Dramatic Differences in the Roles in Lipid Metabolism of Two Isoforms of Diacylglycerol Kinase[†]

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ABSTRACT: Lipid species changes for SV40-transformed fibroblasts from wild-type or from diacylglycerol kinase-ε (DGKε) or diacylglycerol kinase-α (DGKα) knockout mice were determined for glycerophospholipids, polyphosphatidylinositides ($GPInsP_n$) and diacylglycerol (DAG) using direct infusion mass spectrometry. Dramatic differences in arachidonate (20:4 fatty acid)-containing lipids were observed for multiple classes of glycerophospholipids and polyphosphatidylinositides between wild-type and DGKε knockout cells. However, no difference was observed in either the amount or the acyl chain composition of DAG between DGKε knockout and wild-type cells, suggesting that DGKε catalyzed the phosphorylation of a minor fraction of the DAG in these cells. The differences in arachidonate content between the two cell lines were greatest for the $GPInsP_n$ lipids and lowest for DAG. These findings indicate that DGKε plays a significant role in determining the enrichment of $GPInsP_n$ with 20:4 and that there is a pathway for the selective translocation of arachidonoyl phosphatidic acid from the plasma membrane to the endoplasmic reticulum. In contrast, no substantial difference was observed in the acyl chain composition of any class of glycerophospholipid or diacylglycerol between lipid extracts from fibroblasts from wild-type mice or from DGKα knockout mice. However, the cells from the DGKα knockout mice had a higher concentration of DAG, consistent with the lack of downregulation of the major fraction of DAG by DGKα, in contrast with DGKε that is primarily responsible for enrichment of $GPInsP_n$ with arachidonoyl acyl chains.

Eukaryotic diacylglycerol kinases (DGKs)[1] make up a family of 10 known isoforms playing important roles in signal transduction (*1*−*3*). In this work, we compare the properties of two isoforms of DGK, DGKα and DGKε. Although these two isoforms catalyze the same reaction, they have very different modes of interaction with membranes and different mechanisms of regulation. DGKα contains two E-F hands and is a Ca^{2+}-dependent enzyme (*4, 5*), although it can also exhibit Ca^{2+}-independent activity when binding to membranes rich in phosphatidylethanolamine (GPEtn) or cholesterol (*6*). DGKα is an amphitropic enzyme that binds to membranes only upon activation (*7, 8*). In contrast, DGKε is the only isoform with a hydrophobic segment that promotes attachment of the protein to membranes (*9*). In addition, DGKε is also unique in being the only isoform of DGK that has specificity for DAG containing arachidonoyl chains (*10, 11*) as well as other polyunsaturated acyl chains (*12*). Hence, the DAG derived from $GPIns(4,5)P_2$ hydrolysis by $GPIns(4,5)P_2$-specific phospholipase C (PLC) will also be arachidonoyl-rich as will the phosphatidic acid (GPA) formed as a product of DGKε-catalyzed phosphorylation. This process is cyclical, and the DAG that is phosphorylated by DGK to produce GPA, in turn, can be converted to $GPIns(4,5)P_2$ by reaction with CDP-inositol. We studied the contribution of DGKε in determining the enrichment of $GPIns(4,5)P_2$ with arachidonic acid.

Studies using DGKε-deficient mice have demonstrated a role for this isoform in regulating seizure susceptibility and long-term potentiation (*13, 14*). It has also been shown that upon electroconvulsive shock there was a reduced accumulation of arachidonoyl-DAG as well as free arachidonic acid (*14*). There is also a smaller reduction in the level of $GPIns(4,5)P_2$ in the knockout mice upon electroconvulsive

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[1] Abbreviations: DGK, diacylglycerol kinase; RT, reverse transcriptase; DAG, diacylglycerol; GPA, phosphatidic acid; GPEtn, phosphatidylethanolamine; GPCho, phosphatidylcholine; GPIns, phosphatidylinositol; GPGro, phosphatidylglycerol; GPSer, phosphatidylserine; PLC, phospholipase C; $GPIns(4,5)P_2$, phosphatidylinositol 4,5-bisphosphate; WT, wild type; KO, DGKε knockout or DGKα knockout; $GPInsP_n$, polyphosphatidylinositides; ESI−LC−MS/MS, electrospray ionization−liquid chromatography−tandem mass spectrometry.

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shock (*14*). In this work, we study the role of DGKε in the distribution of arachidonoyl side chains among different lipids in the basal state without stimulation. Another isoform, DGKα, is a negative regulator of antigen-mediated triggering of the T-cell receptor (*15*). This isoform has chemotactic, proliferative, and angiogenic activity (*16*). Studies with DGKα knockout mice demonstrated an impairment of anergy induction (*17*). T-cells from these knockout mice produce more interleukin-2 and show an increased level of proliferation in response to T-cell receptor activation (*17*). DGKα is expressed in several human melanoma cell lines but not in noncancerous melanocytes (*18*). It has also recently been found that this isoform can play an important role in the metastasis of certain breast cancers (*19*). Hence, DGKα and DGKε appear to have different biological functions. We have undertaken a lipidomics study to determine if there are also differences in lipid processing resulting from the deletion of each of these two DGK isoforms.

