Minireview

Expression and function of phospholipase A_2 in brain

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Abstract Phospholipase A_2 (PLA_2) appears to play a fundamental role in cell injury in the central nervous system. We have investigated PLA_2 expression in the astrocytoma cell line 1231N1, and found that GIVA, GIVB, GIVC and GVI PLA_2 messages are expressed. PLA_2 activity is increased by inflammatory/injury stimuli such as interleukin-1β and lipopolysaccharide in these cells but with very different time courses. The arachidonic acid liberated is converted to prostaglandin E_2, possibly by cyclooxygenase-2, which is induced by inflammatory stimuli. This cell system emerges as a model to study injury/inflammation-related activation of the new PLA_2 forms GIVB and GIVC.

Key words: Brain injury; Cytokines; Nervous system; Human astrocytoma 1231N1; Inflammation; Phospholipase A_2

1. Introduction

Phospholipase A_2 (PLA_2) acts on the *sn-2* position of phospholipids to generate a free fatty acid and a lysophospholipid [1]. PLA_2 has been implicated in inflammatory processes because it regulates the generation of pro-inflammatory mediators (prostaglandins, leukotrienes, etc.) through cyclooxygenase (COX) and lipoxygenase pathways [2]. In the last decade a large effort has been made to purify, sequence, and characterize enzymes with PLA_2 activity. To date, 14 different groups of PLA_2s have been described [3,4]. From a functional point of view, PLA_2s from mammalian sources have been classified in secreted (groups IB, IIA, IIC, IID, IIE, IIF, III, V, X, and XII), Ca^{2+}-dependent cytosolic (groups IVA, IVB and IVC), and Ca^{2+}-independent cytosolic PLA_2s (groups VIA-1, VIA-2 and VIB) [3]. Of these, only a few have been studied in sufficient detail to elucidate their role in arachidonic acid (AA) release and eicosanoid production in mammalian cells [5–7].

The role of PLA_2 in the nervous system is not yet well defined, which is due, at least in part, to the complexity in the expression of PLA_2 groups in this system, to the lack of specific inhibitors for each of these PLA_2 groups and to the system itself, which presents a complex network of cellular interrelations. Under physiological conditions, PLA_2 is involved in phospholipid turnover, membrane remodeling, exocytosis, detoxification of phospholipid peroxides, and neurotransmitter release. However, under pathological situations, increased PLA_2 activity results in the loss of essential membrane glycerophospholipids, resulting in altered membrane permeability, ion homeostasis, increased free fatty acid release, and the accumulation of lipid peroxides [8]. These processes may culminate in subsequent neuronal injury found in ischemia and spinal cord injury, and in neurodegenerative diseases such as Alzheimer's disease [8]. Evidence of PLA_2's role in brain injury comes from a murine transgenic model carrying a targeted mutation of the GIVA PLA_2 [9]. Animals homozygous for the mutation (PLA_2−/−) are significantly resistant to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced neurotoxicity and had reduced injury after cerebral ischemia [9,10].

2. Expression of PLA_2 in the mammalian nervous system

2.1. Rodent nervous system

There is limited information about PLA_2 expression in the nervous system [2,11]. Brain PLA_2 activity was identified and characterized in the 1970s by several researchers [12–14], but the assignment of these activities to specific PLA_2 groups did not begin until the 1990s. Among these recent assignments, Oka and Arita found the GIIA PLA_2 to be induced by inflammatory factors in cultured rat astrocytes [15]. Others have studied the expression of PLA_2 mRNAs in defined areas of the rat brain [11]. The GIIA, GIIC, GIVA, and GVIA PLA_2 mRNAs appear to be ubiquitously expressed as shown by PCR techniques. No trace of GIB PLA_2 has been observed. GIIA PLA_2 was found at very high levels in brainstem and midbrain. GIIC PLA_2 was detected exclusively in brain with no expression in peripheral tissues. Its expression was very high in all brain regions except brainstem, thalamus, and cerebellum, where levels were lower compared to other regions of

