The Differential Regulation of Group II\textsubscript{A} and Group V Low Molecular Weight Phospholipases \textit{A}_2 in Cultured Rat Astrocytes*

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Ginette Thomas‡§, France Bertrand¶, and Bertrand Saunier‡

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† To whom correspondence should be addressed: Bât. INSERM, Hôpital de Bicêtre, 80 rue du Général Leclerc, 94276 Le Kremlin-Bicêtre Cedex, France.

‡ The abbreviations used are: sPLA\textsubscript{2}, low molecular weight or secretory phospholipase \textit{A}_2; TNF\textalpha{}, tumor necrosis factor; IL-\textit{b}, interleukin-\textit{b}; MAP, mitogen-activated protein; Erk, extracellular signal-regulated kinase; Jnk, c-Jun NH\textsubscript{2}-terminal kinase; PI 3-kinase, phosphatidylinositol 3-kinase; PCR, polymerase chain reaction; ZAL, \textit{N}-benzoyloxycarbonyl-Ile-Glu(O-t-buty1)-Ala-leucinal; DMEM, Dulbecco’s modified Eagle’s medium.

In astrocytes, cytokines stimulate the release of secretory phospholipase \textit{A}_2 (sPLA\textsubscript{2}) activity and group II\textsubscript{A} sPLA\textsubscript{2} expression. This paper reports that two sPLA\textsubscript{2} isoforms, group II\textsubscript{A} and group V, are in fact expressed by astrocytes. Our studies showed that tumor necrosis factor \alpha{} (TNF\textalpha{}) enhanced the mRNA of both isoforms, but the time courses of enhancement differed; group \textit{V} was induced much faster than group II\textsubscript{A}. Moreover, TNF\textalpha{} stimulated both the NF-\kappa{}B and mitogen-activated protein (MAP) kinase (extracellular signal-regulated kinase, c-Jun NH\textsubscript{2}-terminal kinase, and p38 MAP kinase) signaling pathways in astrocytes. Interestingly, PI 3-kinase activity also was enhanced by TNF\textalpha{}, and NF-\kappa{}B pathway was involved in mediating its effect. Specific inhibitors were used to show that both extracellular signal-regulated kinase and p38 MAP kinase may contribute to the effect of TNF\textalpha{} and that blocking phosphatidylinositol 3-kinase activity fully reversed the effect of TNF\textalpha{}. Furthermore, in astrocytes, TNF\textalpha{}-induced release of sPLA\textsubscript{2} activity was partially reversed by thyroid hormone and almost abolished by growth factors. This phenomenon was accompanied by a less marked increase in both group II\textsubscript{A} and group V sPLA\textsubscript{2} mRNA. In the presence of growth factors, the increase in group V mRNA was inhibited early and transiently, in contrast to what was observed with group II\textsubscript{A}, which was more persistently inhibited. Although a transcriptional effect of thyroid hormone or growth factors in astrocytes cannot be definitively excluded, both types of factor interfered with sPLA\textsubscript{2} expression in a manner suggesting the existence of regulation of post-transcriptional events.

The low molecular weight phospholipases \textit{A}_2 belong to a mixed family of phospholipase \textit{A}_2 fatty acyl ester bond hydrolytic enzymes. They were first identified in snake venom (group I\textsubscript{A}) and mammalian secretions (group I\textsubscript{B}), and are also referred to as the secretory phospholipases \textit{A}_2 (sPLA\textsubscript{2}).\textsuperscript{1} Additional sPLA\textsubscript{2} isoforms have been identified in venoms (groups I\textsubscript{B} and III\textsubscript{I}) and in mammals: group II\textsubscript{A}, group II\textsubscript{C} (in rodents only), group \textit{V}, and group X. The latter two isoforms were the most recently discovered. The sPLA\textsubscript{2} isoforms share sequence homology with a high cysteine content, several of them (10–16) bonded by disulfide bridges, and millimolar Ca\textsuperscript{2+} requirement for their enzyme activity (1). The sPLA\textsubscript{2} thus belong to a growing family of enzymes that have been conserved throughout the evolutionary process. In mammals, a tissue-dependent preeminence of expression has been reported for all sPLA\textsubscript{2} isozymes. Nevertheless, their function has yet to be fully elucidated.

In man, elevated group II\textsubscript{A} sPLA\textsubscript{2} activity has been demonstrated, for example, in rheumatoid arthritis exudates and in the serum of patients presenting with septic shock (2). The catalytic activity of this enzyme is known to have the potential to release arachidonic acid from membrane phospholipids (3). The arachidonic acid may then act as a precursor in the synthesis of mediators of the inflammatory reaction (prostaglandins, leukotrienes, etc.). Group II\textsubscript{A} sPLA\textsubscript{2} are also known to be rapidly released from the secretory granules of activated platelets, neutrophils, or mast cells. Group II\textsubscript{A} sPLA\textsubscript{2} can also be synthesized by mesangial cells, chondrocytes and astrocytes, which have also been implicated in immuno-inflammatory processes (4). For all of those reasons, the group II\textsubscript{A} isoform is still referred to as the inflammatory sPLA\textsubscript{2}.

Group \textit{V} sPLA\textsubscript{2} was first identified in cardiac and placental tissue, but is also found in the lungs and liver in man (5) and rats (6). More recently, that isoform, and not group II\textsubscript{A} as was previously thought, was reported to be expressed by PD388D\textsubscript{1} macrophages (7). The functional distinction between group II\textsubscript{A} and group \textit{V} sPLA\textsubscript{2} remains unclear. However, it would appear that not only group II\textsubscript{A}, but also group \textit{V} sPLA\textsubscript{2}, could be involved in immuno-inflammatory diseases and processes, leading some authorities to the conclusion that the two isoforms may have redundant function.

An increase in phospholipase \textit{A}_2 activities and prostaglandin \textit{E}_2 production has been reported in numerous brain diseases, such as the neurodegenerative diseases, and transient ischemia (8, 9). Moreover, recent results suggest that phospholipases \textit{A}_2 are involved in brain diseases (10, 11). Nevertheless, little is known about the pathophysiology of sPLA\textsubscript{2} in the brain. Although neuronal cells have been reported to produce group II\textsubscript{A} sPLA\textsubscript{2} (12), in primary neuron cultures, the presence of sPLA\textsubscript{2} has been shown to potentiate the neurotoxicity of glutamate (13). Most of the data on cerebral sPLA\textsubscript{2} have been obtained \textit{ex vivo}, in astrocyte cultures (14). Moreover, sPLA\textsubscript{2} production has only been clearly demonstrated in astrocytes (15), although cells crossing the blood-brain barrier presumably also produce sPLA\textsubscript{2}. In cultured astrocytes, group II\textsubscript{A} sPLA\textsubscript{2} secretion is stimulated by pro-inflammatory cytokines (IL-1\textbeta{} and TNF\textalpha{}), bacterial lipopolysaccharide, intracellular cAMP elevating agents, and phorbol ester tumor promoters, probably through transcriptional activation (16). Much less is

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known about inhibition of sPLA₂. In astrocytes, dexamethasone and kinase inhibitor H-7 inhibit the effect of endotoxin while the effect of phorbol esters is only obviously antagonized by protein kinase C inhibitors (16).

