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ONTOGENY OF CYTOSOLIC PHOSPHOLIPASE A_2 ACTIVITY IN RAT BRAIN

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SUMMARY: We investigated developmental changes in the activity of cytosolic phospholipase A_2 ($cPLA_2$) in the rat brain. When the cytosolic fractions from rat brain of various ages were examined by gel filtration chromatography, $cPLA_2$ activity was detected at about 100 kDa in all developmental stages. However, the magnitude of $cPLA_2$ activity differed significantly. The $cPLA_2$ activity was highest in the brain of day-12 embryo, gradually decreased toward birth, and retained a constant level into adulthood. This result suggests that $cPLA_2$ plays an important role in the early development of the nervous system.

Phospholipase A_2 (PLA_2) is a rate-limiting enzyme in the arachidonic acid cascade in a variety of cells and tissues including brain (1). The arachidonic acid released is subsequently converted to eicosanoids such as prostaglandins, thromboxanes and leukotrienes (2). These eicosanoids act as neuroregulators in various physiological and pathological events occurring in the central nervous system (3-6).

We previously reported the presence of a novel type of PLA_2 ($cPLA_2$) in rat brain, a cytosolic 100-kD enzyme, which is activated by micromolar concentrations of Ca^{2+}, and translocates from cytosol to membrane in a Ca^{2+}-dependent manner (7). Recently, Clark *et al.* (8) and Sharp *et al.* (9) independently cloned and sequenced a cDNA encoding the high molecular weight cytosolic PLA_2 from a U937 human monocyte cell line cDNA library.

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The abbreviations used are: $cPLA_2$, cytosolic phospholipase A_2; EGTA, ethylene glycol bis(β-aminoethylether)-N,N,N',N'-tetraacetic acid.

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Based on several properties such as Ca^{2+}-sensitivity, molecular weight and Ca^{2+}-induced translocation, $cPLA_2$ in rat brain cytosol appears to be identical with that in U937 cells. In the present study, we have investigated the changes in $cPLA_2$ activity during development of the central nervous system of the rat.

MATERIALS AND METHODS

Materials: L-α-1-stearoyl-2-(1-^{14}C)arachidonoyl phosphatidylcholine (1.85 GBq/mmol) was purchased from Amersham. Superose 6 HR 10/30 column was from Pharmacia; and Centriprep, from Amicon. All other chemicals were of reagent grade from Nacalai Tesque.

Partial Purification of $cPLA_2$ from Rat Brain: Brains from male Wistar rats of various ages (from embryonic day 12 to postnatal day 80) were homogenized in 10 vol. of 10 mM Tris-HCl (pH 7.4) containing 1 mM EGTA (buffer A), and centrifuged at 100,000 x g for 60 min. The supernatant was concentrated to 0.5 ml with a Centriprep 10 device and applied to a Superose 6 HR 10/30 column equilibrated with buffer A containing 100 mM NaCl. An aliquot of each fraction was assayed for PLA_2 activity.

Assay of PLA_2 Activity: PLA_2 activity was measured essentially according to Teramoto *et al.* (10). The standard incubation mixture (100 μl) contained 100 mM Tris-HCl (pH 8.3), 4 mM $CaCl_2$, 50 % glycerol and 500 pmol of L-α-1-stearoly-2-(1-^{14}C)arachidonoyl phosphatidylcholine. The reaction was carried out at 37°C for 30 min and stopped by the addition of 400 μl of Dole's reagent. The fatty acid released was extracted and its radioactivity was counted in liquid scintilation counter.

RESULTS AND DISCUSSION

In a previous paper (7), we demonstrated the existence of two types of PLA_2 in rat brain cytosol. One is the higher molecular weight PLA_2 (PLA_2-H) which appeared as a broad peak of 200 - 500 kDa and whose activity was partially inhibited by the addition of Ca^{2+}. The other is a 100 kDa-PLA_2 ($cPLA_2$ or PLA_2-L) that is activated by Ca^{2+} at μM concentrations and translocates from cytosol to membrane. In the present study, 50 % glycerol was included in the assay medium, because the latter $cPLA_2$ activity was greatly enhanced by 50 % glycerol (Fig. 1A). On the other hand, the activity of PLA_2-H was strongly inhibited by 50 % glycerol (Fig. 1B).

