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Differential Expression of Rat Brain Phospholipase C Isozymes in Development and Aging

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Phosphoinositide-specific phospholipase C (PLC) is a key enzyme in signal transduction. In the present study we examined developmental and aging changes in three PLC isozymes (β1, γ1, and δ1) in the rat brain. Enzyme assays and immunoblot analyses after gel filtration chromatography of brain extracts from embryonic day 19 and postnatal 4- and 48-week rats indicated that γ1-specific activity was highest in fetal brain and decreased with aging, that β1-specific activity was high at 4 weeks but essentially undetected in fetal brain, and that δ1-specific activity was high at both 4 and 48 weeks with faint detection in fetal brain. Our results suggest that the γ1 isozyme may be particularly involved in cell division and growth during the histogenesis of the central nervous system, while β1 and δ1 isozymes may take part in processes of its maturation and maintenance.

An extracellular signal such as a neurotransmitter or growth factor initiates signal transduction through the cell membrane by binding to a specific receptor on its surface. Receptors coupled to the phosphoinositide signal transduction mechanism are coupled to phospholipase C (PLC). PLC catalyzes the breakdown of three phosphoinositides, phosphatidylinositol, phosphatidylinositol 4-monophosphate, and phosphatidylinositol 4,5-bisphosphate, to generate diacylglycerol and three inositol phosphates, among which diacylglycerol and inositol 1,4,5-trisphosphate serve as intracellular messengers for protein kinase C (PKC) activation and intracellular Ca^{2+} mobilization (1–4). Thus, PLC is a key enzyme in the phosphoinositide signal transduction mechanism. Comparison of amino acid sequences has indicated that the PLC can be divided into three types (PLC-β, -γ, and -δ) and that each type includes multiple subtypes. To date, at least 10 PLC isozymes have been identified by protein chemistry or cDNA cloning methods (5).

Alzheimer's disease (AD) is a neurodegenerative disease characterized by the progressive deterioration of cognitive function and memory (6). We previously have demonstrated that a PLC isozyme, PLC-δ1, accumulates abnormally in neurofibrillary tangles, neurites surrounding senile plaque cores, and neuropil threads, all characteristic pathologic hallmarks of AD (7–9). Moreover, we have shown using gel filtration chromatography that among the isozymes that PLC-γ1 activity is significantly decreased and PLC-δ1 activity is significantly increased in AD brains compared with controls (10). As AD is an aging-associated disease, it is important to consider such changes in the context of normal developmental and aging-associated changes of PLC isozymes, but these are scarcely understood. Although Yamada et al. (11) have demonstrated early developmental changes of PLC-β, -γ, and -δ in the rat brain mainly by immunocytochemical methods, developmental and aging contributions of their phosphoinositide hydrolysis function remain unknown.

In the present study we examined the developmental and aging changes of PLC isozymes on their phosphoinositide hydrolysis activities and protein levels in the cerebral cortex from the embryonic (E19) to 96-week-old

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Abbreviations used: PLC, phospholipase C; PKC, protein kinase C; AD, Alzheimer's disease; PMSF, phenylmethylsulfonyl fluoride; DFP, diisopropylfluorophosphate; BSA, bovine serum albumin; EGTA, ethylene glycol tetraacetic acid; DTT, dithiothreitol; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; GFAP, glial fibrillary acidic protein; PBS, phosphate buffered saline; HRP, horseradish peroxidase; EGF, epidermal growth factor; PDGF, platelet-derived growth factor; FGF, fibroblast growth factor.

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Wistar rats, and found the unique and differential expression of each isozyme during development and aging.

