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REVIEW ARTICLE

Regulation and roles of neuronal diacylglycerol kinases: a lipid perspective

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Abstract

Diacylglycerol kinases (DGKs) are a class of enzymes that catalyze the ATP-dependent conversion of diacylglycerol (DAG) to phosphatidic acid (PtdOH), resulting in the coordinate regulation of these two lipid second messengers. This regulation is particularly important in the nervous system where it is now well-established that DAG and PtdOH serve very important roles in modulating a variety of neurological functions. There are currently 10 identified mammalian DGKs, organized into five classes or "Types" based upon similarities in their primary sequences. A number of studies have identified eight of these isoforms in various regions of the mammalian central nervous system (CNS): DGK-α, DGK-β, DGK-γ, DGK-η, DGK-ζ, DGK-ι, DGK-ε, and DGK-θ. Further studies have provided compelling evidence supporting roles for these enzymes in neuronal spine density, myelination, synaptic activity, neuronal plasticity, epileptogenesis and neurotransmitter release. The physiological regulation of these enzymes is less clear. Like all interfacial enzymes, DGKs metabolize their hydrophobic substrate (DAG) at a membrane-aqueous interface. Therefore, these enzymes can be regulated by alterations in their subcellular localization, enzymatic activity, and/or membrane association. In this review, we summarize what is currently understood about the localization and regulation of the neuronal DGKs in the mammalian CNS.

Keywords: Diacyglycerol kinases, lipid metabolizing enzymes, regulation, diacylglycerol, phosphatidic acid

Introduction

It has been long recognized that lipids play important roles in a number of physiological functions. The discovery of the phosphatidylinositol (PtdIns) cycle in neurons accelerated research on the role of lipids in neurobiology. The canonical view of the PtdIns cycle involves the stimulated hydrolysis of phosphatidylinositol-(4,5) bisphosphate PtdIns(4,5)P_2 which leads to the generation of inositol (1,4,5) trisphosphate (IP_3) and diacylglycerol (DAG). This system, along with an influx of extracellular calcium provides a mechanism for stimulated increases in calcium and DAG during neuronal stimulation. It is now well-established that DAG serves a very important role in modulating the release of small-molecule neurotransmitters via vesicular exocytosis, and this discovery helped link the PtdIns cycle to neurotransmitter release. The DAG generated in this cycle is converted to phosphatidic acid (PtdOH) which is subsequently used for the re-synthesis of PtdIns. In addition, PtdOH has been implicated in neurotransmitter release (Humeau *et al.*, 2001), as well as its lyso analogue via a lysoPtdOH receptor (Shiono *et al.*, 1993). However, the cumulative body of research on the PtdIns hydrolysis in neurons clearly indicates that there are other metabolic connections for the production and metabolism of DAG and PtdOH that are independent of the PtdIns cycle which play in important roles in neurons.

Given the above, there is increasing interest in understanding the mechanisms involved in regulating the relative cellular levels of DAG and PtdOH. Enzymes capable of coordinately regulating the levels of these two lipids are the diacylglycerol kinases (DGKs). These enzymes are organized into five classes or "Types" based upon similarities in their primary sequence. All DGKs catalyze

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the transfer of the γ-phosphate of ATP to the hydroxyl group of DAG thereby generating PtdOH while reducing DAG. The notion that these enzymes play important neuronal roles is supported by the observations that eight of the 10 mammalian DGK isozymes are readily detected in the mammalian central nervous system (CNS): DGK-α, DGK-β, DGK-γ, DGK-η, DGK-ζ, DGK-ι, DGK-ε, and DGK-θ (Table 1). Neuronal roles for the other two DGKs, DGK-δ and DGK-κ are less clear. In this review, we will outline the roles and regulation of the DGKs found in the CNS.

Localization and functions of DGKs in the brain

During the initial characterization of the DGK isoforms it became apparent that most of these isoforms exhibited high mRNA expression in the brains of rodents and humans. Newer technologies, such as electronic Northern analysis and serial analysis of gene expression, have supported this finding, and it is clear that this family of enzymes plays a number of roles in regulating lipid signaling and phosphatidylinositol PtdIns turnover in the brain. One observation of interest is that DGK isoforms fall into two general categories with respect to localization in the brain– those that are clustered (densely expressed) within particular regions, and those that display a diffuse expression pattern. This is quite evident in composite images of brain slices, in which the ISH (*in situ* hybridization) image is overlaid with a gene expression pattern that displays the relative intensity of mRNA expression within a brain region (Lein *et al.*, 2007). This method can be quite helpful for comparing and interpreting DGK isoform localization (Figure 1). Using this method, strong clustering is evident for DGK-β, -ι, ε, and ζ in the cortex, hippocampal pyramidal layer, and granular layer of the dentate gyrus. In addition, DGKs -β, -ι, and -ε show strong clustering in the caudate putamen, while DGK-ζ shows moderate clustering in this region. It is interesting to note that DGK-ζ, -ι and–η are the isoforms with the highest

Table 1. Relative mRNA expression levels of mammalian DGKs.

DGK Isoform	Brain	Heart	Liver	Eye / retina	Thymus	Placenta	Testes	Spleen	Intestine	Kidney	Skeletal muscle	References
α												
Pig	++	Trace	0	n.d.	++++	n.d.	n.d.	++	n.d.	Trace	n.d.	Sakane *et al.*, 1990;
Rat	++	n.d.	Trace	n.d.	++++	n.d.	n.d.	++++	n.d.	0	n.d.	Goto *et al.*, 1992
β												
Human	++++	0	0	n.d.	n.d.	Trace (uterus)	trace	0	n.d.	Trace	0	Caricasole *et al.*, 2001;
Rat	++++	0	0	n.d.	0	n.d.	0	0	n.d.	0	0	Sakane *et al.*, 1990
γ												
Rat	++++	0	0	+	0	n.d.	0	0	0	0	n.d.	Goto *et al.*, 1994;
Human	+	n.d.	0	++++	n.d.	n.d.	Trace	Trace	n.d.	Trace	n.d.	Kai *et al.*, 1994
δ1	0	0	0	n.d.	Trace	0	++	Trace	+ (Colon)	0	++++	Sakane *et al.*, 1996;
δ2 Human	Trace	Trace	Trace	n.d	+++	++	++++	++	++	Trace	0	Sakane *et al.*, 2002
η												
Hamster	+++	+	Trace	n.d.	n.d.	n.d.	++++	+++	n.d.	Trace	+	Baum *et al.*, 2008
κ												
Human	0	0	0	n.d.	0	+	+++	Trace	0	0	0	Imai *et al.*, 2005
ε												
Human	Trace	0	0	n.d.	0	0	++++	0	0	0	Ttrace	Tang *et al.*, 1996;
Rat	++	+	0	++++	n.d.	n.d.	Trace	+	n.d.	Trace	0	Kohyama-Koganeya
Mouse	++++	++	0	n.d.	n.d.	n.d.	Trace	0	n.d.	Trace	Trace	*et al.*, 1997; Rodriguez de Turco *et al.*, 2001
ζ												
Human	+++	+++	++	n.d.	n.d.	++	n.d.	n.d.	n.d.	++	++++	Bunting *et al.*, 1996
ι												
Rat	++++	0	0	+	0	n.d.	++	0	0	0	0	Ito *et al.*, 2004;
Human	++	n.d.	n.d.	++++	n.d.	0	n.d.	n.d.	n.d.	0	0	Ding *et al.*, 1998
θ												
Rat	++++	0	0	Trace	n.d.	n.d.	n.d.	Trace	+	Trace	n.d.	Houssa *et al.*, 1997

A qualitative representation of described message levels for the various DGK isoforms is presented across a range of tissues. DGK-δ and DGK-κ, which are poorly expressed in nervous tissue and not a part of this review, have been included for completeness. Comparison is valid across tissues for each individual isoform, but not for a single tissue across isoforms since there is no common normalization factor to relate experiments. Animal species are indicated for each isoform.
++++Highest relative mRNA expression detected for that isoform within the represented Northern blot.
n.d. = not done; trace = detectable.

expression in the thalamus. While all DGK isoforms show relatively strong staining by ISH in the cerebellum (probably due to the very high density of cells in this structure), expression is clustered in the cerebellar Purkinje layer for DGK-γ, -ζ and -ι. There is also differential isoform expression in the microstructures of the olfactory bulb, with DGK-ε and DGK-γ expressed preferentially in the glomerular layer, and DGKs -β, -ζ and -ι clustered within the granule and mitral layers. (The interested reader is referred to gene expression level maps available at http://mouse.brain-map.org for more detailed views). This type of analysis will help to identify potential isoform redundancy within particular regions of the brain, and may provide clues to physiological roles. For example, in the basal ganglia (which includes the caudate-putamen, striatum, and globus pallidus) medium spiny neurons comprise approximately 90% of the neuronal population. Based on the expression levels described above, it is reasonable to conclude that DGK-β, -ι, -η and -ζ will be present in medium spiny neurons. Indeed, recent studies have shown that DGK-ζ and -β both play a role in this cell type (see below). In addition, comparison of expression profiles of potential major signaling partners can provide useful, correlative information. For example, we have viewed the expression profiles for PI-PLC-β1 and protein kinase C (PKC)-α, and find them to be similar to DGK-ζ and DGK-θ, respectively. These examples underscore the potential utility of gene expression pattern analysis with respect to the ongoing task of determining the physiological roles of the various DGK isoforms.