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Abbreviations: AA, arachidonic acid; COX, cyclooxygenase; IL-1, interleukin 1; LPS, lipopolysaccharide; MAFP, methyl arachidonylfluorophosphonate; PGE_2, prostaglandin E_2; PLA_2, phospholipase A_2; TNF-α, tumor necrosis factor-α

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the brain [11]. In contrast, GV PLA_2 mRNA was found at low levels in most areas of the brain except the hippocampus, where it was expressed at very high levels. The GIVA PLA_2 was also expressed in all brain regions, the highest levels found in the brainstem, hippocampus, striatum and midbrain, and in contrast at relatively low levels in the cerebellum. Finally, GVIA PLA_2 had a very high expression in all brain regions. [11]. By measuring activity levels, we have found that Ca^{2+}-independent PLA_2 constitutes the dominant activity in adult rat brain, with the highest levels located in the hippocampus and striatum [16]. Moreover, this activity is subject to developmental changes, increasing during development after birth and peaking at 12 weeks [16].

When an injury occurs in the brain, such as after transient forebrain ischemia, a dramatic increase in expression of GIIA PLA_2 mRNA has been reported. This enhanced expression is localized in ischemia vulnerable regions such as the hippocampus and neocortex [17]. GIIA PLA_2 expression is enhanced also after endotoxic shock, giving very high level of expression in a large number of brain regions [17]. GIVA PLA_2 expression has been studied in acute injury in the rat nervous system. After focal or global ischemia, or facial nerve axotomy (FNA), immuno-histochemistry for the GIVA revealed staining in glial cells within the region of neuron loss, with astrocytes being the most consistent cell type immunoreactive for GIVA PLA_2 following injury. [18]. After ischemia, the staining overlapped with neurodegeneration areas, whereas after FNA, GIVA PLA_2 was observed surrounding the axotomized motor neurons where synaptic loss was seen [18].

In contrast to the rat brain, mouse brain expresses fewer PLA_2s. mRNA for GXII, the most recent secreted PLA_2 group described, has been detected using RT-PCR [19,20] and very recently, another secreted PLA_2, GIIF, has been found in brains of mice injected with lipopolysaccharide (LPS) intraperitoneally, to simulate an inflammatory reaction [21]. On the other hand the GVI PLA_2 has been shown to immunolocalize in astrocytes from the spinal cord under chronic injury in an amyotrophic lateral sclerosis transgenic model where neuropathological degeneration of motor neurons occurs [18].

2.2. Human nervous system

Our knowledge of PLA_2s in the human nervous system is also very limited. The first identification of a PLA_2 group in human brain was done by immuno-histochemistry studies performed on brain slices [22]. It was shown that the majority of brain GIVA PLA_2 is present in astrocytes from the gray matter [22]. The two new human paralogs of GIVA PLA_2, GIVB and GIVC, are expressed in whole brain without regional differences between brain areas [23]. Other recent studies have shown that GVIA, GVIB and GXII PLA_2s are also expressed in human brain [4,24]. Under neurodegeneration conditions such as Alzheimer's disease, there is an increase in immunoreactivity to GIV PLA_2 in astrocytes from the cortex of patients [18].

Interestingly, there are some PLA_2 forms that have not been found to date to be expressed in the mammalian nervous system (mouse, rat or human) such as the GIID, GIIE and GX forms [20,25,26], suggesting that each PLA_2 group has a distinct role in the different tissues.

The fact that astrocytes express PLA_2 and that they are immunoactive cells involved in defense mechanisms responding to external injury, makes them an attractive model system to study PLA_2 expression, regulation, and function in the human brain, specifically under inflammatory/injury conditions. Studies in the human astrocytoma cell line 1231N1 have shown the expression of GIV PLA_2 by Western-blot techniques and its stimulation by carbachol, thrombin, exogenous human PLA_2, and tumor necrosis factor (TNF)-α [27,28]. All of these stimuli promote the phosphorylation of GIV PLA_2 and release of AA to the extracellular medium [27,28].