EXPERIMENTAL PROCEDURES

Tissue Culture. Mouse fibroblasts were obtained from embryos of mice that were made deficient in DGKε (−/−) (*14*) or in DGKα (−/−) (*17*). In each experiment, these cells were compared with wild-type embryonic fibroblasts obtained from siblings of the (−/−) mice. These cells derived from DGKε (+/+) or DGKα (+/+) embryos are designated WT. All cells were immortalized by transfection with the SV40 large T antigen. Cells were cultured in DMEM supplemented with 10% fetal bovine serum, 25 mM HEPES, and a 1% penicillin/streptomycin solution at 37 °C in a humidified atmosphere with 5% CO_2.

Expression Levels of DGK in Cell Lines. We compared the level of mRNA of DGKε or DGKα from the mouse embryonic fibroblast cell lines described above using reverse transcriptase (RT) PCR. These levels were compared with those of actin mRNA. Briefly, total RNA was isolated from each of the four cell lines, and 1 μg of RNA was reverse transcribed using Moloney-murine leukemia virus (MMLV) reverse transcriptase. Using 3% of the cDNA, DGKα, DGKε, and actin were amplified for 36 cycles by annealing at 64 °C for 1 min and extending at 72 °C for 1 min. The primers used for DGKα were 5′-ATGGCCAAAGAGAAGGGCCTC and 5′-GCAGTGGCACTGTGGTAGCCC, and those for DGKε were 5′-CTCAGTTCGAGTCCTTGTTTG and 5′-ATAGACGCCAACGATTTCCAG. Equal aliquot of the resulting reaction mixture was subjected to agarose gel electrophoresis.

Glycerophospholipid Analysis. Global lipid extracts were prepared via a modified Bligh−Dyer extraction procedure as described previously (*20*). Briefly, cells were scraped in 800 μL of an ice-cold 0.1 N HCl/methanol mixture (1:1), and 400 μL of $CHCl_3$ was added to the suspension. The samples were vortexed for 1 min; layers were separated by centrifugation at 18000*g* for 5 min at 4 °C, and 20 μL of an internal standard containing equimolar amounts of 22:0 GPCho (1,2-diundecanoyl-*sn*-glycero-3-phosphocholine) and 28:0 GPA (1,2-dimyristoyl-*sn*-glycero-3-phosphate) was added to the organic layer before the sample was dried in vacuo. Dried lipids were redissolved in 80 μL of a CH_3OH/$CHCl_3$ mixture (9:1). Mass spectral analysis was performed on a Finnigan TSQ Quantum triple-quadrupole mass spectrometer (ThermoFinnigan, San Jose, CA) equipped with a Harvard Apparatus syringe pump and electrospray source. Samples were analyzed at an infusion rate of 10 μL/min in both positive and negative ionization modes over the range of *m*/*z* 350−1200. Data were collected with the Xcalibur software package (ThermoFinnigan) and analyzed with software developed in our laboratory as described in ref *21*. Identification of individual glycerophospholipids was accomplished by tandem mass spectrometry (ESI−LC−MS/MS). Extracted lipids were dissolved in 100 μL of a 58:40:2 2-propanol (IPA)/hexane/100 mM $HCOONH_4$(aq) mixture (mobile phase A). We utilized an Applied Biosystems/MDS SCIEX 4000 Q TRAP hybrid triple quadrupole/linear ion trap mass spectrometer (Applied Biosystems, Foster City, CA). Coupled to this instrument was a Shimadzu (Shimadzu Scientific Instruments, Inc., Columbia, MD) HPLC system consisting of a SCL 10 AVP controller, two LC 10 ADVP pumps, and a CTC HTC PAL autosampler (Leap Technologies, Carrboro, NC). All samples were separated on a Phenomenex (Torrance, CA) Luna Silica column (2 mm × 250 mm, 5 μm particle size) using a 20 μL sample injection. Lipids were separated using a binary gradient program consisting of a 58:40:2 IPA/hexane/100 mM $HCOONH_4$(aq) mixture (mobile phase A) and a 50:40:10 IPA/hexane/100 mM $HCOONH_4$(aq) mixture (mobile phase B). The following LC gradient was used: 50% B from 0 to 5 min, 50 to 100% B from 5 to 30 min, 100% B from 30 to 40 min, 100 to 50% B from 40 to 41 min, and 50% B from 41 to 50 min (delivered at a flow rate of 0.3 mL/min) This analysis results in class separation and fragmentation of the individual species within the class,thus allowing for a more precise identification (*20, 22*).

