Protein Kinase C \(\delta\) (PKC\(\delta\)) Inhibits the Expression of Glutamine Synthetase in Gliial Cells via the PKC\(\delta\) Regulatory Domain and Its Tyrosine Phosphorylation* \\

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Protein kinase C (PKC) plays an important role in the proliferation and differentiation of glial cells. In a recent study we found that overexpression of PKC\(\delta\) reduced the expression of the astrocytic marker glutamine synthetase (GS). In this study we explored the mechanisms involved in the inhibitory effect of PKC\(\delta\) on the expression of glutamine synthetase. Using PKC chimeras we first examined the role of the catalytic and regulatory domains of PKC\(\delta\) on the expression of glutamine synthetase. We found that cells stably transfected with chimeras between the regulatory domain of PKC\(\delta\) and the catalytic domains of PKC\(\alpha\) or \(\epsilon\) inhibited the expression of GS, similar to the inhibition exerted by overexpression of PKC\(\delta\) itself. In contrast, no significant effects were observed in cells transfected with the reciprocal PKC chimeras between the regulatory domains of PKC\(\alpha\) or \(\epsilon\) and the catalytic domain of PKC\(\delta\). PKC\(\delta\) has been shown to undergo tyrosine phosphorylation in response to various activators. Tyrosine phosphorylation of PKC\(\delta\) in response to phorbol 12-myristate 13-acetate and platelet-derived growth factor occurred only in chimeras which contained the PKC\(\delta\) regulatory domain. Cells transfected with a PKC\(\delta\) mutant (PKC\(\delta\)mut), in which the five putative tyrosine phosphorylation sites were mutated to phenylalanine, showed markedly diminished tyrosine phosphorylation in response to phorbol 12-myristate 13-acetate and platelet-derived growth factor and normal levels of GS. Our results indicate that the regulatory domain of PKC\(\delta\) mediates the inhibitory effect of this isoform on the expression of GS. Phosphorylation of PKC\(\delta\) on tyrosine residues in the regulatory domain is implicated in this inhibitory effect. 

Protein kinase C (PKC)\(^1\) is a family of phospholipid-dependent serine-threonine kinases that play important roles in the signal transduction and in the regulation of cell growth and differentiation (1, 2). At least eleven isozymes of PKC have been isolated so far, showing diversity in their structures, cellular distributions, and biological functions (3, 4). PKC is also the receptor for the potent tumor-promoting phorbol esters, which can substitute for DAG in PKC activation (5). The “classical” cPKCs \(\alpha, \beta, \beta, \gamma\) are \(\text{Ca}^{2+}\)-dependent and PMA-responsive. The “novel” nPKCs \(\delta, \epsilon, \gamma, \text{and } \theta\) are \(\text{Ca}^{2+}\)-independent but PMA-responsive, whereas the atypical PKC isoforms \(\zeta\) and \(\lambda\) do not depend on \(\text{Ca}^{2+}\) or respond to PMA. PKC\(\delta\), a member of the novel PKCs, has been associated with proliferation and differentiation of various cell types (6, 7). For example, overexpression of PKC\(\delta\) inhibited the proliferation of fibroblasts and induced monocytic differentiation of the myeloid progenitor cell line 32D (8). PKC\(\delta\) has also been shown to be downstream in the signaling pathway of the PDGF receptor (9). PKC\(\delta\) has been reported to undergo tyrosine phosphorylation in response to various stimuli such as PMA, epidermal growth factor, and PDGF (10–12). The phosphorylation site(s) and the role of tyrosine phosphorylation of PKC\(\delta\) in its activity and in its functional role are just beginning to be defined. 

All PKC isoforms consist of an N-terminal regulatory domain and a C-terminal catalytic domain with serine-threonine kinase activity (1). In the classical PKC isoforms the regulatory domain contains a \(\text{Ca}^{2+}\)-binding domain, and in both the classical and novel PKC isoforms the regulatory domain contains a pseudosubstrate region near the N terminus that is thought to inhibit the activity of the catalytic domain (13–15). The regulatory domain also contains a pair of highly conserved zinc fingers that bind phorbol esters (16). One of the approaches for dissecting the role of the regulatory and catalytic domains of different PKC isoforms is the use of PKC chimeras between the regulatory and catalytic domains of the different isoforms. 

