

## Interleukin-1 enhances pain reflexes. Mediation through increased prostaglandin E<sub>2</sub> levels

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### Abstract

Interleukin-1 (IL-1) has been shown to induce inflammatory reactions in part through increased prostaglandin production. Prostaglandins of the E- and I-type sensitize nociceptors in peripheral tissues. We have therefore investigated the effect of IL-1 perfusion in the isolated rabbit ear, a model which allows the assessment of peripheral pain. Natural IL-1 from human monocytes, IL-1 from glioblastoma cells as well as recombinant IL-1  $\alpha$  or  $\beta$ , increased the pain reflex induced by acetylcholine in a concentration dependent manner. The PGE<sub>2</sub> levels were measured in the perfusate and were found to be enhanced more than 10-fold after the infusion of IL-1 $\alpha$  or IL-1 $\beta$ . This effect was paralleled by the enhanced pain reflexes and persisted for at least one hour after cessation of the IL-1 perfusion. Both the increased pain reflexes as well as the enhanced PGE<sub>2</sub> levels were abolished by addition of the cyclooxygenase inhibitor diclofenac-Na (Voltaren®) to the perfusion fluid. These results show that besides the numerous known physiological functions of IL-1, it may also play a role in peripheral pain sensations.

### Introduction

Interleukin-1 (IL-1) is a polypeptide synthesized in response to microbial invasion, as well as in several disease processes and under some physiological stresses. IL-1 which is produced by both phagocytic leukocytes as well as non-leucocytic cells has emerged as a major mediator of the acute phase response. Injection of IL-1 into animals results in fever, increased slow wave sleep, neutrophilia and stimulation of hepatic acute phase protein synthesis. Experimental data also point to IL-1 as a mediator of local inflammatory and immune mediated diseases eg. chronic inflammatory joint diseases (reviewed in [1]). Many of these conditions are

associated with pain. Therefore, regulation of target cell responses and pharmacological intervention of IL-1 effects are an issue of considerable clinical interest. At least in part the diverse effects observed with IL-1 may be mediated by prostaglandins. IL-1 at picomolar concentrations has been reported to induce PGE<sub>2</sub> synthesis in synovial lining cells [2], brain hypothalamic minces [3], fibroblasts and astrocytes [4].

The role of PGE<sub>2</sub> in pain is well established by the fact that it sensitizes nociceptors to a variety of noxious stimuli, but has very little intrinsic painful effect when applied alone to nerve endings [5]. This activity of PGE<sub>2</sub> on the pain-threshold as well as the peripheral antinociceptive effect of non-steroidal anti-inflammatory drugs has also been demonstrated in the perfused isolated rabbit ear model [6, 7].

The present study was undertaken to test the hypothesis whether IL-1 is able to sensitize the nociceptors and thus enhance the neurotransmitter-induced pain-reflexes via a prostaglandin-dependent mechanism.

## Material and methods

### IL-1 Preparations

*Human monocyte IL-1* (pI 7.0) was purified from supernatants of adherent human blood monocytes stimulated with heat-killed *Staphylococcus epidermidis* as previously described by Dinarello and Bernheim [3]. The specific activity of the IL-1 was approximately  $5 \times 10^6$  half-maximal units per mg as measured on thymocytes.

*Human glioblastoma cell derived IL-1* was purified from the conditioned medium of the cultured human glioblastoma cell line 308 as described previously by Fontana et al. [8]. The IL-1 preparation obtained by gel chromatography was further purified by immune affinity chromatography using a polyclonal anti-human IL-1 antiserum [3].

*Recombinant human IL-1 $\alpha$  and  $\beta$*  were obtained from Biogen SA, Geneva, Switzerland. They were expressed in *E. coli* and their purity and biological activity has been recently reported by Wingfield et al. [9]. Using murine thymocytes, the specific activity was approximately  $10^8$  units/mg.

### Pain reflex assay on the isolated rabbit ear model

The isolated perfused rabbit ear model first described by Juan and Lembeck [6] for studying the effect of prostaglandins on paravascular pain receptors was modified for testing peripherally acting nonsteroidal antiphlogistics [7]. In the present study the following modifications were used: in-

stead of continuous infusion of prostaglandin E<sub>2</sub>, arachidonic acid (Fluka, Switzerland) 500 ng/ml dissolved in Tyrode's solution was perfused for 30 minutes 2 hours prior to the IL-1 perfusion. A stock solution of 0.5 mg/ml dissolved in hexane was stored at  $-20^\circ\text{C}$  under nitrogen. The IL-1 preparation was stored at  $-70^\circ\text{C}$  in 100 mM Tris-HCl buffer pH 7.8 containing 2 mM NaN<sub>3</sub> and diluted in Tyrode's solution just before the perfusion.