We have extended the studies on astrocyte PLA_2s by analyzing the expression of the different PLA_2 groups by RT-PCR in the human astrocytoma cell line 1231N1. The primers used were designed from the human gene sequences (Table 1). As indicated in Fig. 1, GIVA PLA_2, GIVB PLA_2, GIVC PLA_2 and GVIA PLA_2 are all present in this cell line. To the contrary, no trace of any secreted PLA_2 tested could be detected in resting cells (GIIA, IID, GV or GX) under our experimental conditions. The products generated in the PCR reactions were analyzed later by sequencing to confirm their identity. The presence of GIVA was further confirmed by Western-blotting (see below).

Very little is known about the expression of GIVB PLA_2 and GIVC PLA_2 in primary cultures and cell lines. Recently, it has been described that GIVC PLA_2 is present

Table 1
Oligonucleotide primers used for amplification of PLA_2 groups from cDNA

PLA_2 group	Primer sequences	Product (bp)
IIA PLA_2	sense 5′-CTTACCATGAAGACCCTCCTACTGTTGGCA-3′ antisense 5′-GAGGGGACTCAGCAACGAGGGGTGCT-3′	443
IVA PLA_2	sense 5′-GAGTTTTGGGCGTTTCTGGT-3′ antisense 5′-ACGGCAGGTTAAATGTGAGC-3′	450
IVB PLA_2	sense 5′-GAGCGAGTTACGAGAATTCC-3′ antisense 5′-CACATGGGATTCCAGATCAG-3′	420
IVC PLA_2	sense 5′-TACTCTCTGACCGACTTCTG-3′ antisense 5′-GTGAGTTCTGACCAGTCTTC-3′	287
V PLA_2	sense 5′-CAAGGAGGCTTGCTGGACCTAA-3′ antisense 5′-CAGAGGATGTTGGGAAAGT-3′	359
VIA PLA_2	sense 5′-AACGTTAACCTCAGGCCTCC-3 antisense 5′-GAGAGTTTCTTCACCTTGTT-3′	217
X PLA_2	sense 5′-GGAATTCGCCTATATGAAATATGGT-3′ antisense 5′-GGAATTCAAGGTAGTCAGTCACACTTG-3′	327

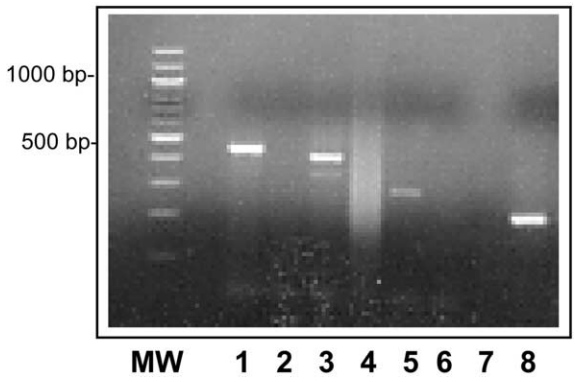

Fig. 1. PCR amplification of PLA_2 groups present in 1231N1 cells. cDNA from resting cells was amplified by PCR with Taq polymerase using the primers listed in Table 1: GIVA (lanes 1 and 2), GIVB (lanes 3 and 4), GIVC (lanes 5 and 6) and GVIA PLA_2 (lanes 7 and 8). Control reactions were run without DNA (lanes 2, 4, 6 and 7). RNA was extracted by the TRIZOL reagent method (Life Technologies), as indicated by the manufacturer and reverse transcription of the first strand cDNA was performed with total RNA (2 μg), oligo(dT)$_{12-18}$ primers (0.5 μg/ml), reverse transcriptase buffer, dNTP mix (10 mM each), 25 U RNasin (RNase inhibitor, Gibco-BRL), MoMLV reverse transcriptase (200 U, Gibco-BRL) and RNase free water to 20 μl, incubated at 70°C for 10 min and 37°C for 90 min. The reaction was stopped by heating at 90°C for 5 min. A 1/5 volume of the generated cDNA was used for amplification. The PCR was performed in a total volume of 100 μl with 1 μM each of the sense and antisense primers, PCR buffer with 2.5 mM magnesium chloride, dNTP (0.2 mM) and Taq polymerase (0.5 U, Roche, Mannheim, Germany)

in human macrophages [29]. However, its role has not yet been defined.

