, DAG accumulation is responsible for the inhibition of insulin secretion by R59949. This is further supported by another finding that the DAG analog DiC8 similarly inhibited
high K⁺-induced [Ca²⁺]i elevation. These results are consistent with an early study by Thomas and Pek in HIT-T15 β-cell lines, in which DiC8 and the DGK inhibitor R59022 inhibited high K⁺-induced [Ca²⁺]i elevation and insulin secretion. 26)

Interestingly, a considerable difference was observed in the effect of R59949 on GSIS between mice and rats: R59949 significantly inhibited GSIS in rat islets as well as MIN6 cells, whereas the DGK inhibitor showed no such inhibitory effect in mouse islets. This discrepancy may be explained by a low expression of phospholipase C (PLC)δ, an isoform activated by Ca²⁺, in mouse β-cells. A study by Zawalich et al. has clearly demonstrated that the expression of PLCδ1 is much higher in rat islets than in mouse islets, and that increases in PLC activity by glucose and high K⁺ are larger in rat islets than in mouse islets. 27) Therefore, the DAG accumulation induced by glucose could be estimated to be higher in rat islets than in mouse islets, which would explain the results that R59949 inhibited GSIS only in rat islets. PKC is unlikely to be involved in the inhibition of insulin secretion by R59949, because Ro 31-8220, a PKC inhibitor, had no effect on the R59949-induced sustained Ca²⁺ decrease. 3)

DGK also acts as an activator of PA-mediated signaling. Inhibition of insulin secretion by R59949 may partly result from the reduction of PA signaling. PA is produced through two major pathways: hydrolysis of phosphatidylcholine by phospholipase D (PLD) and phosphorylation of DAG by DGK. The expression of PLD and PLC is detected in β-cells, and these activities increase in response to glucose and other stimulants in β-cells. 27,28) Moreover, the inhibition of PLD by butan-1-ol has been shown to suppress GSIS, suggesting that PA is a positive regulator of insulin secretion. 28) PA has also been reported to promote the trafficking and membrane association

Fig. 2. Scheme for the Insulin Secretion Regulated by Type I DGK in Pancreatic β-Cells
DAG: diacylglycerol, PA: phosphatidic acid, DGK: diacylglycerol kinase, PKC: protein kinase C, KATP channel: ATP sensitive potassium channel, VDCC: voltage dependent calcium channel, PLC: phospholipase C.

Fig. 3. The Difference between Normal and Abnormal (Diabetic) Conditions in the Regulation of Insulin Secretion by Type I DGK
DAG, diacylglycerol; DGK, diacylglycerol kinase; PA, phosphatidic acid.
of the small guanosine 5′-triphosphate (GTP) binding protein Rac, which promotes insulin granule exocytosis in β-cells. It is likely, therefore, that PA produced by type I DGK facilitates insulin secretion by accelerating the exocytosis of insulin secretory granules in β-cells. Interestingly, a recent report has demonstrated that DGKγ only has an intense PA-binding activity among DGK isoforms; therefore, DGKγ is likely to act as a PA binding protein and participate in the PA-mediated insulin secretion signaling pathway.30)

In short, DGKα and γ, present in pancreatic β-cells, participate in a physiological regulation of insulin secretion, possibly by changing the intracellular lipid balance. Depression of these DGK isoforms, possibly under pathological conditions, would lead to an accumulation of DAG and a decrease in PA, thereby decreasing insulin secretion (Fig. 3).

5. DGK AND TYPE 2 DIABETES

In type 2 diabetes, chronic DAG elevation through de novo synthesis and subsequent abnormal PKC activation initiated by hyperglycemia have been observed in various tissues.31) Abnormal activation of PKC may evoke tissue dysfunction in diabetic complications, including vascular dysfunction31) and diabetic neuropathy and nephropathy.32) Increased DAG levels by DGK downregulation or DGK inhibitor treatment would block insulin signaling by preventing insulin-induced tyrosine phosphorylation of insulin receptor substrates (IRS), which is mediated by PKC-induced serine phosphorylation of IRS.4,33) Chibalin et al. have demonstrated that DGKδ is highly expressed in skeletal muscle and contributes to the regulation of glucose uptake, and further, that DGKδ deficiency causes the development of insulin resistance.4) Thus, DGKδ is suggested to be a key molecule in preventing the development of type 2 diabetes. Conversely, overexpression of DGKε has been shown to diminish tyrosine phosphorylation of IRS-1 in skeletal muscle cells, while reducing the activation of PKC.34) The differences between these two DGK isoforms on insulin action may be explained by differences in their cellular distribution and/or substrate specificity for DGK. DGKε shows substrate specificity for DAG with an arachidonoyl acyl chain at the sn-2 position, whereas DGKδ preferentially phosphorylates palmitic acid-containing DAG species.35) Further studies are needed to elucidate the involvement of DGK in the development of insulin resistance.

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In conclusion, type I DGK seems to participate in the regulation of β-cell function by metabolizing DAG and/or generating PA. Further studies on the regulation of lipid signaling in β-cells will provide additional implications for the role of DGK in maintaining β-cell function. Targeting DAG or PA signaling regulated by DGK in β-cells may provide a novel therapeutic strategy to control and maintain insulin secretion against β-cell dysfunction in type 2 diabetes.

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Conflict of Interest The authors declare no conflict of interest.

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