Protein Kinase C Activity Correlates with the Growth Rate of Malignant Gliomas: Part II. Effects of Glioma Mitogens and Modulators of Protein Kinase C

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THE PROLIFERATION RATES of gliomas may be modulated by the protein kinase C (PKC) signal transduction system. The present study was undertaken to further examine the role of PKC system in growth regulation of gliomas in vitro by measurement of PKC activity over various phases of tumor growth and by assessing its potential role as a signal transduction system induced by serum mitogens and the known glioma mitogens epidermal growth factor and fibroblast growth factor. All human glioma lines examined, and the rat glioma C6, displayed high PKC activity relative to nonmalignant glial cells, which correlated with their proliferation rates over their respective growth phase. Frozen surgical human malignant glioma specimens also displayed high PKC activity. The relatively selective PKC inhibitor staurosporine (SP) reduced PKC activity and corresponding growth rates in a dose-related manner. Stimulation of PKC with phorbol esters under different concentrations of serum in the growth medium indicated that the high PKC activity, which correlated with their rapid growth rates, is highly susceptible to down-regulation by these agents. Epidermal growth factor and fibroblast growth factor increased both PKC activity and the growth rate of glioma line A172; addition of SP reduced the growth rate to levels observed in SP-treated control tumors, indicating that PKC may be a common signal transduction system induced by these mitogens. These results implicate PKC as an important signal transduction system regulating glioma growth, and offers a potential target for tumor inhibition. (Neurosurgery 31:717–724, 1992)

Key words: Brain neoplasm, Glioma, Growth factor, Phorbol ester, Protein kinase C

Protein kinase C (PKC) is a signal transduction system with multifunctional capabilities in different cell types (13, 14). Recent work in our laboratory with established glioma cell lines, and the data to be presented in the current report using frozen human surgical specimens, demonstrates that malignant gliomas possess high intrinsic activity of the enzyme compared with non-transformed glia, which correlates with their growth rates in vitro, and that this activity is susceptible to inhibition by a relatively specific inhibitor of PKC, staurosporine (SP) (5). This study was undertaken to further explore the role of the PKC system in the growth regulation of gliomas by determining PKC activity during different phases of growth of a series of rapidly growing malignant gliomas (both human and rat) in vitro, and by comparing the effects of the PKC inhibitor SP on PKC activity and growth rates within an individual tumor. The serum dependency of glioma growth and PKC activity was examined, as was the effect that activators of PKC have on both of these parameters under different serum conditions. We also investigated the PKC system as a potential signal transduction system used by identified glioma mitogens epidermal growth factor (EGF) (11, 20) and fibroblast growth factor (FGF) (8, 9), whose receptors are expressed (and in the case of EGF receptor, often with gene amplification [6, 11]) on these tumors.

MATERIALS AND METHODS

Glioma cell lines

The cell lines A172, U178-MG, U373-MG, and U563-MG are characterized malignant glioma lines established elsewhere (courtesy of V. P. Collins, M.D., Ludwig Institute for Cancer Research, Stockholm, Sweden). The rat glioma C6 was also used in this study (obtained from the American Type Culture Collection, Rockville, MD). These lines were maintained in 25-ml Falcon tissue culture flasks in medium consisting of Ea-

gle's minimal essential medium supplemented with 10% fetal calf serum (FCS), gentamicin (20 μg/ml), glutamine, pyruvate (1 mmol/L), dextrose (0.1%), and essential amino acids buffered to a pH of 7.0 (all medium constituents were purchased from GIBCO, Grand Island, NY). At confluency the tumors were passaged regularly by gentle trypsinization (0.05% for 10 min), followed by serum inactivation of trypsin, repeated washes with phosphate-buffered saline (PBS), and replating. Cells were grown at 37°C in a humidified 5% CO_2 incubator. All lines in our laboratory were routinely tested for mycoplasma contamination. For measurement of PKC activity and growth rates during the glioma growth phase, glioma cultures at different stages of growth (corresponding to early logarithmic, mid-log, or late logarithmic growth phases, respectively) adherent to culture plates were harvested as below. For PKC activity and growth measurements under various treatment conditions, cell cultures were treated in mid-logarithmic growth phase.

Frozen malignant glioma specimens

PKC activity was measured in specimens obtained from patients undergoing surgery for diagnosis and/or cytoreduction for malignant glioma. The specimens were snap frozen in liquid nitrogen and stored at −70°C. The specimens obtained for this analysis consisted of histologically proven glioblastoma multiforme (four cases) or anaplastic astrocytoma (one case).