Glutamine synthetase (GS) is an important enzyme in the conversion of the excitatory amino acid glutamate to glutamine (17). GS has been used as an astrocytic marker, and its regulation has been studied especially with regard to cAMP (18) and glucocorticoids (19). A recent study suggested an important role for GS as a mediator of interactions between neurons and glial cells under pathological conditions (20). Thus, understanding the factors or mechanisms involved in the regulation of this enzyme has significant implications in understanding pathological processes in the central nervous system. 

Recently, we reported that overexpression of PKC\(\delta\) in the C6 glial cell line reduced the expression of the astrocytic marker GS, whereas overexpression of PKC\(\alpha\) and PKC\(\epsilon\) did not significantly affect the expression of this protein (21). We have now characterized the inhibitory effect of PKC\(\delta\) on GS expression using chimeras between the regulatory and catalytic domains of PKC\(\delta\), \(\alpha\), and \(\epsilon\). We found that the regulatory domain of PKC\(\delta\) mediates its inhibitory effect on GS expression. Furthermore, tyrosine phosphorylation in the regulatory domain of PKC\(\delta\) is implicated in this inhibition. 

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‡ The abbreviations used are: PKC, protein kinase C; PMA, phorbol 12-myristate 13-acetate; GFAP, glial fibrillary acidic protein; GS, glutamine synthetase; PAGE, polyacrylamide gel electrophoresis; PDGF, platelet-derived growth factor; Bryo, bryostatin 1.
EXPERIMENTAL PROCEDURES

Materials—Monoclonal anti-glutamine synthetase antibody, monoclonal anti-GFAP antibody, and anti-PKCα, β, γ, δ, η, θ, ι, and μ were obtained from Transduction Laboratories (Lexington, KY). Protein AG-Sepharose was purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA). An affinity purified polyclonal anti-PKCδ antibody against a polypeptide corresponding to amino acids 726–737 of PKCδ was purchased from Life Technologies, Inc. Leupeptin, aprotonin, phenylmethylsulfonyl fluoride, and sodium vanadate were obtained from Sigma. Polyclonal rabbit antibody against GFAP was purchased from DAKO Corp. (Carpinteria, CA).

Generation of PKC Chimeras—The PKC chimeras were generated by exchanging the regulatory and catalytic domains of PKCα, δ, and ε as described by Asc et al. (22). PKCαδ refers to the chimera with the PKCα regulatory domain and the PKCδ catalytic domain, and PKCδδ refers to the reciprocal chimera. The PKC εDNAs were subcloned into the mammalian e-epitope tagging mammalian expression vector MTH described in detail by Ohal et al. (23). The vector was attached to the end of the C-terminal 12-amino acid tag, originally derived from the C-terminal sequence of PKCε. The e-tag or the overexpression did not affect the localization, translocation, or phosphotyrosine expression of the wild type constructs relative to the respective endogenous isoforms (22, 24).

Site-directed Mutagenesis of PKCδ—Mouse PKCδ was subcloned into the pGEM-T vector (Promega, Madison, WI) as described previously (25). This plasmid served as our “master” vector for the site-directed mutagenesis, using the Transformer site-directed mutagenesis kit from CLONTECH (Palo Alto, CA). Conversion of tyrosine residues at sites 52, 155, and 565 into phenylalanine was performed as described (25). The additional mutations of tyrosine residues at positions 64 and 187 to phenylalanine were introduced into the triple tyrosine-phenylalanine mutant of PKCδ. The following oligonucleotides served as primers for selection and mutagenesis. The selection primer GCGCTGAGGTCGACATTATGGGAG was used to change back the formerly introduced NcoI restriction site (25) in the pGEM-T vector in position 75 into the underlined Sall restriction site to facilitate selection. The primer AGGCCCAATCTTCCAGAGGCCGTGTATTAC was used to mutate the tyrosine residue at site 64 into phenylalanine (bold letter) and to create a BsrBI restriction site (underlined) to facilitate the detection of mutant plasmids. The primer CTCAGAAAGCTTATACTGCGGACATG was used to mutate the tyrosine residue at site 187 into phenylalanine (bold letter) and to create a Dral restriction site (underlined) to facilitate the detection of the mutant plasmids. The mutations were confirmed by direct sequencing (Paragon Biotech Inc., Baltimore, MD).

Cell Cultures and Cell Transfection—C6 cells of early passage (C6–30 cells) were used in this study. Conditions for growth and transfection were as described previously (21). Experiments were routinely carried out on pools of transfected cells, but all the results were confirmed on two different individual clones for each of the different isoforms and chimeras.

Preparation of Cell Homogenates and Immunoblot Analysis—Preparation of lysates from cells and the analysis of GS and of PKC by Western blotting were performed as described previously (21, 26).

