Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

HHS Public Access

Author manuscript

Adv Biol Regul. Author manuscript; available in PMC 2021 January 01.

Published in final edited form as:

Adv Biol Regul. 2020 January ; 75: 100688. doi:10.1016/j.jbior.2019.100688.

Roles of DGKs in neurons: postsynaptic functions?

Casey N. Barber[a], **Daniel M. Raben**[a,b]

[a]The Department of Biological Chemistry, The Johns Hopkins University School of Medicine, 725 North Wolfe Street, Baltimore MD, 21205

Abstract

Diacylglycerol kinases (DGKs) contribute to an important part of intracellular signaling because they generate phosphatidic acid (PtdOH). Recent research has led to the discovery of ten DGK isoforms, all of which are found in the mammalian brain. Many of these isoforms have studied functions within the brain, while others lack such understanding in regards to neuronal roles, regulation, and structural dynamics. However, while previously a neuronal function for DGKθ was unknown, it was recently found that DGKθ is required for the regulation of synaptic vesicle endocytosis and work is currently being conducted to elucidate the mechanism behind this regulation. Here we will review some of the roles of all DGKs and hypothesize additional roles. We will address the topic of redundancy among the ten DGK isoforms and discuss the possibility that DGKθ, among other DGKs, may have unstudied postsynaptic functions. We also hypothesize that in addition to DGKθ's presynaptic endocytic role, DGKθ might also regulate the endocytosis of AMPA receptors and other postsynaptic membrane proteins.

Keywords

Diacylglycerol Kinase; Neurons; Synapse; Neurotransmission; AMPARs; Synaptic Plasticity

1. Introduction

Phosphatidic acid (PtdOH) is an important lipid second messenger involved in numerous cellular functions. It is a component of biological membranes, the target and the product of enzymatic reactions, and a critical mediator in many signaling pathways. As a cone-shaped lipid, its role within biological membranes is continuously studied, with reports implicating its involvement in both exo- and endocytosis. This is especially noted in neurons, where synaptic vesicles are continually cycling through release and regeneration. The shape of PtdOH promotes negative membrane curvature, which is necessary for vesicle fusion with another membrane. PtdOH also binds to the SNARE protein syntaxin-1A, is the metabolic

[b]Corresponding Author: Department of Biological Chemistry, The Johns Hopkins University, School of Medicine, 725 North Wolfe St., Baltimore, MD 21205, draben@jhmi.edu.

We are submitting this manuscript, "Role of DGKs in neurons: postsynaptic function?" by Casey Barber and Daniel M. Raben. There are no conflicts of intersts.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

precursor to phosphatidylinositol 4,5-bisphosphate (PIP_2), and activates a phosphatidylinositol (PI) kinase. These and other roles make PtdOH a vital regulator of neurotransmission. While it can be made *de novo,* a large portion of PtdOH is produced by the metabolism of other molecules by lipid-metabolizing enzymes (50, 51).

Diacylglycerol kinase (DGK) phosphorylates diacylglycerol (DAG) to produce PtdOH. Because its product is such a valuable second messenger, the study of DGK structure, function, and regulation is of high concern as well. To date, ten mammalian DGKs (α, β, γ, δ, η, κ, ε, ζ, ι, θ) have been identified, although DGKs have also been found in *Escherichia coli, Drosophila melanogaster,* and *Caenorhabditis elegans.* This review will discuss solely the roles of the mammalian isoforms. These ten mammalian isoforms are categorized into five classes according to their structure. Type I DGKs (α, β, γ) contain two EF hand motifs, followed by two C1 domains and the catalytic domain. Type II DGKs (δ, η) have an N-terminal PH domain, followed by two EF hand motifs and two catalytic domains (δ also has a C-terminal EPH homology domain). The Type III DGK, ε, simply contains two EF hand motifs followed by the catalytic domain. Type IV DGKs (ζ, ι) have two C1 domains followed by a myristolated alanine rich protein kinase C substrate phosphorylation site like region (MARCKS homology domain), a catalytic domain, ankyrin repeats, and a C-terminal PDZ binding site. Finally, the Type V DGK, θ, contains three C1 domains and a central PH domain, followed by the catalytic domain. These classifications likely point to functional divisions as well. Type I DGKs are all calcium responsive to some extent because of the calcium binding EF hand motifs. Type II DGKs contain the PH domain, which some evidence suggests may bind PIs. Although DGKε doesn't have any additional distinctive domains, it is the only DGK to show substrate specificity-diacylglycerol with an arachidonoyl group at the *sn*-2 position. The Type IV DGKs have specific C-terminal ankyrin repeats and the Type V DGKθ is the only DGK with three C1 domains (52,53, 54). These structural similarities and distinctions produce isoforms with complex and diverse cellular functions.

Research on DGKs has been conducted for several decades, resulting in known functions for some isoforms, while others still remain unclear in certain cellular contexts. It is interesting that one group of proteins with the same primary function, phosphorylating DAG, can have such extensive roles in various tissues. Why are there ten redundant isoforms of the same primary molecule? How do their similarities and distinctions create such diverse functions? In this review, we will consider these questions while discussing some of the known roles of the DGK isoforms. Additionally, we will specifically consider DGKθ and its recently discovered role in the presynaptic bouton of neurons and discuss the possibility of a similar role in the postsynaptic bouton in the recycling of neurotransmitter receptors and other proteins.