Isolation of nontransformed glia

Control normal human glia were obtained from patients undergoing surgery to ameliorate intractable epilepsy. Samples were obtained separate from the epileptic focus, and glia were isolated as previously described (21, 22). In brief, brain cubes were incubated with 0.25% trypsin and 20 μg/ml deoxyribonuclease for 1 hour at 37°C. The dissociated cells were then passed through a nylon mesh of 130 μm pore size, and the filtrate then centrifuged in Percoll at 15,000 rpm for 30 minutes. The visible cell layer was collected, washed, and resuspended in feeding medium described above for glioma cells. These cells were placed in Falcon flasks of 25 ml capacity. Twenty-four hours later, floating oligodendrocytes were removed and plated as enriched (>80%) oligodendrocyte preparations for use in other studies. Adherent cells, consisting of astrocytes and microglia, were allowed to develop morphologically, and were subsequently used for the enzyme assay and isozyme analysis described below.

In vitro glioma proliferation assay

Our method for determining rates of glioma proliferation has been previously published (4). After passage, each cell line was seeded at a density of 10^4 cells in 40 μl of medium on 12-mm glass coverslips placed in 24-well culture plates. Coverslips were previously coated with 10 μg/ml poly-*l*-lysine to facilitate cell adherence. Twelve hours later, after adequate time for cells to adhere to the coverslips, the wells were flooded and washed with PBS. After addition of 1 ml of feeding medium to each well, each of the PKC modulators or mitogens was then added to the wells at predetermined concentrations in replicates of three. At specified intervals, wells were pulsed with 1 μCi [^{3}H]thymidine/ml for a period of 6 hours before harvest. For the short time-course study of phorbol-12-myristate-13-acetate (PMA) on glioma line A172, the above technique was modified to use a short pulse of [^{3}H]thymidine (30 min) at the concentration above to 35-mm Petri dishes containing four coverslips. To measure thymidine incorporation for both of these methods, the coverslips with adherent cells were washed four times with PBS and placed in scintillation vials containing 5 ml of scintillation fluid (Cytoscint, ICN Chemical, Irvine, CA) for determination in a beta counter. Mean [^{3}]thymidine uptake values and corresponding standard errors were then determined from the triplicate readings obtained. Control uptake values were compared with treatment groups using a one-way analysis of variance (ANOVA) and Duncan's multiple comparison ($\alpha = 0.05$). To demonstrate that uptake of [^{3}H]thymidine into cells is a reliable index of DNA synthesis, a control experiment was performed in which synthesis of DNA in the glioma cells was stopped by treating the cells with 50 μg/ml of Mitomycin-C (Sigma Chemical Co., St. Louis, MO) for 30 minutes; cells were then washed four times with fresh medium and pulsed as above with [^{3}H]thymidine. Resulting background [^{3}H]thymidine counts were negligible.

Protein kinase C assay

Cells grown in 150-mm plates were rinsed twice with ice-cold PBS, followed by a rinse with homogenizing buffer containing 50 mmol/L Tris-HCl, 2 mmol/L dithiothreitol, 1 mmol/L phenylmethylsufonylfluoride, and 2 mmol/L ethyleneglycol-bis-(β-aminoethyl ether) N,N,N′,N′-tetraacetic acid (EGTA). The cells were then scraped off the culture plates into 2 ml of the above solution and homogenized (30 strokes) in a glass homogenizer. The homogenate was then centrifuged at 100,000 × *g* for 1 hour. The supernatant was designated the cytosolic fraction. The pellet was resuspended in 2 ml of the above buffer containing 1% Triton X-100, homogenized (30 strokes), and mixed slowly for 30 minutes. This resuspension was then centrifuged at 100,000 × *g* for 1 hour, and the supernatant designated as the particulate fraction. All procedures were performed at 4°C. The enzyme fractions were stored at −70°C before assay for PKC.

The method used to assay for PKC activity (phosphate transfer into lysine-rich histone) is modified from the procedure of Neary et al. (12), after having determined the correct Michaelis-Menten conditions for all cofactors and substrate. The reaction mixture contains (final concentrations): 50 mmol/L Tris-HCl, 100 μmol/L free calcium, 250 μg/ml lysine-rich type IIIS histone, 500 μg/ml phosphatidylserine, 25 μg/ml diacylglycerol (dioctanoyl-sn-glycerol), 10 μmol/L ATP (mixed with [^{32}P]ATP, 0.5–1 × 10^6 cpm), 10 mmol/L $MgCl_2$, and 4 μl of enzyme preparation. The pH of the reaction mixture is 7.4 in a final volume of 40 μl. The enzyme preparation (particulate or cytosolic fraction) is preincubated with the reaction mixture minus ATP and $MgCl_2$ at 30°C for 2 minutes. Phosphorylation of histone is initiated by adding ATP and $MgCl_2$, and allowed to proceed at 30°C for 1 minute. The reaction is terminated by placing a 25-μl aliquot of the mixture onto a 1 × 2 cm phos-