Phosphatidylinositol Phosphate (GPInsP_n) Determination. Polyphosphatidylinositide extraction was performed as described previously (*23*), with the following modifications. Dried lipids were rapidly redissolved in 55 μL of a 1:1:0.3 $CHCl_3$/CH_3OH/H_2O mixture. Before analysis, 5 μL of 300 mM piperidine(aq) and 1 μL of (0.11 M) internal standard 1,2-dioctanoyl-*sn*-glycero-3-phosphoinositol-4,5-bisphosphate were added to each sample, and the sample was vortexed and centrifuged briefly prior to mass spectral analysis by direct infusion on a MDS SCIEX 4000 Q TRAP hybrid triple quadrupole/linear ion trap mass spectrometer (Applied Biosystems).

DAG Analysis. Analysis and quantification of DAG species from cell extracts were performed according to the procedure described in ref *24*. After phospholipid extraction by the modified Bligh−Dyer procedure, diacylglycerols were isolated by separation using normal phase column chromatography and isocratic elution with a 65:35:0.7 $CHCl_3$/CH_3OH/H_2O mixture. 1,2-Dilauroyl-*sn*-glycerol (100 ng) was used as an internal standard and added to each sample before column separation.

RESULTS

RT-PCR of DGK. The results of the RT-PCR analysis confirm that the wild-type (WT) cells express both DGKα and DGKε. However, no mRNA for DGKα or for DGKε was detected in the respective knockout (KO) cell lines

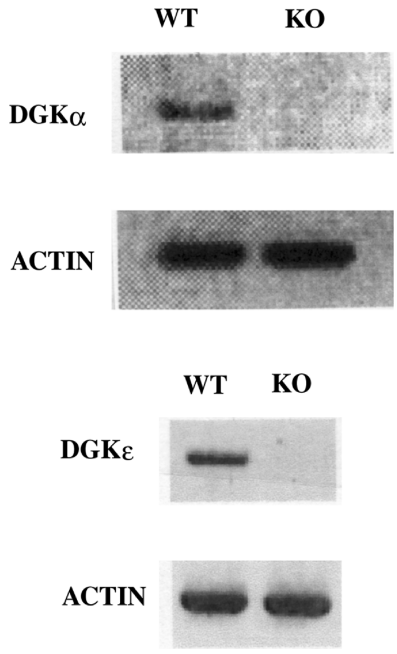


FIGURE 1: RT-PCR detection of mRNA for DGKα and actin in DGKα WT and KO cells and of mRNA for DGKε and actin in DGKε WT and KO cells.

(Figure 1). As a positive control, we show that mRNA for actin is detected in comparable amounts in the KO and WT cell lines.

Phospholipid Composition of Wild-Type versus DGKε Knockout Cells. Glycerophospholipid profiles for six major classes show significant differences in arachidonate-containing species between the WT and KO cells. The arachidonate-containing lipids are compared to all others that do not possess this acyl substituent between WT and KO cells (Figure 2). The lipid class showing the greatest difference in arachidonoyl content between WT and KO cells is polyphosphoinositides ($GPInsP_n$), which is the sum of all phosphatidylinositols, including GPIns, GPInsP, $GPIns(4,5)P_2$, and $GPIns(3,4,5)P_3$. More specifically, the deletion of DGKε decreases the 38:4 GPIns content from approximately 33 to 24% of the GPIns lipid pool. To compensate for this, it appears that the KO cells increase their level of incorporation of oleate (18:1 fatty acid) into the inositol lipid fraction (Figure 3). The identity of each glycerophospholipid component was resolved by tandem mass spectrometry, and their fatty acyl composition was determined. These data are compiled in the tables provided as Supporting Information. In the case of 36:2 GPIns, for example, 18:1/18:1 fatty acids are the predominant species. The 36:2 GPIns seems to have a significantly higher content in the KO fibroblasts with an increase from ~6 to ~11% of the class. Within the $GPInsP_n$ lipids, a separate analysis showed that there was a marked change in arachidonate- and oleate-containing lipids following knockout of DGKε for GPIns, GPInsP, and $GPIns(4,5)P_2$ (Figure 4). Due to the low abundance of basal $GPIns(3,4,5)P_3$, neither 36:2 nor 38:4 $GPIns(3,4,5)P_3$ was detectable in these experiments.

The glycerophosphatidic acid (GPA) lipids had a pattern similar to that observed in the GPIns lipid pool, although the differences between KO and WT were somewhat reduced. Again in this class, 38:4 GPA was the most affected by the deletion of DGKε, with the KO cells having reduced levels of 38:4 GPA. To balance this loss, the KO cells have increased amounts of saturated and mono-unsaturated fatty acid-containing species such as 36:2 GPA. Glycerophosphatidylethanolamine (GPEtn) lipids have a more complex lipid pattern due to the presence of plasmanyl-containing (ether) and plasmenyl-containing (vinyl ether) lipids in addition to the usual diacyl lipids. Numerous arachidonate-containing GPEtn lipids (38:4, 38:5, 38:6, and the 38:4 and 38:5 plasmenyl/plasmanyl GPEtn for example) all contribute less to the KO fibroblast GPEtn lipid pool than to the WT cells. However, overall, the difference in arachidonoyl-containing GPEtn between KO and WT cells is much smaller than the differences observed for GPIns. Glycerophosphatidylcholines (GPCho) also had easily observable differences in arachidonate-containing lipid profiles following DGKε knockout. The 38:4 GPCho contributes more to the total GPCho lipid pool in the WT cells compared to the KO cell type. To compensate for the loss of 38:4 GPCho, it appears that the KO cells had a higher fraction of saturated and mono-unsaturated fatty acid-containing GPCho (Figure 5). Thus, in summary, most lipid classes exhibit more 38:4 than 36:2 lipids in the WT than in the KO cells, indicating a loss of arachidonoyl-containing lipids.