It is important to note that isoforms that do not display expression clustering (e.g. DGK-α, -ε, and -θ in Figure 1) are simply more uniformly expressed in the brain, suggesting that they may be involved in ubiquitous neuronal function(s). In addition, it is not clear to what extent DGK mRNA expression is influenced by signaling pathways, development, or physiological state, and some isoforms may show different distributions under other conditions. While most DGKs (with the exception of DGK-δ and DGK-κ are found in the CNS, some isoforms show a broader tissue distribution than others, and there are species-specific differences in tissue distribution and spliceforms for individual isoforms as well (see Table 1). This should be kept firmly in mind, as it could reveal differences in isoform function across mammalian species.

To date, there are a number of studies documenting the direct involvement of DGK in brain functions including PI recycling, dendritic spine maintenance, and presynaptic neurotransmitter release. The following sections will be dedicated to outlining the individual roles that have been demonstrated for these enzymes in neuronal processes.

DGK-α: a non-neuronal DGK in the CNS

In contrast to the other DGKs found in the CNS, DGK-α is localized in oligodendrocytes (glial cells) rather than neurons (Goto & Kondo, 1999b). Oligodendrocytes produce the myelin sheath, which wraps around and insulates neuronal axons, and therefore are critical for proper neuronal function. Loss (demyelination) or defective (dysmyelination) myelination results in neurological disorders such as multiple sclerosis, leukodystrophies, and schizophrenia (Krämer-Albers *et al.*, 2006; Matalon *et al.*, 2006; Tkachev *et al.*, 2007; Compston & Coles, 2008). In this context, it is significant that DGK-α co-localizes with myelin basic protein in the CNS, and active DGK-α has been detected in highly purified myelin fractions (Kahn & Morell, 1989). More recently, DGK activity in purified

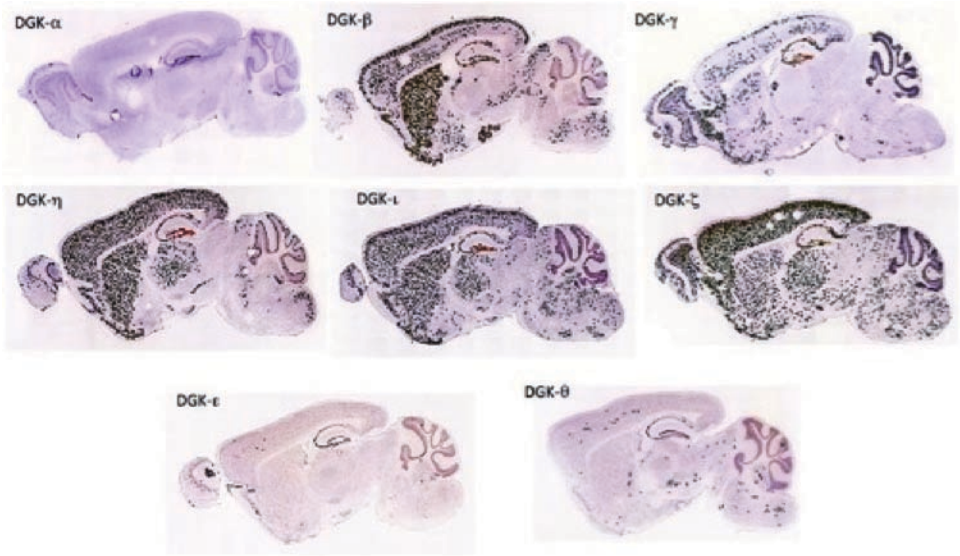

Figure 1. Gene expression profiling reveals differential clustering of DGK isoforms. High throughput ISH images of sagittal sections from male C57BL/6J mice were overlaid with gene expression images producing composite images that reveal regions of high gene expression. All images and gene expression data are from the Allen Institute of Brain Expression (Allen Mouse Brain Atlas [Internet]. Seattle, WA): Allen Institute for Brain Science. ©2009. Available from: http://mouse.brain-map.org.

myelin was shown to be activated by interleukin (IL)-2 and inhibited by PKC-dependent manner (Chakraborty *et al.*, 2003), though the DGK isoform was not confirmed to be DGK-α in that study. PKC, which is regulated by the DGK substrate DAG, has been shown to be important for myelin formation during normal oligodendrocyte differentiation (Asotra & Macklin, 1993) and remodeling (Yong *et al.*, 1994), and pharmacological stimulation of PKC has been shown to impede the expression of the late myelin proteins MBP (myelin binding protein) and PLP (proteolipid protein) (Baron *et al.*, 2000). This has led some to suggest that DGK-α may be involved in regulating myelin formation by regulating the levels of DAG. Additionally, oligodendrocytes play an essential role in the *in vivo* regulation of glutamate released by unmyelinated neurons in the developing cerebral white matter in both human and rat corpus collusum, with similar uptake capacity and affinity for glutamate as that observed in postnatal astrocytes. However, uptake by oligodendrocytes is via the EAAC1 transporter, which is typically downregulated in mature oligodendrocytes (DeSilva *et al.*, 2009). However, cultured oligodendrocytes have been shown to constitutively express the EAAC1 glutamate transporter. Consistent with this, glutamate uptake has been shown to be increased in cultured glial cells by stimulation of PKC with 12-*O*-tetradecanoyl-phorbol-13-acetate or by inhibition of DGK with R59022, but not in neuronal cultures (Casadó *et al.*, 1991). Taken together, these data suggest a significant role for DGK-α in glial cell functions, particularly during early development. Importantly, with respect to this review, the physiological roles of DGK-α in oligodendrocytes has not been rigorously defined.

Neuronal DGK-β

DGK-β is found at low levels in a number of tissues, but is predominantly expressed in brain. In the CNS expression is quite diverse, and includes the cerebral cortex, hippocampal pyramidal cells, dentate gyrus, olfactory tubercle, main olfactory bulb, olfactory nucleus, caudate-putamen, accumbens nucleus, amygdala and hippocampus [see (Caricasole *et al.*, 2001; Goto & Kondo, 1999a) and Figure 1]. This isoform is one of the few whose developmental expression pattern has been determined. Protein expression was very low through P7 (postnatal day 7), thereafter increasing until it peaked between P14 and P28, which corresponds with the time course of synaptogenesis in the rat model used. This regulation appears to be transcriptional, since mRNA and protein expression followed the same approximate time course (Adachi *et al.*, 2005). It was originally proposed that DGK-β may play a role in the dopaminergic system since expression of this isoform is high in regions associated with dopaminergic neurons, suggesting that DGK-β may regulate pathways that impact on cognitive function and emotions. In support of this, of the 16 potential human splice variants of DGK-β identified by Caricasole and coworkers, (Caricasole *et al.*, 2001), spliceforms with a 35-amino-acid C-terminal deletion were detected in some patients with bipolar disorder (Hozumi *et al.*, 2008). While intriguing, there is currently insufficient data to support this hypothesis, and it is noteworthy that the expression patterns of GAD65/67 (glutamic acid dehydrogenase—a marker for GABAergic neurons), and of PI-PLCβ show even more striking homology to the expression pattern of DGK-β. There is stronger evidence to support a role for DGK-β in spine density formation, which is also implicated in cognitive function (Hozumi *et al.*, 2009; Kim *et al.*, 2010; Shirai *et al.*, 2010). As indicated above, the endogenous expression of DGK-β in mice parallels the time course of synaptic development, and overexpression of DGK-β has been shown to promote neurite outgrowth (Hozumi *et al.*, 2009; Shirai *et al.*, 2010) while loss of this DGK results in a reduction in hippocampal and cortical spine density with respect to wild type mice (Shirai *et al.*, 2010) and (Kakefuda *et al.*, 2010).