phocellulose paper (Whatman P81). Each paper is quickly transferred to a beaker containing 75 mmol/L phosphoric acid. After three washes in phosphoric acid (5 min each) to remove unreacted [^{32}P]ATP, the strips are dehydrated with absolute methanol, air-dried, placed in vials containing 5 ml of scintillation fluid, and counted for radioactivity. Negative blanks (non-PKC-dependent reaction) consist of reaction mixture minus calcium (in presence of EGTA), phosphatidylserine, and diacylglycerol. PKC-dependent reaction is calculated as the difference between activity in the presence and absence of calcium, phosphatidylserine, and diacylglycerol. Each enzyme preparation is analyzed in triplicate, with subsequent determination of mean values and corresponding standard errors. Results are expressed as pmol ATP transferred/min/mg protein. Variability between triplicates, and between assays, is less than 10% of the mean in most cases. All reagents were purchased from Sigma Chemical Co., with the exception of [gamma-^{32}P]ATP, which was purchased from New England Nuclear (Wilmington, DE).

To reliably compare activity between cell types, all samples were run at 1 µg of protein in the reaction mixture. When results are expressed as total PKC activity, this represents the sum of the cytosolic and particulate activity within the same cell line.

Modulation of glioma growth in vitro

Serum and PKC modulators

To assess the effects of serum on growth rates and PKC activity, tumors were grown in medium containing varying amounts of FCS for use in the assays described above. The effects of inhibition of PKC activity on the growth rate was assessed by using the PKC inhibitor staurosporine (SP) (19) (Calbiochem, La Jolla, CA) which was dissolved in ethanol and further diluted in PBS; it was used over a range of concentrations from 1 to 100 nmol/L. 4-β-Phorbol-12,13-dibutyrate (PDB) and PMA (Sigma), which are phorbol esters and activators of PKC (1, 10), were administered at doses of 100 nmol/L each. As a phorbol ester control, 4-α-phorbol-12, 13-didecanoate (α-PDD) (Sigma), a phorbol ester that binds but does not activate the enzyme, was used at a concentration of 100 nmol/L.

Glioma mitogens

To assess the effects of known mitogens on PKC activity and glioma growth, the glioma mitogens FGF (Boehringer Mannheim, Indianapolis, IN), isolated from bovine brain, using the technique of Gospodarowicz et al. (7), and EGF (Boehringer Mannheim) were added to glioma medium to achieve final concentrations of 20 and 100 ng/ml each.

RESULTS

PKC activity varies with the growth phase of the tumor

Figure 1*A* depicts total PKC activity (expressed as pmol ATP transferred/min/mg protein in the reaction mixture) in several human gliomas and the rat C6 tumor grown in 10% FCS-containing medium. Activity levels increased in logarithmic stages of tumor growth. Thymidine uptake was noted to follow a similar pattern to the PKC activity when measured over the same intervals during the growth phase in vitro. As shown in Figure 1*B*, frozen surgical tumor specimens (1–88, 2–88, 5–88, 6–88, 1–158) displayed high PKC activity in the same range as the maximal PKC activity obtained in the glioma cell lines (A172, U178, U373, U563, C6). Specimens 1–88, 2–88, 5–88, and 6–88 are histologically diagnosed glioblastoma multiforme, and 1–158 an anaplastic astrocytoma. All tumors expressed high levels of PKC activity in comparison with non-transformed glial controls (astrocyte/microglial total activity was 42 pmol ATP/min/mg protein, or 10- to 100-fold less than the activity measured in the tumors).

PKC inhibition and growth rates

The administration of the relatively selective PKC inhibitor SP produced a dose-related decrease in PKC particulate fraction activity in glioma line A172 (Fig. 2, *top*). The cytosolic activity remained unchanged; therefore total activity decreased as with the particulate fraction. When thymidine uptake was measured over the same concentration range, the proliferation rate was found to decrease in a similar manner as the total and particulate PKC activities (Fig. 2, *bottom*).