[^H]PDBu Binding—[^H]PDBu binding was measured using the polyethylene glycol precipitation assay (27). Briefly, cell lysates (4–60 μg of protein/assay) were incubated with 20 nM[^H]PDBu in the presence of phosphatidylserine. Non specific binding, determined in the presence of 30 μM nonradioactive PDBu, was subtracted to give specific binding. Data represent triplicate determinations in each experiment.

PKC Kinase Assay—PKC activity was assayed by measuring the incorporation of [32P]P from [γ-32P]ATP into substrate in the presence of 100 μM phosphatidylserine and 1 μM PMA as described previously (27).

Immunoprecipitation—Immunoprecipitation of PKCδ was performed as described previously (22). Briefly, C6 cells overexpressing PKCδ or PKCε were serum-starved overnight and treated for 30 min with PMA (10 nM) or with PDGF (100 ng/ml). The samples were preabsorbed with 25 μL of protein AG-Sepharose (50%) for 10 min, and immunoprecipitation was performed using 4 μg/ml anti-PKCδ antibody and 30 μL of protein AG-Sepharose at 4 °C. Following washes, the pellets were resuspended in 25 μL of SDS sample buffer and boiled for 5 min. Before SDS-PAGE, samples were centrifuged again as described above, and all of each supernatant was subjected to Western blotting. Membranes were probed with anti-phosphotyrosine antibody or anti-PKCδ antibody.

RESULTS

Differential Effects of PMA and bryostatin 1 on GS and PKCδ Expression—Recently, we have reported that overexpression of PKCδ reduced the level of GS expression in C6 cells, whereas it did not affect the expression of the astrocytic marker GFAP (21). To further examine the role of PKCδ in this effect we utilized the two PKC activators PMA and bryostatin 1 because these two activators have been reported to differentially affect the activation and down-regulation of PKCδ in other cell types (26, 29). For these studies we used the C6–30 cells because they express low levels of GS and GFAP and can be differentiated to either oligodendrocytes or astrocytes (30). Treatment of the cells with PMA (100 nM) for 24 h did not induce significant changes in the expression of GS, as described previously. In contrast, bryostatin 1 (100 nM) induced a marked decrease in the expression of GS (Fig. 1A), whereas it did not affect the expression of GFAP (data not shown). As illustrated in Fig. 1B, under these conditions 100 nM PMA induced down-regulation of all the major classical and novel PKC isoforms expressed in C6–30, whereas bryostatin 1 induced down-regulation of all these PKC isoforms except PKCδ. PKCε and μ were not affected by either of the compounds. Similar down-regula-
the role of PKC and Western blot analysis. The membranes were probed with anti-PKC antibody that recognizes the \( \varepsilon \)-tag. The immunoreactive bands for the different PKC chimeras were visualized as described under “Experimental Procedures.” Results are from one representative experiment; similar results were obtained in each of three additional experiments. IB, immunoblot.

**Overexpression of PKC Chimeras**—To further characterize the role of PKC\(\delta\) in the inhibition of GS expression, we examined the relative contributions of the regulatory and catalytic domains of this isoform. For these studies, we used chimeras between the regulatory and catalytic domains of PKCa, \(\delta\) and \(\varepsilon\), combined at the highly conserved hinge region. The chimeras were engineered in a way that allowed similar construction of the wild type PKC isoforms, thus providing us with exact positive controls (22). The presence of the \(\varepsilon\)-tag on the different constructs allowed better detection on Western blots and provided a good epitope for immunoprecipitation.

Cells were transfected with the different chimeras, PKC \(\alpha/\delta\), \(\delta/\varepsilon\), \(\varepsilon/\alpha\), \(\varepsilon/\delta\), and \(\delta/\varepsilon\) and with the PKC wild type isoforms PKC\(\alpha/\beta\), \(\beta/\delta\), and \(\varepsilon/\varepsilon\). To examine the level of protein expression, we analyzed by Western blotting three pooled cultures and five different overexpressing clones for each of the chimeras as well as for the vector controls. Fig. 2 illustrates a representative Western blot of C6 cells overexpressing the different PKC chimeras and the vector control. Using the previously described tagging system (23), we were able to detect specifically the transfected PKC isoforms with the antibody against PKCe. The band corresponding to the endogenous PKC\(\varepsilon\) can also be seen. To establish that the overexpressed PKC chimeras were functionally active we measured specific \(^{3}H\)PDBu binding and kinase activity on cell lysates. All PKC chimeras exhibited increased \(^{3}H\)PDBu binding and kinase activity as compared with the control cells transfected with the empty vector (Table 1). Moreover, binding and kinase activity were further enhanced in cells treated for 24 h with 20 \(\mu M\) ZnCl\(_{2}\), an inducer of the metallothionein expression vector (data not shown).