The tissue-specific distribution of the different PLA_2 groups suggests that the different forms may play distinct functional roles. It is intriguing to explore the function of PLA_2s, especially in the nervous system where knowledge regarding regulation both in normal and injured cells is limited. It is interesting to note that the part of the nervous system that has the widest and highest expression of PLA_2 groups is the hippocampus [11,16]. Since the hippocampus is related to memory and the generation of neurons, the elucidation of the role of PLA_2 in that part of the brain will be of great interest.

3. PLA_2: implication for inflammation/injury in brain

Neuronal survival is dependent on glial function, which can exert both neuroprotective and neurotoxic influences. Glial cells (including astrocytes) are a primary target of cytokines and are activated in response to many of them, including TNF-α and interleukin (IL)-1 [30]. This activation can trigger further release of cytokines that might enhance or suppress local inflammatory responses and neuronal survival. IL-1, the prototypic inflammatory cytokine, comprises a family of proteins that are the products of separate genes. Brain cells can express all members of the IL-1 family, albeit at low levels, in the healthy nervous system [31]. Following experimental injury, infection or inflammation, microglia (brain macrophages) appear to be the early primary source of IL-1 [32]. Astrocytes also produce IL-1, usually slightly later than microglia after an acute insult [32]. It remains unclear whether endogenous IL-1 plays a physiological role in normal, healthy brain, since its expression is barely detectable. There is evidence suggesting that endogenous IL-1 influences sleep patterns and synaptic plasticity [31], but the majority of the actions of exogenous and endogenous IL-1 are associated with responses to local or systemic insult. IL-1 is also believed to act as a mediator of neuroimmune responses to disease and neurodegeneration [32].

TNF-α has a more conflicting role than IL-1 in the nervous system, because it can enhance and inhibit neuronal injury, depending on the time-course and level of expression [11]. TNF-α is a 17 kDa protein with both secretory and membrane-bound forms, the active form usually found as a homotrimer [33]. Neurons appear to be the principal cell type producing TNF-α under physiological conditions. TNF-α interacts with multiple neurotransmitters, and participates in synaptic activity. In addition to its effects as an endogenous pyrogen on hypothalamic neurons, TNF-α may modulate the