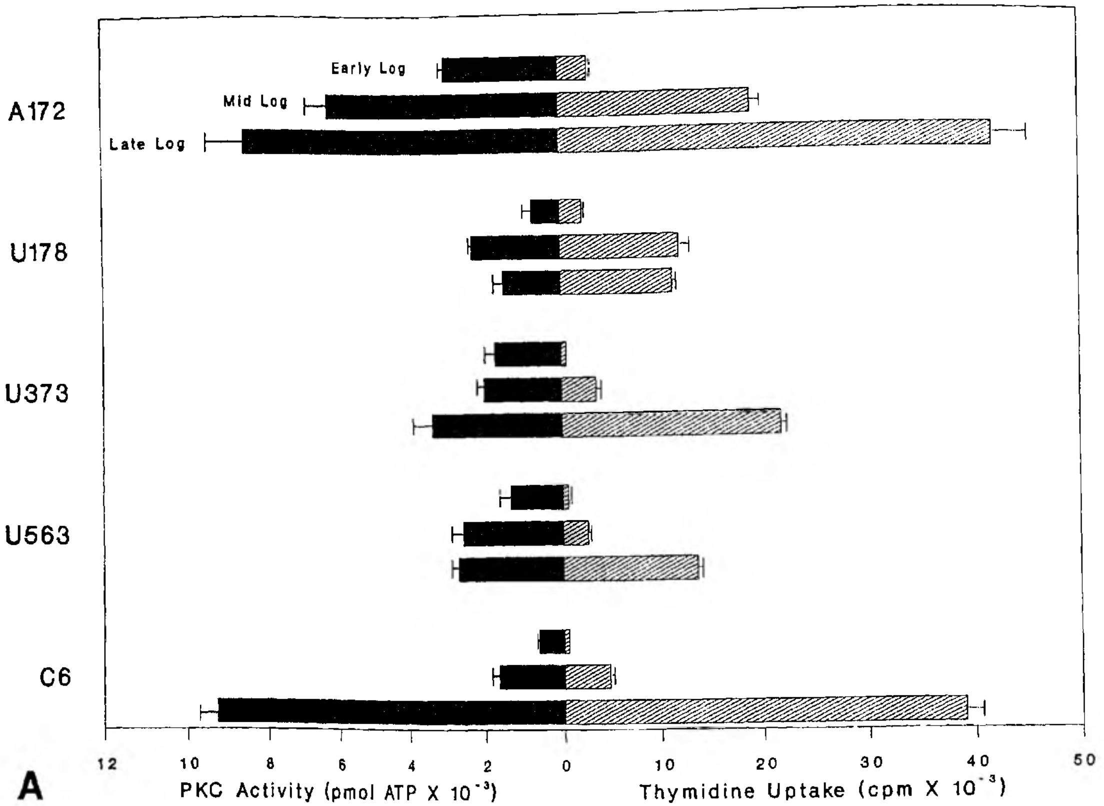
Serum effects on glioma growth

To further establish the relation between tumor growth rate and PKC activity, selected tumors were grown in reduced (1–5%) or serum-free media. As expected, reducing the amount of serum in the medium reduced the amount of thymidine uptake in the same tumor line under control conditions (measured with a 6-hr pulse 48 hr after the medium was changed) (Fig. 3*A*). Direct measurement of PKC activity in glioma line A172 under different serum conditions revealed a marked reduction in control PKC activity in cultures grown in serum-free medium as compared with cultures grown in 10% FCS-containing medium (Fig. 3*B*, compare time 0 at *top* and *bottom*). The activity measured in the tumor grown under serum-free conditions, however, was still far greater than activity in nontransformed glia.

Effects of PKC activation in gliomas under different growth conditions

In contrast to the decrease in cell proliferation seen with the administration of an inhibitor of PKC, the effect of the PKC activators PDB and PMA depended upon the basal growth rate of the tumor as determined by the serum concentration of the glioma medium (glioma line A172; Fig. 3*A*). In serum-free medium, where the growth of the tumor is much slower, the activators enhanced proliferation (thymidine uptake measured 24 hr after the addition of 100 nmol/L PMA), whereas in the presence of 5% serum, the basal rate of proliferation was greater and treatment with phorbol esters produced a decrease in growth rate.

Measurement of PKC activity (Fig. 3*B*) revealed that after the addition of PMA in serum-free medium, an initial activation phase, characterized by a rapid and marked increase in particulate and total activity, was followed by a diminution of total and particulate activity (*top*). In contrast, measurement of ac-