We have extensively characterized the glycerophospholipid species from both WT and DGKε KO cell extracts by ESI−LC−MS/MS (Tables 1 and 2 of the Supporting Information). As a result of this analysis, 366 species were identified in WT and 405 species in DGKε KO cell extracts. Only two of the phospholipid classes show marked differences in terms of the number and variety of identified species. More phosphatidylserine (GPSer) (50 species vs 33) species and a rare phospholipid, phosphatidylthreonine (GPThr) (*25*) (20 species vs 7), were ascertained in the DGKε KO cell extracts.

We also determined the ratio of GPA to GPIns for both WT and DGKε KO cells (Figure 6). This ratio was significantly higher for the DGKε KO cells ($p = 0.0014$ by ANOVA with random effects). The analysis of DAG species from both genotypes reveals no significant differences. The lack of measured changes in DAG might have resulted because there were only trace levels of 38:4 DAG in both WT and KO cell lines. The majority of the lipids present were from the 32, 34, and 36 series of DAGs.

Phospholipid Composition of Wild-Type versus DGKα Knockout Cells. Analysis of the glycerophospholipids from WT and DGKα KO cells was also performed. The cellular phospholipid pattern did not have broad, statistically significant differences in glycerophospholipid species between the WT and KO cells. In GPIns, only 36:4 has a modest but significant decrease in class percentage (Figure 7). The difference in the ratio of GPA to GPIns between the DGKα KO and WT cells was marginally significant ($p = 0.0498$ by ANOVA with random effects) (see Figure 6).

Comprehensive ESI−LC−MS/MS analysis of the glycerophospholipid species in these two phenotypes did not reveal any significant differences. Four hundred seven phospholipid species were identified in WT and 348 species in DGKα KO cell extracts (Tables 3 and 4 of the Supporting Information). In contrast, the analysis of DAG species revealed some interesting differences between WT and KO cells. Here too, the majority of the DAG species were from the 32, 34, and 36 series, but unlike in the DGKε KO case,

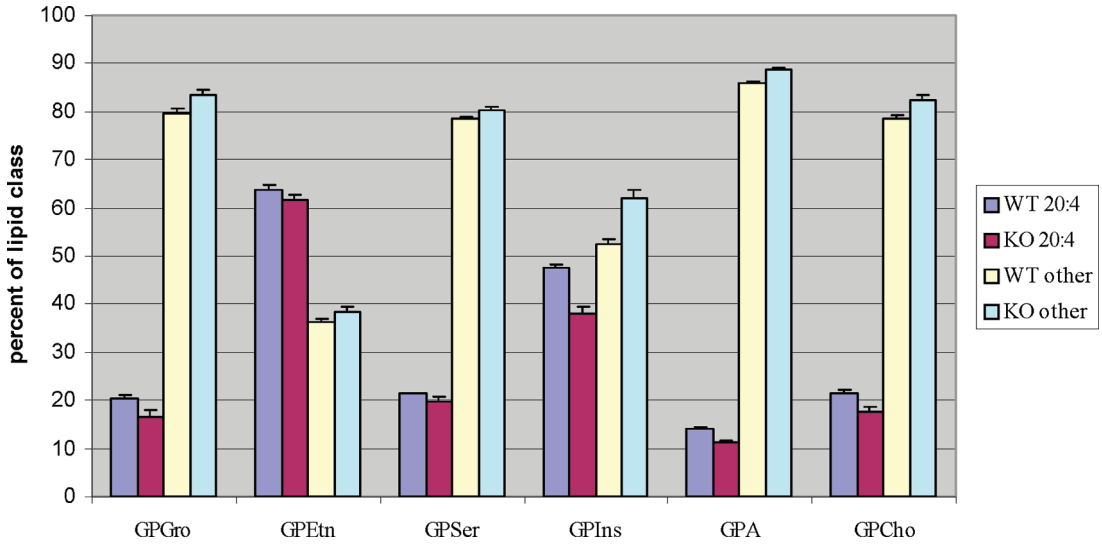


FIGURE 2: Lipid profiles for the six major glycerophospholipid classes. DGKε knockout and WT cells have statistically significant differences in arachidonate-containing lipids.

