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Neuroscience Letters 340 (2003) 177–180

Neuroscience Letters

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Cyclooxygenase-2 mediates platelet-activating factor-induced prostaglandin E_2 release from rat primary astrocytes

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Received 20 September 2002; received in revised form 9 January 2003; accepted 9 January 2003

Abstract

The phospholipid mediator platelet-activating factor (PAF), and its non-hydrolyzable analog methylcarbamyl-PAF (mc-PAF) increase prostaglandin E_2 (PGE_2) release from astrocyte-enriched cortical cell cultures. Cyclooxygenase (COX) enzymes – of which there are two known isoforms – convert arachidonic acid to prostaglandin (PG) H_2 (PGH_2), which is further metabolized to various PGs, including PGE_2. COX-1 is generally considered to contribute to cell homeostasis, whereas COX-2 is thought to mediate inflammatory/immune PG formation. In this study we examined the involvement of the COX isoforms in PAF-induced PGE_2 release. Treatment of cells with the non-specific COX inhibitor indomethacin, or the specific COX-2 inhibitor NS-398, prior to mc-PAF stimulation completely blocked the PAF-induced release of PGE_2; treatment with more selective COX-1 inhibitors (i.e. piroxicam and SC-560) failed to significantly do so. These data suggest that COX-2 is responsible for PAF-mediated PGE_2 release in primary astrocytes.

Keywords: Astrocytes; Cyclooxygenase; Immune system; Inflammation; Platelet-activating factor; Prostaglandins

Prostaglandins (PGs) have important functions in brain cells, and may mediate a variety of neuropathologic phenomena, including such inflammation-associated disorders as Alzheimer's disease (AD) [3] and amyotrophic lateral sclerosis (ALS) [1]. When cells and tissue are exposed to various pro-inflammatory stimuli, arachidonic acid (AA) is liberated from membrane phospholipids and is converted to PGs, by the action of cyclooxygenase (COX) enzymes. Two related but unique isoforms of COX, cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) have been identified; both catalyze identical reactions, a cyclooxygenation to form PGG_2, and a peroxidation which reduces PGG_2 to PGH_2, the precursor of all other PGs, including PGE_2. COX-1 is constitutively expressed by most cells and is generally considered to be involved in maintaining cell homeostasis; in contrast the mitogen-inducible COX-2 is implicated in inflammatory and immune responses [18].

A number of observations suggest that astrocytes have an important role in central nervous system (CNS) inflammation/immune responses. Following CNS injury or an immune/inflammatory challenge, astrocytes undergo a phenotypic alteration – a response known as activation. The activated astrocytes then release cytokines and other pro-inflammatory mediators, including PGs. These released substances communicate with (and ultimately affect the function of) such neighboring cells as neurons and microvascular cells. Astrocytes are a major source of PGs in the CNS; in culture these cells synthesize up to 20 times more PGs than do neurons [16]. PGE_2 is the major AA metabolite involved in modulation of immuno-inflammatory responses [18].

The acute or immediate phase of inflammation is the earliest response to tissue injury, as well as to immunological or pro-inflammatory challenges. It has been shown in several cell types that COX-1 is often responsible for the immediate increases in PGs produced by various types of inflammatory stimuli, and COX-2 for the increased levels characteristic of the delayed phase of inflammation [7]. However other data suggest that the degree to which each COX isoform contributes to particular acute inflammatory responses depends upon such factors as the nature of the inflammatory stimulus and the cell type involved. We have recently shown that the pro-inflammatory mediator platelet-

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0304-3940/03/$ - see front matter © 2003 Elsevier Science Ireland Ltd. All rights reserved.
doi:10.1016/S0304-3940(03)00129-0

activating factor (PAF) increases the release of PGE_2 from cortical astrocytes [17]. This effect is observed within minutes of PAF stimulation and PGE_2 accumulation peaks at 30 min, suggesting that PAF induces an acute inflammatory reaction in astrocytes. In the present study we examine the participation of two COX isozymes in PAF-induced PGE_2 mobilization, using COX inhibitors with varying degrees of selectivity for COX-1 and COX-2.