**GS Levels in Cells Overexpressing PKC Chimeras**—The effect of the PKC chimeras on the level of GS was examined. In cells overexpressing PKC\(\delta/\varepsilon\), the level of GS was low as compared with control cells and similar to that reported previously for cells overexpressing wild type PKC\(\delta\). In contrast, the level of GS was unchanged in cells overexpressing PKCa/\(\delta\) and \(\varepsilon/\alpha\) (Fig. 3A) or in cells overexpressing PKCa/\(\varepsilon\) (data not shown).

**Expression of glutamine synthetase in C6 cells overexpressing PKC chimeras.** C6 cells expressing different chimeras or the empty vector (M) were incubated in the absence or presence of 10 \(\mu M\) PMA for 48 h (A). Results are from one representative experiment; similar results were obtained in each of four additional experiments. The different transfectants were incubated for 24 h with 20 \(\mu M\) ZnCl\(_{2}\) (B). Densitometric analysis of the immunoblots was performed on five different clones and three pooled cultures. The results represent the means ± S.E. of three separate experiments. White bars, medium; shaded bars, zinc.

| PKC chimeras | \(^{3}H\)PDBu bound | Kinase activity |
|--------------|---------------------|-----------------|
| M            | 1.98 ± 0.09         | 100             |
| PKCa/\(\varepsilon\) | 6.21 ± 0.03         | 251 ± 28        |
| PKC\(\delta/\beta\) | 5.67 ± 0.20         | 231 ± 29        |
| PKC\(\varepsilon/\delta\) | 8.5 ± 0.12         | 377 ± 32        |
| PKC\(\delta/\alpha\) | 7.78 ± 0.09         | 396 ± 48        |
| PKC\(\delta/\varepsilon\) | 8.15 ± 1.02         | 364 ± 41        |
| PKC\(\delta/\varepsilon\) | 6.23 ± 0.12         | 238 ± 37        |
| PKC\(\delta/\varepsilon\) | 6.23 ± 0.13         | 254 ± 29        |
GS levels similar to those of cells expressing PKCa/α or PKCe/ε (Fig. 3A).

Treatment of the cells with 10 nM PMA, which induces activation of PKC without the marked down-regulation that occurs at 100 nM PMA, enhanced the response observed in untreated cells (Fig. 3A). Thus, PMA further reduced the expression of GS in cells overexpressing PKC δδ or the chimeras δα and δε, which contain the δ regulatory domain. Similar to what was observed in the vector control cells, transfectants expressing PKC α/α or ε/ε or chimeras that contain the δ catalytic domain showed a smaller decrease in GS expression presumably mediated by the endogenous PKCδ. Similar results were obtained in transfectants expressing PKC α/ε and ε/α (data not shown).

We also examined the expression of GS in cells treated for 24 h with 20 μM ZnCl2. Under these conditions, cells overexpressing PKCδ/δ or chimeras containing the regulatory domain of PKCδ showed a larger decrease in GS expression as compared with PMA-treated cells, whereas cells expressing chimeras that contain the δ catalytic domain had levels of GS similar to those of the control vector cells (Fig. 3B).

Tyrosine Phosphorylation of PKCδ Occurs in the Regulatory Domain—The translocation and activation of PKCδ in response to PMA and physiological stimuli such as epidermal growth factor and PDGF have been shown to be associated with tyrosine phosphorylation of this isoform (10–12). However, the sites of phosphorylation and the role of this process in the activity and function of PKCδ are still unclear.

We first examined which domain is involved in the tyrosine phosphorylation of PKCδ. Cells overexpressing PKCδ/δ or the various chimeras containing either the regulatory or the catalytic domains of PKCδ were stimulated with PMA and PDGF. Following immunoprecipitation of PKCδ and Western blotting, membranes were probed with anti-phosphotyrosine antibody or with anti-PKCδ antibody. As can be seen in Fig. 4, PKCδ/δ, PKCα/α, and PKCε/ε underwent tyrosine phosphorylation in response to PMA. In contrast, no tyrosine phosphorylation was observed in cells overexpressing PKCα/δ or PKCe/δ, thus suggesting that tyrosine phosphorylation in response to these stimuli occurred on the regulatory domain of the enzyme.