Neuronal DGK-γ

Of all the DGK isoforms, DGK-γ appears to present the most diverse behavior between rodent and human forms. In rats, it is strongly expressed in cerebellar Purkinje cells and the hippocampus, while full length human DGK-γ is primarily detected in the retina (Kai *et al.*, 1994). Rat DGK-γ is found predominately associated with the cytoskeleton (Goto *et al.*, 1994; Kai *et al.*, 1994), while human DGK-γ is distributed between the membrane and soluble fractions, with approximately 60% membrane bound prior to agonist stimulation (Kai *et al.*, 1994). Perhaps most curious, while both species of DGK-γ contain calcium-binding EF hands, the rat form exhibits Ca^{2+} sensitive activity while the human form does not. Importantly for this review, there is very little clear evidence outlining a functional role for this isoform in neurons. It has been suggested that DGK-γ may compete for Rac1 binding with DGK-ζ (see below) to assist in modulating the formation of growth cones and neuronal processes (Tsushima *et al.*, 2004). In addition, DGK-γ has been observed to associate with PKC-γ in CHO cells and *in vitro* (Yamaguchi *et al.*, 2006). DGK-γ is phosphorylated and activated by PKC-γ in this system, though colocalization in neurons has not been shown, and the functional implications are not clear. However, PKC-γ has been shown to be involved in learning and memory (Abeliovich *et al.*, 1993), and it has been suggested that DGK-γ may be involved in memory and learning through this apparent association. The developmental expression of DGK-γ has been determined in rat brain, but unlike DGK-β it is detectable at P1. Of note is the observation that although total DGK-γ protein did increase over time to P28, there was a differential expression between the medial geniculate nucleus (MGN) where protein peaked at P14 and disappeared by P21, and the hippocampus, where protein increased several fold between P14 and P21, peaking between P21 and P28 (Adachi *et al.*, 2005). The expression trend observed in the MGN suggested to the authors that DGK-γ may play a role

in the development of the auditory system, though this has not yet been tested. Coincidentally, the loss of DGK-γ from the MGN roughly correlates with increased soma size and decreased cell density in that region (Clerici & Coleman, 1998), and kinase-dead nuclear DGK-γ has been shown to increase the size of cells and delay progress through S-phase of the cell cycle (Matsubara *et al.*, 2006). Clearly, much more work is needed to build on these findings to define the neuronal role(s) of DGK-γ.

Neuronal DGK-η

DGK-η is perhaps one of the least studied of the 10 known mammalian DGKs. DGK-η protein is found predominantly in the brain and testes (Klauck *et al.*, 1996) but differential localization of mRNA within the brain has not been reported. However, expression databases contain sufficient information on the regional distribution and expression density of DGK-η mRNA in the mouse brain to reveal a clustering pattern similar to DGK-ι (Figure 1 and see below), though the significance of this is unknown. Like DGK-β, mutations in DGK-η were reported to correlate with bipolar disease. This observation was based on chromosomal linkage and a genetic analysis of patients who suffered from this condition (Baum *et al.*, 2008). While subsequent efforts to validate and expand on this study found no relationship between DGK-η and bipolar disorder (Squassina *et al.*, 2009; Tesli *et al.*, 2009), an approximately 25% increase in DGK-η mRNA levels has been reported for some individuals with bipolar disorder or schizophrenia (Moya *et al.*, 2010). In Hela cells, DGK-η appears to be involved in regulating the Ras/B-Raf/C-Raf/MEK/ERK signaling cascade in an activity-independent manner (Yasuda *et al.*, 2009). Such studies have not been conducted in neurons and the functional roles of this DGK remain a mystery.

We should mention that a neuronal role for the other two Type II DGKs, DGK-δ and DGK-κ is uncertain. DGK-κ is expressed predominately in the testes and is, therefore, unlikely to play a role in the CNS. While there is a significant body of work on DGK-δ signaling in non-neuronal cells, there is very little on its function in the brain. DGK-δ has been implicated in the generation of seizures, although it is important to note that this conclusion relies on a study of a single patient with a genetic disruption of DGK-δ who suffered from other complications as well (Leach *et al.*, 2007).

Neuronal DGK-ε

DGK-ε is the lone member of this class of DGKs. Like DGK-θ below, DGK-ε shows a uniform distribution within the CNS. Gene expression levels appear to be highest in the Purkinje cells of the cerebellum, pyramidal cells of the hippocampus, mitral cells of the olfactory bulb, and neurons of the substantia nigra (Rodriguez de Turco *et al.*, 2001). Mice in which DGK-ε was ablated by targeted gene disruption displayed an increased resistance to electroconvulsive shock with shorter tonic seizures and faster recovery than their wild type counterparts. Additionally, long-term potentiation was attenuated in granular cell synapses. These data led the investigators to conclude that DGK-ε likely modulates neuronal synaptic activity, neuronal plasticity, and epileptogenesis (Musto & Bazan, 2006; Rodriguez de Turco *et al.*, 2001).

This isoform is unique in that it is the only isoform of all mammalian DGKs that shows a distinct preference for 1-stearoyl-2- arachidonyl diacylglycerols that are enriched in cellular PtdIns (Lung *et al.*, 2009). It is this specificity that has led to suggestions that this isoform is involved in the recycling of DAGs derived from hydrolysis of PtdIns(4,5)P_2. Surprisingly, the PtdIns cycle appears to be depressed in the cerebral cortex of the DGK-ε knockout mice described above, as evidenced by reduced levels of 20:4-DAG and free 20:4 fatty acid with respect to their wild type counterparts (Rodriguez de Turco *et al.*, 2001). These data suggest that DAG recycling by DGK-ε is important to maintain flux through the PI cycle.

Neuronal DGK-ζ

This well-studied isoform is expressed in many tissues, and is strongly expressed in the CNS. This DGK-ζ was originally identified in the cortices of the cerebellum and cerebrum, the olfactory bulb, and pyramidal cells of the hippocampal CA1 and CA3 (Goto & Kondo, 1996; Hozumi *et al.*, 2003), though expression is also evident in the caudate-putamen, thalamus, hypothalamus of mice (Figure 1). Insights into the neuronal roles of DGK-ζ are beginning to accumulate. Early reports suggested that this isoform plays a role in hypoxic responses in the hippocampus (see Goto *et al.*, 2006). It should be noted, however, that this interpretation largely relies on alterations in DGK-ζ subcellular distribution. Other evidence associates this DGK with leptin receptor signaling in the hypothalamus (Liu *et al.*, 2001). Leptin is an adipocyte-derived hormone that has been implicated in regulating food intact and energy metabolism. In mice, a high-fat diet stimulates leptin production and increases hypothalamic DGK-ζ mRNA levels. This correlation has led to the proposal that this DGK isoform may be involved in leptin-mediated signaling, although the precise mechanism remains unclear. Recently, new evidence indicates that DGK-ζ plays an important role in the maintenance, but not establishment, of neuronal spine density. Kim *et al.* (2009) demonstrated that knockdown of this isoform in rat hippocampal neurons decreased spine density in a PKC-independent manner, while overexpression of this enzyme increased spine density. Consistent with this, spine density, as well as PtdOH production, is reduced in DGK-ζ knockout mice. Reduction in spine density depends on catalytic activity as overexpression of a catalytically inactive enzyme did produce this effect. This appears to be a largely postsynaptic mechanism which is dependent on the interaction of DGK-ζ, with the PDZ domains of a family of postsynaptic PSD-95 proteins (PSD-95, SAP97, PSD-93/chapsyn-110, and SAP102) and subsequent localization of DGK-ζ to the postsynaptic

membrane. A more precise mechanism is certain to emerge in the near future.

Another interesting observation that currently sets DGK-ζ apart from many other isoforms is that it is often found to be localized to the nucleus. Nuclear localization has been shown to change in response to different physiological and pathophysiological stimuli (primarily ischemia), as well as during different developmental stages (Hozumi *et al.*, 2003; Nakano *et al.*, 2006; Nakano *et al.*, 2009). It is unclear at this point whether nuclear DGK-ζ is active or inactive. Finally, DGK-ζ may have additional neuronal functions that do not depend on its catalytic activity. For example, DGK-ζ interacts with syntrophin and RacGDP to localize and regulate Rac1 at growth cones and sites of sites of early process (dendrite) formation in an activity-independent manner (Yakubchyk *et al.*, 2005).

Neuronal DGK-ι

This DGK isoform is localized to the brain and retina (Ding *et al.*, 1998), and ISH analysis shows moderate levels in the cortex, caudate-putamen, thalamus, and cerebellar cortex, with high expression in the hippocampal calcium region and dentate gyrus (Sommer *et al.*, 2001). In what appears to be a recurring theme, DGK-ι has been implicated in behavioral regulation. A differential study comparing expression levels from the brain of alcohol-preferring (AA) and alcohol-avoiding (ANA) rats identified DGK-ι as one of only two genes to show a significant difference in expression levels, although no link between DGK-ι activity and behavior was demonstrated (Sommer *et al.*, 2001). More recently, work with a DGK-ι mouse knockout model revealed that this isoform may be involved in regulating presynaptic glutamate release during DHPG (3,5-dihydroxyphenylglycine)-induced long-term potentiation (Yang *et al.*, 2010). These data suggest that DGK-ι mediates long-term depression induced by a group I metabotropic glutamate receptor(s) (mGluR1 and mGluR5).