Dissociated astrocytes were cultured from cortices of postnatal day 1–2 rat pups as previously described [10] with minor modifications [17]. In brief, cells from dissociated cortices were plated onto poly-L-lysine coated 35 or 100 mm culture dishes. All cell culture constituents were purchased from Gibco-Life Technologies (Rockville, MA). The initial culture media, minimal essential medium (MEM) containing 15% horse serum, were aspirated 2–5 h after plating to remove unattached cells and debris, and replaced with MEM containing 10% fetal bovine serum (FBS). Medium was replaced with MEM/10% FBS every 3–4 days. Astrocytes were kept at 37°C in a humidified 5%CO_2/95% air incubator for 9–15 days, by which time the cultures were confluent and could be used for experiments. Most of the cells in this preparation (approximately 85% of cultured cells) were immunopositive for glial fibrillary acidic protein, the astrocyte-specific intermediate filament protein, and had the characteristics of flat type 1-like astrocytes. The only other cells we were able to identify immunologically in this preparation were microglia (approximately 5% of cultured cells were immunopositive for CD-45). No neurons were detected using neurofilament-specific antibodies. Many of the remaining cells exhibited morphology reminiscent of radial glia that have not yet assumed the genetic program of mature astrocytes [8].

Methylcarbamyl-PAF (mc-PAF) (Cayman Chemical, Ann Arbor, MI) was dissolved in ethanol at a stock concentration of 10 mM. Indomethacin, piroxicam, NS-398 (Biomol; Plymouth Meeting, MA), and SC-560 (Cayman Chemical) were dissolved in 45% hydroxy-β-cyclodextrin (HBC; Sigma, St. Louis, MO). Cells were serum-deprived for 24 h prior to experimental treatments to induce quiescence. Where treatment with inhibitors is indicated, these compounds were added 30 min prior to the addition of mc-PAF.

Direct assay of the PGE_2 concentration in cell-conditioned medium was used as an index of PGE_2 secretion by primary astrocytes. PGE_2 levels were measured by ELISA according to manufacturer's instructions (Cayman Chemicals, Ann Arbor, MI), as described previously [17]. Results are derived from at least three separate experiments, assayed in duplicate or triplicate ($n = 6–8$).Data are expressed as means ± SEMs. Statistical analyses were performed using ANOVAs for comparisons between groups, followed by Fischer's PLSD post-hoc comparisons by means contrast. *P*values <0.05 were considered statistically significant.

Addition of mc-PAF (0.001–1 μM) to the astrocyte-enriched cortical cell cultures resulted in concentration-dependent increases in the release of PGE_2 into the conditioned media (Fig. 1), confirming previous results [17]. As these primary astrocytes express both COX-1 and COX-2 according to Western blot analyses (data not shown), we next assessed the involvement of each isozyme in the PAF effect.

Prior exposure of cells to lower concentrations (1 or 10 μM) of piroxicam (which is considered to be more specific for COX-1 than for COX-2; [13]) had no effect on mc-PAF-induced PGE_2 release (Fig. 2A). A higher concentration (50 μM) attenuated some of this PGE_2 release; this effect was not statistically significant. The COX-1 selective inhibitor SC-560 similarly failed to significantly influence mc-PAF-induced PGE_2 release (Fig. 2B). These results suggest that COX-1 activity is not required for PAF-mediated PGE_2 release from astrocytes, even though COX-1 is expressed in these cells.

Prior exposure of astrocytes to the non-selective COX inhibitor indomethacin [12] (1, 10, and 50 μM) attenuated the mc-PAF-induced PGE_2 release in a concentration-dependent manner without affecting basal PGE_2 release (Fig. 3A). The COX-2 selective inhibitor NS-398 [9] completely abolished mc-PAF-induced PGE_2 release (Fig. 3B); highest concentrations (10 and 50 μM) also prevented basal PGE_2 release. These results suggest that the COX-2 isozyme is required for PAF-induced PGE_2 release from astrocytes.