MATERIALS AND METHODS

Materials. L-α-phosphatidyl [myo-inositol 2-^{3}H(N)] (1.0 Ci/mmol) was purchased from American Radiolabeled Chemicals (St. Louis, MO). L-α-Phosphatidylinositol (soybean, ammonium salt), phenylmethylsulfonyl fluoride (PMSF), and diisopropylfluoro-phosphate (DFP) were obtained from Sigma Chemical. (St. Louis, MO). Superdex 200 pg HiLoad columns were obtained from Pharmacia LKB Biotechnology (Uppsala, Sweden). Standard proteins used for calibration of the Superdex 200 pg columns were obtained from Boehringer Mannheim (Germany). All other chemicals were of reagent grade and were obtained commercially.L-α-phosphatidyl [myo-inositol 2-^{3}H(N)] (1.0 Ci/mmol) was purchased from American Radiolabeled Chemicals (St. Louis, MO). L-α-Phosphatidylinositol (soybean, ammonium salt), phenylmethylsulfonyl fluoride (PMSF), and diisopropylfluoro-phosphate (DFP) were obtained from Sigma Chemical. (St. Louis, MO). Superdex 200 pg HiLoad columns were obtained from Pharmacia LKB Biotechnology (Uppsala, Sweden). Standard proteins used for calibration of the Superdex 200 pg columns were obtained from Boehringer Mannheim (Germany). All other chemicals were of reagent grade and were obtained commercially.

Brain samples. Wistar rats pregnant for 19 days (E19) were anesthetized under ether inhalation, and rat fetuses were removed from uteri by Cesarean section. Brains were taken from E19 fetuses as well as from 1-, 2-, 4-, 8-, 24-, 36-, 48-, 72-, 96-week-old male Wistar rats purchased from Japan SLC (Kyoto). The animals were treated in accordance with the guidelines published in the NIH Guide for the Care and Use of Laboratory Animals.Wistar rats pregnant for 19 days (E19) were anesthetized under ether inhalation, and rat fetuses were removed from uteri by Cesarean section. Brains were taken from E19 fetuses as well as from 1-, 2-, 4-, 8-, 24-, 36-, 48-, 72-, 96-week-old male Wistar rats purchased from Japan SLC (Kyoto). The animals were treated in accordance with the guidelines published in the NIH Guide for the Care and Use of Laboratory Animals.

Preparation of brain extracts. Brain tissue samples from the cerebral cortex (1 g wet weight) were homogenized with a Teflon-glass homogenizer in 4 volumes of 10 mM Hepes buffer (pH 7.0) containing 0.32 M sucrose, 0.05 % NaN_3, 100 μM orthovanadate, 0.1 mM PMSF, 0.5 mM DFP, 1 mM dithiothreitol (DTT), 10 μg/mL aprotinin, 5 μg/mL pepstatin A, 5 μg/mL leupeptin, 5 mM benzamidine, and 4 mM ethylene glycol tetraacetic acid (EGTA). The homogenate was centrifuged at 105,000 $\times$ g for 60 min and the supernatant thus obtained was used as the cytosolic fraction. The pellet was washed twice, suspended in homogenization buffer, and used as the particulate fraction for further experiments.Brain tissue samples from the cerebral cortex (1 g wet weight) were homogenized with a Teflon-glass homogenizer in 4 volumes of 10 mM Hepes buffer (pH 7.0) containing 0.32 M sucrose, 0.05 % NaN_3, 100 μM orthovanadate, 0.1 mM PMSF, 0.5 mM DFP, 1 mM dithiothreitol (DTT), 10 μg/mL aprotinin, 5 μg/mL pepstatin A, 5 μg/mL leupeptin, 5 mM benzamidine, and 4 mM ethylene glycol tetraacetic acid (EGTA). The homogenate was centrifuged at 105,000 $\times$ g for 60 min and the supernatant thus obtained was used as the cytosolic fraction. The pellet was washed twice, suspended in homogenization buffer, and used as the particulate fraction for further experiments.

Gel filtration of brain extracts. Brain extract was loaded onto a Superdex 200 pg column (1.6 $\times$ 60 cm) equilibrated and eluted with 25 mM Hepes buffer (pH 7.0) containing 1 mM DTT, and 0.1 M NaCl. Two-milliliters fractions were collected, assayed for PLC activity, and used for immunochemical detection. Apparent molecular weights of PLC activity fractions were estimated by using an elution profile of standard proteins (ferritin, molecular weight 450 kDa; catalase, molecular weight 240 kDa; sweet potato acid phosphatase (12), molecular weight 110 kDa; bovine serum albumin (BSA), molecular weight 68 kDa) on the same Superdex 200 pg column used in gel filtration chromatography of brain extracts after washing the column thoroughly with the elution buffer. Brain extract was loaded onto a Superdex 200 pg column (1.6 $\times$ 60 cm) equilibrated and eluted with 25 mM Hepes buffer (pH 7.0) containing 1 mM DTT, and 0.1 M NaCl. Two-milliliters fractions were collected, assayed for PLC activity, and used for immunochemical detection. Apparent molecular weights of PLC activity fractions were estimated by using an elution profile of standard proteins (ferritin, molecular weight 450 kDa; catalase, molecular weight 240 kDa; sweet potato acid phosphatase (12), molecular weight 110 kDa; bovine serum albumin (BSA), molecular weight 68 kDa) on the same Superdex 200 pg column used in gel filtration chromatography of brain extracts after washing the column thoroughly with the elution buffer.