Many contrasting effects of pro-inflammatory cytokines and growth factors on the brain have been described, as evidenced by neuron degeneration and survival data. This could also be true for other soluble factors, such as thyroid hormones, the presence of which is crucial during the development of the brain (17). The contribution of thyroid hormone to brain homeostasis and neuron survival after the development period is currently emerging. Since astrocytes are involved in neuron survival and function, it was of interest to determine whether growth factors and thyroid hormone could interfere with sPLA₂ induction in astrocytes. We report herein that the two types of factor inhibited the increase in sPLA₂ activity induced by TNFα. We have demonstrated that TNFα, in addition to inducing group II A sPLA₂, as has previously been reported in this journal (16), also induces the group V isoform in astrocytes. The mRNA induction of both isoforms was decreased in the presence of thyroid hormone or growth factors. For group II A sPLA₂, our data are compatible with the existence of an inhibitory effect occurring at a post-transcriptional level.

**EXPERIMENTAL PROCEDURES**

**Materials**—1-Hexadecanoyl-2-(1-pyrenedecanoyl)-sn-glycero-3-phosphophorylcholine (pyrene-phosphatidyl glycerol) was purchased from Interchim. Bovine serum albumin (essentially fatty acid- and thyroid hormone-free), wortmannin, LY294002, forskolin, and epidermal growth factor (EGF) were supplied by Sigma-Aldrich. Recombinant TNFα was obtained from Peprotech. Basic fibroblast growth factor (bFGF) was obtained from Biovalley. Anti-phospho-MAP kinase antibodies were obtained from Peprotech. Basic fibroblast growth factor (bFGF) was obtained from Biovalley. Anti-phospho-MAP kinase antibodies were obtained from Peprotech. Basic fibroblast growth factor (bFGF) was obtained from Biovalley. Anti-phospho-MAP kinase antibodies were obtained from Peprotech.

**Cell Culture and Incubation Condition**—(a) DTNC1 and CTXNT2 astrocytes (18) were cultured as recommended by ATCC. (b) Primary culture astrocytes were obtained from brains of 2- to 3-day-old Harlan Sprague Dawley rats and cultured as has been previously described (19). The cells were cultured in a 1:1 mixture of Dulbecco’s modified Eagle’s medium (DMEM) containing 4.5 g/liter glucose and supplemented with antibiotics and 10% fetal calf serum. At the end of the culture period, 90–95% of the cells were glial fibrillary acidic protein-positive. (c) Rabbit chondrocytes were purchased from Roche and cultured, as has been previously described (20). (d) Mouse C127 cells expressing recombinant group II A sPLA₂, under the control of mouse metallothionein I promoter, and human metallothionein I A gene (21), conferring resistance to 10 μM CdCl₂, were cultured in DMEM-F12 containing antibiotics and supplemented with 10% fetal calf serum. (e) Incubation of astrocytes in the presence of hormones, growth factors, and cytokines was performed in DMEM-F12 containing 4.5 g/liter glucose and antibiotics, supplemented with 0.1% essentially thyroid hormone-free bovine serum albumin. The other cells were incubated in the same medium except that the glucose content was 1 g/liter only. Unless otherwise specified, the culture medium was changed at the beginning of the period of incubation with the factors. (f) For the mRNA studies, the culture medium was changed for the incubation medium (see above) once, 18 h before initiating incubation as in e. In consequence, both group II A and group V sPLA₂ mRNA were at a steady state under resting conditions.

**Phospholipase A₂ Assay**—After centrifugation of the culture medium, in order to pellet the floating cells, sPLA₂ activity was measured by incubating an aliquot of the supernatant with 2 nmol of fluorescent substrate, pyrene-phosphatidylglycerol, in 100 mM Tris-HCl, pH 8.5, containing 10 mM CaCl₂, 500 mM NaCl, and 0.1% bovine serum albumin. The fluorescence (measured at 397 nm with excitation at 345 nm) of full substrate hydrolysis was determined using 0.1 unit of sPLA₂ activity derived from Naja naja venen. The initial hydrolysis rate of the samples was determined using a spectrofluorometer (model 430, Turner) by calculating the linear increase in fluorescence as a function of time at 24 °C (Ministat, Huber).

**Reverse Transcriptase-PCR Analysis**—After incubation of astrocytes with factors, total RNA was isolated from the cells using guanidinium thiocyanate denaturation and phenol-chloroform extraction. RNA concentration was quantified by spectrophotometry and its integrity assessed by agarose gel electrophoresis with ethidium bromide labeling. The synthesis of cDNA was conducted using reverse transcriptase with random hexamer priming. The cDNA was amplified, after determining the optimum PCR conditions (25–30 cycles), with Taq polymerase, using specific pairs of primers for each of the three rat sPLA₂ cDNAs studied: for group II A, 5′-GTTGCGAGAGATCTCCGAC-3′ and 5′-GCAAATGCGCTTTTCTCTGTA-3′; for group V, 5′-CAOGG-GGGCTGGCTAGAATGGA-3′ and 5′-AAGACGTGTGTTAATCTGCGA-3′; for group II V, 5′-GGGATTGCGATTCCTGTCT-3′ and 5′-T-AAGCTTGTGAGCAGGAC-3′. The relative amounts of mRNA were determined by scanning the amplified products, normalized to the amount of glyceraldehyde 3-phosphate dehydrogenase amplified and calibrated by the band intensity, to ensure the conformity of the step. The radiolabeled DNA was incorporated into the amplified fragments by Cerenkov radioautography. When all the amplifiers had been shown to lie within the linear PCR range at the same number of cycles, the count values were compared with each other for each sPLA₂ isoform.