Cells are thought to have ample basal capacity for COX-catalyzed formation of PGE_2 by expressing either COX-1 or COX-2, or both. However some data suggest that pro-inflammatory stimuli can induce the de novo synthesis of

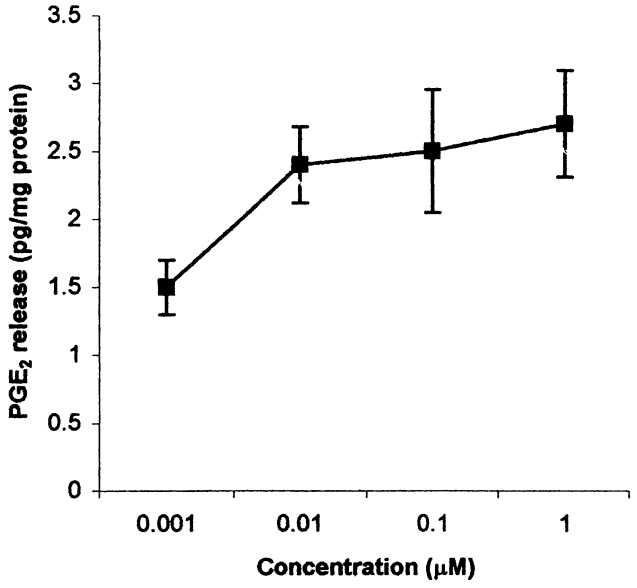

Fig. 1. PGE_2 release from primary cortical astrocytes exposed to the non-hydrolyzable analog of PAF, mc-PAF. Cells were incubated at 37 °C with various mc-PAF concentrations for 30 min, at which time the media was collected and assayed for PGE_2. Each point represents the mean ± SEM of at least three independent experiments, carried out in triplicate. The mean mean ± SEM for control cultures was 0.8 ± 0.011.

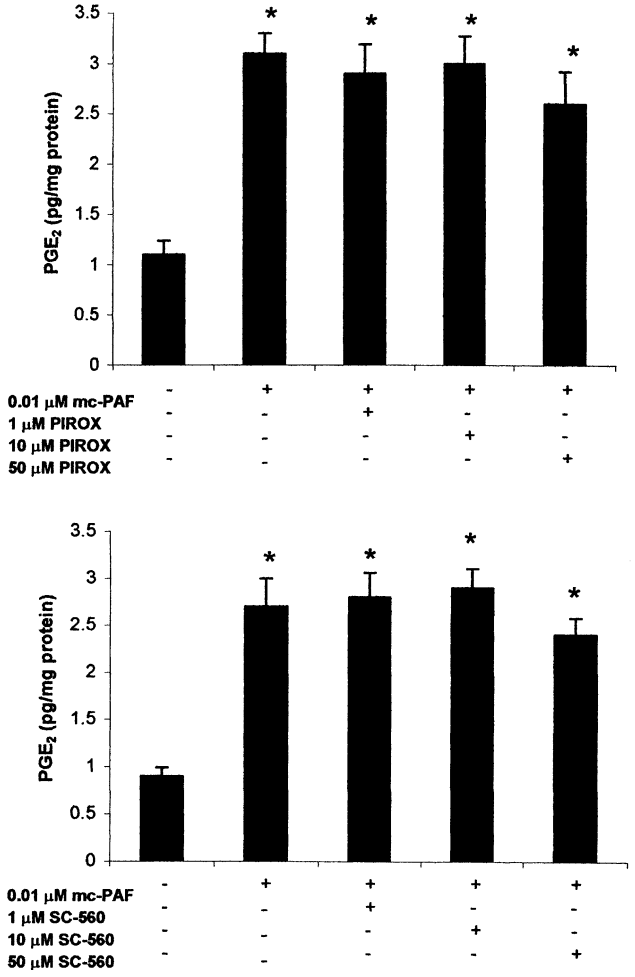

Fig. 2. Preferential COX-1-selective inhibitors have minimal influence on the mc-PAF-induced PGE_2 release from astrocytes. Cells were incubated at 37 °C with various concentrations of (A) piroxicam or (B) SC-560 for 30 min prior to addition of mc-PAF (0.1 μM) for 30 min, at which time the media was collected and assayed for PGE_2. Each point represents the mean ± SEM of at least three independent experiments, carried out in triplicate. *Statistically significant ($P < 0.05$) difference relative to control and **relative to mc-PAF.

COX-2 protein within minutes in astrocytes [6]. We do not believe this to be the case for PAF-induced astrocytic PGE_2 release, for several reasons. First, COX-1 and COX-2 protein levels did not increase within 30 min of mc-PAF stimulation (as assessed by immunocytochemical and Western blot analyses; data not shown). Second, pre-treatment with either a transcription inhibitor (actinomycin D; 5 μg/ml) or a protein translation inhibitor (cyclohexamide; 10 μg/ml) had no effect on mc-PAF-induced PGE_2 release (data not shown). Indeed basal expression of COX-2 appears to be sufficient to sustain the PAF-induced response.