Fig. 2 shows typical elution profiles of rat brain cytosol of embryonic day 15 (A) and postnatal day 80 (B) from a Superose 6 HR 10/30 gel filtration column. In both cases, $cPLA_2$ activity appeared as a single peak at about 100 kDa, and absolutely required Ca^{2+} for the activity (data not shown). However, the

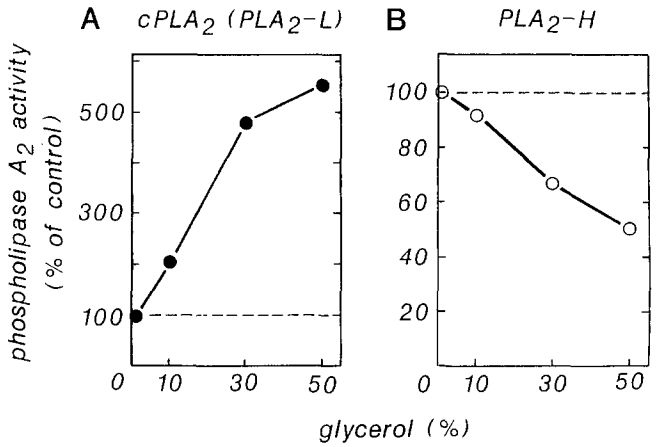

Fig. 1. Effect of Glycerol on $cPLA_2$ (PLA_2-L) and PLA_2-H Activities.
$cPLA_2$ and PLA_2-H were partially purified from rat brain cytosol by gel filtration chromatography according to Yoshihara and Watanabe (7), and assayed for PLA_2 activity with various concentrations of glycerol. PLA_2 activity is represented as % of control (without glycerol). Specific activities of $cPLA_2$ and PLA_2-H without glycerol were 2.26 and 0.74 nmol arachidonic acid liberated /30 min/ mg protein at pH 8.3, respectively.

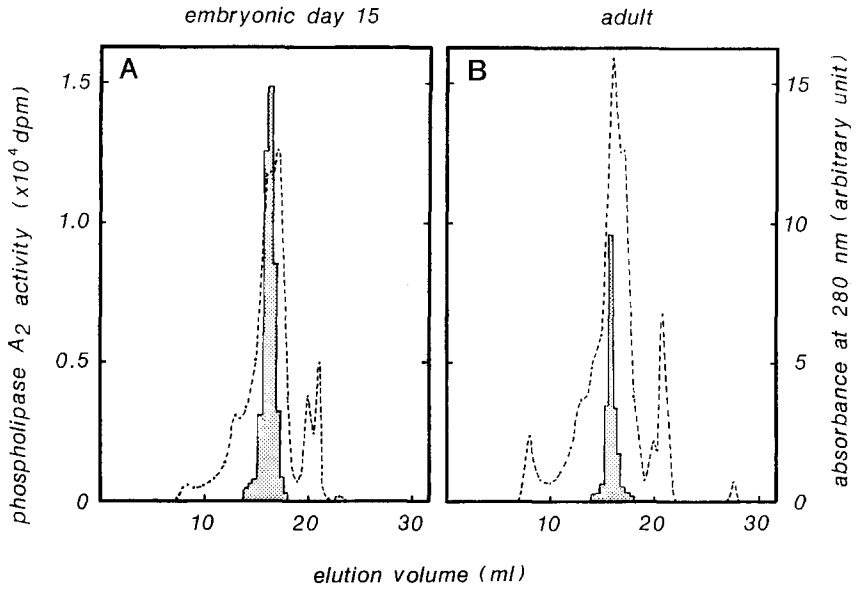

Fig. 2. Typical Elution Profiles of $cPLA_2$ Activity obtained by Superose 6 HR 10/30 Column Chromatography.
Concentrated cytosol (0.5 ml) from rat brain of embryonic day 15 (A) or postnatal day 80 (B) was loaded onto a gel filtration column equilibrated with buffer A containing 100 mM NaCl. Flow rate was 0.4 ml/min, and 0.4 ml fractions were collected. An aliquot (28 μl) of each fraction was assayed for PLA_2 activity (hatched column). Dashed lines indicate the absorbance at 280 nm.

magnitude of $cPLA_2$ activity significantly differed between embryonic (A) and adult (B) brain.