2. Functions of DGKs

DGKα

Similar to many of the other DGK isoforms, DGKα seems to have roles in the central nervous system (CNS), cancer malignancies, and a few other unrelated roles. It is the only isoform found in the CNS selectively in glial cells and not in neurons. Specifically, DGKα is

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

expressed in oligodendrocytes, the cells in the CNS responsible for producing myelin. DGKα was found to co-localize with myelin basic protein (MBP), one of the main components of myelin, and DGKα is found in fractions of very purified myelin (1). This suggests that DGKα may be involved in the production, regulation, or maintenance of myelin in oligodendrocytes possibly by regulating the amounts of DAG available, although more research is required to confirm this hypothesis. Nonetheless, DGKα is a unique isoform of DGK because of its expression in glial cells. It would be interesting to consider if DGKα is expressed in any other glial cell types, or if that potential expression influenced neurotransmission.

In addition to oligodendrocytes, DGKα is also highly expressed in kidney and T-cells. While overexpression of DGKα has been correlated with increased NF-κ B signaling in melanoma cells, its inhibition has shown to upregulate the activity of natural killer cells, which could have an anticancer role. In summary, DGKα plays a role in T-cell anergy and T-cell activation can be rescued by knockdown or inhibition of DGKα. Accordingly, DGKα knockout (KO) mice have overactive T-cells resistant to anergy. It has also been shown that inhibition of DGKα in T-cells increases Ras-Erk signaling, which leads to promotion of T-cell activity. It is hypothesized that DGKα resides in the nucleus until its activation, upon which it translocates to the plasma membrane inner leaflet. It then metabolizes DAG to PtdOH, which regulates various oncogenic signaling pathways. Lack of DGKα can impact cancer cell viability and angiogenesis and might also act as an important anticancer protein by increasing T-cell activation (2).

This nuclear DGKα has also been shown to affect cell cycle progression. In 2016, Poli *et al.* found that changes in activity or expression of DGKα can alter the phosphorylation of the Retinoblastoma protein, which regulates the G1/S transition of the cell cycle. Thus, halting this progression induces cell cycle arrest, often apoptosis or autophagy. In this study, Poli *et al.* found DGKα is highly expressed in the nuclei of human erythroleukemia cell line K562 and its activity in the nucleus ensures successful progression from G1 to S phase (3). Similarly, DGKα expression is also found to be upregulated in human hepatocellular carcinoma (HCC) cell lines compared to expression in healthy liver. When DGKα expression is knocked down in these cell lines, cell proliferation is significantly decreased (4).

Additionally, DGKα is a potential target of immunotherapy for diabetic neuropathy (DN). DN is caused by an upregulation of DAG production which then leads to aberrant protein kinase C activation. By metabolizing DAG, DGK can ameliorate DN. However, Hayashi *et al.* specifically found that vitamin E mitigates several symptoms of DN in mice by activating DGKα, and this was not seen in DGKα−/− mice. Therefore, vitamin E activates DGKα specifically *in vitro* and *in vivo,* which leads to improvement of symptoms associated with DN (5).

DGKβ

While several isoforms of DGK are found in the brain, DGKβ is perhaps one of the most well-studied. Most of the literature on DGKβ is within the context of the nervous system, with downstream implications in neurological diseases. In 2001, 16 splice variants of the

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

human DGKβ were discovered, although splice variants have been found for other DGK isoforms as well. One DGKβ spliceform in particular, a 35 amino acid deletion from the C-terminus, has been correlated with bipolar disorder (BD) (7,8).

DGKβ is located at excitatory synapses of pyramidal neurons and also interneurons. DGKβ+ puncta are found on MAP2+ dendrites, adjacent to vGlut1+ and VGAT+ puncta and overlap with PSD95+ puncta, suggesting that it is expressed postsynaptically. Endogenous DGKβ expression increases with synaptogenesis and development, while overexpression of DGKβ leads to increased dendrite outgrowth at DIV7 and increased spine maturation at DIV14. It is expressed throughout different regions of the brain including the hippocampus, olfactory bulb, striatum, and the nucleus accumbens (6, 49).

DGKβ KO mice have been useful in studying the function of DGKβ in the CNS and its potential role in numerous neurological diseases. These mice have several psychomotor behavioral abnormalities, including reduced anxiety and depression, and hyperactivity. When given lithium as a mood stabilizer, the anxiety and hyperactive symptoms were reversed (9). DGKβ KO mice also show deficits in cognitive functions, exemplified by the Y-maze and Morris water-maze tests. These impairments in spatial and long-term memories are caused by reduced long-term potentiation (LTP) in the hippocampal CA1 region of DGKβ KO mice. Some morphological abnormalities have been observed as well, such as impairments in cortical spine formation. Primary cultures of hippocampal neurons from the DGKβ KO mice exhibit less dendritic branching and spine density than WT, and these phenotypes are rescued by the overexpression of DGKβ. In the hippocampal CA1 region, alterations in the PA and DAG content were also observed (10, 49).

DGKγ

Unfortunately, not much is known regarding a role for DGKγ in the CNS. However, DGKγ is expressed in the rat brain at birth and gradually increases throughout development, located in cerebellar Purkinje cells and the hippocampus. It is suggested that DGKγ might play a role in learning and memory because of its association with PKCγ, although more research is required to confirm this hypothesis (13). In humans, however, DGKγ is most highly expressed in the retina and interestingly, the rat form of DGKγ is calcium sensitive while the human form is not (12).