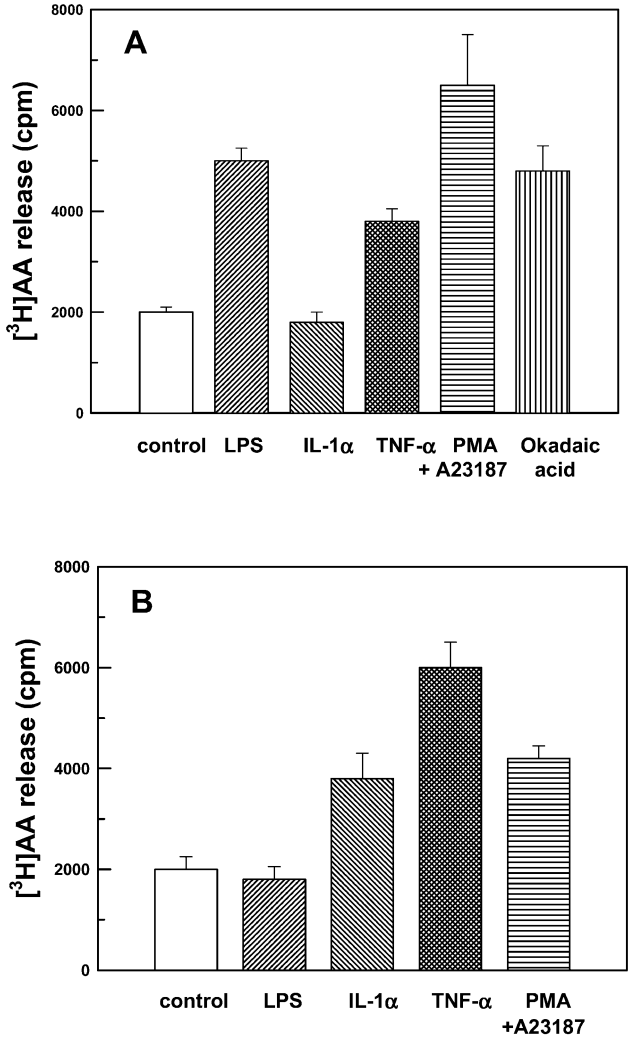

Fig. 2. [^{3}H]AA release by stimulated 1231N1 cells. Cells labeled with 0.5 μCi/ml [^{3}H]AA overnight were stimulated for 90 min (A) or 24 h (B) with different stimuli: 40 ng/ml TNF-α, 40 ng/ml IL-1α, 200 ng/ml LPS, 50 ng/ml phorbol myristate acetate plus 1 μM A23187 (Ca^{2+} ionophore), or 100 nM okadaic acid, in the presence of 0.1 mg/ml bovine serum albumin. The supernatants were removed, and assayed for radioactivity by liquid scintillation counting, as previously described [33].

responsive state of α_2-adrenergic receptors and potentiate the release of norepinephrine. TNF-α also increases the frequency of spontaneous synaptic current through a presynaptic mechanism, augments evoked synaptic activity, and inhibits long-term potentiation in hippocampal neurons [33]. After injury, activated microglia are a major source of TNF-α, contributing to early neuronal damage, but improving neuronal recovery at later times (2–4 weeks) [30,33]. It is now known that TNF-α participates in apoptotic processes in the nervous system by activating Fas receptors and signaling mechanisms that are involved in this type of death [11]. Both IL-1 and TNF-α have been described as modulators of PLA_2 activity and/or expression in cells from the nervous system [34].

Since astrocytes are able to respond to external injury we studied PLA_2 activation by injury/inflammation-related factors such as IL-1α or bacterial LPS, a lipid present in gram-positive bacteria. Experiments with [^{3}H]AA labeled cells were carried out, and after 1.5 h treatment a significant release of [^{3}H]AA was achieved by LPS as well as the control stimuli TNF-α, phorbol myristate acetate plus A23187, and okadaic acid (Fig. 2A). However, at 24 h, the stimulation with IL1-α resulted in cellular responses higher than those of LPS (Fig. 2B). AA release by IL-1α has also been shown in primary cultures of neonatal murine astrocytes [35] and in primary cultures of ovine astroglia [36]. Our studies on the time-course of [^{3}H]AA release in response to LPS and IL-1α revealed that the response to optimal LPS doses (200 ng/ml) reached a plateau after 2 h treatment. Interestingly, AA release from cells stimulated with 40 ng/ml IL-1α plateaued after 20 h of incubation (data not shown). This behavior suggests the intriguing possibility that IL-1 needs the induction and expression of different factors to release AA from the phospholipid stores. In this sense, it has been described that in primary cultures of mice striatal astrocytes IL-1β treatment for 24 h enhances ATP-evoked release of AA [37]. The molecular mechanisms involved include both the induction of the purinergic receptor P_{2Y2} and an increase in the amount of GIV PLA_2 protein. P_{2Y2} receptors appear to be absent in untreated astrocytes but their expression is induced by IL-1 [37]. Thus stimulation by IL-1 would induce the appearance of P_{2Y2} receptors in the membrane of stimulated astrocytes. Under such conditions, ATP coming from damaged neurons in the location of inflammation or injury could then further activate the astrocytes contributing to the events that end in AA release. Further experimentation is required to conclude if the astrocytoma 1231N1 cell line has the same behavior and requirements as the striatal mouse astrocytes after IL-1 stimulation.

