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Induction by Interleukin-1β Peptide of Prostaglandin E_2 Formation via Enhanced Prostaglandin H Synthase-2 Expression in 3T6 Fibroblasts

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ABSTRACT. Several synthetic interleukin-1 (IL-1) peptides were tested *in vivo* for pyrogenic activity and *in vivo* for their ability to stimulate prostaglandin production. Only the IL-1β fragment (208–240) enhanced body temperature, although both IL-1β (208–240) and IL-1α (223–250) stimulated prostaglandin E_2 (PGE_2) production *in vitro*. We report here that the IL-1β fragment (208–240) did not have the capacity to induce arachidonic acid (AA) mobilization by 3T6 fibroblasts. However, this peptide was able to increase the expression of the inducible prostaglandin H synthase isoform (PGHS-2; EC 1.14.99.1.), which is related to its ability to stimulate prostaglandin E_2 synthesis. BIOCHEM PHARMACOL **56**;6:759–761, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. IL-1β peptide; prostaglandins; arachidonic acid mobilization; prostaglandin H synthase-2; inflammation

IL-1‡ is the name given to a group of cytokines that possess similar biologic activities and are derived from two distinct gene products, IL-1α and IL-1β. Within the last few years, it has become evident that the biologic activity of IL-1 is not restricted to lymphocyte proliferation. Indeed, this peptide can modify the growth and secretory activity of a large number of cell types, including fibroblasts, hepatocytes, endothelial cells, chondrocytes, natural killer cells, synovial cells, and osteoclasts [1, 2], which share a common involvement in immune or inflammatory responses. The mechanism of action by which the cytokine exerts its effects on these parameters is still not understood, although it has recently been proposed that stimulation of AA metabolism by IL-1β may explain several aspects of its action [1]. Using anti-IL-1 antibodies, Giri *et al.* [3] demonstrated that murine IL-1 is initially synthesized as a 33,000 mol. wt precursor that is cleaved enzymatically to the lower molecular weight forms. There have been several attempts to define the structural requirements for IL-1 activities by testing synthetic fragments of IL-1 in various biologic assay systems. Thus, in some cases, IL-1 fragments with biologic activities have been described [4–6]. Recently, an IL-1β fragment, IL-1β (208–240), was shown to have the capacity *in vivo* to enhance non-rapid-eye-movement sleep, to induce cerebral pial arteriolar dilatation and to increase prostanoid periarachnoid cerebral spinal fluid levels, and was also found to be pyrogenic. *In vitro*, it was able to stimulate PGE_2 production by rheumatoid synovial fibroblasts but did not stimulate T-cell proliferation [4, 5].

The aim of the present study was, therefore, to examine the pro-inflammatory mechanism of the IL-1β fragment (208–240). Thus, we measured the effect of this peptide on AA mobilization and the expression of the inducible isoform of PGHS (EC 1.14.99.1.), an enzyme involved in AA metabolism.

MATERIALS AND METHODS

Materials

IL-1β, IL-1β peptide (163–171), and IL-1β peptide (208–240) were obtained from Bachem Feinchemikalien AG. RPMI 1640, FCS, penicillin G, streptomycin and trypsin/EDTA were purchased from Life Technologies. [^{3}H]AA (180–240 Ci/mmol) was obtained from DuPont/NEN. Sheep PGHS-2 purified from ovine placenta and polyclonal antiserum directed against PGHS-2 (synthetic peptide from murine PGHS-2) were from Cayman Chemicals Co. The ECL kit was purchased from Amersham. PMA and all other reagents were acquired from Sigma.

Cell Culture

Murine 3T6 fibroblasts were supplied by Dr. N. Suesa (Lab. Menarini SA, Badalona, Spain), and were grown and routinely maintained as previously described [7] in RPMI 1640 containing 10% FCS. Cells were harvested with

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‡ *Abbreviations*: AA, arachidonic acid; FCS, fetal calf serum; IL-1, interleukin-1; PGE_2, prostaglandin E_2; PGHS-2, prostaglandin H synthase-2; and PMA, 4β-phorbol-12-myristate acetate.