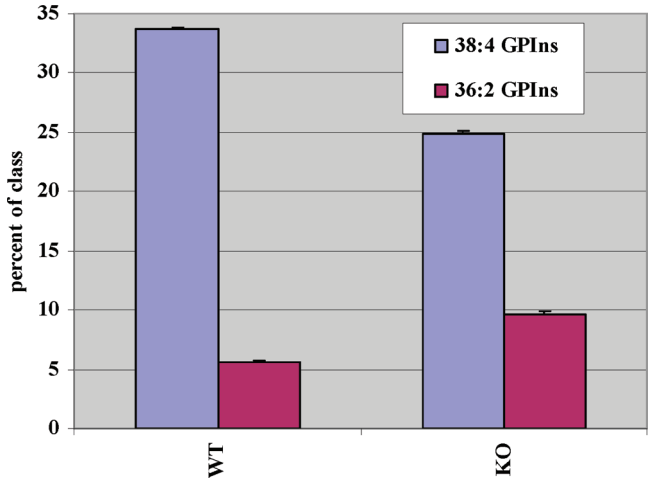


FIGURE 3: Lipid profiles for two major phosphatidylinositol lipids from triplicate samples of WT and KO cells. DGKε knockout and WT cells have statistically significant differences in arachidonate-containing 38:4 GPIns. WT 38:4 GPIns comprises a higher percentage of the GPIns lipid class than does DGKε knockout 38:4 GPIns. The opposite trend is observed for 36:2 GPIns. The data shown are from one of three representative experiments that were performed. All three experiments had statistically significant differences in lipid profiles similar to those shown.

there were marked changes in the quantity of DAG species in the extracts from DGKα KO cells. The amount of DAGs is almost double in the KO samples compared to the WT samples. The largest differences occur in saturated and mono- and di-unsaturated DAG species (Figure 8).

DISCUSSION

The two isoforms of DGK that we studied in this work are known to have different biological functions. One reason for this is that these enzymes are more strongly expressed in different organs. DGKε is found principally in brain, retina, and cardiac muscle, while DGKα is found mainly in white blood cells, spleen, and thymus. Both DGKα and DGKε are expressed in the WT cell lines that we used, but they are not expressed in the corresponding KO cell line (Figure 1). This work demonstrates that these two isoforms of DGK also have very different properties on a biochemical level. We suggest that DGKε is involved more specifically in GPInsP$_n$ cycling while DGKα contributes to regulating the bulk concentration of DAG in the cell from all sources.

DGKε contributes significantly to the arachidonoyl enrichment of GPIns(4,5)P$_2$ since the KO cells have less arachidonoyl-GPIns(4,5)P$_2$ (Figures 3 and 4). This is the case in spite of the fact that the specificity of DGKε for arachidonoyl-containing forms of DAG is only ~10-fold using in vitro assays (*9*). Even more surprising is the increase in the level of arachidonoyl enrichment between the formation of GPA by DGKε and its conversion to GPIns(4,5)P$_n$ (Table 1). In this work, we observed that the arachidonoyl content of DAG is only ~3% and there is little difference between KO and WT cells. This increases to an arachidonoyl content of GPA of slightly more than 10%. The increase is smaller for KO cells than for WT cells, indicating that DGKε contributes to the arachidonoyl enrichment of GPA, which is expected because of the arachidonoyl specificity for substrates phosphorylated by this DGK isoform. There are also other sources of GPA, in addition to DGKε, including other DGK isoforms as well as that from phospholipase D-catalyzed reactions. In the case of phospholipase D, substrates with an arachidonoyl group at the *sn*-2 position of the glycerol backbone are not favored by the enzyme (*26*), indicating that this is not a source of arachidonoyl enrichment.

The GPA that is formed can be converted back into GPIns(4,5)P$_2$. The first step in this conversion is the synthesis of GPIns, catalyzed by CTP:phosphatidate cytidylyltransferase. This is the rate-limiting step in the synthesis of GPIns(4,5)P$_2$ (Scheme 1). This step takes place in the endoplasmic reticulum and not in the plasma membrane. The enzyme exhibits some specificity for 1-stearoyl-2-arachidonoyl phosphatidic acid as substrate under certain in vitro conditions (*27*). However, to explain the large increase we observe in arachidonoyl content upon going from GPA to GPIns(4,5)P$_2$ (Table 1), there must be some segregation of arachidonyl-GPA from other forms of GPA. In other words, the lipids involved in inositol cycling must be segregated from other lipids. There are two membranes involved in this process, the endoplasmic reticulum and the plasma membrane (Scheme 1). The process starts in the plasma membrane, as