Neuronal DGK-θ

DGK-θ is the sole representative of the Type V class, and is enriched in the nervous system over other tissues. Like DGK-ε, DGK-θ is expressed in a uniform pattern throughout multiple regions of the brain. mRNA expression is highest in the cerebellum and hippocampus, but moderate signal is detected throughout the brain (Houssa *et al.*, 1997). While there are currently no published studies on neuronal DGK-θ, work in several immortalized cells lines of neuronal origin (PC-12 and N2a) indicates that a subpopulation of this enzyme is found in speckle domains of the nucleus (Tabellini *et al.*, 2003). Speckle domains are regions that contain high levels of splicing factors, and the presence of DGK-θ in these domains suggests that this enzyme may play a role in RNA processing. Stimulation of cells with NGF (nerve growth factor) resulted in an increase in the amount of DGK-θ nuclear protein and activity, further supporting this notion. In a separate study, Li and colleagues demonstrated a role for DGK-θ in the activation of the nuclear receptor steroidogenic factor 1 (SF-1), and showed that or knock down of DGK-θ but not DGK-ζ, or expression of kinase-dead DGK-θ eliminated SF-1 induced gene expression in response to agonist (Li, 2007). Based on this data it is tempting to speculate that DGK-θ may participate in gene regulation in neurons.

Regulation of DGKs

General considerations

DGKs are members of a class of enzymes known as interfacial enzymes. These enzymes interact with at least one substrate that resides in an organized lipid structure in an aqueous environment. These enzymes may be regulated by (A) alterations in their subcellular distribution without affecting catalytic activity, (B) alterations in their intrinsic catalytic activity, or (C) both.

A number of studies have examined the agonist-induced redistribution of various DGKs in response to a variety of signals. These studies generally involved cell fractionation followed by Western blot analysis, immunohistochemistry or immunofluorescence of particular DGKs, or by following the distribution of fluorescently-tagged DGKs. As outlined below, these approaches have provided valuable information regarding the effect of cellular stimulation on the subcellular distribution of these enzymes.

Examination of the effect of agonist on the specific activity of various DGKs is less studied. Except for the effect of calcium on the Type I DGKs, studies providing a connection between agonist stimulation and the intrinsic catalytic activity of these enzymes remain scarce. There are two major approaches for the analysis of DGK activity: "surface dilution kinetics" (Carman *et al.*, 1995), a kinetic model first developed to explain the dependence of enzyme activity on the surface composition of mixed detergent micelles (the "dilution" effect), and "interfacial kinetic analysis", a multifaceted mathematical analysis of enzyme kinetics at interfaces which attempts to account, where possible, for all the kinetic constants involved in interfacial binding and catalysis (Berg & Jain, 2002). In both approaches, the dependence of the enzyme on the bulk and surface concentrations of substrates and products is analyzed. At the extremes, these analyses have shown that interfacial enzymes behave as either "scooters" or "hoppers". Scooters have a long interfacial residence time, while hoppers bind transiently to an interface and are less processive than scooters.

With the exception of DGK-ε, DGKs are likey to exhibit hopping characteristics based on cellular distribution. Of the mammalian DGKs, however, only DGK-ε and DGK-θ have been subjected to kinetic studies (Tu-Sekine *et al.*, 2006; Dicu *et al.*, 2007; Tu-Sekine *et al.*, 2007; Lung *et al.*, 2009; Tu-Sekine & Raben, 2009; Tu-Sekine & Raben, 2010), and more study is necessary to determine if the

mammalian DGK family exhibits common kinetic characteristics. The remainder of this review discusses the current data on the regulation of the individual DGK isoforms in neuronal and non-neuronal systems.

Regulation of DGK-α

While studies designed to examine the regulation of DGK-α have not been restricted to oligodendrocytes, they have provided valuable clues to essential regulatory factors. Like all Type I DGKs, this DGK possesses two EF-hand domains at the *N*-terminus. The EF hands bind calcium with a K_d 0.3 μM in the intact enzyme and 9.9 μM for the EF-hand peptide expressed in *Escherichia coli*. Calcium binding alters the conformation of the enzyme and exposes a hydrophobic region as determined by 2-*p*-toluidinylnaphthalene 6-sulfonate (TNS) binding and gel migration (Yamada *et al.*, 1997) suggesting that calcium binding may promote membrane association. (Mérida *et al.*, 2007; Topham, 2006; Topham & Prescott, 1999). A deletion analysis of the *N*-terminus of this enzyme revealed that the upstream recoverin homology (RVH) interacts with the EF hands to inhibit enzyme activity and obstruct a hydrophobic membrane-associating region, and that binding of calcium or deletion of the RVH domain activates the enzyme (Jiang *et al.*, 2000) and promotes membrane association (Sanjuán *et al.*, 2001). DGK-α is also sensitive to particular phospholipids, and is activated by phosphatidylserine (PtdSer) in a calcium-dependent manner (Abe *et al.*, 2003), and may be activated by phosphatidylethanolamine (PtdEth) and cholesterol in a calcium independent manner (Fanani *et al.*, 2004). In addition, there is some evidence that the purified form of this enzyme is stimulated by phosphatidylcholine (PtdCho) and, to a lesser extent, by PtdEth and PtdSer, but inhibited by PtdIns and unaffected by fatty acids. However, in this pioneering report the substrate and activating lipids were added as separate dispersions, and co-sonication of DG and PtdCho to form mixed vesicles did not produce activation over DG dispersions alone (Kanoh *et al.*, 1983). It is not clear whether this is due to the lack of Ca^{2+} in these experiments, or whether this indicates that PtdCho is not a physiologic activator; however, the evidence that PtdSer is an activator remains strong. There is also strong evidence showing that DGK-α activity is regulated by phosphorylation. DGK-α can be activated by PCK-ε mediated phosphorylation *in vitro* and *in vivo,* and by epidermal growth factor receptor and src-mediated phosphorylation in Cos cells or hepatocytes. (Kanoh *et al.*, 1989; Schaap *et al.*, 1993; Cutrupi *et al.*, 2000).

Agonist-induced subcellular redistribution likely plays a role in the regulation of DGK-α. For example, localization of this isoform to the plasma membrane of peripheral T-cells is important for downregulation of the T-cell receptor (Sanjuán *et al.*, 2001). Similarly, arachidonic acid promotes association of DGK-α with the plasma membrane and Golgi of CHO-K1 cells, while purinergic stimulation results in its translocation to the plasma membrane only. There is also compelling evidence that PI(3,4)P2 and PI(3,4,5)P3 both activate this enzyme and recruit it to intracellular membranes in the presence of calcium, further expanding the understanding of DGK-α translocation and activation (Ciprés *et al.*, 2003). The effect of purinergic stimulation on DGK-α translocation is particularly interesting in the context of CNS function, since oligodendrocyte migration and extension of glial processes to neuronal axons has been proposed to be regulated in part by the $P2Y_{12}$ purinergic receptor on these cells (Amadio *et al.*, 2006), and development of oligodendrocytes is mediated by activation of P1 receptors (Stevens *et al.*, 2002). In a similar vein, localization of DGK-α to internal membranes appears to be necessary for regulating the secretion of exosomes containing the FasL-ligand from activated T-cells (Alonso *et al.*, 2005), which also has a functional CNS counterpart. While oligodendrocytes do not express FasL they have been observed to secrete exosomes, and analysis of these exosomes and their functions is a new area of study (Krämer-Albers *et al.*, 2007; Bakhti *et al.*, 2011). It would be interesting indeed to determine if DGK-α was involved in exosome secretion by oligodendrocytes. Finally, this isoform has exhibited agonist-dependent nuclear translocation in various cells including rat thymocytes, peripheral T-lymphocytes, and CTLL-2 cells (Wada *et al.*, 1996). IL-2 stimulates the translocation of DGK-α to the nuclear matrix of T-cells, while activation of the T-cell receptor leads to the translocation of this isoform to a perinuclear region (Wada *et al.*, 1996). While the nuclear function of DGK-α is unknown, nuclear translocation appears to be a common theme for DGKs, and may suggest a general role for this enzyme family in a nuclear function such as gene transcription. Examination of the subcellular distribution of DGK-α in oligodendrocytes should provide clues to the role of this enzyme in these cells.

Regulation of DGK-β

Like DGK-α, this Type I DGK also contains EF-hand domains in the *N*-terminus, however, its activity does not appear to be regulated *in vitro* by calcium (Caricasole *et al.*, 2001). This is somewhat surprising since Ca^{2+} binding has been measured using an *E. coli*-expressed DGK-β EF-hand peptide and found to have a higher affinity for calcium (K_d = 0.9 μM) than DGK-α (K_d = 9.9 μM). In addition, TNS (2-p-toluidinyl-naphthalene-6-sulphonate) binding and gel migration analysis of this EF-hand peptide demonstrated a conformational change upon calcium binding (Yamada *et al.*, 1997). Unfortunately, calcium affinity has not been measured in the context of the whole protein, and calcium labeling has not been done *in vivo*, so it is unclear whether the DGK-β is constitutively bound to Ca^{2+}, or whether the EF hands do not respond to calcium. It is still possible that regulation of DGK-β occurs by a calcium-mediated modulation of its localization or intrinsic catalytic activity. What is more evident is that this isoform can also be stimulated by PtdSer (Caricasole *et al.*, 2001). As indicated, the activity

of DGK-β, like other interfacial enzymes, may be modulated by substrate availability or membrane association. In this regard, DGK-β is one of two DGKs (the other being DGK-γ) with C1 domains that bind phorbol ester with high affinity (Shindo *et al.*, 2003), and translocates to membranes in response to phorbol esters. (Caricasole *et al.*, 2001). The notion that this may serve a regulatory role is highlighted by the observation that a truncated version of DGK-β, which still contains an intact phorbol ester-binding C1 domain, shows impaired membrane translocation (Caricasole *et al.*, 2001). This finding may indicate that the C1 domain is dependent on the conformation of the enzyme for access to phorbol ester. Clearly, more research on the neuronal regulation of this DGK is needed.