As several isoforms of DGK are associated with malignancies, there is evidence for a role of DGKγ in colorectal cancer as a tumor suppressor. Kai *et al.* 2017 analyzed methylation of the promoter CpG islands of DGK genes in colorectal cancer (CRC) cell lines and found that *DGKG,* the gene that encodes DGKγ, which is not methylated in normal, healthy colonic tissue, was hypermethylated in all CRC cell lines analyzed. Accordingly, *DGKG* expression is decreased in the CRC cell lines as compared to healthy tissue and is rescued in the cells upon treatment with the DNA methyltransferase inhibitor 5-aza-2′-deoxycytidine (5-aza-dC). Also, this methylation of *DGKG* was detected in colorectal adenomas, evidence that this methylation may occur during the early stages of colorectal tumorigenesis. Interestingly, the addition of both kinase-dead and constitutively active forms of DGKγ to CRC cell lines resulted in the slight inhibition of cell proliferation, migration, and invasion, and all forms of

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

DGKγ decreased the activity of Rac1 in CRC cells (11). A significant amount of additional research is required to learn more about the potential roles of DGKγ in other environments.

DGKδ

Similar to other DGK isoforms, DGKδ is correlated with several human diseases such as type II diabetes and epilepsy. DGKδ KO mice die shortly after birth, so much of the research is conducted with KO cell lines or human tissue. Early research discovered DGKδ KO mice embryos had decreased EGF receptor expression and activity. DGKδ knockdown or KO cells exhibited DAG accumulation, increased threonine phosphorylation of EGF receptor and increased phosphorylation of other PKC substrates, and increased PKC autophosphorylation. This work was among the first to suggest that DGKδ regulates EGF receptors by PKC signaling (14).

Later, it was discovered that DGKδ could play a role in type II diabetes. In skeletal muscle from human type II diabetes patients, DGKδ activity and expression are significantly reduced, a phenotype also seen in mouse models of the disease. In mice, this phenotype is reversed with the correction of hyperglycemia. The use of a DGKδ specific inhibitor in rat skeletal muscle resulted in a reduction in glucose transport through PKC signaling. DGKδ heterozygous mice exhibited an accumulation of DAG, reduced sensitivity to peripheral insulin and insulin signaling, and also glucose transport. These mice also displayed age-dependent obesity (15). These defects in glucose homeostasis suggest strong evidence for the involvement of DGKδ in type II diabetes.

In the rodent nervous system, DGKδ is significantly expressed in the midbrain and forebrain as early as E12.5. It is particularly expressed in pyramidal neurons of the cortex and hippocampus and granule cell neurons in the cerebellum. Leach *et al.* 2007 generated a DGKδ mutant “knockdown” mouse that resulted in the absence of DGKδ from the midbrain and forebrain, but preserved trace amounts of DGKδ in the cerebellum. These mice were viable and fertile with no obvious morphological or behavioral phenotypes. Fifty percent of the mutant mice studied displayed abnormal epileptic discharges and electrographic seizures, which lead the authors to suggest that this phenotype, together with DGKδ’s expression in the areas of the brain known to produce seizures, support a role for DGKδ in epilepsy (16).

There is also evidence to support a role for DGKδ in adipogenesis. There is an upregulation of DGKδ expression during the differentiation of 3T3-L1 preadipocyte cells to adipocytes, and inhibiting DGKδ expression also inhibits adipogenesis. It is hypothesized that DGKδ phosphorylates a specific pool of DAG required for differentiation (17, 18).

DGKη

A vast majority of the literature available on DGKη discusses its possible involvement with BD while many others focus on its correlation with cancer. In 2008, Baum *et al.* performed a genome-wide association study on over 550,000 single nucleotide polymorphisms (SNPs) in two independent case-control matched samples of BD. As a result, the strongest association signal was detected within the first intron of DGKη. This was the first study to propose a relationship between DGKη and BD, reporting a strong association with BD and three SNPs

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

in the *DGKH* gene, the gene encoding the DGKη protein (19). Another study also reported a relationship between BD in a Finnish family and one of the three previously reported SNPs (20). Similar studies describe associations between BD and DGKη in Sardinian and Chinese samples at the haplotype level (21, 22). Although these studies suggest DGKη expression is increased in patients with BD, another study has shown that DGKη expression is 25% higher in individuals with BD and schizophrenia, suggesting this phenotype isn't specific to BD (23).

Many other studies have associated DGKη with various malignancies. Yasuda *et al.* showed that DGKη was significantly expressed in stomach cancers as compared with adjacent healthy stomach tissue. In the same study, the deficiency of DGKη via knockdown resulted in reduced proliferation of HeLa cells (24). Crotty *et al.* also found that DGKη has a regulatory role in the Ras/B-Raf/C-Raf/MEK/ERK signaling pathway and that DGKη's overexpression could activate this pathway, evidence that DGKη serves as a scaffolding or adaptor protein in the complex (14). DGKη has also been found to be highly expressed in cancerous epithelium from mutant EGFR mice and mice with K-Ras lung cancer. Additionally, DGKη expression has been observed in human lung cancer cell lines having EGFR or K-Ras mutations, and the reduction of DGKη expression resulted in a growth deficiency of one of the K-Ras mutant cell lines (25).

DGKκ

DGKκ is another DGK isoform that has not received much attention. Its roles and regulation are less clear than other DGKs, with little to no expression in the CNS, but its expression has shown to be highest in testes and some expression in placenta. When expressed in HEK293 cells, DGKκ localizes primarily to the cell membrane (26).