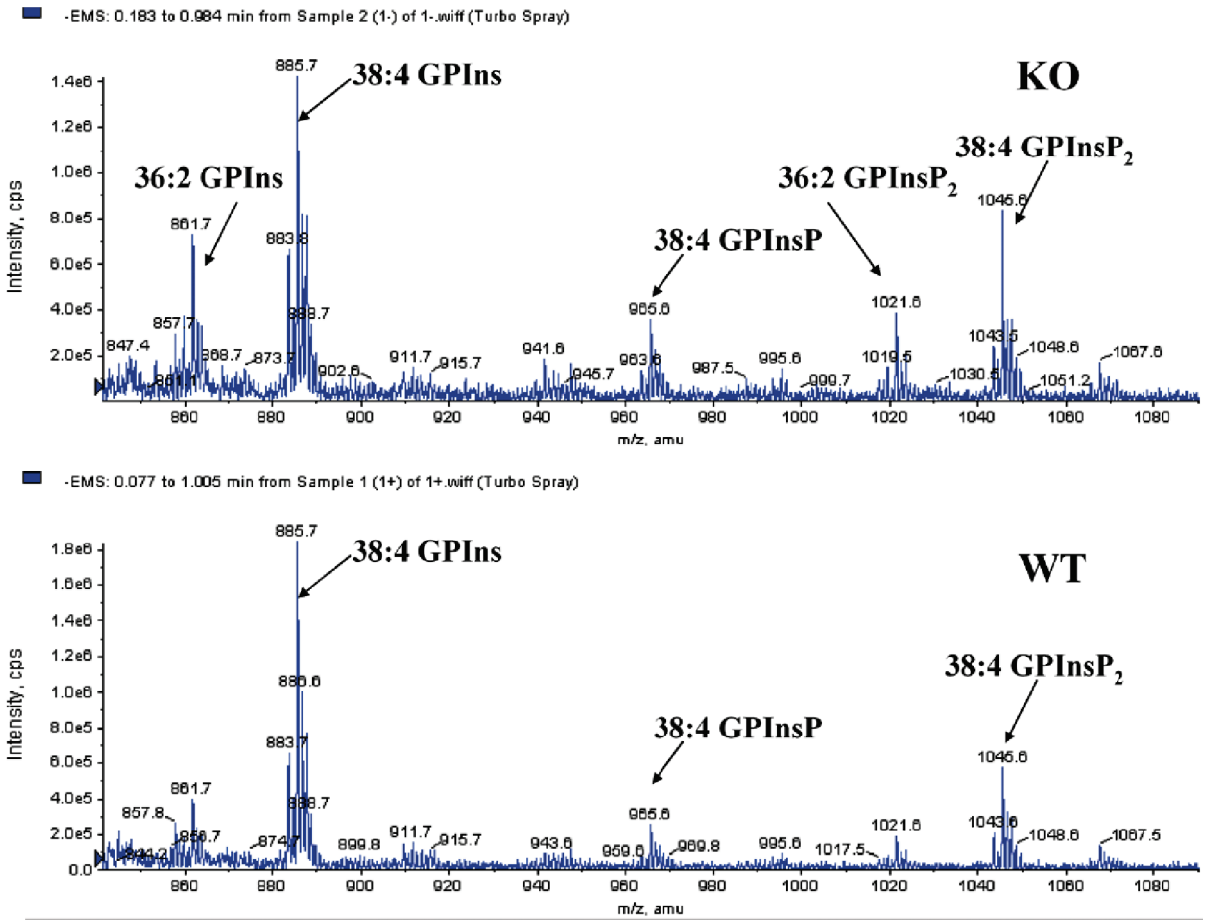


FIGURE 4: Phosphatidylinositol polyphosphate mass spectral region of KO and WT mouse fibroblast extracts. DGKε knockout leads to a dramatic increase in the level of non-arachidonate-containing lipid species.

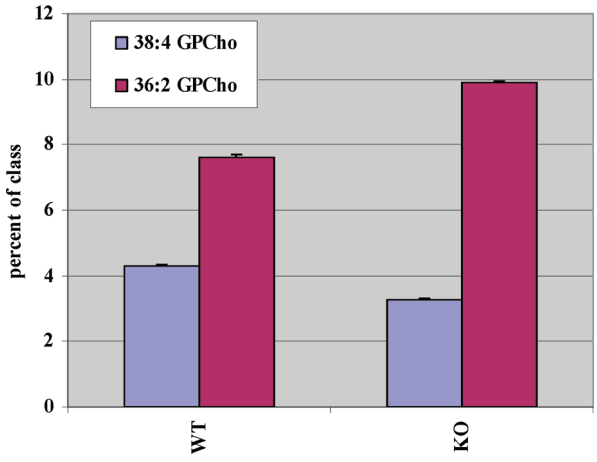


FIGURE 5: Lipid profiles for two major phosphatidylcholine lipids from one experiment (triplicate samples of WT and KO). DGKε knockout and WT cells have statistically significant differences in levels of arachidonate-containing 38:4 GPCho. WT 38:4 GPCho comprises a higher percentage of the GPCho lipid class than does DGKε knockout 38:4 GPCho. The opposite trend is observed for 36:2 GPCho. Two additional experimental sets were also analyzed (both of which had statistically significant differences in lipid levels similar to those shown).