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TABLE 1. Effect of IL-1β and IL-1β synthetic peptides on [^{3}H]AA release and PGE_2 production by 3T6 fibroblasts

	% [^{3}H]AA release		PGE_2 release (ng/mL)	
	2 hr	6 hr	2 hr	6 hr
Control	9 ± 1	10 ± 2	11 ± 1	12 ± 1
PMA (1 μg/mL)	54 ± 4*	68 ± 3*	196 ± 7*	ND
IL-1β (10 ng/mL)	14 ± 2	28 ± 3*	173 ± 6*	122 ± 4*
IL-1β(1 ng/mL)	12 ± 1	19 ± 2*	109 ± 5*	89 ± 3*
IL-1β (163–171) (12 μg/mL)	9 ± 2	12 ± 1	16 ± 2	15 ± 1
IL-1β (208–240) (12 μg/mL)	12 ± 2	13 ± 1	137 ± 6*	116 ± 4*
IL-1β (208–240) (4 μg/mL)	10 ± 1	12 ± 1	87 ± 4*	73 ± 3*

Preconfluent (2000 cells/cm^2) fibroblasts were labeled with [^{3}H]AA as we described in Materials and Methods, and incubated with agonists for 2 or 6 hr at 37°. Then, the supernatants were removed and the percentage release of [^{3}H]AA was calculated and the amount of PGE_2 measured by EIA. Each group represents the mean ± SEM of determinations made from four separate dishes.

*$P < 0.05$ (Student's *t*-test).

ND = not determined.

trypsin/EDTA and seeded into a 12-well tissue culture cluster (Costar). Cell cultures were maintained at 37° with 95% air, 5% CO_2. Cell viability tests were performed under all experimental conditions.

Incorporation and Release of [^{3}H]Arachidonic Acid

After a period of fibroblast replication (2 days) and a period of FCS starvation (8 hr), [^{3}H]AA was incorporated to subconfluent culture cells to study the [^{3}H]AA released as we described previously [7, 8].

PGE_2 Production by Fibroblasts in Culture

An aliquot of culture supernatant medium (0.25 mL) was acidified with 1 mL of 1% formic acid. PGE_2 was extracted and determined using a PGE_2-monoclonal enzyme immunoassay kit (Cayman Chemicals) as we described previously [9].

Protein Determination

Total protein was measured by the Bradford method [10] by means of the Bio-Rad protein assay, using BSA as standard.

Western Blot Analysis

Cells were washed twice in PBS and scraped off in PBS containing 2 mM EDTA and pelleted. Finally, cell pellets were solubilized in PBS containing 2 mM EDTA, 20 μg/mL of phenylmethylsulfonylfluoride, 20 μg/mL of aprotinin, 20 μg/mL of leupeptin, 200 μg/mL of dimethyldithiocarbamic acid and 1% Tween 20. Cell lysates (20–30 μg of protein) were separated by a 10% SDS-PAGE gel [11], blotted and PGHS-2 immunodetected as we described previously [8].

Statistics and Data Analysis

Results are expressed as means ± SEM. Differences between control cultures and treated cultures were tested by using either Student's *t*-test or one-way ANOVA followed by the least significance difference test as appropriate.

RESULTS AND DISCUSSION

Relatively small (4 kDa) peptides with IL-1-like activities were purified from the plasma of febrile patients [12] and from the urine of healthy humans [13]. These observations initiated experiments aiming to elucidate the structure-activity relationship for IL-1 and eventually to produce active IL-1-like peptides. Obal *et al.* [4] showed that an IL-1β fragment (163–171) is active in thymocyte proliferation, whereas IL-1β peptide (208–240) stimulates PGE_2 production.

Our results show that IL-1β and the IL-1β fragment (208–240) induced PGE_2 synthesis in a concentration-dependent manner (Table 1) whereas IL-1β peptide (163–171) failed to produce PGE_2, in agreement with the results obtained by Obal *et al.* [4] in synovial and dermal fibroblasts. These results also indicate that the IL-1 fragment (208–240) is less potent than the whole IL-1β molecule. Furthermore, time-course experiments showed an early onset of IL-1β fragment (208–240)-induced release of PGE_2 (Fig. 1). Thus, there was a rapid rise in PGE_2 levels, which reached a maximum 2 hr after the addition of IL-1β or IL-1β peptide.