One of the latest additions to the slim literature on DGKκ describes a potential relationship between DGKκ and hypospadias. Xie *et al.* found five SNPs in *DGKK,* the gene that encodes DGKκ, associated with hypospadias. This study was the first to propose a relationship between DGKκ and hypospadias among a Han Chinese population, specifically to mild or moderate cases of the disease (27).

DGKε

DGKε is a unique isoform in that it is the only one reported to exhibit substrate specificity. It prefers to phosphorylate DAG with an arachidonoyl acyl chain at the *sn-2* position *in vitro.* This preference is hypothesized to affect DAG insertion into the membrane and is the first piece of evidence to suggest that a DGK isoform discriminates between DAG substrates (28).

Some early research involving DGKε suggested a role in epilepsy and cardiac hypertrophy. Musto and Bazan used DGKε KO mice to investigate the role of DGKε in susceptibility to seizures. They placed tripolar electrode units into the hippocampi of male DGKε KO and WT mice and stimulated 6 times daily for 4 days with a subconvulsive electrical stimulation for 30-minute intervals to achieve kindling. The result was DGKε KO mice, compared to WT, experienced significantly fewer motor seizures and epileptic events beginning on the second day of stimulation. The authors hypothesize that DGKε regulates kindling

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

epileptogenesis, related to its substrate specificity. Because DGKε specifically phosphorylates arachidonate-containing DAG, which is blocked in DGKε KO mice, the deficiency of arachidonoyl-moiety inositol lipids contributes significantly to the signaling involved in epileptogenesis (29). Additionally, there is evidence that DGKε can restore cardiac dysfunction. $G\alpha_q$ protein-coupled receptor (GPCR) signaling plays a key role in cardiac hypertrophy, which incorporates both DAG and PKC signaling. Niizeki *et al.* created DGKε transgenic mice that overexpressed DGKε specifically in the heart, and these mice exhibited no difference in cardiac function or morphology compared to WT. To induce cardiac hypertrophy, DGKε transgenic mice and WT mice were subjected to continuous phenylephrine infusion or thoracic transverse aortic constriction (TAC). Afterwards, WT mice experienced increases in heart weight, cardiac dysfunction, and a decrease in survival rate. However, all of these phenotypes were abolished in the DGKε KO mice. This was the first report to suggest that DGKε has a role in cardiac function, which may be specifically related to the DAG populations and levels created by DGKε (30).

DGKε may also have a role in Huntington's disease (HD). DGK was identified after screening a kinase inhibitor library for molecules that block the cellular toxicity of Htt. Furthermore, after knocking down 4 of the DGK isoforms expressed in the brain (β, γ, ε, and ζ), DGKε specifically blocked Htt toxicity. Also, in a fly model of HD, a mutant DGKε with reduced function significantly improved motor dysfunction. Together, this evidence led to the hypothesis that DGKε is involved in the pathogenesis of HD (31).

DGKζ

DGKζ is also one of the most well-studied DGK isoforms, with studied roles in T-cells and the brain. There is also evidence of alternative splicing that creates multiple spliceforms of DGKζ, similar to other isoforms (32). An early study correlated DGKζ with T-cell maturation in the thymus by reporting that DGKζ works synergistically with DGKα. T-cell maturation occurs through a progression of CD4-CD8- double negative to CD4+CD8+ double positive to CD4+CD8- or CD4-CD8+ single-positive cells. Double KO of DGKζ and DGKα resulted in a significant decrease in the number of CD4+CD8- and CD4-CD8+ thymocytes accompanied by increased DAG accumulation and signaling in mice. This reduction is the product of impaired positive selection, but not negative selection, of these cells in the double KO mice. This developmental deficiency can be partially rescued by the exogenous addition of PtdOH. Also, a reduction in DGK activity is correlated with the promotion of thymic lymophomagenesis. Together, these data suggest that DGKζ activity, together with DGKα, is important for positive selection during T-cell maturation and development and tumor suppression (33).

There is also a report of what may be a key DGKζ interactor. This proteomic study identified sorting nexin 27 (SNX27), which is involved in intracellular trafficking and contains a PDZ and phox homology domain. Immunoprecipitation and two-hybrid analysis confirmed the interaction is direct and physically dependent on the PDZ domains of both proteins. In T lymphocytes, where DGKζ is highly expressed, SNX27 co-localizes with transferrin receptor-positive vesicles. When DGKζ shRNA was used to knockdown its expression in T lymphocytes, the transport of the transferrin receptor from the vesicle to the

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

plasma membrane was accelerated, highlighting DGKζ's role in vesicle trafficking (34). At the time, this was the only report that a DGK regulates vesicle recycling or membrane trafficking.

It is also known that DGKζ interacts with a family of proteins called syntrophins through both of the proteins' PDZ domains. Syntrophins and DGKζ are both highly abundant in cortical neurons and neuroblastoma N1E-115 cells throughout the cell body, neurites, and growth cones. Culturing N1E- 115 cells with serum prevents neurite outgrowth, but the overexpression of DGKζ in these cells induces neurite growth, presumably through DGKζ's association with the GTPase Rac1. DGKζ and Rac1 colocalize in neurites and together with syntrophin, form a complex. It is hypothesized that this complex regulates neurite outgrowth in neuronal cells (35).