**Northern Blot Analysis**—Group II A and V sPLA₂ specific radiolabeled probes were synthesized using the PCR method. After radioactive synthesis, and after a purification step, each PCR product was sequenced in both directions (Applied Biosystems). The sequences were then compared with those theoretically corresponding in the nucleotide data bank (GenBank) and were found to fully match those expected. The radiolabeled probe was synthesized by PCR by amplifying the specific DNA template (that sequenced) of each isoform of rat sPLA₂, for each of the three rat sPLA₂ genes, in the presence of the corresponding primers (see above) and 100 μCi of [32P]dCTP. Before the Northern blot analysis, the radiolabeled probes were purified, incubated at 95 °C for 5 min, and then immediately plunged into iced water for a few minutes. Total RNA (15–20 μg/lane), extracted as described above, were analyzed using 1% agarose, 2.2 M formaldehyde gel electrophoresis and blotted onto a nylon filter. The filter was incubated for 15 min in prehybridization buffer, then incubated overnight at 65 °C in the same buffer containing sPLA₂ radiolabeled probe (see above). The filter was washed several times with SSC (1× SSC is 150 mM NaCl plus 17 mM sodium citrate) at various concentrations and temperatures, until the background radioactivity was sufficiently low. Autoradiography of the filter was then conducted at 80–200 mCi/mg/ml for 18–72 h.

**Immunoblot Analysis**—The cells were scraped into 80 mM β-glycerophosphate, pH 7.4, containing 20 mM EGTA, 15 mM MgCl₂, 1 mM phenylmethylsulfonfluoride, 50 μg/ml aprotinin, 4 μg/ml leupeptin, 10 μg/ml antipain, 1 mM trypsin inhibitor, 1 mM benzamidine, 10 μg/ml pepstatin, and 1 mM Na₃VO₄. The cells were then homogenized by sonication for 3 × 30 s on ice and centrifuged at 105,000 × g and 4 °C for 35 min. The supernatants were aliquoted and stored at −80 °C. Protein quantification was conducted using the method described by Bradford (22). Cytosolic proteins were analyzed with SDS-polyacrylamide gel (gradient 7–15%) electrophoresis, and blotted onto a nitrocellulose membrane. The membrane was then incubated in 50 mM Tris-HCl, pH 7.4, containing 150 mM NaCl and 1% bovine serum albumin, in the presence of primary antibody (1/200). The antigen-antibody complexes were detected by further incubation with peroxidase-coupled second antibody (1/30,000), chemiluminescence reaction, and autoradiography.

**NF-κB Gel-shift Assay**—Nuclear extracts from treated or control cells were prepared as in Ref. 23. Briefly, cells were scraped into phosphate-buffered saline, centrifuged, and then resuspended in 10 mM Hepes, pH 7.9, 10 mM EDTA, 1 mM dithiothreitol, 0.5 mM phenylmethylsulfonfluoride, 2 μg/ml aprotinin, 2 μg/ml leupeptin, 1 μg/ml pepstatin A. After centrifuging the cells at 21,000 × g, the pellets were incubated at 4 °C in 20 mM Hepes-NaOH, pH 7.9, 400 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 2 μg/ml aprotinin, 2 μg/ml leupeptin, and 1 μg/ml pepstatin A. After centrifuging at 21,000 × g, the...
supernatants were aliquoted and stored at −80 °C. Protein quantification was performed as above. Synthetic double-stranded 31-mer oligonucleotide, containing the b sequences of the human immunodeficiency virus long terminal repeat (24), was 5’-end-labeled with [γ-32P]ATP using T4 kinase. Then, 10 μg of nuclear protein was incubated at 37 °C for 15 min with 0.1 μCi of radiolabeled probe in 40 mM Tris-HCl, pH 7.5, containing 4 mM MgCl2, 200 mM NaCl, 2 mM EDTA, 2 mM dithiothreitol, 16% glycerol, and 200 ng/ml poly(dI-dC). Nonspecific binding was determined by incubation in the presence of 0.1 μg/ml non-labeled probe. The samples were analyzed using native polyacrylamide gel (4%) electrophoresis at 150 V for 2 h in 90 mM Tris borate, pH 8.0, containing 2 mM EDTA. The gel was dried immediately afterward, and autoradiography conducted (Instant Image, Packard).

**PI 3-Kinase Activity**—PI 3-kinase activity was measured as described in Auclair et al. (66). Briefly, cells were harvested in a lysis buffer containing protease and phosphatase inhibitors. 500 μg of protein from the lysates were incubated for 60 min at 4 °C with 1.5 μg of anti-p85 PI 3-kinase-subunit antibody, then further for 16 h with protein A-Sepharose beads. After washing twice in phosphate-buffered saline, twice in 100 mM Tris-HCl, pH 7.4, containing 500 μM LiCl, then twice in 10 mM Tris-HCl, pH 7.5, containing 100 mM NaCl and 1 mM EDTA, the beads were resuspended in 50 μl of 10 mM Tris-HCl, pH 7.5, containing 100 mM NaCl, 1 mM EDTA, 20 μg of phosphatidylserine, and 2.5 μg of phosphatidyserine, and were incubated for 25 min at 4 °C. Following incubation of those cells with 100 nM TNFα, the sPLA2 activity in the culture medium in the presence of cells, i.e., the presence of cells, was dependent on NF-κB binding, and a β-galactosidase mammalian cell expression vector, to evaluate the yield of transfection in each well. After 24 h, the cells were incubated for 8 h with the test agents, then luciferase and β-galactosidase activity were determined. Luciferase/β-galactosidase activity ratio was calculated for each well.

**RESULTS**

**Inhibition of sPLA2 Activity by Thyroid Hormone in DITNC1 Astrocytes**—As has been previously reported (25), sPLA2 activity was detected in the culture medium from DITNC1 astrocytes (Fig. 1). The amount of sPLA2 activity detected with resting DITNC1 cells varied from one experiment to another (from about 10–100 pmol/min/mg of protein), increasing with cell confluence. Following incubation of those cells with 100 ng/ml TNFα, the sPLA2 activity in the culture medium increased after a lag time of at least 6 h, peaking (from 15-fold up to 100-fold the basal activity) between 24 and 48 h after addition (data not shown). However, short duration incubation (<1 h) of DITNC1 cells with TNFα was sufficient for an increase in sPLA2 activity to be observed at 24 h (data not shown). In the presence of (3,5,3')-triiodo-l-thyronine (T3), the active form of thyroid hormone, an increase in protein content was observed. This has already been reported in primary cultured astrocytes (26). An increase was also observed with T3 alone, known to induce astrocyte proliferation (27). However, in contrast to TNFα, with T3 (1 μM or higher), the sPLA2 activity released was 25% lower, in comparison to that of resting cells. Moreover, concentration-dependent inhibition (over the range of one to two thirds) of the TNFα-induced sPLA2 activity was observed with T3 (Fig. 1). This inhibitory effect was maximum for 1–10 nM T3 where activity was 50% lower on average.