The formation and release of prostanoids from 3T6 fibroblasts is a multi-step process requiring a series of enzymatic conversions of AA to the final product. The amount of free AA in resting cells is maintained at a low level by a multitude of phospholipid-remodeling pathways and upon stimulation, AA is made abundantly available for eicosanoid biosynthesis. Because AA is found predominantly esterified in the sn-2 position of membrane phospholipids, phospholipase A_2 is the most likely enzyme to release AA [14]. Previously, we reported that phospholipase A_2 and the subsequent AA mobilization is enhanced by several agonists in 3T6 fibroblast cultures [7, 15]. To determine whether there was a specific alteration in arachidonate mobilization, cells prelabeled with [^{3}H]AA were treated with IL-1β and IL-1β peptides. Our result shows that the IL-1β (208–240) was not able to induce a significant [^{3}H]AA mobilization (Table 1). Then, we focused on the second step of the arachidonate cascade.

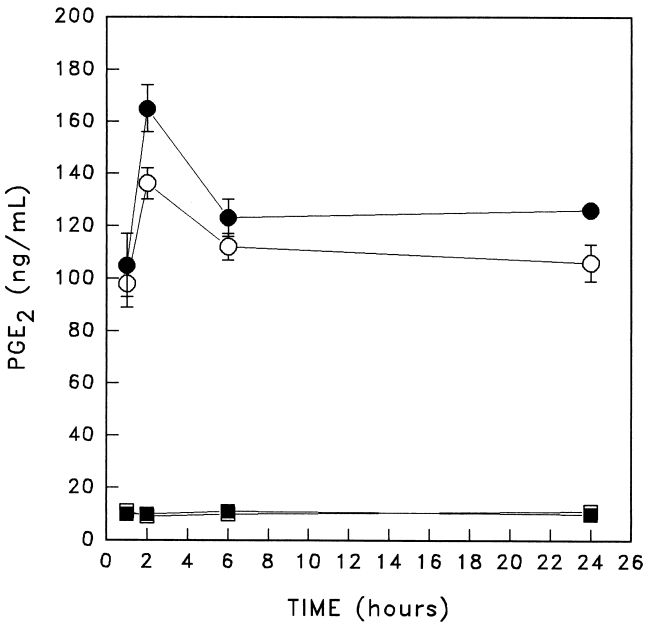

FIG. 1. Time course of PGE_2 formation by 3T6 fibroblasts. Preconfluent fibroblasts were incubated with RPMI 1640 (□) or RPMI 1640 containing IL-1β (10 ng/mL, ●), IL-1β (163–171) (12 μg/mL, ■) or IL-1β (208–240) (12 μg/mL, ○). Then, the supernatant was removed and the amount of PGE_2 was measured by EIA. Each group includes the mean ± SEM of measurements made from four separate dishes.

Prostaglandin H synthase utilizes AA as a substrate to produce prostanoids. Two isoforms of PGHS presently known are regulated in a cell-type and stimulus-specific manner [16]. Recently, we observed that 3T6-stimulated fibroblasts induce PGHS-2 expression [8, 17]. Here, we show that IL-1β peptide (208–240) has a significant effect on PGHS-2 expression, which is detectable early at 2–3 hr (Fig. 2). Given that IL-1β (208–240) was unable to significantly increase AA release, and that IL-1β (208-240) stimulated the expression of PGHS-2, this effect on cellular PGHS-2 levels is likely to be the mechanism of prostaglandin induction by IL-1β (208–240). These data are consistent with the capacity to induce PGE_2 synthesis and the pro-inflammatory effects *in vivo* attributed to IL-1β fragment (208–240).

In conclusion, the present results suggest that the capacity of IL-1β peptide (208–240) to produce PGE_2 and to induce pro-inflammatory effects could be related to its action on PGHS-2 expression.

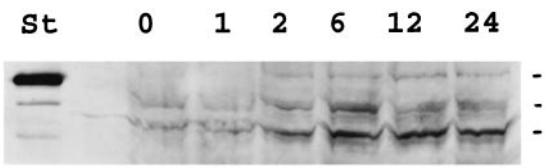

FIG. 2. Western blot analysis of PGHS-2 in cultured murine 3T6 fibroblasts. Ovine PGHS-2 (50 ng) was used as standard. The effect of IL-1β peptides on PGHS-2 expression was studied in fibroblast lysate (20–30 μg of protein) from cells incubated for 0, 1, 2, 6, 12 or 24 hr in RPMI 1640 with FCS and IL-1β (208–240) (1 μg/mL). Data are representative of three experiments.

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