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INTERLEUKIN-1β INDUCES CYTOSOLIC PHOSPHOLIPASE A2 GENE IN RAT C6 GLIOMA CELL LINE

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SUMMARY: Treatment of the rat C6 glioma cell line with interleukin-1β (IL-1β) resulted in an accumulation of cytosolic phospholipase A2 (cPLA2) in mRNA level. The increase of cPLA2 in mRNA level was observed at 6 hours after treatment of IL-1β and reached to the maximal level at 12 hours. The accumulation also remained sustained for up to 72 hours. Dose response curve of IL-1β showed that as little as 40 units/ml concentration induced the accumulation of mRNA and 100 units/ml concentration showed the maximal effect. In contrast, cyclooxygenase 2 gene was not affected with treatment of IL-1β.

Phospholipase A2 (PLA2) liberates fatty acids from the sn-2 position of phospholipids. This reaction is of particular significance when the fatty acid is arachidonic acid, since it serves as a precursor for eicosanoids such as prostaglandins and leukotrienes (1). Arachidonic acid and eicosanoids are not only modifying factors under the pathogenesis such as cerebral ischemia (2), hypoglycemia (3) and seizures (4, 5) but also exert neuromodulatory roles in the control of behavior (6), sleep (7), regulation of blood flow (8), thermoregulation (9) and control of neuroendocrine (10, 11) in the central nervous system. In view of these facts, regulation of an arachidonic acid specific PLA2, that is cytosolic phospholipase A2 (cPLA2), especially plays important roles in various physiological and pathological responses (12).

Interleukin-1 (IL-1) is a cytokine with a range of biological activities and is suggested to be a key mediator of inflammation (13). In the immune system, by releasing prostaglandin E, macrophages can lead to the production of soluble factors such as IL-1 or some growth factors, whereas, in the central nervous system, astrocytes take place of macrophage to play important roles in various responses. For example, intracerebro-

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Abbreviations: cPLA2, cytosolic phospholipase A2; IL-1β, Interleukin-1β; COX2, cyclooxygenase 2.

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ventricular administration of IL-1 induces the febrile response, acute-phase-glycoprotein synthesis, slow-wave sleep, loss of appetite and sickness syndrome (13-16). Furthermore IL-1 also stimulates astrocyte growth after brain injury (17). IL-1 expression changes during the development (18). In spite of these physiological effects of IL-1 described above, the molecular mechanism by which IL-1 can activate cells of neuronal and glial origin remains unclear.

The brain contains significant PLA2 activity and is rich in arachidonic acid-containing phospholipids (19). Elevated levels of free fatty acids including arachidonic acid and arachidonic cascade metabolites have been observed following cerebral ischemia, hypoglycemia and seizures (2-5). IL-1 has been known to increase expression of cPLA2 in HeLa cells (20), monocytes (21) and fibroblast cell lines (22, 23). In this report, we investigated the relationship of IL-1β and cPLA2 on the mRNA expression level using rat C6 glioma cell lines. We show here that IL-1β induces the accumulation of cPLA2 in C6 cells.

MATERIALS AND METHODS

Cell Culture and IL-1β Treatment : Rat C6 glioma cells were cultured in Dulbecco's modified Eagle's medium (GIBCO BRL Life Technologies Inc., Grand Island, NY, USA) containing 10 %(v/v) fetal calf serum. At three days after plating the cells at 1 x 10^5 cells in 150 mm dish, the medium was replaced with culture medium supplemented with various doses of human recombinant IL-1β (Genzyme Co., Cambridge, MA, USA). The cells ($5x10^7$-$1x10^8$ cells) were collected after 0 to 72 hours.

RNA Blotting: Total RNA was extracted from C6 cells by the standard method (24). mRNA was purified from total RNA using Oligotex-dT30 (Takara Shuzo Co.,ltd., JAPAN). The mRNA (2-5 μg) was run on 1 % agarose-2.2M formaldehyde gel and then transferred onto a nylon membrane (Hybond N+, Amersham Co., UK). After being baked, the membrane was subjected to hybridization under the conditions described elsewhere (24). Rat cPLA2 and cyclooxygenase 2 (COX2) cDNA were labeled with [α-^{32}P] UTP (Amersham Co. UK) by transcribed from T7 promoter. After hybridization at 55 °C, the membrane was washed several times with 2 x standard saline containing sodium phosphate and EDTA (SSPE), 0.1 %(v/v) SDS, two times each for 10 min at room temperature, 1 x SSPE, 0.1 % (v/v) SDS for 15 min at room temperature and 0.1 x SSPE, 0.1 %(v/v) SDS for 10 min at 55 °C. The same membrane was used for reprobing with glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) and COX2 genes. GAPDH probe was labeled by random priming method. After the exposure onto an imaging plate overnight, the signal was detected and analyzed by BAS2000 imaging analyzer (Fuji Photo Film Co.,ltd., Japan).