Overexpression of a PKCδ Mutant That Lacks Five Tyrosine Residues—Studies in vitro (25) and in vivo (12) suggested five putative phosphorylation sites in PKCδ, one in the catalytic domain (Tyr585) and four in the regulatory domain (Tyr264, Tyr267, Tyr135, and Tyr187). Because four of the putative phosphorylation sites in PKCδ are located in the regulatory domain, we examined the role of tyrosine phosphorylation of PKCδ in its inhibitory effect on GS expression.

For these experiments we utilized a PKCδ mutant in which the five putative tyrosine phosphorylation sites were mutated to phenylalanine (δ5 mutant). Cells were transfected with PKCδ or PKCδ5, and the level of expression was determined using Western blot analysis. Using the previously described tagging system (23), we were able to detect specifically the transfected PKCδ isoforms with the antibody against PKCe. Fig. 5A illustrates a representative Western blot of C6 cells overexpressing PKCδ, PKCδ5, and the vector control. The levels of the overexpressed PKCδ and PKCδ5 in the transfected cells were about 7–9-fold higher than the endogenous PKCδ as determined using an anti-PKCδ specific antibody (data not shown). Cells overexpressing PKCδ or PKCδ5 showed an increased level of [3H]PDBu binding from 1.45 ± 0.04 pmol/mg protein in the vector control cells to 7.29 ± 0.21 pmol/mg protein in cells transfected with PKCδ and 8.82 ± 0.23 pmol/mg protein in cells transfected with PKCδ5. Although they had similar levels of [3H]PDBu binding, cells overexpressing PKCδ had an increased kinase activity of 465 ± 49% over the vector control.
cells, whereas cells overexpressing PKCδ5 showed an increase of only 87 ± 11% over control cells. The transfected PKCδ and δ5 displayed similar localization in untreated cells and translocated similarly to the membrane in response to PMA (data not shown).

Tyrosine Phosphorylation of PKCδ Plays a Role in the Inhibitory Effect on GS Expression—We first examined the degree of tyrosine phosphorylation of PKCδ and PKCδ5 in response to PMA and PDGF. Cells were treated with either compound for 30 min, PKCδ was immunoprecipitated using the e-tag specific antibody, and membranes were stained with anti-phosphotyrosine antibody. As illustrated in Fig. 5B, PMA and PDGF induced marked tyrosine phosphorylation of PKCδ within 5–30 min. In contrast, these compounds induced negligible tyrosine phosphorylation of the PKCδ5 mutant. We then examined the level of GS in cells that were overexpressing PKCδ or PKCδ5 and were either untreated or treated with PMA (10 nM) or PDGF (100 ng/ml). PMA (10 nM) activates PKC without the marked down-regulation that occurs at a concentration of 100 nM. We found that cells overexpressing the PKCδ5 mutant displayed a slightly higher level of GS compared with the control vector cells and markedly higher levels compared with cells overexpressing PKCδ wild type. Treatment of cells overexpressing PKCδ with PMA (10 nM) or PDGF (100 ng/ml) induced a marked decrease in GS expression. In contrast, similar treatments of cells expressing the δ5 mutant did not exert a significant effect on GS expression (Fig. 5C).

DISCUSSION

Protein kinase C comprises a family of eleven closely related isoforms that are involved in the differentiation and proliferation of various cell types (1–3). In a recent study, we have shown differential roles of specific PKC isoforms in the proliferation and differentiation of glial cells (21). Specifically, PKCα and PKCδ inhibited astrocytic differentiation by reducing the expression of the astrocytic markers GFAP and GS, respectively. In the present study we have explored the mechanisms involved in the inhibitory effect of PKCδ on GS expression. We found that the regulatory domain of PKCδ is responsible for the inhibitory effect of this isoform on the expression of GS. Chimeras have been used to dissect the contribution of individual PKC domains to the specific functions of different PKC isoforms, and both the catalytic and the regulatory domains of PKC have been implicated in determining isoform-specific functions. For example, the catalytic domain of PKCβ was found to confer isoform-specific function in the differentiation of erythroleukemia cells (32), and the catalytic domain of PKCδ in reciprocal δ- and ε-chimeras mediated PMA-induced macrophage differentiation of mouse promonocytes (33). In contrast, the regulatory domain of PKCε enhanced cell growth induced colonies in soft agar in NIH 3T3 cells, and the catalytic domain played a role in determining the saturation density of cell growth (34). Thus, it appears that either the regulatory or the catalytic domain can confer isoform-specific effects and that this depends on the cell type, the specific PKC isoform and the specific function studied.