Regulation of DGK-γ

DGK-γ is the final member of the class I DGKs, and like its sister isoforms, possesses EF hands. In the same study that measured the calcium affinity for DGK-α and DGK-β, DGK-γ was shown to have the highest affinity for calcium ($K_d = 0.4$ μM). However, like DGK-β, the activity of this isoform is independent of calcium *in vitro* assays (Tsushima *et al.*, 2004). While little is known about the regulation of DGK-γ activity, there are data suggesting that PKC-γ may be involved in regulating this enzyme in Purkinje cells. Both DGK-γ and PKC-γ are expressed in these cells, and stimulation by purinergic agonist results in the localization of both enzymes to the plasma membrane (Shirai *et al.*, 2000). Following this localization, there appears to be an association between DGK-γ and PKC-γ which leads to the phosphorylation of the accessory domain of DGK-γ, resulting in its activation (Yamaguchi *et al.*, 2006). Aside from this, direct biochemical analyses of DGK-γ's catalytic activity and regulation have not been presented.

Regulation of DGK-ε

DGK-ε is the smallest of all 10 mammalian DGK isoforms (64 kD). This isoform lacks all of the known regulatory motifs. It has been suggested that this enzyme is constitutively active, with substrate availability and specificity as its primary regulators. There is compelling evidence, however, that the primary mode of regulation is inhibitory in nature. DGK-ε is inhibited by a variety of anionic phospholipids, including its product PtdOH, as well as PtdSer and the PI-PLC substrate PtdIns(4,5)P_2, (Thirugnanam *et al.*, 2001). The rather potent inhibition of DGK-ε by PtdIns(4,5)P_2 is particularly interesting as it lends support to the notion that this DGK isoforms is involved in regulating the PtdIns cycle. For example, a reduction in PtdIns(4,5)P_2 resulting from the phosphatidylinositol-phospholipase C-mediated cleavage of this phospholipid would stimulate DGK-ε and result in the subsequent phosphorylation of the liberated DAG. The DGK-ε-mediated generation of PtdOH could then inhibit this enzyme which would effectively "reset" the PtdIns cycle. Epand and colleagues have added evidence to further support this notion. They recently showed that the selectivity for DAG substrate recognition and PtdOH inhibition both depend on the fatty acid species at the sn-1 and sn-2 position. In addition to the substrate DAG specificity (Tang *et al.*, 1996; Thirugnanam *et al.*, 2001), PtdOH-mediated inhibition is almost strongest with sn-1 stearoyl and sn-2 arachidonoyl species (Lung *et al.*, 2009). Finally, the enzyme is also inhibited by 2-arachidonyl-glycerol, as is DGK-ζ (Gantayet *et al.*, 2010) although the role of this inhibition has not been established.

Regulation of DGK-ζ

The regulation of DGK-ζ in neurons is just emerging. The C-terminal PDZ-binding domains are known to be critical for localization to the postsynaptic density (PSD). Deletion of this region results in a generalized distribution of the protein within neurons (Kim *et al.*, 2009). Studies in non-neuronal systems have revealed activators of DGK-ζ activity, including anionic phospholipids (Thirugnanam *et al.*, 2001), interaction with the retinoblastoma (R_b) protein (Los *et al.*, 2006), and src kinase (Davidson *et al.*, 2004). With respect to src, it is not clear whether a src-mediated phosphorylation of DGK-ζ is involved in this process. Importantly, PKC-α and DGK-ζ appear to be reciprocal regulators of one another. PKC-α-mediated phosphorylation of DGK-ζ in the MARCKs domain has been shown to reduce both its nuclear accumulation and its catalytic activity, as evidenced by *in vitro* and *in vivo* measurements (Luo *et al.*, 2003). On the other hand, DGK-ζ has been shown to regulate PKC-α activity *in vivo*, presumably by eliminating DAG, an important cofactor for this PKC isoform. This relationship is particularly interesting since the mRNA expression pattern of DGK-ζ is very similar to that of PKC-α in mouse brain by gene expression analysis (Allen Mouse Brain Atlas [Internet]. Seattle (WA): Allen Institute for Brain Science. ©2009. Available from: http://mouse.brain-map.org). Somewhat surprisingly, in contrast to the Type I DGKs, calcium inhibits this enzyme.

Regulation of DGK-ι

Like many DGKs discussed here, the neuronal regulation of DGK-ι remains unresolved. Ding *et al.*, (1998) demonstrated that the human isotype is found in both the cytosol and nucleus, and that phosphorylation by PKC-α or PKC-γ reduces DGK-ι's localization in the nucleus of Cos 7 cells, though regulation of human DGK-ι by PKC is not yet established in neurons. Curiously, overexpression of the rat form of this enzyme in hippocampal neurons revealed a primarily cytoplasmic distribution indicating a differential regulation of rat DGK-ι and DGK-ζ by PKC in neurons. DGK-ι shares significant homology with DGK-ζ and also contains a bipartite nuclear localization signal within a MARCKs domain. Limited work has been done on the kinetics of DGK-ι, but Ito *et al.* (2004) have reported

that a truncated spliceform of rDGK-ι (termed DGK-ι -r3) exhibited reduced catalytic activity over the full length form (approx 50%), due primarily to a three-fold increase in the K_M for ATP (Ito *et al.*, 2004). A rigorous analysis of the regulation of DGK-ι's activity, however, has not been reported and the physiological relevance of the various spliceforms remains undetermined.

Regulation of DGK-θ

DGK-θ is also activated by anionic phospholipids, including PtdSer and PtdOH, the product of the DGK reaction. Interestingly, PI(4,5)P_2 does not appear to activate this pH domain-containing isoform (Tu-Sekine *et al.*, 2007). Similar experiments have not examined the effect of PI(3,4,5)P_3 or other phosphoinositides, and it is not known whether this pH domain binds lipid. It is interesting to note that interaction with PtdSer appears not only to stimulate catalytic activity, but also to broaden the pH profile of this enzyme. While the mechanism of this effect has not been determined, there is speculation that interaction with PtdSer may stabilize the enzyme at the interface, minimizing interaction with water and sensitivity to pH.

Protein partners for DGK-θ have been identified and include: the constitutively active form of RhoA (V14RhoA), which has been shown to inhibit activity both *in vitro* and *in vivo* (Houssa *et al.*, 1999); PKC-ε, which phosphorylates DGK-θ *in vitro* and promotes translocation of the enzyme to the plasma membrane (van Baal *et al.*, 2005); and Akt, which has been shown to stimulate catalytic activity, though its ability to phosphorylate DGK-θ is not known (Clarke *et al.*, 2007). Finally, DGK-θ has been shown to interact with and regulate DNA binding activity of nuclear SF-1, though regulation of DGK-θ activity has not been tested.

This isoform appears to be particularly sensitive to deletion and point mutations, making it difficult to study individual regulatory regions (Los *et al.*, 2004). However, the presence of the C1 domains and the C-terminal accessory domain both seem to be important for activity. In addition, disruption or deletion of the C1 domains prevents membrane association of DGK-γ and DGK-ζ (Santos *et al.*, 2002; Shirai *et al.*, 2000), and cysteine to alanine mutations known to interfere with DAG binding by DGK C1 domains eliminates membrane association in response to DiC8 (Los *et al.*, 2004). DGK-θ translocation and activation also appear to be subject to regulation by PI3K in an aterial system, though the mechanism of this regulation is unknown (Walker *et al.*, 2001; Clarke *et al.*, 2007). Unfortunately, none of the described work was conducted in a neuronal system. It is unclear, therefore, as to the extent that the described systems represent neuronal regulatory mechanisms for mammalian DGK-θ.

Conclusion

Interest in the physiological and pathophysiological roles of DGKs is increasing. Much has been accomplished regarding the location of these enzymes within the CNS. We are now gaining a better understanding of the role of these enzymes in modulating neuronal spine density, myelination, synaptic activity, neuronal plasticity, and epileptogenesis and neurotransmitter release. There are, however, major gaps in our understanding of the subcellular localization of these enzymes and, importantly, the regulation of their subcellular localization and enzymatic activity. Clearly, this is an emerging area of research that is likely to have an impact on our understanding of neurophysiology and the mechanisms of various neurological diseases.