Enzyme assay. The activity of PLC was measured as described previously (13,14). In brief, reaction mixtures contained 280 μM phosphatidylinositol, 30,000 dpm of L-α-phosphatidyl-[myo-inositol 2-^{3}H(N)], 1 mg/mL sodium deoxycholate, 1.5 mM $CaCl_2$, 50 mM Hepes (pH 7.0), and brain extract (10 μg protein) or eluted sample from the column (20 μL). After incubation for 60 min at 37° C, the reaction was stopped with 1 mL of chloroform/methanol/concentrated HCl (50:50:0.3), followed by the addition of 0.3 mL of 1 N HCl containing 5 mM EGTA. After centrifugation for 10 min at 3,000 $\times$ g, a 700-μL aliquot of the supernatant was removed for liquid scintillation counting. Protein concentration was determined by the method of Bradford (15) with BSA as the standard.The activity of PLC was measured as described previously (13,14). In brief, reaction mixtures contained 280 μM phosphatidylinositol, 30,000 dpm of L-α-phosphatidyl-[myo-inositol 2-^{3}H(N)], 1 mg/mL sodium deoxycholate, 1.5 mM $CaCl_2$, 50 mM Hepes (pH 7.0), and brain extract (10 μg protein) or eluted sample from the column (20 μL). After incubation for 60 min at 37° C, the reaction was stopped with 1 mL of chloroform/methanol/concentrated HCl (50:50:0.3), followed by the addition of 0.3 mL of 1 N HCl containing 5 mM EGTA. After centrifugation for 10 min at 3,000 $\times$ g, a 700-μL aliquot of the supernatant was removed for liquid scintillation counting. Protein concentration was determined by the method of Bradford (15) with BSA as the standard.

Antibodies. A specific antibody against PLC-δ1 was prepared using PLC-δ1 protein produced by an E. coli expression system (16). The characterization and specificity of the antibody against PLC-δ1 have been fully described previously (7). This antibody recognizes rat PLC-δ1 as an 85 kDa protein after sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) as reported previously (7,10). Specific antibodies against PLC-β1 and PLC-γ1 were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-glial fibrillary acidic protein (GFAP) antibody was obtained from Dako Japan (Tokyo).A specific antibody against PLC-δ1 was prepared using PLC-δ1 protein produced by an E. coli expression system (16). The characterization and specificity of the antibody against PLC-δ1 have been fully described previously (7). This antibody recognizes rat PLC-δ1 as an 85 kDa protein after sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) as reported previously (7,10). Specific antibodies against PLC-β1 and PLC-γ1 were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-glial fibrillary acidic protein (GFAP) antibody was obtained from Dako Japan (Tokyo).

Immunochemical detection. The samples from developing and aging rat brains and eluted samples from gel filtration chromatography in Laemmli sample buffer were subjected to 4-20 % SDS-PAGE, blotted onto Immobilon (Millipore, Bedford, MA), blocked with phosphate-buffered saline (PBS) containing 0.1% Tween 20 (TPBS) for 1 hr, and incubated with anti-PLC-β1 (1:500), -γ1 (1:500), -δ1 (1:5,000), and -GFAP (1:3,000) antibodies in PBS containing 3 % BSA for 18