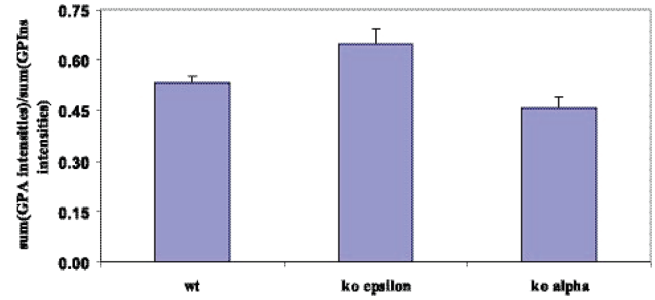


FIGURE 6: Ratio of GPA to GPIns lipids in WT, DGKα knockout, and DGKε knockout cells. The DGKε knockout GPA:GPIns ratio was higher than that of the WT samples. Knockout of DGKα led to a decrease in the GPA:GPIns ratio compared to the WT cells.

a consequence of hormone stimulation, activating a GPIns-(4,5)P_2-specific phospholipase C that will convert an arachidonoyl-rich GPIns(4,5)P_2 to an arachidonoyl-rich DAG. The arachidonoyl-rich DAG is phosphorylated preferentially by DGKε to form an arachidonoyl-rich GPA. The arachidonoyl-GPA must then be selectively transformed into GPIns lipids. This requires the transfer of GPA between the two membranes, a process that is not dependent on soluble lipid carriers or on vesicular transport (*28*) but may occur at membrane contact sites formed between the plasma membrane and endoplasmic reticulum (*29*). There is evidence in yeast that such contact sites are enriched in synthases for GPSer and GPIns lipids (*31*). The close approach of these two membranes is also indicated by the fact that the tyrosine phosphatase, PTP-1B, in the endoplasmic reticulum acts on protein substrates of the plasma membrane (*32*). A possible explanation of our results is that DGKε at these contact sites would preferentially transfer arachidonoyl-containing GPA. This transfer occurs more readily in the presence of DGKε since the increase in arachidonoyl content between GPA and GPIns lipids is greater for the WT cells than for the KO

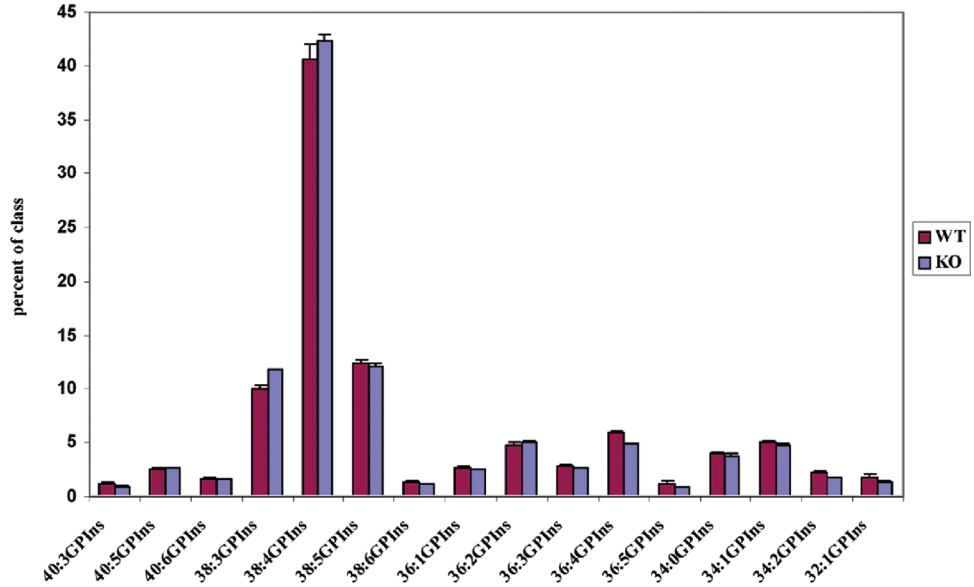


FIGURE 7: Lipid profiles for the major phosphatidylinositol lipids. DGKα knockout and WT cells have virtually no statistically significant differences in any GPIns lipids. The same is true for almost all glycerophospholipids.