RESULTS

Northern hybridization analysis was performed to examine whether the mRNA expression of cPLA2 is changed by IL-1β in C6 cells or not. At three days after plating the C6 cells at 1 x 10^5 cells in 150 mm dish, the medium was replaced with culture medium supplemented with 100 units/ml of IL-1β. As shown in Fig. 1, IL-1β induced approximately 3-fold increase of cPLA2 mRNA. From the data of repetitive experiments, induction of 3- to 5-fold was observed. We also tested the effect of IL-1β on the expression level of cyclooxygenase 2 (COX2) gene, since COX2 is required for the conversion of arachidonic acid to PGH2 and has been shown to be regulated by a number of agents, including IL-1. Cyclooxygenase gene was not significantly affected with

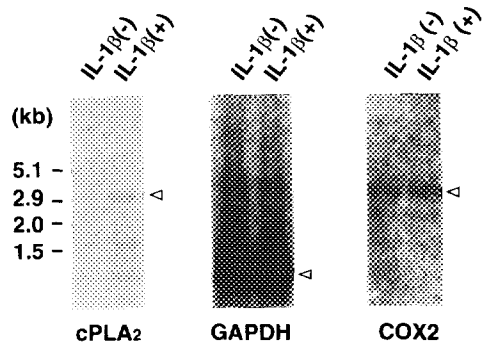

Figure 1. *The effect of IL-1β on cPLA2, COX2 and GAPDH mRNA level in C6 glioma cells.* Cellular mRNA from C6 cells was purified and analyzed by Northern hybridization using a ^{32}P-labeled specific probe for cPLA2, COX2 and GAPDH. IL-1β(-) and IL-1β(+) mean to incubate for 24 hr with control media and IL-1β (100 units/ml), respectively. Two micrograms of mRNA per lane was loaded. IL-1β induced approximately 3-fold increase of cPLA2 mRNA without affecting expression level of cyclooxygenase gene. The positive bands corresponding to mRNA for cPLA2, COX2 and GAPDH are indicated by arrowheads.

treatment of IL-1β under the present experimental conditions. No significant change in mRNA levels for the intracellular gene, glyceraldehyde-3-phosphate-dehydrogenase (GAPDH), was found between control and treated cells.

To investigate the time course of the induction of cPLA2 in the C6 cells, mRNA was purified at each time point after treatment of IL-1β (100 units/ml) and Northern hybridization analysis was carried out. The increase of cPLA2 in mRNA level began to be observed at 6 hours after treatment of IL-1β and reached to its maximum at 12 hours. The accumulation remained sustained for up to 72 hours (Fig. 2). Dose response relationship of IL-1β showed that as little as 40 units/ml concentration began to induce the accumulation of mRNA (Fig. 3). One hundred units/ml concentration was required for the maximal effect.

DISCUSSION

The data presented here demonstrated that IL-1β induces the accumulation of cPLA2 mRNA. The effect of IL-1β reached to approximately two in three of maximal level in cells treated for 6 hours and to maximum for 12 hours. Previously IL-1β has been reported to activate transcription factor NFκB in C6 glioma cells (25). The activation was detectable from 20 min and maintained for up to 24 hours. The activation of NFκB in glial cells may represent in an early step of IL-1β signaling in the brain and be likely to have consequences for gene, for example cPLA2, induced by IL-1β in these cells. As for the enzyme activity, we checked that C6 cells show the PLA2 enzyme activity maintained at low level (about 100 pmoles /mg protein /20 min). IL-1β treatment of C6 cells resulted in the increase of approximately 2- to 3-fold in the PLA2 enzyme activity (data not shown).

Dose response relationship of IL-1β showed that 40 units/ml (4.6 pM) concentration began to induce the accumulation of mRNA and 100 units/ml (11.5 pM) concentration showed the maximal effect. The effect of 40 units/ml indicated less than half-maximal effect. High affinity receptor of IL-1 exists with a dissociation constant (Kd)