PKCδ has been shown to become tyrosine phosphorylated in response to various stimuli. In NIH 3T3 cells, PKCδ is tyrosine phosphorylated upon PMA and PDGF stimulation (10, 12), in response to carbachol, substance P, and PMA stimulation in parotid acinar cells (35) and through activation of the IgE receptor in RBL-2H3 cells (36). Tyrosine phosphorylation of PKCδ was also observed in response to epidermal growth factor stimulation in keratinocytes (11) and in ras-transformed keratinocytes (37). It appears that the sites of tyrosine phosphorylation and the effect of this phosphorylation on the activity of PKCδ or on its function depend somewhat on the specific system. PKCδ contains 19 tyrosines located in both the regulatory and catalytic domains, and different tyrosines have been implicated as possible phosphorylation sites. Thus, Tyr187 has been shown to be phosphorylated in 3T3 and NIH 3T3 cells in response to PMA or PDGF stimulation (12). However, a mutation in this tyrosine failed to alter either the activity of PKCδ or the ability of PKCδ to induce monocytic differentiation (12). In another study, Tyr252 was suggested to mediate the tyrosine phosphorylation of PKCδ upon stimulation of the IgE receptor in RBL-2H3 cells but not upon stimulation with PMA (25).

We found, similar to what has been recently described by Acs et al. in fibroblasts (34), that tyrosine phosphorylation of PKCδ was confined to the regulatory domain of this isoform, suggesting that the tyrosines phosphorylated in response to PMA and PDGF are located on this domain. The role of the tyrosine phosphorylation of PKCδ in its inhibitory effect on GS expression was examined using a PKCδ mutant in which five putative sites of tyrosine phosphorylation were mutated to phenylalanine (δ5 mutant). This mutant was constructed based on in vitro assessment of the activity as tyrosine kinase substrates of the oligopeptides corresponding to the sequences around each of the 19 tyrosines in PKCδ (25) as well as on in vivo studies (12). Our results indicate that the δ5 mutant had a markedly diminished tyrosine phosphorylation in response to PMA and PDGF, indicating that at least some of these tyrosines are functional phosphorylation sites in this system. In addition, the cells overexpressing the PKCδ5 mutant showed normal levels of GS as compared with cells overexpressing PKCδ. These results suggest that tyrosine phosphorylation of PKCδ plays a role in the inhibitory effect exerted by this isoform on GS expression. We found that although treatment of cells overexpressing PKCδ5 with PMA induced a similar pattern of translocation to that of PKCδ, the kinase activity of these cells was significantly lower, at least as evaluated using myelin basic protein as a substrate. It is currently not clear what is the effect of the tyrosine phosphorylation of PKCδ on the activity of the enzyme toward its different substrates in this system. Different effects have been reported in different systems, suggesting changes in substrate specificity rather than simply in level of absolute activity (25, 38).

The astrocytic glutamine synthetase is an important enzyme in the removal of the toxic substances NH₃ and glutamate and in the conversion of these substances into glutamine (39). Changes in GS expression have been reported with human pathology (40), and GS has been reported to protect against neuronal degeneration following trauma or ischemia (20). GS expression has been reported to be regulated by cAMP, glucocorticoids, and insulin (18, 19, 41). GS mRNA is regulated by the glucocorticoid response element, NF-κB, and by contact with neuronal cultures (40, 42, 43). Because GS has also been used as an astrocytic marker, understanding its regulation provides important information on astrocytic differentiation as well as on pathological conditions in the central nervous system. Our results imply a negative role for PKCδ in GS expression, with a modulatory effect via tyrosine phosphorylation. A possible mechanism by which PKCδ reduces the expression of GS may be through the induction of c-Jun, which has been implicated in negative regulation of GS transcription (42).

In conclusion, our results demonstrate that the regulatory but not the catalytic domain of PKCδ determines the inhibitory effect of this isoform on the expression of GS. Our findings further emphasize a potentially important role of tyrosine phosphorylation of PKCδ in this system. It is currently not clear which tyrosine kinases are involved in this process; however, tyrosine kinases of the Src family have been implicated in both the tyrosine phosphorylation of PKCδ and the function of
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gial cells (28). Studies are currently underway to identify the specific tyrosine(s) involved in the phosphorylation of PKChs in this system and the tyrosine kinases that phosphorylate it at these sites.

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