T3-induced inhibition of sPLA2 activity was observed only in the presence of cells, i.e., no inhibitory effect (even at 1 μM T3) was observed by incubating this hormone with conditioned medium from resting or TNFα-treated cells (data not shown). Moreover, several hours were required for T3 to induce inhibition of both basal and TNFα-induced sPLA2 activity (data not shown). We next used C23425, a pharmacological compound structurally related to thyroid hormones, and designed to simulate the effect of T3 by binding to its receptors (28). A dose-inhibitory response effect on TNFα-induced sPLA2 activity was also observed with C23425 (data not shown).

**Inhibition of sPLA2 Activity by Growth Factors in DITNC1 Astrocytes**—Three groups of MAP kinases have been identified: Erk, Jnk, and p38 MAP kinase. The various isoforms of MAP kinases known to be activated through dual threonine/tyrosine phosphorylation by MAP kinase kinases (29). The stimulation of Erk activity by EGF and bFGF has already been characterized in primary cultured astrocytes (30). Exposure of DITNC1 cells to EGF or bFGF was followed by an increase in dual phosphorylation of Erk1 and Erk2. The stimulation induced by bFGF was less potent (Fig. 2A). The phosphorylation was clearly correlated with an increase in the phosphotyrosine content of Erk1 and Erk2 (Fig. 2A). EGF also stimulated the phosphorylation of Jnk1 and Jnk2, but more transiently (Fig. 2A). A basal level of dual phosphorylation of p38 MAP kinase was observed, but neither EGF nor bFGF enhanced it (data not shown).

Both basal and TNFα-induced sPLA2 activity were decreased by incubation of DITNC1 cells with the growth factors (Fig. 2B). The magnitude of inhibition was 60–85% in the presence of EGF, 70–85% in the presence of bFGF, and always more than 90% with both together. In order to check whether this growth factor effect was specific of cytokine-specific pathway, or not, we incubated cells with forskolin, known to stimulate the adenyl cyclase catalytic subunit. Indeed, cAMP has been reported to modulate group IIA sPLA2 expression in various cell types, including primary cultured astrocytes (16). This was accompanied by a decrease (more than 2-fold) in sPLA2 activity in DITNC1 cells. Fig. 2B shows that the effect of forskolin was also decreased by incubation with growth factors. Furthermore, although the growth factor inhibition of TNFα-induced sPLA2 activity was very potent, the inhibition was additive when cells were exposed to both T3 and growth factors, simultaneously (Fig. 2C).
With the aim of determining whether growth factors exerted their inhibitory effect via the Erk pathway, we incubated DITNC1 cells with PD98059 (31), which is an inhibitor of MEK, the MAP kinase kinase activating Erk (32). The incubation of DITNC1 cells with 30 μM PD98059 for 1 h effectively blocked the growth factor-induced phosphorylation of Erk (data not shown). The pretreatment, which partially inhibited the effect of TNFα, failed, however, to free sPLA2 activity from inhibition by EGF and/or bFGF (data not shown). Phosphatidylinositol 3-kinase (PI 3-kinase) has been reported to mediate growth factor signaling in astrocytes (33). When pre-incubated with DITNC1 cells, LY294002, a PI 3-kinase inhibitor, the increase in sPLA2 activity induced by TNFα was fully reversed. Wortmannin is another PI 3-kinase inhibitor in astrocytes (34).

Although less than with LY294002, an inhibitory effect of wortmannin was also observed. Part of the discrepancy between the magnitude of effect of the two inhibitors might be related to the molecular unstability reported for wortmannin with cells (35).

Inhibition of sPLA2 Activity by Thyroid Hormone in Primary Cultured Astrocytes and Chondrocytes—In order to determine whether the inhibitory effect of thyroid hormone, which has never been described before, was restricted to DITNC1 cells, we next prepared primary cultures of rat brain astrocytes (19). TNFα was discovered to stimulate sPLA2 activity and expression of the group IIα sPLA2 gene in rat brain astrocytes (16). Although potent in those cells, TNFα-induced stimulation of sPLA2 activity peaked (7–15-fold the basal activity) later (30–32 h) and was less marked (Fig. 3A) than in DITNC1 cells (Fig. 1). The TNFα-stimulated release of sPLA2 activity by primary cultured astrocytes decreased in the presence of T3 (concentrations > 0.1 nm) (Fig. 3A). However, in contrast to what was observed with DITNC1 cells, potentiation (up to 2-fold) of the TNFα-induced effect was observed with lower T3 concentrations (Fig. 3A). Another astrocyte cell line, derived from another brain area was then cultured (18). Despite the incubation of CTXTNAA2 cells with and without TNFα, no sPLA2 activity was detected in the conditioned medium from these cells (data not shown). This illustrates the heterogeneity of the various subtypes of astrocytes, as has already been reported. Since different cell subtypes were identified in the primary cultured astrocytes, part of the discrepancies in the results with DITNC1 cells may be explained by the heterogeneity of the subtypes (Figs. 1 and 3A).

As already mentioned, the chondrocyte is another cell type in which cytokine stimulation of group IIα sPLA2 gene expression has been reported. Rabbit chondrocytes were primary cultured (20) up to confluence. In this model, IL-1β has been reported to increase sPLA2 activity the most (36), while TNFα only has a limited effect or is devoid of effect. Our studies showed that IL-1β induced a 7–11-fold increase in sPLA2 activity, with that activity culminating over an incubation period of 24 h (Fig. 3B). Chondrocytes were then incubated, in the presence and in the absence of IL-1β, with various concentrations of T3, as described previously. Incubation in the presence of T3 alone did not give rise to inhibition of basal sPLA2 activity (Fig. 3B). However, T3 did induce inhibition of IL-1β-induced sPLA2 activity, and the inhibitory response was concentration-dependent. A concentration of 1 μM T3 induced an approximately 40% reduction in IL-1β-induced sPLA2 activity (Fig. 3B).