Within neurons, DGKζ has been shown to be expressed specifically at excitatory synapses via interactions between its PDZ domain and the postsynaptic scaffolding protein PSD-95. Overexpression of DGKζ leads to a significant increase in dendritic spines, while knockdown causes a reduction in dendritic spines in culture. This effect is dependent on DGKζ catalytic activity and binding to PSD-95. *In vivo,* DGKζ KO mice exhibit reduced spine density and reduced excitatory transmission. Live imaging experiments suggested that DGKζ is involved in spine maintenance but not spine formation. Altogether, these data propose that DGKζ-PSD-95 binding couples PA production to dendritic spine maintenance (42).

DGKι

DGKι is another DGK isoform predominantly expressed in the CNS, so many published studies have aimed to study the role of DGKι in neurons. For example, DGKι is highly expressed in small-diameter dorsal root ganglia (DRG) neurons, which process pruritogenic (itch-causing) and algogenic (pain-causing) stimuli. DGKι KO mice possessed heightened sensitivity to histamine, but not other pruritogens, and *in vivo* calcium responses in DGKι KO DRG neurons were increased. However, basal pain sensitivity and responses to other pruritogenic or algogenic agents were unaffected. This suggests that DGKι is involved in regulating sensory and behavioral responses to histamine (36).

Because DGKι also contains a PDZ domain, it has also been shown to interact with PSD-95 family proteins localized at the postsynapse. DGKι KO mice exhibit a slight increase in presynaptic release possibility. Additionally, DGKι KO synapses, at neonatal stages, display a decrease in metabotropic glutamate receptor-dependent long-term depression (mGluR-LTD). This reduction incorporates a suppression of a decrease in presynaptic release probability. When the activity of PKC is inhibited, release probability and mGluR-LTD are normalized (37). These findings suggest that the presynaptic localization of DGKι, and therefore presynaptic changes in DAG signaling, affect mechanisms in the postsynapse such as LTD.

There is also some evidence that DGKι may work together with DGKη to affect manic and anxiety symptoms in mice. While 85% of pups born to WT mothers survive to weaning, only 30% of pups born to DGKι/DGKη double KO mothers survive to weaning, because of

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

poor maternal care. For example, pups raised by double KO mothers had significantly smaller or completely absent milk spots, pointing to a deficiency in the mothers' nursing. Dissimilar to WT, double KO mothers exhibited volatile and frightened behavior during cage handling. Significantly, double KO mothers displayed phenotypes of anxiety and mania, and these were not observed in any single KO mothers. These data suggest that DGKι and DGKη interact to affect maternal behavior during pup raising and may regulate mania and anxiety in female mice (38).

DGKθ

While known to be expressed in the CNS, a role for DGKθ in the brain was unknown until very recently, although much was known about its expression and some regulation. Cai *et al.* 2014 performed genome-wide DNA microarray analysis to find that eliminating DGKθ gene expression changed the expression levels of numerous other genes. For example, eliminating DGKθ suppressed the expression of sterol regulatory element binding proteins (SREBPs) and their downstream targets, while the expression of genes involved in the sphingolipid metabolic pathway such as acid ceramidase and sphingosine kinases was upregulated (39).

While the role of DGKθ and DGKθ-interacting proteins were previously unknown, Tu-Sekine *et al.* 2013 described evidence that DGKθ is regulated by or interacts with proteins with high percentages (10–20%) of basic amino acids and is also regulated by magnesium and zinc (40). Importantly, Goldschmidt *et al.* 2016 characterized and identified a role for DGKθ within the CNS. DGKθ is found throughout the adult mouse brain in cortex, hippocampus, cerebellum, olfactory bulb, and midbrain, and expression increases significantly after birth from P0 to P25. DGKθ is predominantly found in excitatory neurons, pre- and postsynaptically, and expression in glia is negligible. When DGKθ expression is eliminated in KO mouse cultures, or suppressed by transfection with a shRNA, rates of synaptic vesicle endocytosis after stimulation are significantly increased in comparison to WT neurons. The rates further increase with higher stimulation and are rescuable by the addition of WT DGKθ (41). Significantly, this was the first report to describe DGKθ's role in regulating the synaptic vesicle cycle.

All DGK Isoforms

The variety and range of functions and expression patterns of all the DGK isoforms is highlighted above (Table 1). In many ways it is incredible that isoforms of the same protein can have such vast differences in their roles and downstream effects. All DGK isoforms are alike in that they contain catalytic domains that phosphorylate DAG in a regulated, effective manner. Their differences reside in their domain variations, which lead to differences in interacting proteins, the proteins that regulate them, and perhaps subcellular localization. However, why are there ten DGK isoforms, some being relatively close in structure? Why and how did nature evolve this family of proteins to include some slight redundancy? Also, there is a lack of evidence that the various isoforms compensate for each other, demonstrated by knocking out one isoform and assaying all the remaining isoforms (41). This suggests that while perhaps structurally redundant to some extent, each isoform remains functionally distinct. How do their domain differences permit this? Most of the DGK isoforms still require much research to completely identify their roles and regulations within certain

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

contexts. It will be interesting in the future to examine the similarities and differences of the DGK isoforms, while considering the redundancy of domain structure and function.

3. New Potential Roles of DGKθ

Clearly, there are likely undiscovered roles for the neuronal DGKs, specifically DGKθ. DGKβ, ε, ζ, ι and θ are the most well-studied isoforms within the CNS. Their functional link to several neurological diseases suggests they are important both pre- and postsynaptically, and studies suggest that DAG and DGKs are involved in synaptic plasticity. DGKζ and ι have previously identified roles in the postsynapse and DGKθ's endocytic role could also be functional in postysynaptic endocytosis (48). Here, we hypothesize that DGKs have critical functions in the postsynapse, specifically DGKθ.