Declaration of interest

The authors report no declarations of interest.

References

Abe T, Lu X, Jiang Y, Boccone CE, Qian S, Vattem KM, Wek RC, Walsh JP. 2003. Site-directed mutagenesis of the active site of diacylglycerol kinase alpha: calcium and phosphatidylserine stimulate enzyme activity via distinct mechanisms. Biochem J 375:673–680.

Abeliovich A, Paylor R, Chen C, Kim JJ, Wehner JM, Tonegawa S. 1993. PKC gamma mutant mice exhibit mild deficits in spatial and contextual learning. Cell 75:1263–1271.

Adachi N, Oyasu M, Taniguchi T, Yamaguchi Y, Takenaka R, Shirai Y, Saito N. 2005. Immunocytochemical localization of a neuron-specific diacylglycerol kinase beta and gamma in the developing rat brain. Brain Res Mol Brain Res 139:288–299.

Alonso R, Rodríguez MC, Pindado J, Merino E, Mérida I, Izquierdo M. 2005. Diacylglycerol kinase alpha regulates the secretion of lethal exosomes bearing Fas ligand during activation-induced cell death of T lymphocytes. J Biol Chem 280:28439–28450.

Amadio S, Tramini G, Martorana A, Viscomi MT, Sancesario G, Bernardi G, Volonté C. 2006. Oligodendrocytes express $P2Y_{12}$ metabotropic receptor in adult rat brain. Neuroscience 141:1171–1180.

Asotra K, Macklin WB. 1993. Protein kinase C activity modulates myelin gene expression in enriched oligodendrocytes. J Neurosci Res 34:571–588.

Bakhti M, Winter C, Simons M. 2011. Inhibition of myelin membrane sheath formation by oligodendrocyte-derived exosome-like vesicles. J Biol Chem 286:787–796.

Baron W, de Jonge JC, de Vries H, Hoekstra D. 2000. Perturbation of myelination by activation of distinct signaling pathways: an *in vitro* study in a myelinating culture derived from fetal rat brain. J Neurosci Res 59:74–85.

Baum AE, Akula N, Cabanero M, Cardona I, Corona W, Klemens B, Schulze TG, Cichon S, Rietschel M, Nöthen MM, Georgi A, Schumacher J, Schwarz M, Abou Jamra R, Höfels S, Propping P, Satagopan J, Detera-Wadleigh SD, Hardy J, McMahon FJ. 2008. A genome-wide association study implicates diacylglycerol kinase eta (DGKH) and several other genes in the etiology of bipolar disorder. Mol Psychiatry 13:197–207.

Berg OG, Jain MK. 2002. Interfacial Enzyme Kinetics. London: John Wiley & Sons, Ltd.

Bunting M, Tang W, Zimmerman GA, McIntyre TM, Prescott SM. 1996. Molecular cloning and characterization of a novel human diacylglycerol kinase zeta. J Biol Chem 271:10230–10236.

Caricasole A, Bettini E, Sala C, Roncarati R, Kobayashi N, Caldara F, Goto K, Terstappen GC. 2002. Molecular cloning and characterization of the human diacylglycerol kinase beta (DGKbeta) gene: alternative splicing generates DGKbeta isotypes with different properties. J Biol Chem 277:4790–4796.

Carman GM, Deems RA, Dennis EA. 1995. Lipid signaling enzymes and surface dilution kinetics. J Biol Chem 270:18711–18714.

Casadó V, Mallol J, Lluis C, Canela EI, Franco R. 1991. Effect of phospholipases and proteases on the [3H]N6-®-phenylisopropyladenosine ([3H]R-PIA) binding to A1 adenosine receptors from pig cerebral cortex. J Cell Biochem 47:278–288.

Chakraborty G, Reddy R, Drivas A, Ledeen RW. 2003. Interleukin-2 receptors and interleukin-2-mediated signaling in myelin: activation of diacylglycerol kinase and phosphatidylinositol 3-kinase. Neuroscience 122:967–973.

Ciprés A, Carrasco S, Merino E, Díaz E, Krishna UM, Falck JR, Martínez-A C, Mérida I. 2003. Regulation of diacylglycerol kinase alpha by phosphoinositide 3-kinase lipid products. J Biol Chem 278:35629–35635.

Clarke CJ, Ohanian V, Ohanian J. 2007. Norepinephrine and endothelin activate diacylglycerol kinases in caveolae/rafts of rat mesenteric arteries: agonist-specific role of PI3-kinase. Am J Physiol Heart Circ Physiol 292:H2248–H2256.

Clerici WJ, Coleman JR. 1998. Postnatal cytoarchitecture of the rat medial geniculate body. J Comp Neurol 399:110–124.

Compston A, Coles A. 2008. Multiple sclerosis. Lancet 372:1502–1517.

Cutrupi S, Baldanzi G, Gramaglia D, Maffè A, Schaap D, Giraudo E, van Blitterswijk W, Bussolino F, Comoglio PM, Graziani A. 2000. Src-mediated activation of alpha-diacylglycerol kinase is required for hepatocyte growth factor-induced cell motility. EMBO J 19:4614–4622.

Davidson L, Pawson AJ, López de Maturana R, Freestone SH, Barran P, Millar RP, Maudsley S. 2004. Gonadotropin-releasing hormone-induced activation of diacylglycerol kinase-zeta and its association with active c-src. J Biol Chem 279:11906–11916.

DeSilva TM, Kabakov AY, Goldhoff PE, Volpe JJ, Rosenberg PA. 2009. Regulation of glutamate transport in developing rat oligodendrocytes. J Neurosci 29:7898–7908.

Dicu AO, Topham MK, Ottaway L, Epand RM. 2007. Role of the hydrophobic segment of diacylglycerol kinase epsilon. Biochemistry 46:6109–6117.

Ding L, Traer E, McIntyre TM, Zimmerman GA, Prescott SM. 1998. The cloning and characterization of a novel human diacylglycerol kinase, DGKiota. J Biol Chem 273:32746–32752.

Fanani ML, Topham MK, Walsh JP, Epand RM. 2004. Lipid modulation of the activity of diacylglycerol kinase alpha- and zeta-isoforms: activation by phosphatidylethanolamine and cholesterol. Biochemistry 43:14767–14777.

Gantayet A, Jegatheswaran J, Jayakumaran G, Topham MK, Epand RM. 2011. Endocannabinoids and diacylglycerol kinase activity. Biochim Biophys Acta 1808:1050–1053.

Goto K, Funayama M, Kondo H. 1994. Cloning and expression of a cytoskeleton-associated diacylglycerol kinase that is dominantly expressed in cerebellum. Proc Natl Acad Sci USA 91:13042–13046.

Goto K, Kondo H. 1996. A 104-kDa diacylglycerol kinase containing ankyrin-like repeats localizes in the cell nucleus. Proc Natl Acad Sci USA 93:11196–11201.

Goto K, Kondo H. 1999a. Diacylglycerol kinase in the central nervous system–molecular heterogeneity and gene expression. Chem Phys Lipids 98:109–117.

Goto K, Kondo H. 1999b. Diacylglycerol kinase: molecular diversity and gene expression in central nervous system. Tanpakushitsu Kakusan Koso 44:976–982.

Goto K, Nakano T, Hozumi Y. 2006. Diacylglycerol kinase and animal models: the pathophysiological roles in the brain and heart. Adv Enzyme Regul 46:192–202.

Goto K, Watanabe M, Kondo H, Yuasa H, Sakane F, Kanoh H. 1992. Gene cloning, sequence, expression and *in situ* localization of 80 kDa diacylglycerol kinase specific to oligodendrocyte of rat brain. Brain Res Mol Brain Res 16:75–87.

Houssa B, de Widt J, Kranenburg O, Moolenaar WH, van Blitterswijk WJ. 1999. Diacylglycerol kinase theta binds to and is negatively regulated by active RhoA. J Biol Chem 274:6820–6822.

Houssa B, Schaap D, van der Wal J, Goto K, Kondo H, Yamakawa A, Shibata M, Takenawa T, van Blitterswijk WJ. 1997. Cloning of a novel human diacylglycerol kinase (DGKtheta) containing three cysteine-rich domains, a proline-rich region, and a pleckstrin homology domain with an overlapping Ras-associating domain. J Biol Chem 272:10422–10428.

Hozumi Y, Fukaya M, Adachi N, Saito N, Otani K, Kondo H, Watanabe M, Goto K. 2008. Diacylglycerol kinase beta accumulates on the perisynaptic site of medium spiny neurons in the striatum. Eur J Neurosci 28:2409–2422.

Hozumi Y, Ito T, Nakano T, Nakagawa T, Aoyagi M, Kondo H, Goto K. 2003. Nuclear localization of diacylglycerol kinase zeta in neurons. Eur J Neurosci 18:1448–1457.