Inhibition of Group IIα sPLA2 mRNA Induction in DITNC1 Cells—In order to determine whether the inhibitory effects on sPLA2 activity could be detected at mRNA level, we first looked for the expression of group IIα sPLA2 mRNA in DITNC1 cells, using Northern blot analysis (cf. “Experimental Procedures”). As shown in Fig. 4A, a band with an apparent molecular weight of about 750 bases was detected, in line with the expected size of group IIα sPLA2 mRNA (37). The intensity of the band showed considerable variation, depending on how the cells were treated. Incubation with supra-nanomolar concentrations of T3, or growth factors, resulted in an apparent decrease in the basal level of the mRNA (Fig. 4A). TNFα induced a dramatic rise in group IIα sPLA2 mRNA (Fig. 4A). The stimulation was half-reversed by concomitant incubation with T3 (Fig. 4A), and that effect was concentration-dependent (data not shown). In the presence of growth factors, TNFα (Fig. 4A) or forskolin (Fig. 4B) had almost no effect on the mRNA.

Inhibition of sPLA2 Activity in Cells Overexpressing Group IIα sPLA2 mRNA—In order to test whether post-transcriptional events were involved in the inhibition, DITNC1 astrocytes were treated with TNFα for several hours, to let group IIα
sPLA₂ mRNA increase prior to addition of growth factors. Fig. 5A shows that inhibition occurred at the same extent as when TNFα and growth factors were added together, simultaneously. Moreover, inhibitory effect occurred also when the cytokine was removed before the incubation with growth factors, once mRNA was induced with TNFα (data not shown). A similar effect was observed in astrocytes with thyroid hormone also (data not shown), albeit to a lesser extent.

In an attempt to confirm that the post-transcriptional events involved in such an inhibition were TNFα-independent, mouse C127 cells expressing recombinant group IIₐ sPLA₂ under the control of the mouse metallothioneine I promoter were cultured. Mouse C127 cells concomitantly and constitutively express human metallothioneine, conferring resistance to cadmium (21). Under basal conditions, the cells spontaneously release large quantities of sPLA₂ activity (500–1000 pmol/min/mg of protein). Incubation of the cells in the presence of growth factors drastically altered the amount of sPLA₂ activity released into the medium in 18–24 h. The fall in activity was greater than 70% (Fig. 5). Although to a lesser extent than with growth factors, incubation in the presence of thyroid hormone or its analogue, CGS23425, was also accompanied by a decrease in sPLA₂ activity in 18–24 h (Fig. 5). However, the T3 concentration required for maximum inhibition (25–50%) was sometimes lower than that with DITNC1 astrocytes, depending on cell confluence. Thyroid hormone was devoid of effect after a cell incubation period of only 4–6 h. Induction of Group V sPLA₂ mRNA by TNFα in DITNC1 Astrocytes—We also tested whether, in addition to group IIₐ, the other two sPLA₂ isoforms detected in the brain (38) were expressed by astrocytes. In resting DITNC1 cells, following a reverse transcription step, the mRNA of group V sPLA₂ was evidenced by PCR (Fig. 6A), but not that of group II C sPLA₂ (data not shown). PCR amplification of group V sPLA₂ cDNA was markedly increased when the cells were incubated with TNFα (Fig. 6A), whereas group II C sPLA₂ cDNA amplimer remained undetectable (data not shown). Northern blot analysis was then performed with a group V sPLA₂ mRNA-specific radiolabeled probe. A band of about 700 bases, the expected size for group V sPLA₂ mRNA (6), was detected, but its intensity was much less than that observed with group IIₐ sPLA₂ mRNA (data not shown). Radioactive reverse transcriptase-PCR was therefore used to evaluate the variation in the expression of group V sPLA₂ mRNA. As shown in Fig. 6B, following TNFα treatment of DITNC1 cells, the amount of group V sPLA₂ cDNA amplified was significantly increased, compared with the quantity under resting conditions. The basal level of cDNA was halved by cell treatment with T3 or growth factors (Fig. 6B). The effect of TNFα was inhibited by treatment with T3 or growth factors. However, for growth factors, the magnitude of the inhibition appeared much less than that observed with group IIₐ sPLA₂ cDNA.

Comparison of Group IIₐ and Group V sPLA₂ mRNA Modulation in DITNC1 Cells—The time courses of mRNA induction of the two sPLA₂ isoforms expressed in DITNC1 astrocytes were then compared. We observed that, even in the absence of TNFα, cell incubation gave rise to a spontaneous increase in the group IIₐ sPLA₂ cDNA detected, peaking at 24 h, while that
of group V remained in a steady state over the same period. The conditions of cell incubation were consequently modified (cf. “Experimental Procedures”), in order to achieve steady-state resting conditions for both sPLA2 mRNA, while maintaining induction with TNFα (Fig. 7, A and B). In contrast to group V sPLA2 mRNA level, which increased early (Fig. 7B), the increase in group IIα sPLA2 mRNA occurred after a lag time of 4–6 h (Fig. 7A). Growth factors only decreased the basal level of group IIα sPLA2 mRNA (Fig. 7A), while partial reversal of the effect of TNFα at 24 h was observed with the mRNA of both isoforms (Fig. 7, A and B). However, in the presence of growth factors, the early induction of group V sPLA2 was fully reversed, but only transiently, thus delaying the TNFα-induced effect by more than 6 h (Fig. 7B). In contrast, with group IIα sPLA2 mRNA, inhibition seemed to occur throughout the incubation period (Fig. 7A).

Stimulation of NF-κB by TNFα in Astrocytes—In both primary cultured (data not shown) and DITNC1 astrocytes (Fig. 8A), cytosolic IκBα isoform levels were markedly decreased under exposure to TNFα, suggesting the existence of TNFα-induced IκBα proteolysis. The decrease had already reached a maximum at 5 min and was fully reversed at 30 min, as is commonly observed in other cell types. Subsequently, a progressive decrease in cytosolic IκBβ, another isoform, was observed up to at least the 6-h time point (Fig. 8A). We therefore attempted to determine whether NF-κB binding in DITNC1 cells was increased by TNFα. In order to do so, a gel-shift assay was conducted using double-stranded oligodeoxynucleotide and nuclear extracts (cf. “Experimental Procedures”). DITNC1 cell incubation with TNFα for only 5 min was sufficient to enable detection of a shift in the electrophoretic mobility of the oligonucleotide (Fig. 8B). The amount of shifted oligonucleotide increased over time, peaking at 15 min. Beyond that time point, a partial decrease occurred, but an electrophoretic shift was still present at 90 min.