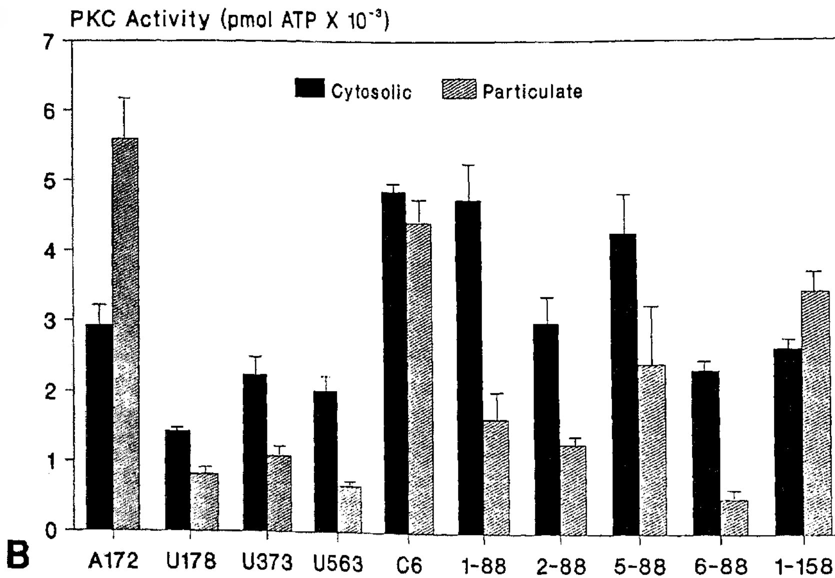


FIGURE 1. PKC activity varies with the growth phase of the glioma. Shown is measurement of total PKC activity (expressed as pmol ATP transferred/min/mg protein) over early logarithmic, mid-log, and late-log phases for a series of human malignant gliomas and the rat C6 glioma (*A, left*). Activity increased in the later phases of tumor growth. The growth rates as measured by [^{3}H]thymidine incorporation over the same phases of growth followed a similar pattern to the PKC activity (*A, right*). Surgical malignant glioma specimens also display high PKC activity within the range as that of the malignant glioma lines (*B*) (surgical specimens designated 1-88, 2-88, 5-88, 6-88, are glioblastoma multiforme by histopathology, 1-158 is an anaplastic astrocytoma; established glioma cell lines are A172, U178, U373, U563, and C6). Values are means of triplicates + SEM.

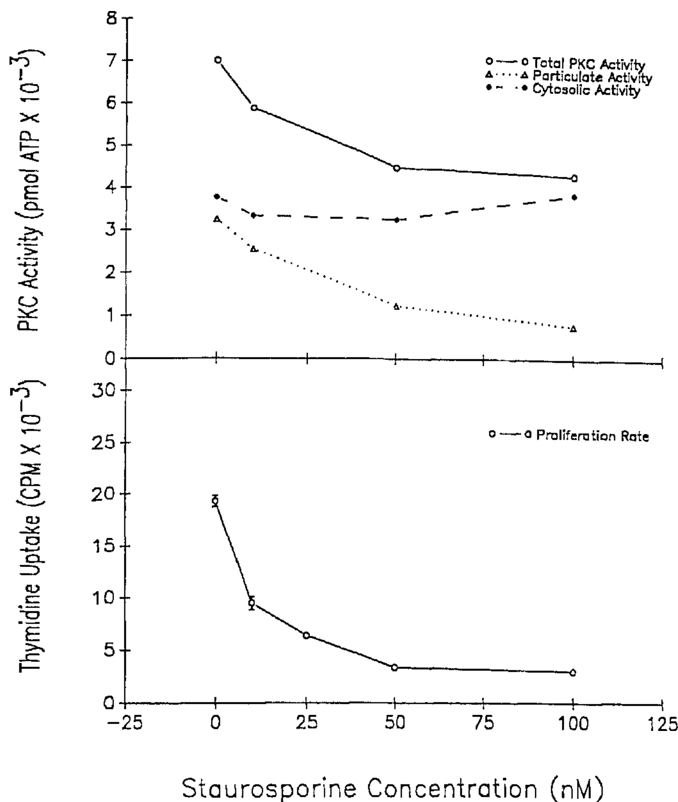


FIGURE 2. PKC inhibition in glioma line A172 by SP. Administration of the PKC inhibitor SP produced a dose-related decrease in PKC particulate fraction activity in glioma line A172 (*top*). Cytosolic activity is unaffected; therefore total activity decreased as with the particulate fraction. Values are mean of triplicates, with SEM <10% of the mean in most cases. When thymidine uptake was measured over the same concentration range (after a 2-day incubation before the thymidine pulse), the proliferation rate was found to decrease in correspondence with the particulate PKC activity (*bottom*). Values are means of triplicates ± SEM.

tivity in the presence of 10% FCS-containing medium indicates a prompt down-regulation of the higher initial PKC activity, which is reflected by an immediate decrease in thymidine incorporation (*bottom*) as previously described (5).

The growth rate of rat glioma C6, in variance to the human tumor, was inhibited by 100 nmol/L PMA in both serum-free and serum-present conditions [Fig. 3*C*, *top* (serum-free) and *bottom* (10% FCS)]. Direct measurement of the PKC activity indicated an immediate down-regulation of the enzyme under both of these conditions. Again, alterations in enzyme activity were associated with similar changes in the growth rate of the tumor.