hr at 4°C. Blots then were washed with TPBS, incubated with horseradish peroxidase (HRP)-linked antibody against rabbit immunoglobulin (Ig) (diluted 1:1,000). Subsequently, membrane-bound HRP-labeled antibodies were detected by an enhanced chemiluminescence detection system (ECL kit, Amersham). Protein bands reacting with antibodies were detected on radiographic film (X-Omat JB-1, Kodak) 5 to 60 sec after exposure. Integrated optical densities for the 150 kDa protein band recognized by the anti-PLC-β1 antibody, for the 145 kDa protein band recognized by the anti-PLC-γ1 antibody, for the 85 kDa protein band recognized by the anti-PLC-δ1 antibody, and for the 40 kDa protein band recognized by the anti-GFAP antibody were measured by a scanning densitometer (Arcus II, Agfa, Germany); these densities were taken to indicate relative quantities of PLC-β1, PLC-γ1, PLC-δ1, and GFAP, respectively.The samples from developing and aging rat brains and eluted samples from gel filtration chromatography in Laemmli sample buffer were subjected to 4-20 % SDS-PAGE, blotted onto Immobilon (Millipore, Bedford, MA), blocked with phosphate-buffered saline (PBS) containing 0.1% Tween 20 (TPBS) for 1 hr, and incubated with anti-PLC-β1 (1:500), -γ1 (1:500), -δ1 (1:5,000), and -GFAP (1:3,000) antibodies in PBS containing 3 % BSA for 18 hr at 4°C. Blots then were washed with TPBS, incubated with horseradish peroxidase (HRP)-linked antibody against rabbit immunoglobulin (Ig) (diluted 1:1,000). Subsequently, membrane-bound HRP-labeled antibodies were detected by an enhanced chemiluminescence detection system (ECL kit, Amersham). Protein bands reacting with antibodies were detected on radiographic film (X-Omat JB-1, Kodak) 5 to 60 sec after exposure. Integrated optical densities for the 150 kDa protein band recognized by the anti-PLC-β1 antibody, for the 145 kDa protein band recognized by the anti-PLC-γ1 antibody, for the 85 kDa protein band recognized by the anti-PLC-δ1 antibody, and for the 40 kDa protein band recognized by the anti-GFAP antibody were measured by a scanning densitometer (Arcus II, Agfa, Germany); these densities were taken to indicate relative quantities of PLC-β1, PLC-γ1, PLC-δ1, and GFAP, respectively.

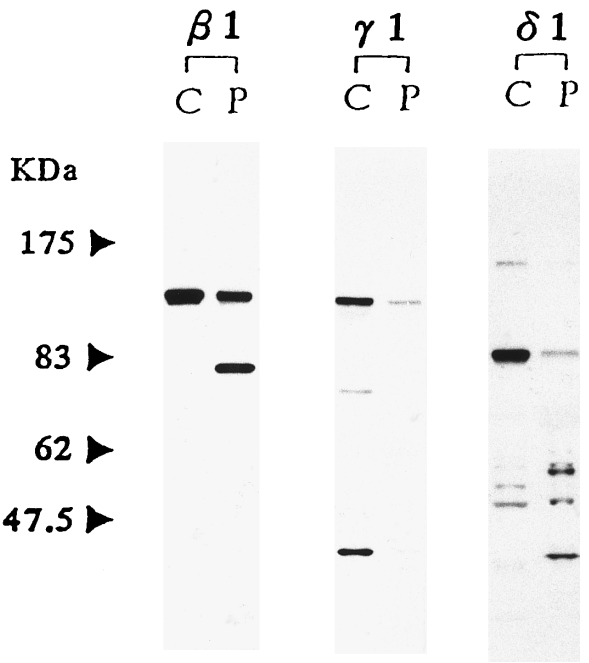

FIG. 1. Expression of phospholipase C (PLC)-β1, -γ1, and $-\delta$1 in the cytosolic and particulate fractions of the cerebral cortex. Specific antibodies immunochemically recognized PLC-β1, -γ1, and $-\delta$1 as the 150-, 145-, and 85-kDa protein bands, respectively, on SDS-PAGE. Both PLC-γ1 and PLC-δ1 were present mainly in the cytosolic fraction, while PLC-β1 also was expressed at a high level in the particulate fraction at 4 weeks age in rat cerebral cortex. C, cytosolic fraction; P, particulate fraction.