Stimulation of MAP Kinases by TNFα in Astrocytes—DITNC1 astrocytes were incubated with TNFα, and the dual phosphorylation of the MAP kinases was checked on the cytosol. A very transient activation of Jnk was detected as early as 5 min, and had already dropped back to the control level at time point 30 min (Fig. 8C). The MAP kinase, Erk, was also mildly stimulated by TNFα in primary cultured (data not shown) and DITNC1 (Fig. 8C) astrocytes. Although a basal level of p38 MAP kinase dual phosphorylation was observed (Fig. 8C, lower panel), this phosphorylation was durably enhanced by TNFα, with stimulation peaking at 10 min. The presence of growth factors did not inhibit the stimulation of p38 MAP kinase by TNFα. Using SB203580, a specific inhibitor that has already been shown to block stimulation of p38 MAP kinase in astrocytes (39), we investigated to determine whether signaling pathway was involved in the stimulation of sPLA2 activity. As was observed with PD98059, incubation with 10 μM SB203580 for 1 h partially prevented the TNFα-induced effect.

Stimulation of PI 3-Kinase by TNFα in Astrocytes and Relationship with NF-κB Pathway—Further experiments were performed to test, first, whether PI 3-kinase is involved in signaling of TNFα in astrocytes. Fig. 8D shows that an incubation of DITNC1 cells with TNFα for 15 min enhances immunoprecipitated PI 3-kinase activity, an effect that is blocked by 10 μM LY294002. Moreover, to address possible issue of causality of PI 3-kinase to NF-κB pathway in TNFα signaling, DITNC1 cells were transfected with a plasmid construct containing a reporter gene under the control of a promoter, the activity of which is dependent on NF-κB binding. As shown in Fig. 8E, TNFα potently stimulated the reporter gene expression. This enhancement was less when either LY294002 or wortmannin was added before adding the cytokine. These results suggest that, in astrocytes, PI 3-kinase activity takes a part in the enhancement of NF-κB transcriptional activity induced by TNFα. We found further that growth factors did not inhibit TNFα effect on this expression in DITNC1 cells at 8 h (data not shown).

Involvement of TNFα Signaling Pathways in sPLA2 Induction in Astrocytes—To address issues of causality of the stimulation of TNFα signaling pathways to induction of group IIα and group V sPLA2 mRNA, we performed additional experiments using pharmacologic inhibitors known to block these pathways. Our data suggest that not only PI 3-kinase/NF-κB (Fig. 9, B and C), but also MAP kinase pathways are involved in the enhancement of sPLA2 mRNA induced by TNFα (Fig. 9A). It is noteworthy that, after an incubation period of 4 h, inhibitors of these pathways prevented, at least partially, TNFα to enhance group V sPLA2 mRNA, whereas none of them
affected group II A sPLA2 mRNA. Nevertheless, this picture was completely the opposite at 24 h (data not shown), group IIA sPLA2 mRNA results correlating with those of sPLA2 activity measured in the culture medium (data not shown).

We also used ZAL (N-benzyloxycarbonyl-Ile-Glu(O-t-butyl)-Ala-leucinal), an inhibitor of 26 S proteasome. 1 μM ZAL did not interfere with sPLA2 catalytic activity, although 20 μM ZAL inhibited this catalytic activity in vitro (in the absence of cells). In the presence of 1 μM ZAL, the enhancement of sPLA2 activity observed with TNFα in DITNC1 astrocytes was fully reversed (Fig. 9C). This is consistent with an involvement of 26 S proteasome in sPLA2 expression and its enhancement with TNFα.

**DISCUSSION**

In astrocytes, the secretion of sPLA2 activity has been reported to be potently stimulated by pro-inflammatory cytokines. However, the mechanism regulating the release of sPLA2 activity in astrocytes has yet to be fully elucidated. Our studies show that the increase in sPLA2 activity observed in astrocytes under exposure to TNFα is at least partially reversed by T3 or growth factors. Since the catalytic activity of sPLA2 was unaffected, the release of sPLA2, or its production, was inhibited by the two types of factors. Several hours were required before the inhibitory effect of T3 was observed. That time interval precludes interference with the early stages of the effect of TNFα. This was not the case with the growth factors, which decreased the release of sPLA2 activity more potently and earlier than thyroid hormone did. The pathway involved in TNFα-induced stimulation of sPLA2 in astrocytes has yet to be elucidated. Because an increase in group IIA sPLA2 mRNA level accompanied the increase in sPLA2 activity in primary cultured (16) and DITNC1 (25) astrocytes, the hypothesis that TNFα stimulated group IIA sPLA2 transcription was formulated. The TNFα-induced increase in sPLA2 activity was reversed by actinomycin D (16). That finding is consistent with the transcription hypothesis. Moreover, upstream of the transcription start site of the rat group IIA sPLA2 gene (40), multiple potential NF-κB binding sites are located within (-191/-111) (Fig. 10). TNFα has already been reported to stimulate NF-κB in astrocytes (41, 42).

Although the NF-κB activation mechanism is not well known in astrocytes, it has been studied in other cell types. The best...
known pathway involves the stimulation of I-κB kinase, which phosphorylates I-κB on two serine residues. This is followed by I-κB ubiquitination and ubiquitin-mediated proteolysis by 26 S proteasome. NF-κB p50/p65 heterodimers, which are retained by I-κB in the cytosol, are thus released and can migrate into the nucleus, where they bind to specific DNA sites. This binding is required in order to modulate the transcription of target genes, such as that of I-κBα itself (43). Consistently with TNFα stimulation of NF-κB in astrocytes, we observed that NF-κB dimer binding increased very early, following a transient decrease in cytosolic I-κBα (Fig. 8). This finding suggested that I-κBα proteolysis was involved in the stimulation of NF-κB. The rapid recovery of I-κBα, the synthesis of which is stimulated by NF-κB (43), further suggested that NF-κB binding did indeed occur in parallel with its transcriptional activity in astrocytes.

Since a short incubation period with the cytokine was sufficient to cause a late increase in sPLA₂ activity (data not shown), early events, such as the decrease in I-κBα, may be involved in TNFα enhancement of group IIA sPLA₂ expression. However, while induction of group IIA sPLA₂ mRNA is observed with TNFα, it is delayed by several hours (Fig. 7). Both the lag time to the increase and its reversal by cycloheximide (data not shown) argue in favor of protein synthesis to enhance group IIA sPLA₂ mRNA. This was difficult to reconcile with the hypothesis that I-κBα proteolysis enhances mRNA directly via NF-κB binding. Stimulation of NF-κB binding without I-κBα proteolysis has already been reported for astrocytes (44). We observed a gradual fall in the I-κBβ detected in the cytosol. The decline began later than the decrease in I-κBα. While we have yet to determine exactly how the I-κBβ isoform is involved in the NF-κB pathway (45), its involvement may explain the prolonged activation of NF-κB binding observed in astrocytes under exposure to TNFα (Fig. 8B). Nevertheless, we observed that 26 S proteasome has been implicated in NF-κB pathway activation (67), our result may suggest that NF-κB is a pathway of TNFα to sPLA₂ in astrocytes. Thus, whether or not TNFα enhances group IIA sPLA₂ expression via NF-κB binding in astrocytes as has been suggested for IL-1β in mesangial cells (4), has yet to be demonstrated.