Postsynaptic Endocytosis

The predominant form of postsynaptic endocytosis involves the endocytosis of neurotransmitter receptors from the synapse and perisynapse. Neurotransmitter receptors such as AMPA, NMDA, and metabotropic receptors reside at the synapse, bind neurotransmitter ligands, and induce signaling pathways to propagate neurotransmission. Synaptic plasticity is the alteration of synaptic properties in response to activity that results in the strengthening or weakening of a synapse. Long-term potentiation (LTP) is the phenomenon in which receptors are trafficked to and inserted into the synapse, resulting in overall strengthening of the synaptic connection. Long-term depression (LTD) is the opposite effect, in which receptors are removed and trafficked away from the synapse, resulting in synaptic weakening. The details of receptor trafficking are not precisely understood, and several models exist that explain the possible mechanism of receptor insertion and removal from the synapse (44, 45, 46). At least one of these models involves the use of synaptic vesicles to transport receptors. These vesicle dynamics presumably function in similar manners to presynaptic endocytosis, in terms of vesicle fusion with the lipid membrane.

Postsynaptic Role for DGKθ

It isn't hard to imagine that DGKθ could have a role in postsynaptic endocytosis as well. At the presynapse, DGKθ regulates endocytosis after stimulation, and the mechanism of this regulation is currently unknown. However, DGKθ could be interacting with key endocytic proteins such as dynamin, it could be localized at very specific points to generate PA rafts, or a number of other mechanisms to regulate endocytosis of vesicles.

The endocytosis of AMPA receptors, in addition to other postsynaptic material, occurs at highly localized sites at the dendritic spine called endocytic zones. The endocytic zones are stable, punctate sites of clathrin within a few hundred nanometers of the postsynaptic density (PSD). At these sites, there is also a physical link between dynamin-3 and the postsynaptic adaptor protein Homer. Together, these proteins function to endocytose material, including the recycling of AMPA receptors in and out of the synapse. Additionally, the actin cytoskeleton not only performs scaffolding functions, but also has a physical link to endocytosis (43, 46, 47). Being as that most of the same endocytic machinery is at work in

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

the postsynapse as the presynapse, DGKθ may regulate or modulate this postsynaptic machinery. Perhaps DGKθ also creates sites of PA on the postsynaptic membrane that lend to the endocytosis of vesicles or further interacts with clathrin to maintain proper rates of endocytosis. Regardless of the mechanism, it is completely conceivable that DGKθ also functions to regulate the endocytosis of postsynaptic material including AMPA receptors, and may contribute to alterations in synaptic plasticity. This would include the θ isoform in the group of DGKs already known to have a function within the context of synaptic plasticity.

4. General Conclusion

The literature continuously adds to what is already known about the roles, expression, and regulation of the ten DGK isoforms in mammals. Conceptually, it is quite amazing that a protein family with slightly different domain characteristics can have such a wide variety of functions. From mitigating symptoms of diabetic neuropathy, to regulating synaptic vesicle dynamics, the roles of DGK seem limitless. Even within the CNS, DGKs function from regulating spine density to neurotransmission. Recent work has also correlated several DGK isoforms with synaptic plasticity, although a role for DGKθ in the postsynapse has not yet been described. However, we propose that DGKθ functions in the postsynapse similarly as it does in the presynapse by regulating the rates of endocytic vesicles that carry AMPA receptors. This could occur by DGKθ altering the lipid composition of the membrane in specific sites or by DGKθ interacting with and regulating a key endocytic protein such as clathrin or dynamin. We hypothesize that DGKθ regulates the endocytosis of AMPA receptors at the postsynapse, thereby offering a previously unacknowledged mechanism of synaptic plasticity regulation. More research is required to shed light on this potential mechanism. Nevertheless, DGK is a critically important signaling molecule within the CNS and beyond.

References

1. Goto K & Kondo H Diacylglycerol kinase in the central nervous system—molecular heterogeneity and gene expression. Chem Phys Lipids 98, 109–117 (1999). [PubMed: 10358933]
2. Purow B Molecular Pathways: Targeting Diacylglycerol Kinase Alpha in Cancer. Clin Cancer Res 21, 5008–5012 (2015). [PubMed: 26420856]
3. Poli A et al. Nuclear Localization of Diacylglycerol Kinase Alpha in K562 Cells Is Involved in Cell Cycle Progression. J Cell Physiol 232, 2550–2557 (2017). [PubMed: 27731506]
4. Takeishi K et al. Diacylglycerol kinase alpha enhances hepatocellular carcinoma progression by activation of Ras-Raf-MEK-ERK pathway. Journal of Hepatology 57, 77–83 (2012). [PubMed: 22425622]
5. Hayashi D et al. Diacylglycerol Kinase alpha is Involved in the Vitamin E-Induced Amelioration of Diabetic Nephropathy in Mice. Sci Rep-uk 7, 2597 (2017).
6. Hozumi Y, Watanabe M, Otani K & Goto K Diacylglycerol kinase β promotes dendritic outgrowth and spine maturation in developing hippocampal neurons. Bmc Neurosci 10, 99 (2009). [PubMed: 19691842]
7. Caricasole A et al. Molecular Cloning and Characterization of the Human Diacylglycerol Kinase β (DGKβ) Gene ALTERNATIVE SPLICING GENERATES DGKβ ISOTYPES WITH DIFFERENT PROPERTIES. J Biol Chem 277, 4790–4796 (2002). [PubMed: 11719522]
8. Hozumi Y et al. Diacylglycerol kinase beta accumulates on the perisynaptic site of medium spiny neurons in the striatum. Eur J Neurosci 28, 2409–2422 (2008). [PubMed: 19087171]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