Detailed changes in $cPLA_2$ activity in rat brain during development were next investigated. Samples of brain cytosol from embryonic day 12, 15, 19, and postnatal day 3, 35, 80 were subjected to gel filtration chromatography, and $cPLA_2$ activity at each age was measured. Elution profiles of $cPLA_2$ activity from various specimens were similar to those in Fig. 2. As shown in Fig. 3, the highest activity was detected at embryonic day 12. A gradual decrease in activity was observed toward birth, and no significant change was seen after postnatal day 35. The $cPLA_2$ activity of the embryonic day-12 brain was 5.5-fold higher than that of the adult brain.

The development of the nervous sysytem includes a variety of events such as neuronal differentiation and migration, neuronal cell death, process outgrowth and guidance, synapse formation, gliogenesis and myelination (11). In particular, the first axonal outgrowth and guidance begin at the middle of embryonic development in mammals (12). $cPLA_2$ may be involved in such developmental events in the embryonic nervous system. Negre-Aminou *et al.* (13) demonstrated that growth cone preparations isolated from embryonic rat brain exhibit elevated levels of free arachidonic acid and that the rate of arachidonic acid turnover is very high in isolated growth cones. These facts may be attributed to the high activity of $cPLA_2$ in embryonic brain. Therefore,

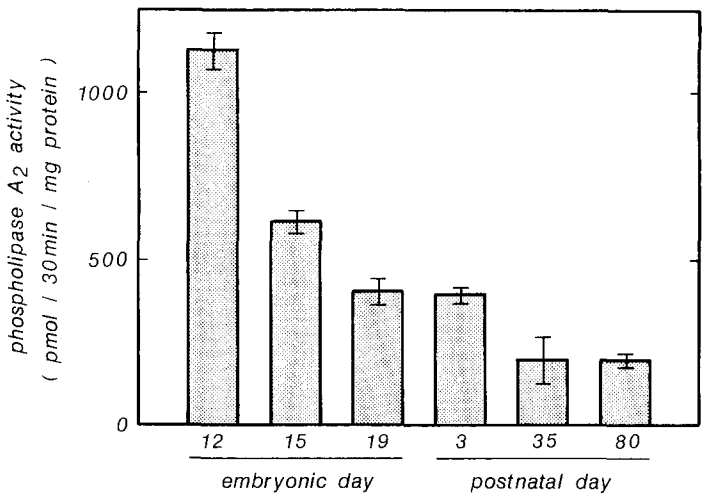

Fig. 3. Developmental Changes in $cPLA_2$ Activity in Rat Brain.
$cPLA_2$ activities in rat brain cytosol of embryonic day 12, 15, 19, postnatal day 3, 35, and 80 were measured after partial purification with a Suprose 6 HR 10/30 column as described in Materials and Methods. Values are expressed as mean ± SEM (n=4 for embryonic day 12, 15, 19, postnatal day 3 and 35; n=7 for postnatal day 80).

$cPLA_2$ might be a part of biochemical machinery that regulates the growth cone motility resulting in axonal guidance.

Though we do not know at present the reason for the higher activity of $cPLA_2$ in embryonic brain, several explanations are plausible. (A) The $cPLA_2$ enzyme itself in the embryonic brain is in greater concentration than in the adult brain. (B) Some unidentified activator of $cPLA_2$ is present in the embryonic brain, or some unidentified inhibitor is present in the adult brain. (C) $cPLA_2$ undergoes some structural change or modification during neural development, such as phosphorylation, dephosphorylation, limited proteolysis, etc. Preliminarily, RT/PCR (reverse transcription/polymerase chain reaction) analysis with $cPLA_2$-specific oligonucleotide primers and Western blot analysis with anti-$cPLA_2$ peptide antibody revealed that there seems to be no difference in the amount of $cPLA_2$ transcript and protein between embryonic and adult brain, thus eliminating possibility "A" above mentioned. Studies are now in progress to elucidate the mechanism and physiological meaning of this ontogenic change in $cPLA_2$ activity.

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