We identified a potential AP-1 binding site (46) at −482/−476, in the rat group IIA sPLA₂ gene (Fig. 10). TNFα was
shown to stimulate the MAP kinase Jnk in astrocytes (47, 48). In other cell types, Jnk activation was reported to involve a protein kinase called MEKK1, which is known to phosphorylate and activate the identified MAP kinase kinases of Jnk, MKK4 and MKK7 (29). Jnk is known to phosphorylate and activate transcription factors, such as c-Jun (49), and enhance their neosynthesis (50). Moreover, TNFα has already been reported to stimulate AP-1 transcriptional activity through stimulation of Jnk (51). It was thus tempting to speculate that the AP-1 site identified on the group IIA sPLA2 gene was involved in the TNFα-induced effect. In DTNC1 astrocytes, TNFα induced stimulation of Jnk dual phosphorylation, in accordance with our hypothesis. The lag time for neosynthesis of a transcription factor capable of binding to the AP-1 site may explain the delayed enhancement of the mRNA. However, since Jnk stimulation was only transient, how AP-1 transcriptional activity would then be stimulated remains to be determined.

In addition, the similarity of the time courses of the decrease in I-kBα and Jnk dual phosphorylation may suggest the existence of a stimulation pathway shared by Jnk and NF-κB. Interestingly, MEKK1 has also been reported to mediate the stimulation of the NF-κB pathway by TNFα (52), while recombinant MEKK1 has been shown to activate the I-kB kinase complex (29, 52). It is therefore possible that MEKK1 contributes, with TNFα, to the activation of those pathways, but it is unlikely that MEKK1 is the only mediator regulating group IIA sPLA2 activity in astrocytes.

The existence of other TNFα signaling pathways has been reported and such pathways may be involved in the effect of TNFα on sPLA2 activity. In other cell types, TNFα stimulation of p38 MAP kinase has already been reported (53). We found that TNFα stimulated the dual phosphorylation of p38 MAP kinase and Erk in astrocytes (Fig. 9). Both pharmacological inhibitors of MEK and p38 MAP partially inhibited the release of sPLA2 activity enhanced by TNFα. This could suggest that MAP kinases play a part in the intracellular signaling of TNFα to group IIA sPLA2, in this case, probably through stimulation of transcriptional activity via the AP-1 binding site identified upstream of its promoter. Since NF-κB and AP-1 binding sites have often been reported to cooperate in response to cell injury or stress (54), this could be the case for the induction of group IIA sPLA2. However, this has yet to be established for astrocytes.

PI 3-kinase has recently been reported to mediate cytokine signals in astrocytes (55). LY294002 and wortmannin are inhibitors of PI 3-kinase catalytic activity, and are known to block the PI 3-kinase pathway in astrocytes (34). Both these inhibitors reversed the effect of TNFα, albeit LY294002 was however more potent, suggesting that the PI 3-kinase pathway could be involved in the increase in sPLA2 activity. Indeed, we observed not only that TNFα enhanced PI 3-kinase activity in astrocytes, but also that this enhancement is, at least in part, involved in increasing NF-κB-dependent transcriptional activity. Thus, in astrocytes, both the MAP kinase and PI 3-kinase/NF-κB pathways are likely to be involved in TNFα stimulation of group IIA sPLA2. However, since PI 3-kinase inhibitors only partially inhibited the enhancement of NF-κB transcriptional activity induced by TNFα (Fig. 5E) and, in the same time, fully reversed sPLA2 induction by TNFα (Fig. 5F), it is likely that PI 3-kinase is also involved in this induction at another step than NF-κB. In conclusion, with respect to our data on TNFα signaling to sPLA2 in astrocytes, the regulation of the release of sPLA2 activity is probably more complex than has hitherto been suggested and we may logically suspect that a large number of steps may potentially be regulated.

The fact that the growth factors inhibited not only the effect of TNFα, but also that of forskolin, in which NF-κB is not involved, in the regulation of group IIA sPLA2 (4), renders interference with a signaling pathway specific to cytokines unlikely. Moreover, the growth factors did not block the identified signaling pathways that TNFα stimulates in astrocytes (data not shown), and even stimulated some of them. Growth factors have already been reported to inhibit group IIA sPLA2 activity in other cell types (56). The presence of a growth factor target located on the gene and inhibiting its transcription may therefore be suspected. However, while cytokine-induced sPLA2 activity was also inhibited by EGF or bFGF in osteoblasts, no decrease in group IIA mRNA level was observed (56), in contrast to what was observed in our model. This finding indicated that the growth factors have a post-transcriptional effect in osteoblasts. In astrocytes, we observed that growth factors exerted an inhibitory effect on the release of sPLA2 activity, even if its mRNA was first induced by the cytokine (Fig. 5A).

Although the existence of a transcriptional effect of EGF and bFGF in astrocytes cannot be excluded, we tested the hypothesis that those factors could inhibit group IIA sPLA2 expression through a post-transcriptional mechanism that is independent of cytokine-induced stimulation. In order to do so, we cultured C127 cells expressing group IIA sPLA2 under the control of mouse metallothioneine 1 promoter (21). Under our culture conditions, group IIA sPLA2 overexpression is constitutive in those cells. In the presence of growth factors, the amount of sPLA2 activity released into the medium sharply decreased.
(Fig. 5). In consequence, unless the growth factors interfered negatively with metallothioneine I promoter activity via another mechanism, our observation indicates that group II\(_A\) sPLA\(_2\) release may be subject to growth factor-dependent post-transcriptional regulation, independently of any effect of TNF\(\alpha\) or other cytokines. Moreover, our data and the description of post-transcriptional regulation of group II\(_A\) sPLA\(_2\) in various cell types (36, 56) suggest that such a cytokine-independent post-transcriptional regulation may also occur in astrocytes. However, the nature of that regulation has yet to be elucidated.