Glioma mitogens and PKC activity

Figure 4 depicts experiments in which two known mitogens for gliomas, FGF and EGF, were added to glioma line A172. Over the 48-hour treatment period, the proliferation rates increased significantly from that of control cultures. The addition of the PKC inhibitor SP at 50 nmol/L to cultures containing these mitogens significantly decreased the growth rates (by 89.6% and 84.9% in EGF- and FGF-stimulated cultures, respectively) (Fig. 4*A*). The absolute growth rate obtained by the addition of SP to EGF- and FGF-treated cultures was not statistically different from that of control cultures exposed to SP, indicating that the maximal reduction of PKC-mediated growth produces a constant basal proliferation rate, independent of the initial rate of growth of the tumor.

PKC activity measured after the addition of EGF, 20 ng/ml, revealed a rapid increase in cytosolic activity in glioma line A172 (Fig. 4*B*). Particulate activity showed a modest increase over the 24-hour treatment period.

DISCUSSION

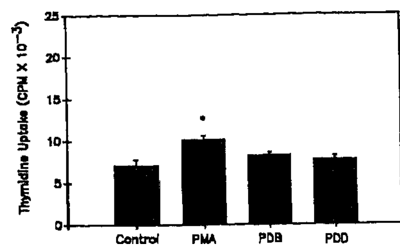
This study extends observations regarding the role of the PKC signal transduction system in modulating glioma growth. As noted previously, all glioma lines tested displayed high levels of PKC activity in comparison with non-transformed glial controls (astrocytes, oligodendroglia, and microglia). In this study, the PKC activity was shown to correlate with [^{3}H]thymidine uptake within given tumors at different phases of tumor growth. Measurement of high PKC activity in human malignant glioma surgical specimens suggests this high PKC activity may also be functional in vivo.

The ability of the relatively selective PKC inhibitor SP to markedly reduce the proliferation rate and PKC activity of glioma line A172 in a dose-related fashion further supports the evidence that intrinsic high PKC activity of glioma cells correlates with their rapid growth rate. The mechanism of staurosporine inhibition of PKC activity has not been precisely resolved, although it has been suggested that it competes with high affinity for the ATP binding site of the enzyme (18, 19). It is an extremely potent inhibitor of PKC, with an IC_{50} of 2.7 nmol/L in isolated enzyme fractions in vitro (19). Recent work, however, suggests that it may also inhibit other tyrosine-specific protein kinases, especially when used in higher concentrations. For example, the tyrosine kinase activity of the platelet-derived growth factor receptor may also be partially inhibited in Swiss 3T3 fibroblasts in concentrations of 10^{-7} mol/L range, which may facilitate the decrease PKC activity by inhibiting the hydrolysis of phospholipase C, the enzyme responsible for the generation of diacylglycerol (the endogenous activator of PKC) (18).

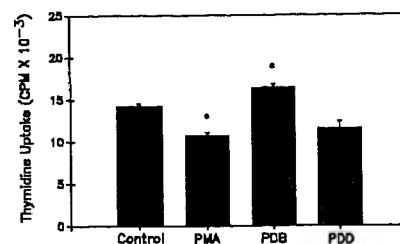
Under reduced serum conditions, both growth rates and PKC activity within a given tumor are decreased, as demonstrated in both the human A172 tumor and the rat C6 glioma. In the A172 tumor line, the low activity is amenable to transient stimulation in the presence of a pharmacological activator such as PMA or PDB, which results in a corresponding transient increase in growth rate. However, in serum-containing medium, PKC activity increases and is associated with a more rapid growth rate; this elevated PKC activity is then susceptible to a more rapid down-regulation by the pharmacological PKC activators (2), producing a paradoxical inhibition of growth. Results from this study are consistent with previous reports which indicate that the alteration of in vitro glioma growth rates induced by phorbol