PLA_2 inhibitors were used to implicate the involvement of GIV PLA_2 in the AA release. Methyl arachidonyl-fluorophosphonate (MAFP) is an active-site directed, irreversible inhibitor of GIV PLA_2 and has been used to implicate GIV PLA_2 in the release of AA in many systems [5]. Different doses of MAFP were preincubated for 30 min with cells prior to stimulation with 200 ng/ml LPS and a profound inhibition of the [^{3}H]AA release with 10–50 μM MAFP treatment was detected (Fig. 3). However, due to the fact that MAFP is not an specific inhibitor, more experimentation needs to be done to conclude which PLA_2 form is important for AA release in this cell line.

By using organotypic cultures of rat hippocampal slices, it has recently been shown that conditions that mimic ischemia,

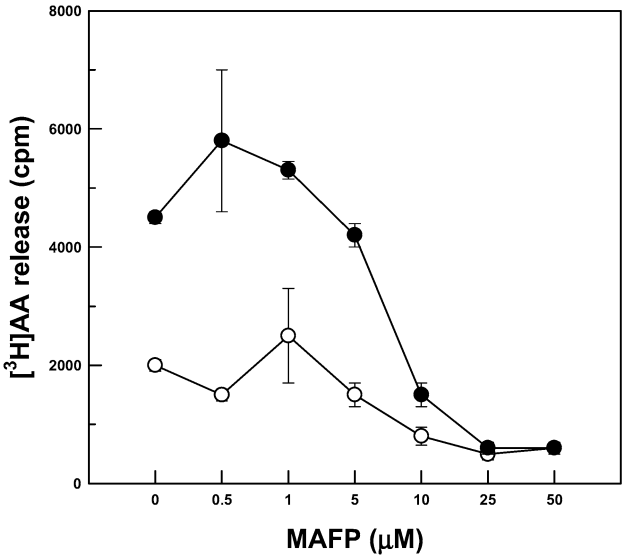

Fig. 3. Inhibition of [^{3}H]AA release by MAFP in LPS stimulated 1231N1 cells. Labeled cells were preincubated with different concentrations of MAFP for 30 min, and then treated with 200 ng/ml of LPS (filled circles) or media (open circles) for 2 h. Supernatants were recovered and assayed for radioactivity by liquid scintillation counting.

like oxygen and glucose deprivation (OGD) cause an increase in hippocampal PLA_2 activity [38]. Those conditions promote neuronal death. However, blockade of PLA_2 activity during OGD exposure improved survival of hippocampal neurons. This effect was observed only with inhibitors for GIV PLA_2 like arachidonyl fluoromethyl ketone, but not with inhibitors for GVI (bromoenol lactone) or for secretory PLA_2 (LY311727) [38], suggesting an adverse effect of GIV PLA_2 on neuron survival under ischemic conditions. It is possible that, at least in the hippocampus, these effects are due to AA metabolism by lipoxygenases, since lipoxygenase inhibitors reduce neural injury in hippocampal rat slices [39].

Besides PLA_2's role during nervous system damage, some evidence exists to allow speculation that PLA_2 can serve a beneficial role in this system. For example, it has been described that PLA_2 expression is developmentally regulated [16], also a role for PLA_2 activation in nerve regeneration has been proposed [40], and finally particularly high PLA_2 activity has been found in neuronal growth cones [41]. Additionally, polyunsaturated fatty acids such AA and linoleic acid have been shown to prevent neuronal death in rodent models of transient global ischemia [42]. Therefore, PLA_2 could have both a protective role and a destructive activity, depending on the local injury/inflammation conditions.