RESULTS

Expression of PLC-β1, -γ1 and $-\delta$1 in the Cytosolic and Particulate Fractions in the Rat Brain

PLC-β1, -γ1 and $-\delta$1 were recognized as 150 kDa-, 145 kDa-, and 85 kDa-protein bands, respectively, on SDS-PAGE. Both PLC-γ1 and PLC-δ1 were present mainly in the cytosolic fraction, while PLC-β1 also was expressed at a high level in the particulate fraction of 4-week rat cerebral cortex (Fig. 1).

Gel Filtration Chromatography of Brain Extracts for PLC-β1, -γ1 and $-\delta$1

Figure 2A shows a typical elution pattern of cortical PLC at E19 on a Superdex 200 pg column. One major peak and one very minor peak of PLC activity were obtained at E19. The first form was detected at the elution position having a molecular weight of about 240 kDa. The second form was detected at the elution position having a molecular weight of about 140 kDa. Immunochemical detection by specific antibodies against PLC isozymes revealed that the first major peak of PLC activity mainly corresponded to PLC-γ1, and the second very minor peak mainly corresponded to PLC-δ1.

Figure 2B shows a typical elution pattern of cerebral cortical PLC at 4 weeks on a Superdex 200 pg column. Two major peaks of PLC activity were obtained at this age. The activity of the first form was higher than that of the second form. The first form was detected at the elution position having a molecular weight of about 240 kDa. The second form was detected at the elution position having a molecular weight of about 140 kDa. Immunochemical detection by specific antibodies against PLC isozymes revealed that the first major peak of PLC activity mainly corresponded to PLC-γ1, and the second major peak mainly corresponded to PLC-δ1. PLC-β1 also was detected as a minor component before the elution position of PLC-γ1.

Figure 2C shows a typical elution pattern of cerebral cortical PLC at 48 weeks of age on a Superdex 200 pg column. Two major peaks of PLC activity were obtained. The activity of the first peak and that of the second peak were almost the same. The first form was detected at the elution position having a molecular weight of about 240 kDa. The second form was detected at the elution position having a molecular weight of about 140 kDa. Immunochemical detection by specific antibodies against PLC isozymes revealed that the first major peak of PLC activity mainly corresponded to