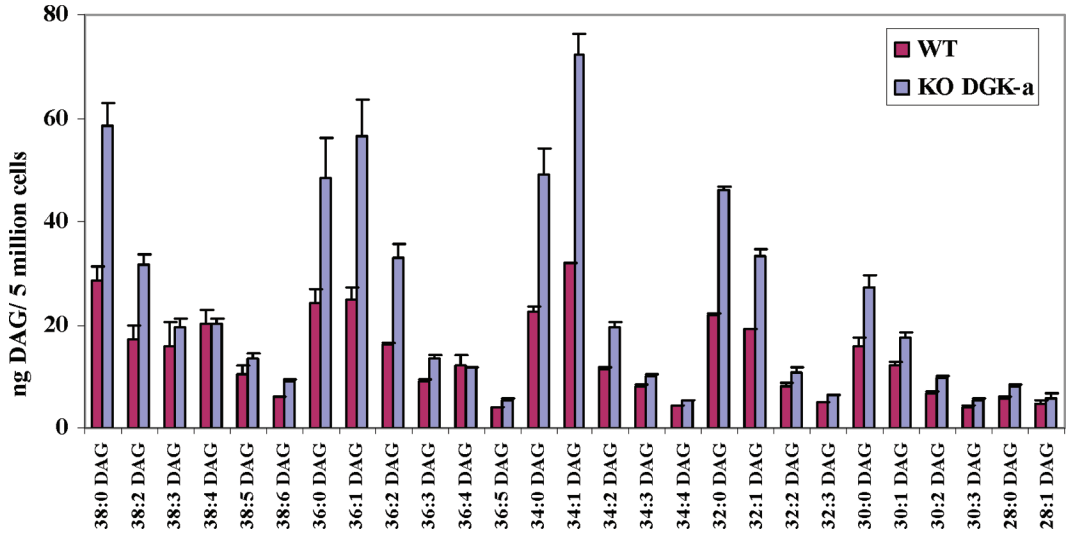


FIGURE 8: Lipid profiles for the major diacylglycerol lipids. DGKα knockout and WT cells had statistically significant differences in multiple DAG lipids. No significant differences were observed in the DGKε knockout experiments.

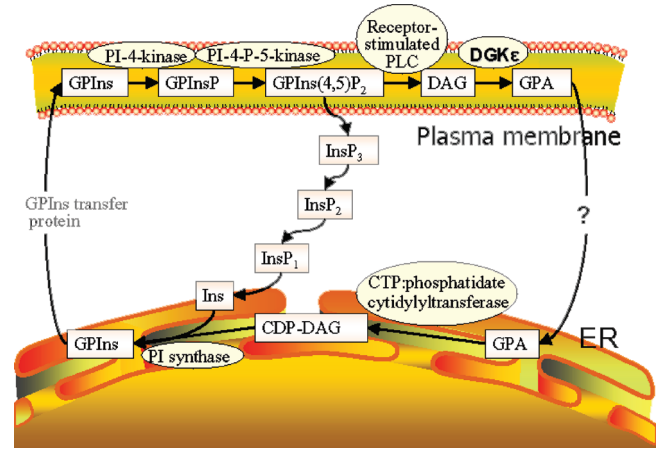
Table 1: Percentages of Lipid with Arachidonoyl Chains in DGKε Cells[a]

lipid	% PL	KO	WT
DAG	−	3	3
GPA	1	11	13
$GPInsP_n$	5−10	38	48

[a] Approximate percentage of the total lipid for each of the phospholipid classes taken from values given in ref *35*. The DAG concentration in a nonstimulated cell is expected to be <1%. KO and WT columns indicate the percentage of each lipid class found to contain arachidonic acid.

cells. The process may also involve the DGKε that is present in the endoplasmic reticulum (not shown in Scheme 1) (*30*), possibly at contact sites where it can directly transfer the arachidonoyl-containing GPA to GPIns synthase, also known to be present at contact sites (*31*). We find that the ratio of GPA to GPIns is higher for DGKε KO cells than for either WT or DGKα KO cells (Figure 6). This is consistent with the suggested promotion of transfer of GPA between membranes by DGKε. In the DGKε KO cells, transfer of

Scheme 1



GPA to the endoplasmic reticulum is slower and hence the concentration of this lipid increases, resulting in the change in the observed GPA:GPIns ratio. The transferred GPA would then be converted into arachidonoyl-rich GPIns and

returned to the plasma membrane. The cycle would also be driven by GPA itself since this lipid regulates the transcription of GPIns synthase and is an allosteric regulator of type I phosphatidylinositol phosphate kinase (*33*). Once formed, it has been found that the $GPInsP_n$ lipids do not undergo remodeling (*34*). Thus, a combination of the selective phosphorylation of arachidonoyl-DAG by DGKε together with the preferential transfer of arachidonoyl-GPA from the plasma membrane to the endoplasmic reticulum, in which DGKε participates in a noncatalytic role, determines the enrichment of $GPIns(4,5)P_2$ with arachidonoyl groups. A fraction of the arachidonoyl chains are also transferred to other phospholipids, resulting in some arachidonoyl enrichment of several lipid classes (Figure 2), but the enrichment of the $GPInsP_n$ lipids is clearly the greatest. Thus, in addition to its arachidonoyl specificity in forming GPA, DGKε must also have a noncatalytic role in increasing the arachidonoyl content of $GPInsP_n$.

The role of DGKα is quite different. This isoform causes little change in the acyl chain composition of any class of phospholipid, including the $GPInsP_n$ (Figure 7) or the DAG. This result is consistent with the lack of acyl chain specificity observed when DGKα is tested in in vitro DAG kinase assays. However, the DAG content of DGKα KO cells increases significantly (Figure 8). Thus, unlike DGKε, DGKα plays an important role in regulating the total DAG in the cells since the absence of this isoform in DGKα KO cells leads to an increase in the level of the DGK substrate, DAG.

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SUPPORTING INFORMATION AVAILABLE

Lists of identified lipid species for DGKε WT (Table 1), DGKε KO (Table 2), DGKα WT (Table 3), and DGKα KO (Table 4) cells. This material is available free of charge via the Internet at http://pubs.acs.org.

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