4. COX: involvement in injury/inflammation processes in the nervous system

In the early 1980s it was reported that prostaglandin E_2 (PGE_2) is produced by mice glial cells treated with LPS [43]. The first step in PGE_2 production is the oxidation of AA, which is catalyzed by a COX. There are two COX isoenzymes, COX-1, that generally is a constitutive form, and COX-2, an inducible enzyme that, in some cases, is also expressed under basal conditions. COX-2 is expressed throughout the forebrain in discrete populations of neurons and is enriched in

the cortex and hippocampus in the rat nervous system [44]. No expression has been detected in glial or vascular endothelial cells in normal brain [44]. However, COX-2 can be induced in those cells by pro-inflammatory/injury factors such as IL-1 or LPS. In cultured mouse astrocytes, COX-2 expression and PGE_2 production is enhanced by IL-1β after 4 h [45]. Other studies have shown that, in rat hypothalamic astrocytes, LPS induces PGE_2 release after only 1 h of incubation, but in this case, 80% of PGE_2 production is accounted for by COX-1 [46]. Recent work has shown that IL-1β coming from peripheral sites of inflammation induces COX-2 expression in the central nervous system, and spinal cord neurons contributing to inflammatory pain hypersensitivity developed in the neighboring uninjured tissue [47]. Moreover, intravenous injection of IL-1β enhances expression of COX-2 and PGE_2 synthase (the terminal enzyme in the synthesis of PGE_2) in mouse cerebral blood vessels (mainly in endothelial cells and perivascular macrophages) [48]. Possibly PGE_2 produced in those sites diffuses into the brain parenchyma and acts on PGE_2 receptors to induce cellular responses [48].

The astrocytoma cell line 1231N1 expresses COX-1 and GIV PLA_2 (Fig. 4). Western-blots performed with proteins from cells stimulated with IL-1α, LPS or TNF-α for 24 h showed an important induction of COX-2 expression above basal levels, without changes in the expression of COX-1 (Fig. 4). We have also studied the production of PGE_2 after cell treatment with IL-1α and LPS. Supernatants from control and stimulated cells were assayed for PGE_2 by a specific radio-immunoassay. As shown in Table 2, both IL-1α and LPS treated cells induced the synthesis of PGE_2 well above the levels found in unstimulated cells. These results point to the possibility that AA released from phospholipids is subsequently metabolized to PGE_2 through a COX-2 enzyme in this cell line.

In summary, the distinct pattern of expression of PLA_2 forms in the brain and their regulation by injury/inflammatory stimuli suggest that this family of lipases may play an important role in brain function and pathology. In this context, the human astrocytoma cell line 1231N1 constitutes an attractive cell model to study, in a defined cellular setting, the precise role of the different GIV PLA_2 forms in glial function and response to damage.

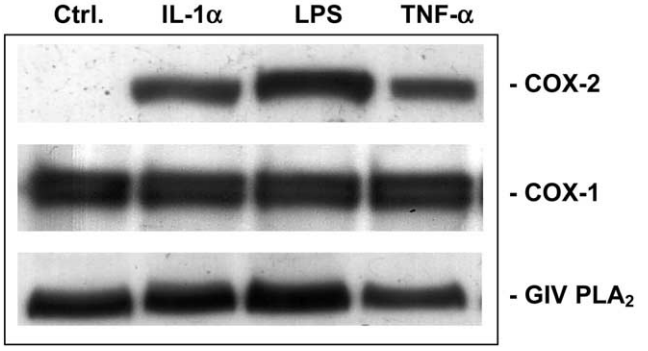

Fig. 4. COX-2, COX-1 and GIVA PLA_2 expression by stimulated 1231N1 cells. Proteins from unstimulated cells (ctrl) or cells stimulated with 40 ng/ml IL-1α, 200 ng/ml LPS or 40 ng/ml TNF-α for 24 h were analyzed for expression of COX-2, COX-1 and GIV PLA_2 by Western-blotting. Anti-COX-2 and -COX-1 antisera were from Cayman Chemical. Antiserum against GIV PLA_2 was a gift of Lilly Research Laboratories. Detection was achieved by ECL reagent (Amersham Pharmacia Biotech).

Table 2
PGE_2 production by the human astrocytoma cell line 1231N1

Stimulus	PGE_2 (nmol 10^{-1})
None	7
IL-1 (40 ng/ml)	28
LPS (200 ng/ml)	42

PGE_2 was measured in the cell supernatants by a specific radio-immunoassay (PerSeptive Biosystems, Framingham, MA, USA).

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