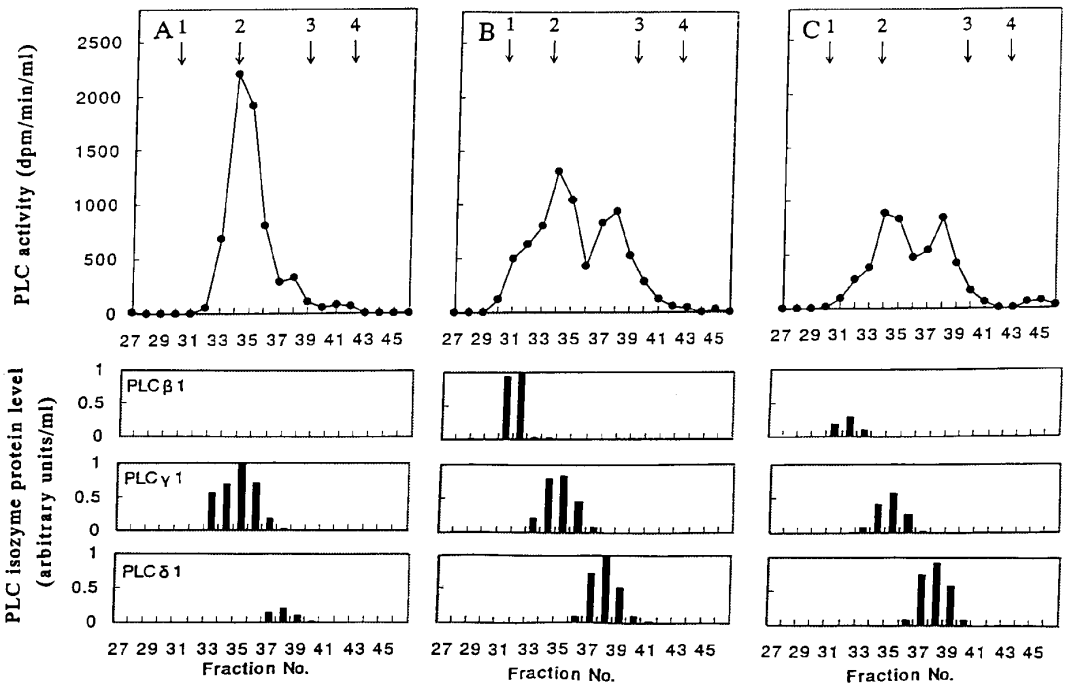

FIG. 2. Separation of rat brain phospholipase C (PLC) by Superdex 200 pg gel filtration chromatography. (A) Typical elution pattern of cortical PLC at 19 days of embryonic life (E19). (B) Typical elution pattern of cortical PLC at 4 weeks of age. (C) Typical elution pattern of cortical PLC at 48 weeks of age. An extract of rat brain cytosols was applied to a Superdex 200 pg column (1.6 × 60 cm), equilibrated, and eluted with 25 mM Hepes buffer (pH 7.0) containing 1 mM dithiothreitol and 0.1 M NaCl. One-milliliter fractions were collected, assayed for PLC activity, and used for immunochemical detection. The assay for PLC activity and the method for immunochemical detection are described in Materials and Methods. Arrows at the top of the figure show the elution positions of standard proteins: 1, ferritin (molecular weight 450 kDa); 2, catalase (molecular weight 240 kDa); 3, sweet potato acid phosphatase (molecular weight 110 kDa); 4, BSA (molecular weight 68 kDa). Lower panel indicates the relative distribution of PLC-β1, PLC-γ1, and PLC-δ1 immunoreactivity, respectively.

PLC-γ1, and the second major peak mainly corresponded to PLC-δ1. PLC-β1 also was detected as a minor component before the elution position of PLC-γ1.

Activity corresponding to PLC-β1 was almost absent at E19, and higher at 4 weeks than at 48 weeks. Activity corresponding to PLC-γ1 was highest at E19, and higher at 4 weeks than at 48 weeks. Activity corresponding to PLC-δ1 was almost the same at 4 and 48 weeks of age, and remarkably low at E19 (Fig. 2, A-C).

Developmental and Aging Changes of Protein Levels for PLC-β1, -γ1 and –δ1 in the Cerebral Cortex

PLC-β1. PLC-β1 was minimally detected at E19 in both cytosolic and particulate fractions. Its protein level increased after birth, being highest at 4 weeks of age, and then gradually decreased with development and aging in the cytosolic fraction. In the particulate fraction it gradually increased after birth, being highest at 24 weeks of age, and then decreased with aging. PLC-β1 was more abundant in the cytosolic fraction than in the particulate fraction until 8 weeks, and was more evident in the particulate fraction than in the cytosolic fraction after 24 weeks (Fig. 3A).

PLC-γ1. The protein level of PLC-γ1 was highest at E19, decreasing after birth in the cytosolic fraction. PLC-γ1 also was highest at E19, decreasing after birth, and remaining constant from 2 to 96 weeks in the particulate fraction. At all stages PLC-γ1 was more abundant in the cytosol than in the particulate fraction (Fig. 3B).

PLC-δ1. PLC-δ1 was expressed slightly at E19; its protein level increased with development, being highest at 24 weeks, and then gradually decreasing in the cytosolic fraction. PLC-δ1 was detected minimally at E19, but increased with postnatal development in the particulate fraction. At all stages PLC-δ1 was much more abundant in the cytosol than in the particulate fraction (Fig. 3C).

Developmental Changes of Protein Levels for GFAP in the Cerebral Cortex

GFAP was expressed in both cytosolic and particulate fractions, more abundantly in the particulate fraction. Total GFAP protein level was minimally detected at E19, and its protein level increased with develop-