The results of this study show that both PAF-induced and constitutive PGE_2 release are predominantly mediated by COX-2 in astrocytes; and that astrocytes express sufficient basal COX-2 activity to mediate the acute inflammatory response to PAF.

COX-2 is the major enzyme responsible for PG production in developing brain, and astrocytes are an important source of PGE_2 in developing brain [14]. Since we used early post-natal (1–2 days of age) rats to make our cell cultures, it appears that COX-2 can synthesize astrocytic PGs early in development; indeed PAF-mediated PGE_2 release from astrocytes may have a role in development. It should be kept in mind that cultured astrocytes express elements of a reactive phenotype in culture [11], including COX-2 expression [5] and may thus provide a model for the activated astrocytes seen in various neurodegenerative and inflammatory-associated disorders. While glial activation can be protective, excess activation can be deleterious [2]. In fact, activated astrocytes are neurotoxic in culture systems [4] and may be involved in neurodegeneration in vivo [2]. Moreover, PGE_2 release has been shown to induce neuronal degeneration [15]. Our findings suggest that PAF may have a significant role in the inflammatory-immune function of astrocytes by affecting COX-2-

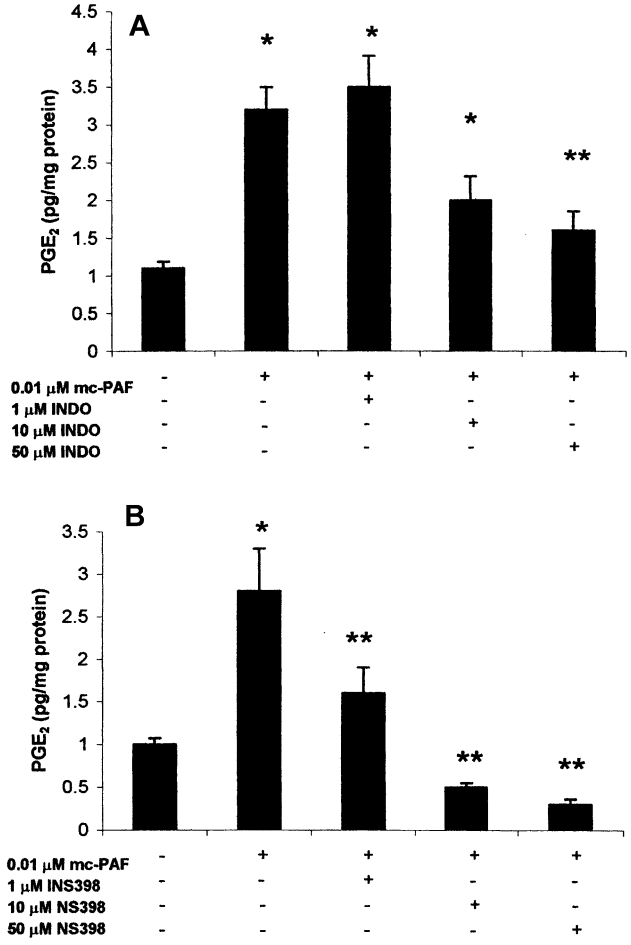

Fig. 3. Inhibition of COX-2 attenuates mc-PAF-induced PGE_2 release from astrocytes. Cells were incubated at 37 °C with various concentrations of (A) the nonselective COX inhibitor indomethacin or (B) the COX-2 selective inhibitor NS-398 for 30 min prior to addition of mc-PAF (0.1 μM) for 30 min, at which time the media was collected and assayed for PGE_2. Each point represents the mean ± SEM of at least three independent experiments, carried out in triplicate. *Statistically significant ($P < 0.05$) difference relative to control and **relative to mc-PAF.

mediated PGE_2 release, and could ultimately have a role in inflammatory-immune-associated diseases.

Acknowledgements

These studies were supported in part by grants from The National Institutes of Mental Health (Grant No. 5-RO1 MH28783-24) and The Center for Brain Sciences and Metabolism Charitable Trust.

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