Serum–Dependency of Activation of PKC in Glioma Line A172

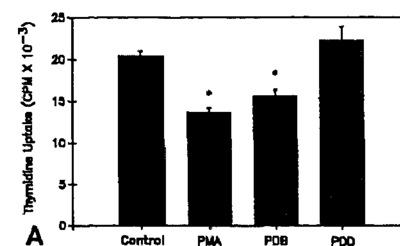
Serum–Free Medium

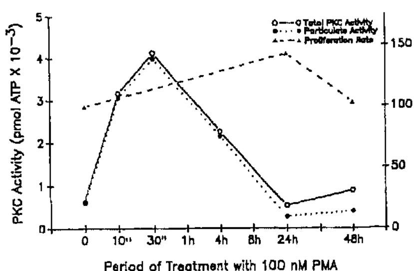


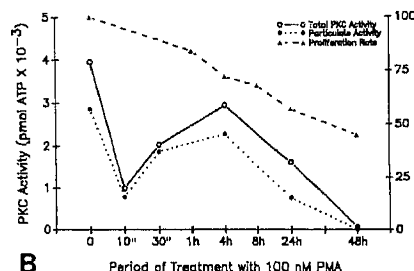
1% FCS Medium

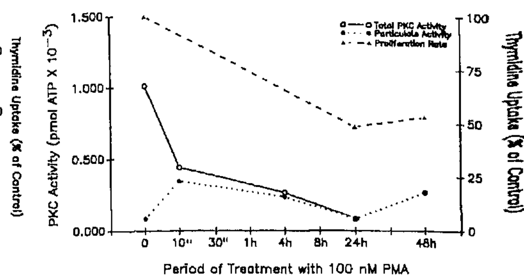


5% FCS Medium









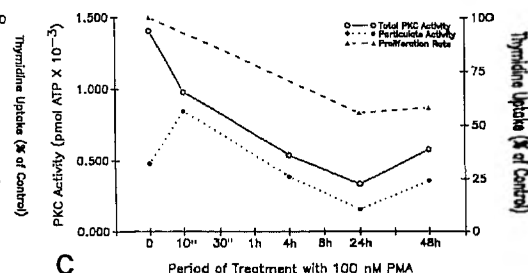
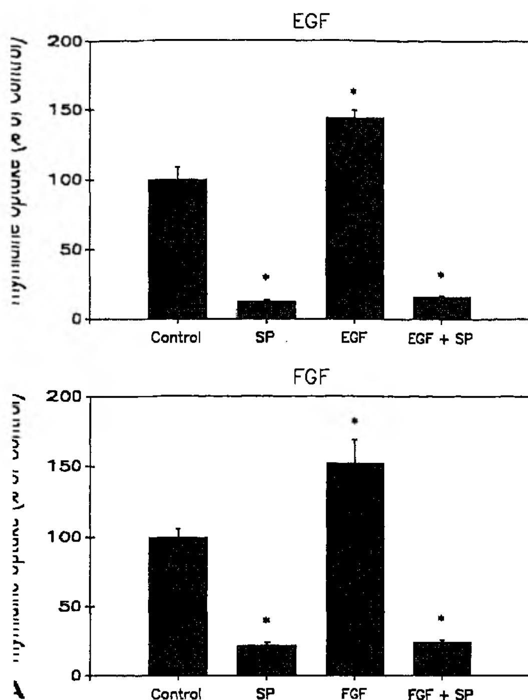


FIGURE 3. Activation or down-regulation of PKC is dependent upon glioma cell line and basal PKC activity. Administration of the phorbol esters and PKC activators PMA and PDB (both at 100 nmol/L) stimulated A172 tumor growth under serum-free medium conditions (*A, top*), but in contrast decreased growth rates in the presence of 5% FCS-containing medium (*A, bottom*). The addition of α-PDD (100 nmol/L), a phorbol ester that binds but does not stimulate PKC, produced no significant change in growth rates in all media tested. Values are means of triplicates + SEM. *, values significantly different from controls (one-way ANOVA, Duncan's multiple comparison, $P < 0.05$). To determine corresponding PKC activity, serial measurements were obtained over several time intervals in tumor line A172 after the administration of 100 nmol/L PMA. Under serum-free conditions (*B, top*), initial low activity was stimulated for several hours (which was associated with a transient stimulation of tumor growth), followed by down-regulation at 24 hours. In 10% FCS-containing medium, the higher initial PKC activity is immediately down-regulated, and was associated with a prompt inhibition of tumor growth (*B, bottom*) as described previously (5). In contrast to the effect of serum upon PKC activation of glioma line A172, the rat glioma C6 was inhibited by 100 nmol/L PMA in both serum-free and 10% FCS conditions (*C, top* and *bottom*). [^{3}H]thymidine counts followed a similar pattern to PKC activity under both conditions. Values are means of triplicates, with SEM <10% of the mean in most cases.

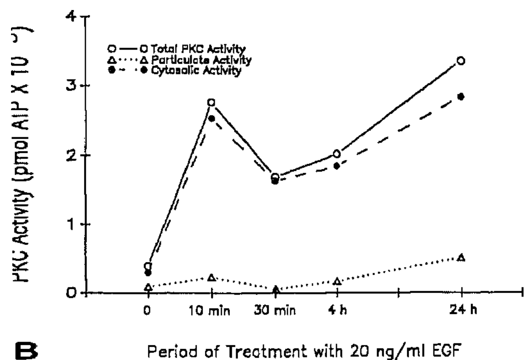
esters is dependent upon the serum concentration used in the culture medium. Under serum-free conditions, PMA and PDB act as mitogens for the A172 tumor (16, 17), while in the presence of high serum concentrations they inhibit growth (3–5). That the PKC system is selectively involved in these growth rate changes induced by the phorbol esters known to activate PKC (PMA and PDB) is supported by the findings that a phorbol ester that binds, but does not activate PKC (α-PDD) (1, 10), does not alter the rates of growth. In contrast to the human A172 line, the rat C6 tumor in this study displayed a higher intrinsic PKC activity in the absence of serum, which was immediately down-regulated by PMA without an intervening activation phase, resulting in only an inhibition of growth under these conditions.