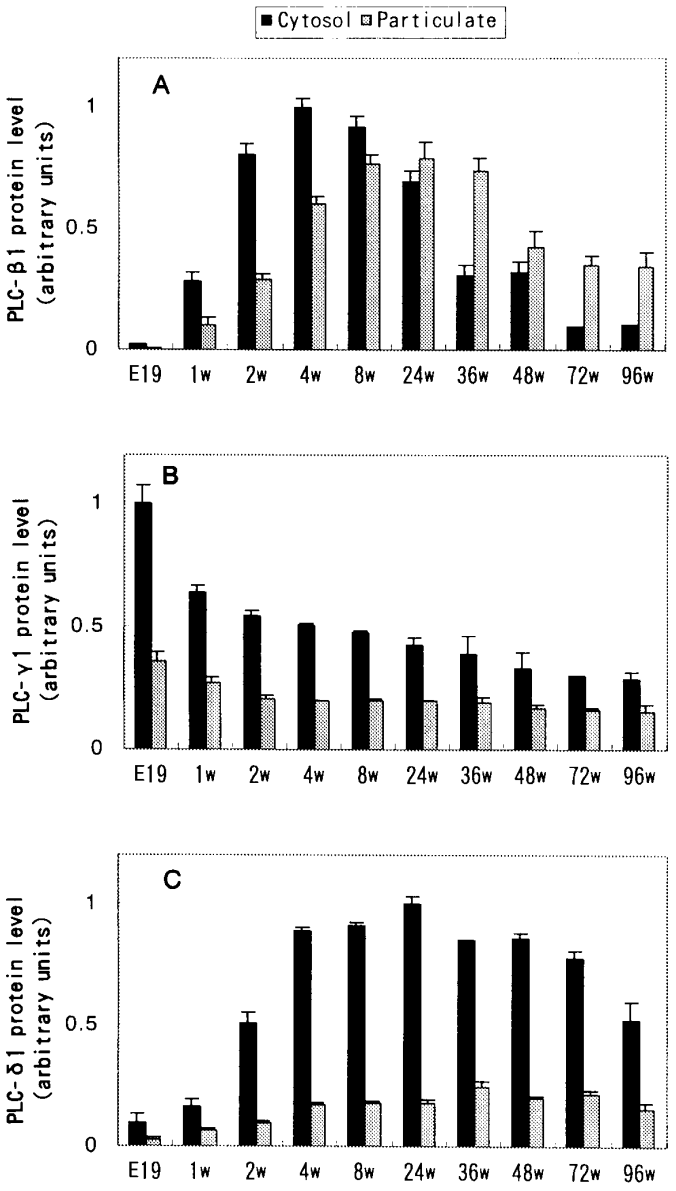

FIG. 3. Developmental changes of protein levels for PLC-β1, -γ1, and $-\delta$1 in the cerebral cortex. (A) PLC-β1 protein level. (B) PLC-γ1 protein level. (C) PLC-δ1 protein level. The method for immunochemical detection is described in Materials and Methods. Bars indicate standard errors of mean (SEM) (n = 4).

ment, being highest at 24 weeks, and then gradually decreasing with aging (Fig. 4).

DISCUSSION

The present study revealed differential contributions of each PLC isozyme to the central nervous system (CNS) development and aging. Gel-filtration chromatography of cortical tissue extracts demonstrated that at E19 PLC activity was almost entirely due to the PLC-γ1 isozyme. At 4 postnatal weeks PLC-γ1 activity remarkably decreased, while PLC-β1 and PLC-δ1 activities drastically increased. At 48 weeks PLC-γ1 activity decreased, while PLC-δ1 activity remained at the same level as at 4 weeks. PLC-β1 activity in relation to the total PLC activity of the cytosolic fraction decreased at 48 weeks compared with 4 weeks.

Immunochemical detection of each isozyme in the cytosolic fraction of the cerebral cortex from E19 to 96 weeks was essentially consistent with the results of gel filtration chromatography. The protein level of PLC-γ1 was highest at E19, and decreased gradually with aging. PLC$-\beta$1 was detected minimally at E19, but increased remarkably postnatally with peak protein level at 4 weeks, then gradually decreasing. Interestingly, the protein level of PLC-δ1 gradually increased from E19 to 24 weeks, and then remained constant during weeks 36 to 72. Considering the function of each PLC isozyme in the CNS, it is important to clarify which enzymes are found in what type of cells. On this point Yamada et al. (11) immunohistochemically investigated expression of PLC-β, -γ and $-\delta$ isozymes, which respectively are considered identical with PLC-β1, -γ1, and $-\delta$1 isozymes, in the Wistar rat nervous system at E17 and through postnatal development to adulthood. They found that PLC-γ immunoreactivity was expressed intensely in radial fibers from the late fetal to the early newborn stage, while a weaker PLC-β reaction also was demonstrated in these structures. PLC-β and PLC-γ immunoreactivity appeared in neurons of various regions after the first postnatal week and then increased until the adult stage. PLC-δ immunoreactivity appeared in astrocytes throughout the brain from the second postnatal week, although weak antigenicity also was present in some neurons. Our immunohistochemical study was essentially identical with these results with the exception that postnatally PLC-δ1 was present in neuronal somas and processes although some glia-appearing cells also showed weak immunore-