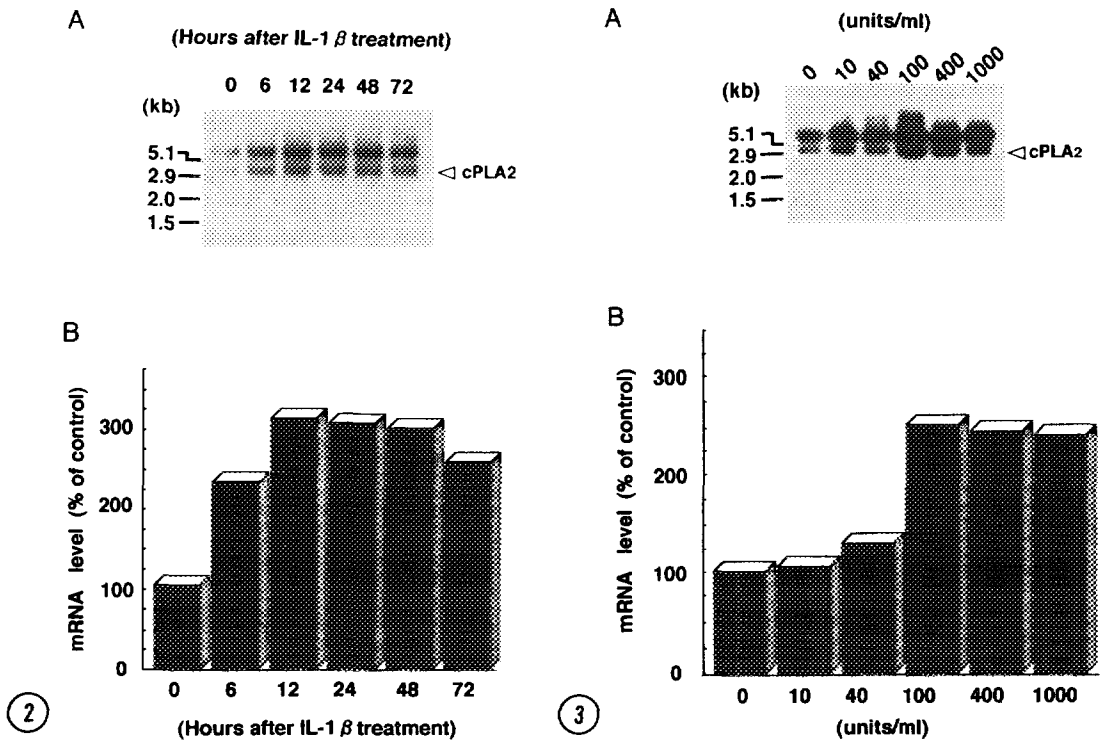

Figure 2. *Time course of induction of cPLA2 by IL-1β.* (A) Autoradiogram of Northern hybridization. mRNA was purified and analyzed by Northern hybridization at each time point after treatment with IL-1β (100 units/ml). Five micrograms per lane were loaded. The probes specific for rat cPLA2 were labeled with [α-^{32}P]UTP by transcribed from T7 promoter. (B) % increase of cPLA2 mRNA in IL-1β treated cells relative to that of control cells. The relative value of mRNA for cPLA2 was determined from the measurement of the band intensity in a fixed area by an imaging analyzer and then was standardized by the GAPDH control. The maximal effect was observed at 12 hr after treatment of IL-1β. The accumulation was retained up to 72 hr. Typical data are shown here. Similar results were obtained from three separate experiments.

Figure 3. *Dose-response relationship of induction of cPLA2 by IL-1β.* (A) Autoradiogram of Northern hybridization. At 24 hours after treatment with IL-1β, mRNA was purified and analyzed at each concentration of IL-1β by Northern hybridization. Five micrograms of mRNA per lane was used. The probes specific for rat cPLA2 were labeled with [α-^{32}P] UTP by transcribed from T7 promoter. (B) % increase of cPLA2 mRNA in IL-1β-treated cells relative to that of control cells. The relative value of mRNA for cPLA2 was determined from the measurement of the band intensity in a fixed area by an imaging analyzer and then was standardized by the GAPDH control. Dose-response relationship showed that as little as 40 units/ml concentration induced the accumulation of mRNA and 100 units/ml concentration is necessary for the maximal effect. Typical data are shown here. Similar results were obtained from three separate experiments.

of 5 to 50 pM, whereas low affinity receptor with 300-500 pM. Therefore IL-1β may bind to the high affinity receptor site to stimulate cPLA2 activity in C6 glioma cells.

In contrast, mRNA level of COX2 did not significantly change in the time-frame of the present study, even at 6 hours after IL-1β treatment. This result is inconsistent with the results in the neurons of rat hippocampus and cerebral cortex which has been shown by Yamagata et al. (26); detected by Northern analysis and *in situ* hybridization, the expression of COX2 mRNA was induced within 30 min after electroconvulsive seizure,

peaked at 1-2 hr, and remained elevated for as long as 8 hr after seizure, and particularly the induction was specific for neurons (26). IL-1α and IL-1β has been shown to cause accumulation of COX1 (or 2) mRNA and/or protein in human umbilical vein endothelial cells (27) and in dermal fibroblasts (28), respectively, whereas IL-1α induced the increase in cPLA2 and prostaglandin E2 content without affecting COX2 level in human lung fibroblast cell line, WI-38 (23). Taken together with the recent report that cPLA2 is localized mostly in the astrocytes of the gray matter, neither in neurons, microglia, nor oligodendrocytes (14), these results suggest that IL-1 regulation of COX2 appears to be specific for cell types. In the central nervous system, IL-1 might act on both astrocytes and neurons dependently with some signal relations or independently and such unknown signals might cause a selective induction of cPLA2 or COX2 expression in these cells. This interpretation is partly supported by our recent finding that induction of COX1 (3 kbp mRNA) and COX2 (4 kbp mRNA) occurs in the neuronal cells by initiation of primary culture essentially through stimulation of glial cell growth (29).

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