9. Kakefuda K et al. Diacylglycerol Kinase β Knockout Mice Exhibit Lithium-Sensitive Behavioral Abnormalities. Plos One 5, e13447 (2010). [PubMed: 20976192]
10. Shirai Y et al. Essential Role of Neuron-Enriched Diacylglycerol Kinase (DGK), DGKβ in Neurite Spine Formation, Contributing to Cognitive Function. Plos One 5, e11602 (2010). [PubMed: 20657643]
11. Kai M et al. Epigenetic silencing of diacylglycerol kinase gamma in colorectal cancer. Mol Carcinogen 56, 1743–1752 (2017).
12. Kai M, et al. 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 (1994). [PubMed: 8034597]
13. Adachi N, et al. Immunocytochemical localization of a neuron-specific diacylglycerol kinase β and γ in the developing rat brain. Mol Brain Res 139, 288–299 (2005). [PubMed: 16019106]
14. Crotty T et al. Diacylglycerol kinase δ regulates protein kinase C and epidermal growth factor receptor signaling. Proc National Acad Sci 103, 15485–15490 (2006).
15. Chibalin A et al. Downregulation of Diacylglycerol Kinase Delta Contributes to Hyperglycemia-Induced Insulin Resistance. Cell 132, 375–386 (2008). [PubMed: 18267070]
16. Leach N et al. Disruption of Diacylglycerol Kinase Delta (DGKD) Associated with Seizures in Humans and Mice. Am J Hum Genetics 80, 792–799 (2007). [PubMed: 17357084]
17. Lowe C et al. Knockdown of diacylglycérol kinase delta inhibits adipocyte differentiation and alters lipid synthesis. Obesity 21, 1823–1829 (2013). [PubMed: 23703849]
18. Shulga Y et al. Diacylglycerol Kinase Delta Promotes Lipogenesis. Biochemistry-us 52, 7766–7776 (2013).
19. Baum A et al. A genome-wide association study implicates diacylglycerol kinase eta (DGKH) and several other genes in the etiology of bipolar disorder. Mol Psychiatr 13, 197 (2008).
20. Ollila HM, et al. Findings from bipolar disorder genome-wide association studies replicate in a Finnish bipolar family-cohort, Mol. Psychiatry 14, 351–353 (2009). [PubMed: 19308021]
21. Squassina A, et al. The diacylglycerol kinase eta gene and bipolar disorder: a replication study in a Sardinian sample, Mol. Psychiatry 14, 350–351 (2009). [PubMed: 19308020]
22. Zeng Z, et al. Common SNPs and haplotypes in *DGKH* are associated with bipolar disorder and schizophrenia in the Chinese Han population, Mol. Psychiatry 16, 473–475 (2011). [PubMed: 20733578]
23. Moya P, Murphy D, McMahon F & Wendland J Increased gene expression of diacylglycerol kinase eta in bipolar disorder. Int J Neuropsychoph 13, 1127–1128 (2010).
24. Yasuda S, et al. Diacylglycerol kinase η augments C-Raf activity and B-Raf/C-Raf heterodimerization, J. Biol. Chem. 284, 29559–29570 (2009). [PubMed: 19710016]
25. Nakano T et al. Diacylglycerol kinase η modulates oncogenic properties of lung cancer cells. Clin Transl Oncol 16, 29–35 (2014). [PubMed: 23572183]
26. Imai S, et al. Identification and characterization of a novel human type II diacylglycerol kinase, DGK kappa. J Biol Chem. 280, 39870–39881 (2005). [PubMed: 16210324]
27. Xie H et al. Association between diacylglycerol kinase kappa variants and hypospadias susceptibility in a Han Chinese population. Asian J Androl 20, 85–89 (2018). [PubMed: 28597849]
28. Shulga Y, Topham M & Epand R Substrate specificity of diacylglycerol kinase-epsilon and the phosphatidylinositol cycle. Febs Lett 585, 4025–4028 (2011). [PubMed: 22108654]
29. Musto A & Bazan N Diacylglycerol Kinase Epsilon Modulates Rapid Kindling Epileptogenesis. Epilepsia 47,267–276 (2006). [PubMed: 16499750]
30. Niizeki T et al. Diacylglycerol kinase-ε restores cardiac dysfunction under chronic pressure overload: a new specific regulator of Gα q signaling cascade. Am J Physiol-heart C 295, H245–H255 (2008).
31. Zhang N et al. Inhibition of Lipid Signaling Enzyme Diacylglycerol Kinase ϵ Attenuates Mutant Huntingtin Toxicity. J Biol Chem 287, 21204–21213 (2012). [PubMed: 22511757]
32. Ding L et al. Alternative splicing of the human diacylglycerol kinase ζ gene in muscle. Proc National Acad Sci 94, 5519–5524 (1997).