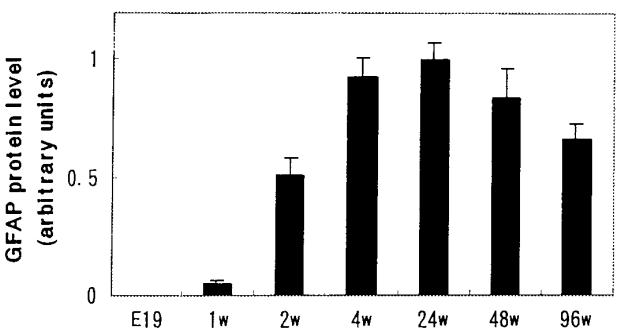

FIG. 4. Developmental changes of protein levels for GFAP in the cerebral cortex. The method for immunochemical detection is described in Materials and Methods. Bars indicate standard errors of mean (SEM) (n = 4).

activity (data not shown). The discrepancy may reflect differences in the antibodies used. Unlike the present study, the previous one did not address developmental and aging changes of enzyme activity and quantitative protein levels of each isozyme.

Numerous growth factor receptors have a protein tyrosine kinase domain, and several protein tyrosine kinases have been demonstrated to associate with and phosphorylate PLC-γ on tyrosine. These include the receptors for epidermal growth factor (EGF), platelet-derived growth factor (PDGF), and fibroblast growth factor (FGF) (17–20). The present findings of high protein levels of PLC-γ1 and its enzyme activity at E19 and early developmental stages suggest that high activities of *src* family kinases linked to EGF-, FGF-, and PDGF-receptors are important in differentiation and development of the CNS (21-26). PLC-γ1 isozyme may play an essential role in cellular division and growth in CNS histogenesis mediated by various growth factors.

Evidence suggests that nontyrosine kinase receptors use G protein to activate members of the PLC-β and possibly PLC-δ families (27). As determined by immunoblotting procedure, overall levels of G-protein increase from the fetal stage through the neonatal and adolescent periods to reach a maximum concentration in young adult tissues (28), consistent with the developmental changes we saw in PLC-β1, suggesting that G-protein and PLC-β1 are functionally coupled in development. It is notable that the protein levels of PLC-β1 in the particulate fraction increased with aging until 36 weeks, suggesting that although PLC-β1 is localized predominantly in the cytoplasm (consistent with the absence of trans-membrane spanning domains) in the early developmental stage, its translocation from cytosol to membrane may be associated with the development and maturation of the CNS. In rat parietal cortex (29), visual cortex (30), and dentate gyrus (31), the bulk of synaptogenesis does not occur until after the first postnatal week. PLC-β1 protein level increased with development from E19 to 4 weeks and PLC-δ1 protein levels increased from E19 to 24 weeks of age in the cerebral cortex, suggesting that PLC-β1 functions in early synaptogenesis and establishment of the neural network, and PLC-δ1 is involved in the maturation and maintenance of the CNS. In addition, immunoblot analysis showed GFAP immunoreactivity to increase during postnatal development of the rat brain, very similar to that of PLC-δ1. Immunohistochemically PLC-δ1 is present in neurons, but also to an extent in glial cells, so PLC-δ1 might play a role in phosphoinositide signal transduction in these cells, although further study will be necessary to clarify this functional significance.

In conclusion, enzyme assay and immunoblot analysis revealed that three PLC isozymes were present in both fetal and adult brains, with strong reactions for β1 and δ1 in adult brain and that of γ1 in fetal brain, suggesting that each PLC isozyme is differently involved in the course of development and aging. PLC-γ1 isozyme may be involved especially in cellular division and growth during CNS histogenesis, while PLC-β1 functions in early synaptogenesis and establishment of the neural network, and PLC–δ1 may be involved in the maturation and maintenance of the CNS.

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