Hozumi Y, Watanabe M, Otani K, Goto K. 2009. Diacylglycerol kinase beta promotes dendritic outgrowth and spine maturation in developing hippocampal neurons. BMC Neurosci 10:99.

Humeau Y, Vitale N, Chasserot-Golaz S, Dupont JL, Du G, Frohman MA, Bader MF, Poulain B. 2001. A role for phospholipase D1 in neurotransmitter release. Proc Natl Acad Sci USA 98:15300–15305.

Imai S, Kai M, Yasuda S, Kanoh H, Sakane F. 2005. Identification and characterization of a novel human type II diacylglycerol kinase, DGK kappa. J Biol Chem 280:39870–39881.

Ito T, Hozumi Y, Sakane F, Saino-Saito S, Kanoh H, Aoyagi M, Kondo H, Goto K. 2004. Cloning and characterization of diacylglycerol kinase iota splice variants in rat brain. J Biol Chem 279:23317–23326.

Jiang Y, Qian W, Hawes JW, Walsh JP. 2000. A domain with homology to neuronal calcium sensors is required for calcium-dependent activation of diacylglycerol kinase alpha. J Biol Chem 275:34092–34099.

Kahn DW, Morell P. 1989. Evidence for the presence of diacylglycerol kinase in rat brain myelin. Neurochem Res 14:541–546.

Kai M, Sakane F, Imai S, Wada I, Kanoh H. 1994. Molecular cloning of a diacylglycerol kinase isozyme predominantly expressed in human retina with a truncated and inactive enzyme expression in most other human cells. J Biol Chem 269:18492–18498.

Kakefuda K, Oyagi A, Ishisaka M, Tsuruma K, Shimazawa M, Yokota K, Shirai Y, Horie K, Saito N, Takeda J, Hara H. 2010. Diacylglycerol kinase ß knockout mice exhibit lithium-sensitive behavioral abnormalities. PLoS ONE 5:e13447.

Kanoh H, Kondoh H, Ono T. 1983. Diacylglycerol kinase from pig brain. Purification and phospholipid dependencies. J Biol Chem 258:1767–1774.

Kanoh H, Yamada K, Sakane F, Imaizumi T. 1989. Phosphorylation of diacylglycerol kinase *in vitro* by protein kinase C. Biochem J 258:455–462.

Kim K, Yang J, Kim E. 2010. Diacylglycerol kinases in the regulation of dendritic spines. J Neurochem 112:577–587.

Kim K, Yang J, Zhong XP, Kim MH, Kim YS, Lee HW, Han S, Choi J, Han K, Seo J, Prescott SM, Topham MK, Bae YC, Koretzky G, Choi SY, Kim E. 2009. Synaptic removal of diacylglycerol by DGKzeta and PSD-95 regulates dendritic spine maintenance. EMBO J 28:1170–1179.

Klauck TM, Xu X, Mousseau B, Jaken S. 1996. Cloning and characterization of a glucocorticoid-induced diacylglycerol kinase. J Biol Chem 271:19781–19788.

Kohyama-Koganeya A, Watanabe M, Hotta Y. 1997. Molecular cloning of a diacylglycerol kinase isozyme predominantly expressed in rat retina. FEBS Lett 409:258–264.

Krämer-Albers EM, Bretz N, Tenzer S, Winterstein C, Möbius W, Berger H, Nave KA, Schild H, Trotter J. 2007. Oligodendrocytes secrete exosomes containing major myelin and stress-protective proteins: Trophic support for axons? Proteomics Clin Appl 1:1446–1461.

Krämer-Albers EM, Gehrig-Burger K, Thiele C, Trotter J, Nave KA. 2006. Perturbed interactions of mutant proteolipid protein/DM20 with cholesterol and lipid rafts in oligodendroglia: implications for dysmyelination in spastic paraplegia. J Neurosci 26:11743–11752.

Leach NT, Sun Y, Michaud S, Zheng Y, Ligon KL, Ligon AH, Sander T, Korf BR, Lu W, Harris DJ, Gusella JF, Maas RL, Quade BJ, Cole

AJ, Kelz MB, Morton CC. 2007. Disruption of diacylglycerol kinase delta (DGKD) associated with seizures in humans and mice. Am J Hum Genet 80:792–799.

Lein ES, Hawrylycz MJ, Ao N, Ayres M, Bensinger A, Bernard A, Boe AF, Boguski MS, Brockway KS, Byrnes EJ, Chen L, Chen L, Chen TM, Chin MC, Chong J, Crook BE, Czaplinska A, Dang CN, Datta S, Dee NR, Desaki AL, Desta T, Diep E, Dolbeare TA, Donelan MJ, Dong HW, Dougherty JG, Duncan BJ, Ebbert AJ, Eichele G, Estin LK, Faber C, Facer BA, Fields R, Fischer SR, Fliss TP, Frensley C, Gates SN, Glattfelder KJ, Halverson KR, Hart MR, Hohmann JG, Howell MP, Jeung DP, Johnson RA, Karr PT, Kawal R, Kidney JM, Knapik RH, Kuan CL, Lake JH, Laramee AR, Larsen KD, Lau C, Lemon TA, Liang AJ, Liu Y, Luong LT, Michaels J, Morgan JJ, Morgan RJ, Mortrud MT, Mosqueda NF, Ng LL, Ng R, Orta GJ, Overly CC, Pak TH, Parry SE, Pathak SD, Pearson OC, Puchalski RB, Riley ZL, Rockett HR, Rowland SA, Royall JJ, Ruiz MJ, Sarno NR, Schaffnit K, Shapovalova NV, Sivisay T, Slaughterbeck CR, Smith SC, Smith KA, Smith BI, Sodt AJ, Stewart NN, Stumpf KR, Sunkin SM, Sutram M, Tam A, Teemer CD, Thaller C, Thompson CL, Varnam LR, Visel A, Whitlock RM, Wohnoutka PE, Wolkey CK, Wong VY, Wood M, Yaylaoglu MB, Young RC, Youngstrom BL, Yuan XF, Zhang B, Zwingman TA, Jones AR. 2007. Genome-wide atlas of gene expression in the adult mouse brain. Nature 445:168–176.

Liu Z, Chang GQ, Leibowitz SF. 2001. Diacylglycerol kinase zeta in hypothalamus interacts with long form leptin receptor. Relation to dietary fat and body weight regulation. J Biol Chem 276:5900–5907.

Los AP, van Baal J, de Widt J, Divecha N, van Blitterswijk WJ. 2004. Structure-activity relationship of diacylglycerol kinase theta. Biochim Biophys Acta 1636:169–174.

Los AP, Vinke FP, de Widt J, Topham MK, van Blitterswijk WJ, Divecha N. 2006. The retinoblastoma family proteins bind to and activate diacylglycerol kinase zeta. J Biol Chem 281:858–866.

Lung M, Shulga YV, Ivanova PT, Myers DS, Milne SB, Brown HA, Topham MK, Epand RM. 2009. Diacylglycerol kinase epsilon is selective for both acyl chains of phosphatidic acid or diacylglycerol. J Biol Chem 284:31062–31073.

Luo B, Prescott SM, Topham MK. 2003. Protein kinase C alpha phosphorylates and negatively regulates diacylglycerol kinase zeta. J Biol Chem 278:39542–39547.

Matalon R, Michals-Matalon K, Surendran S, Tyring SK. 2006. Canavan disease: studies on the knockout mouse. Adv Exp Med Biol 576:77–93; discussion 361.

Matsubara T, Shirai Y, Miyasaka K, Murakami T, Yamaguchi Y, Ueyama T, Kai M, Sakane F, Kanoh H, Hashimoto T, Kamada S, Kikkawa U, Saito N. 2006. Nuclear transportation of diacylglycerol kinase gamma and its possible function in the nucleus. J Biol Chem 281:6152–6164.

Mérida I, Avila-Flores A, Merino E. 2008. Diacylglycerol kinases: at the hub of cell signalling. Biochem J 409:1–18.

Moya PR, Murphy DL, McMahon FJ, Wendland JR. 2010. Increased gene expression of diacylglycerol kinase eta in bipolar disorder. Int J Neuropsychopharmacol 13: 1127–1128.

Musto A, Bazan NG. 2006. Diacylglycerol kinase epsilon modulates rapid kindling epileptogenesis. Epilepsia 47:267–276.

Nakano T, Hozumi Y, Ali H, Saino-Saito S, Kamii H, Sato S, Kayama T, Watanabe M, Kondo H, Goto K. 2006. Diacylglycerol kinase zeta is involved in the process of cerebral infarction. Eur J Neurosci 23:1427–1435.

Nakano T, Iseki K, Hozumi Y, Kawamae K, Wakabayashi I, Goto K. 2009. Brain trauma induces expression of diacylglycerol kinase zeta in microglia. Neurosci Lett 461:110–115.