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

33. Guo R et al. Synergistic control of T cell development and tumor suppression by diacylglycerol kinase α and ζ. Proc National Acad Sci 105, 11909–11914 (2008).
34. Rincón E et al. Proteomics Identification of Sorting Nexin 27 as a Diacylglycerol Kinase ζ-associated Protein New Diacylglycerol Kinase Roles in Endocytic Recycling. Mol Cell Proteomics 6, 1073–1087 (2007). [PubMed: 17351151]
35. Yakubchyk Y et al. Regulation of neurite outgrowth in N1E-115 cells through PDZ-mediated recruitment of diacylglycerol kinase zeta. Molecular and Cellular Biology 25, 7289–7302 (2004).
36. Bartsch V, Niehaus J, Taylor-Blake B & Zylka M Enhanced histamine-induced itch in diacylglycerol kinase iota knockout mice. Plos One 14, e0217819 (2019). [PubMed: 31167004]
37. Yang J et al. DGKι regulates presynaptic release during mGluR-dependent LTD. The EMBO Journal 30, 165–180 (2011). [PubMed: 21119615]
38. Bartsch V, Lord J, Diering G & Zylka M Mania- and anxiety-like behavior and impaired maternal care in female diacylglycerol kinase eta and iota double knockout mice. Genes Brain Behav e12570 (2019). doi:10.1111/gbb.12570 [PubMed: 30985063]
39. Cai K, Lucki N & Sewer M Silencing diacylglycerol kinase-theta expression reduces steroid hormone biosynthesis and cholesterol metabolism in human adrenocortical cells. Biochimica Et Biophysica Acta Bba - Mol Cell Biology Lipids 1841, 552–562 (2014).
40. Tu-Sekine B, Goldschmidt H, Petro E & Raben D Diacylglycerol kinase θ: Regulation and stability. Adv Biological Regul 53, 118–126 (2013).
41. Goldschmidt H et al. DGKθ Catalytic Activity Is Required for Efficient Recycling of Presynaptic Vesicles at Excitatory Synapses. Cell Reports 14, 200–207 (2016). [PubMed: 26748701]
42. Kim K et al. Synaptic removal of diacylglycerol by DGKϵζ and PSD-95 regulates dendritic spine maintenance. Embo J 28,1170–1179 (2009). [PubMed: 19229292]
43. Lu J et al. Postsynaptic Positioning of Endocytic Zones and AMPA Receptor Cycling by Physical Coupling of Dynamin-3 to Homer. Neuron 55, 874–889 (2007). [PubMed: 17880892]
44. Huganir RL & Nicoll RA AMPARs and Synaptic Plasticity: The Last 25 Years. Neuron 80, 704–717 (2013). [PubMed: 24183021]
45. Roth R, Zhang Y & Huganir R Dynamic imaging of AMPA receptor trafficking in vitro and in vivo. Curr Opin Neurobiol 45, 51–58 (2017). [PubMed: 28411409]
46. Malenka R & Bear M LTP and LTD: An Embarrassment of Riches. Neuron 44, 5–21 (2004). [PubMed: 15450156]
47. Rosendale M, Jullié D, Choquet D & Perrais D Spatial and Temporal Regulation of Receptor Endocytosis in Neuronal Dendrites Revealed by Imaging of Single Vesicle Formation. Cell Reports 18, 1840–1847 (2017). [PubMed: 28228251]
48. Lee D, Kim E & Tanaka-Yamamoto K Diacylglycerol Kinases in the Coordination of Synaptic Plasticity. Frontiers Cell Dev Biology 4, 92 (2016).
49. Hozumi Y & Goto K Diacylglycerol kinase β in neurons: Functional implications at the synapse and in disease. Adv Biological Regul 52, 315–325 (2012).
50. Tu-Sekine B, Goldschmidt H & Raben D Diacylglycerol, phosphatidic acid, and their metabolic enzymes in synaptic vesicle recycling. Adv Biological Regul 57, 147–152 (2015).
51. Barber C, Huganir R & Raben D Phosphatidic acid-producing enzymes regulating the synaptic vesicle cycle: Role for PLD? Adv Biological Regul 67, 141–147 (2018).
52. Shirai Y, Ikeda M & Saito N Regulation of diacylglycerol kinase by phosphorylation. Adv Biological Regul 52, 239–247 (2012).
53. Shirai Y & Saito N Diacylglycerol kinase as a possible therapeutic target for neuronal diseases. J Biomed Sci 21, 28 (2014). [PubMed: 24708409]
54. Topham M & Prescott S Mammalian diacylglycerol kinases, a family of lipid kinases with signaling functions. The Journal of Biological Chemistry 274, 11447–11450 (2099).

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Table 1.

summarizes the location and role for each DGK isoform that is described in this review. When appropriate, the specific location within the brain is listed (neurons vs. glia). Roles with question marks denote roles that require significant additional research for confirmation.

DGK Isoform	Location	Role
α	brain- glia kidney T-cells	myelin production? T-cell anergy and activation cell cycle progression
β	brain- neurons	cognition psycomotor behavior LTP
γ	rat- brain human- retina	colorectal tumorigenesis?
δ	skeletal muscle brain	glucose homeostasis? adipogenesis seizures?
η	brain	bipolar disorder? cancer cell proliferation
κ	testes placenta	hypospadias?
ε	brain	seizures cardiac function pathogenesis of HD?
ζ	T-cells brain- neurons	T-cell maturation vesicle trafficking neurite outgrowth spine maintenance
ι	brain- neurons	histamine sensing mGluR-LTP rodent maternal behavior
θ	brain- neurons	synaptic vesicle endocytosis