Several growth factor signaling pathways have been studied in astrocytes. Our findings show that both EGF and bFGF stimulate MAP kinase Erk in DTNC1 cells. However, in PC12 cells, the stimulation of Erk by nerve growth factor, EGF, or bFGF appeared related to mRNA stabilization rather than destabilization (57). The mechanism involved in PC12 cells therefore cannot explain the decrease in group II\(_A\) sPLA\(_2\) mRNA observed with EGF or bFGF in astrocytes. Moreover, Erk was probably not involved in the growth factor-mediated inhibition of the effect of TNF\(\alpha\) in astrocytes, since that inhibition was maintained in the presence of an inhibitor of MEK. In PB-3c mast cells, the MAP kinase, Jnk, was involved in the reversal of ionomycin-induced IL-3 mRNA stabilization (58). However, Jnk is unlikely to mediate the inhibition of sPLA\(_2\) activity observed with EGF and bFGF in astrocytes. First, TNF\(\alpha\), which enhanced group II\(_A\) sPLA\(_2\) expression, stimulated Jnk and, second, only EGF transiently stimulated this pathway, whereas both EGF and bFGF were separately inhibitors of sPLA\(_2\) activity. Thus, we have yet to determine which growth factor signaling pathway(s) mediated inhibition in astrocytes. The inhibition mechanism is likely to involve post-transcriptional regulation of group II\(_A\) sPLA\(_2\), probably through destabilization of its mRNA.

T3 inhibition of the cytokine-induced increase in sPLA\(_2\) activity has not only been observed with TNF\(\alpha\) in astrocytes, but also with IL-1\(\beta\) in chondrocytes, suggesting that T3 interferes with a step common to, or downstream of, the effects of both TNF\(\alpha\) and IL-1\(\beta\). T3 is known to exert most of its effects through binding to specific DNA-bound nuclear receptors regulating the transcription of target genes. It was, however, disturbing that no potential as-yet-listed T3 receptor (T3R) was identified (40, 46). This potential binding site is, in fact, neither a direct repeat nor a palindrome. Thus, we do not know whether it could be a functional target for either thyroid hormone, which decreased the TNF\(\alpha\)-induced increase in group II\(_A\) sPLA\(_2\) mRNA, or retinoic acid, which strongly potentiated the increase in sPLA\(_2\) activity (data not shown).

We tested the alternative hypothesis that thyroid hormone inhibited group II\(_A\) sPLA\(_2\) expression indirectly. In order to do so, we used the C127 cell line constitutively expressing group II\(_A\) sPLA\(_2\). Incubation of those cells in the presence of thyroid hormone or its analogue, CGS23425, affected the amount of sPLA\(_2\) activity released in the medium. Unless thyroid hormone interfered with metallothioneine promoter-dependent transcriptional activity directly, which has never been reported, this finding indicated that group II\(_A\) sPLA\(_2\) release may be subject to thyroid hormone-dependent post-transcriptional regulation, which may be independent of any effect of TNF\(\alpha\).

The existence of such a mechanism in astrocytes has yet to be demonstrated. It might explain the T3-induced inhibition of both basal and TNF\(\alpha\)-induced group II\(_A\) sPLA\(_2\), when no transcriptional effect of T3 could be demonstrated. Moreover, in astrocytes, the group II\(_A\) sPLA\(_2\) inhibitions induced by thyroid hormone and growth factors were additive (Fig. 2), showing that their mechanisms were likely to be distinct. The existence of post-transcriptional cooperation between thyroid hormone and growth factors has already been reported for other cell types (59, 60). However, the cooperation resulted in mRNA stabilization, and not the destabilization that our findings on group II\(_A\) sPLA\(_2\) mRNA in astrocytes tend to indicate.

One of our findings is complementary to results already published in this journal (16), namely that, among the sPLA\(_2\) mRNA detected in the brain (38), group II\(_A\) is not the only isoform expressed by cultured astrocytes: group V sPLA\(_2\) mRNA is also expressed. TNF\(\alpha\), which potently enhanced group V sPLA\(_2\) expression, did so much earlier than it enhanced group II\(_A\) sPLA\(_2\). We have yet to determine how the expression of rat group V sPLA\(_2\) is regulated. We are also not aware of the DNA sequence responsible for modulation of transcription. Nevertheless, the very early TNF\(\alpha\)-induced stimulation of expression would appear to suggest direct stimulation of group V sPLA\(_2\) expression, i.e. enhancement of transcription by the cytokine. As was the case with group II\(_A\) sPLA\(_2\), both thyroid hormone and growth factors partially reversed the effect of TNF\(\alpha\) on group V sPLA\(_2\). However, with the growth factors, the kinetic profile was consistent with transient inhibition of the early TNF\(\alpha\)-induced effect on group V mRNA, which was at variance with that observed with group II\(_A\).

The roles of the multiple sPLA\(_2\) isoforms expressed in the rat brain (38) are largely unknown. The functional redundancy reported for group II\(_A\) and group V sPLA\(_2\) in other systems, might provide a tentative explanation for the stimulation of both isoforms by TNF\(\alpha\) in astrocytes. Using pyrene-phosphatidylcholine as a substrate, we did not detect any group V sPLA\(_2\) activity in astrocyte supernatants. Group V sPLA\(_2\) activity, when reported, has most frequently been determined using purified recombinant protein (61, 62) or transfected cells over-expressing it (63), and with relatively low substrate hydrolysis rates. Because the level of group V sPLA\(_2\) mRNA was probably low in our cells, the level of secretion might be below the limit of sensitivity of currently available methods. Catalytic activity is not, however, the only way sPLA\(_2\) isoforms can act on cells (64).

Both endogenous and exogenous sPLA\(_2\) isoforms have been reported to bind to specific receptors (38), including receptors in the central nervous system. Thus, in an astrocytoma model, group II\(_A\) sPLA\(_2\) has been reported to stimulate intracellular signaling pathways via catalytic activity-independent and receptor binding-dependent mechanism (65). The description of high affinity sPLA\(_2\) binding sites (K\(_d\) ~ nm) could be consistent with a “hormonal” effect of the very low quantities of sPLA\(_2\) released. Under this hypothesis, it is tempting to speculate that the role of release of both group II\(_A\) and group V sPLA\(_2\) might be functional complementarity rather than redundancy, which would be supported by their differential regulation observed with TNF\(\alpha\) in astrocytes.

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The Differential Regulation of Group II$_A$ and Group V Low Molecular Weight Phospholipases A$_2$ in Cultured Rat Astrocytes

Ginette Thomas, France Bertrand and Bertrand Saunier

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