Rodriguez de Turco EB, Tang W, Topham MK, Sakane F, Marcheselli VL, Chen C, Taketomi A, Prescott SM, Bazan NG. 2001. Diacylglycerol kinase epsilon regulates seizure susceptibility and long-term potentiation through arachidonoyl-inositol lipid signaling. Proc Natl Acad Sci USA 98:4740–4745.

Sakane F, Imai S, Kai M, Wada I, Kanoh H. 1996. Molecular cloning of a novel diacylglycerol kinase isozyme with a pleckstrin homology domain and a C-terminal tail similar to those of the EPH family of protein-tyrosine kinases. J Biol Chem 271:8394–8401.

Sakane F, Imai S, Kai M, Yasuda S, Kanoh H. 2007. Diacylglycerol kinases: why so many of them? Biochim Biophys Acta 1771:793–806.

Sakane F, Imai S, Yamada K, Murakami T, Tsushima S, Kanoh H. 2002. Alternative splicing of the human diacylglycerol kinase delta gene generates two isoforms differing in their expression patterns and in regulatory functions. J Biol Chem 277:43519–43526.

Sakane F, Yamada K, Kanoh H, Yokoyama C, Tanabe T. 1990. Porcine diacylglycerol kinase sequence has zinc finger and E-F hand motifs. Nature 344:345–348.

Sanjuán MA, Jones DR, Izquierdo M, Mérida I. 2001. Role of diacylglycerol kinase alpha in the attenuation of receptor signaling. J Cell Biol 153:207–220.

Santos T, Carrasco S, Jones DR, Mérida I, Eguinoa A. 2002. Dynamics of diacylglycerol kinase zeta translocation in living T-cells. Study of the structural domain requirements for translocation and activity. J Biol Chem 277:30300–30309.

Schaap D, van der Wal J, van Blitterswijk WJ, van der Bend RL, Ploegh HL. 1993. Diacylglycerol kinase is phosphorylated *in vivo* upon stimulation of the epidermal growth factor receptor and serine/threonine kinases, including protein kinase C-epsilon. Biochem J 289(Pt 3):875–881.

Shindo M, Irie K, Masuda A, Ohigashi H, Shirai Y, Miyasaka K, Saito N. 2003. Synthesis and phorbol ester binding of the cysteine-rich domains of diacylglycerol kinase (DGK) isozymes. DGKgamma and DGKbeta are new targets of tumor-promoting phorbol esters. J Biol Chem 278:18448–18454.

Shiono S, Kawamoto K, Yoshida N, Kondo T, Inagami T. 1993. Neurotransmitter release from lysophosphatidic acid stimulated PC12 cells: involvement of lysophosphatidic acid receptors. Biochem Biophys Res Commun 193:667–673.

Shirai Y, Kouzuki T, Kakefuda K, Moriguchi S, Oyagi A, Horie K, Morita SY, Shimazawa M, Fukunaga K, Takeda J, Saito N, Hara H. 2010. Essential role of neuron-enriched diacylglycerol kinase (DGK), DGKbeta in neurite spine formation, contributing to cognitive function. PLoS ONE 5:e11602.

Shirai Y, Segawa S, Kuriyama M, Goto K, Sakai N, Saito N. 2000. Subtype-specific translocation of diacylglycerol kinase alpha and gamma and its correlation with protein kinase C. J Biol Chem 275:24760–24766.

Sommer W, Arlinde C, Caberlotto L, Thorsell A, Hyytia P, Heilig M. 2001. Differential expression of diacylglycerol kinase iota and L18A mRNAs in the brains of alcohol-preferring AA and alcohol-avoiding ANA rats. Mol Psychiatry 6:103–8; 5.

Squassina A, Manchia M, Congiu D, Severino G, Chillotti C, Ardau R, Piccardi M, Zompo MD. 2009. The diacylglycerol kinase eta gene and bipolar disorder: a replication study in a Sardinian sample. Mol Psychiatry 14:350–351.

Stevens B, Porta S, Haak LL, Gallo V, Fields RD. 2002. Adenosine: a neuron-glial transmitter promoting myelination in the CNS in response to action potentials. Neuron 36:855–868.

Tabellini G, Bortul R, Santi S, Riccio M, Baldini G, Cappellini A, Billi AM, Berezney R, Ruggeri A, Cocco L, Martelli AM. 2003. Diacylglycerol kinase-theta is localized in the speckle domains of the nucleus. Exp Cell Res 287:143–154.

Tang W, Bunting M, Zimmerman GA, McIntyre TM, Prescott SM. 1996. Molecular cloning of a novel human diacylglycerol kinase highly selective for arachidonate-containing substrates. J Biol Chem 271:10237–10241.

Tesli M, Kähler AK, Andreassen BK, Werge T, Mors O, Mellerup E, Koefoed P, Melle I, Morken G, Wirgenes KV, Andreassen OA, Djurovic S. 2009. No association between DGKH and bipolar disorder in a Scandinavian case-control sample. Psychiatr Genet 19:269–272.

Thirugnanam S, Topham MK, Epand RM. 2001. Physiological implications of the contrasting modulation of the activities of the epsilon- and zeta-isoforms of diacylglycerol kinase. Biochemistry 40:10607–10613.

Tkachev D, Mimmack ML, Huffaker SJ, Ryan M, Bahn S. 2007. Further evidence for altered myelin biosynthesis and glutamatergic

dysfunction in schizophrenia. Int J Neuropsychopharmacol 10:557–563.

Topham MK. 2006. Signaling roles of diacylglycerol kinases. J Cell Biochem 97:474–484.

Topham MK, Prescott SM. 1999. Mammalian diacylglycerol kinases, a family of lipid kinases with signaling functions. J Biol Chem 274:11447–11450.

Tsushima S, Kai M, Yamada K, Imai S, Houkin K, Kanoh H, Sakane F. 2004. Diacylglycerol kinase gamma serves as an upstream suppressor of Rac1 and lamellipodium formation. J Biol Chem 279:28603–28613.

Tu-Sekine B, Ostroski M, Raben DM. 2006. Analysis of two diacylglycerol kinase activities in mixed micelles. Adv Enzyme Regul 46:12–24.

Tu-Sekine B, Ostroski M, Raben DM. 2007. Modulation of diacylglycerol kinase theta activity by alpha-thrombin and phospholipids. Biochemistry 46:924–932.

Tu-Sekine B, Raben DM. 2009. Regulation of DGK-theta. J Cell Physiol 220:548–552.

Tu-Sekine B, Raben DM. 2010. Characterization of cellular DGK-theta. Adv Enzyme Regul 50:81–94.

van Baal J, de Widt J, Divecha N, van Blitterswijk WJ. 2005. Translocation of diacylglycerol kinase theta from cytosol to plasma membrane in response to activation of G protein-coupled receptors and protein kinase C. J Biol Chem 280:9870–9878.

Wada I, Kai M, Imai S, Sakane F, Kanoh H. 1996. Translocation of diacylglycerol kinase alpha to the nuclear matrix of rat thymocytes and peripheral T-lymphocytes. FEBS Lett 393:48–52.

Walker AJ, Draeger A, Houssa B, van Blitterswijk WJ, Ohanian V, Ohanian J. 2001. Diacylglycerol kinase theta is translocated and phosphoinositide 3-kinase-dependently activated by noradrenaline but not angiotensin II in intact small arteries. Biochem J 353:129–137.

Yakubchyk Y, Abramovici H, Maillet JC, Daher E, Obagi C, Parks RJ, Topham MK, Gee SH. 2005. Regulation of neurite outgrowth in N1E-115 cells through PDZ-mediated recruitment of diacylglycerol kinase zeta. Mol Cell Biol 25:7289–7302.

Yamada K, Sakane F, Matsushima N, Kanoh H. 1997. EF-hand motifs of alpha, beta and gamma isoforms of diacylglycerol kinase bind calcium with different affinities and conformational changes. Biochem J 321(Pt 1):59–64.

Yamaguchi Y, Shirai Y, Matsubara T, Sanse K, Kuriyama M, Oshiro N, Yoshino K, Yonezawa K, Ono Y, Saito N. 2006. Phosphorylation and up-regulation of diacylglycerol kinase gamma via its interaction with protein kinase C gamma. J Biol Chem 281:31627–31637.

Yang J, Seo J, Nair R, Han S, Jang S, Kim K, Han K, Paik SK, Choi J, Lee S, Bae YC, Topham MK, Prescott SM, Rhee JS, Choi SY, Kim E. 2010. DGKiota regulates presynaptic release during mGluR-dependent LTD. J EMBO 30:165–180.

Yasuda S, Kai M, Imai S, Takeishi K, Taketomi A, Toyota M, Kanoh H, Sakane F. 2009. Diacylglycerol kinase eta augments C-Raf activity and B-Raf/C-Raf heterodimerization. J Biol Chem 284:29559–29570.

Yong VW, Dooley NP, Noble PG. 1994. Protein kinase C in cultured adult human oligodendrocytes: a potential role for isoform alpha as a mediator of process outgrowth. J Neurosci Res 39:83–96.

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