The observations of the expression of messenger RNA for the platelet-derived growth factor receptor (15), and amplification of the EGF receptor gene (11) in malignant gliomas poses the question of how signals from factors acting through such receptors may be transduced into altered proliferation rates; in this regard intracellular signal transduction systems are likely to play a pivotal role (20). In the present study, the ability of SP to totally block the mitogenic effect of the known glioma mi-

Proliferative Effects of Glioma Mitogens EGF and FGF are Blocked by the PKC Inhibitor Staurosporine



Protein Kinase C Activity of Glioma Line A172 Following Treatment With EGF



E 4. Inhibition of PKC blocks proliferative effects of glioma mitogens EGF and FGF. Under serum-free ons, the addition of EGF (*top*) and FGF (*bottom*) at a f 100 ng/ml increases the proliferative rate of glioma 72 (*A*). Values are means of triplicates + SEM. *, val- nificantly different from controls (one-way ANOVA, n's multiple comparison, $P < 0.05$). Concurrent ad- ation of SP (50 nmol/L) reduces the proliferative rate produced by SP alone (values not significantly differ- x = 0.05). PKC activity measured after addition of ogen EGF at 20 ng/ml to glioma A172 shows an im- e increase in PKC activity, predominately in the cy- fraction (*B*). A similar pattern, but of less magnitude, en in the particulate fraction. Values are means of tes, with SEM <10% of the mean in all cases.

togens FGF and EGF, and the ability of EGF to increase PKC activity implicate PKC as a potential common signal transduction mechanism induced by these mitogens.

Whether the high intrinsic PKC activity of these tumors represents a primary defect in the PKC enzyme system *per se*, or an altered regulation of the enzyme activity secondary to the aberrant growth factor or receptor genes known to exist in these tumors, remains to be determined. Regardless of the resolution of this important question, this overexpressed enzyme activity may represent a potential future site for chemotherapeutic targeting in these tumors.

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COMMENTS

The role of protein kinase C (PKC) in the signal transduction pathway in the growth of human gliomas has been of significant interest. It would appear that PKC is at higher levels in gliomas than in non-malignant glial cells. Epidermal growth factor (EGF) and fibroblast growth factor (FGF) appear to play a part in growth regulation of gliomas. FGF is a highly consistent and effective stimulator of growth. EGF at times can down regulate certain tumors. FGF appears to be an autocrine growth factor in gliomas.

In this paper, the interrelationship between these potentially important growth factors and the PKC pathway are explored. The data suggests strongly that EGF and FGF increase PKC activity as well as the growth rate of glioma lines. A selective PKC inhibitor, staurosporine reduced PKC levels and corresponding growth rates. The findings therefore support the hypothesis that the PKC signal transduction pathway is part of the system that regulates glioma growth.

This type of research effort, which focuses on specific peptide modulators of glioma growth and the targets of their effects, is very interesting and provides the types of insights needed to identify new avenues for altering the growth of malignant gliomas.

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In this paper Couldwell et al. have expanded upon their previous work published in this journal regarding growth regulation of gliomas and the PKC system. In this study, as in their previous study (1), they have shown increased PKC activity in a number of human glioma cell lines, in rat glioma C6, and in frozen human malignant glioma specimens. They have shown decreases in PKC activity and in tumor growth in dose related fashion with the known PKC inhibitor staurosporine, and this is postulated as a potential therapeutic agent for human investigation. Much of this encouraging work was alluded to in their last paper. When attention is turned to the phorbol esters PDB and PMA (which are activators of PKC) the implications are not as clear. With these agents, tumor growth is enhanced under slow growth conditions (serum-free) but is decreased under high growth conditions for the human A172 cell line. In rat glioma C6, phorbol esters inhibit growth under both conditions. This paradox is explained by a down-regulation hypothesis as outlined in their previous paper, and I would direct the reader to Dr. Sawaya's previous comments on it.

The last new finding in this study that differs from the previous work is the demonstration that glioma mitogens FGF and EFG stimulate PKC activity effectively in these tumors. The fact that this occurs, and the ability to reverse this effect with staurosporine, does lend credence to the authors' contention that PKC is an important signaling mechanism in tumor growth, and that anti-PKC therapies may some day be useful in clinical neuro-oncology.

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