All other chemicals were handled as described previously [7]. A pain reflex was elicited every 20 minutes by bolus injections of 10  $\mu\text{g}$  acetylcholine into the perfusion system of the ear [7]. The short-lasting depression of the systemic blood pressure was taken as a quantitative pain-reflex parameter, whereas the "head-flick" response was taken for qualitative judgment only.

In separate experiments 200, 300 and 1000 pg/ml PGE<sub>2</sub> were infused in order to determine the threshold concentration of PGE<sub>2</sub> which increased the pain reflex. The concentration of the prostaglandin in the eluate was monitored at the same time.

### Chemical determinations

PGE<sub>2</sub>, 6-Keto-PGF<sub>1 $\alpha$</sub>  and LTC<sub>4</sub> concentrations were measured by radioimmunoassay [10, 11].

## Results

### 1. Effects of increasing concentrations of perfused PGE<sub>2</sub>

PGE<sub>2</sub> at concentrations of 200, 300 and 1000 pg/ml infused into the rabbit ear for 2 hours led to an enhancement of the acetylcholine-induced pain reflex in a dose-dependent manner. The PGE<sub>2</sub> concentration which was determined in

#### Figure 1

Pain reflex induced by acetylcholine after perfusion of IL-1 $\beta$  into the isolated rabbit ear ■: Tyrode's solution, ▲: 200 ng/ml IL-1 $\beta$ , ●: diclofenac-Na 10  $\mu\text{g}/\text{ml}$ .  $\bar{x} \pm \text{SE}$ , number of animals shown at each time-point.

#### Figure 2

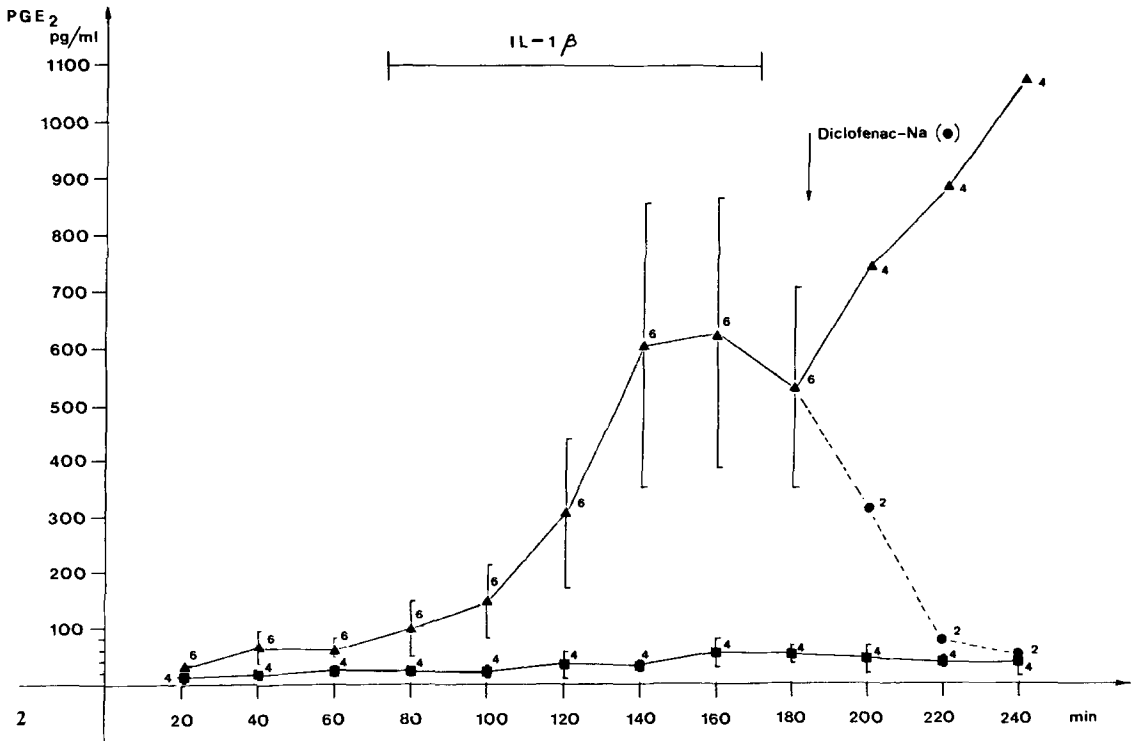
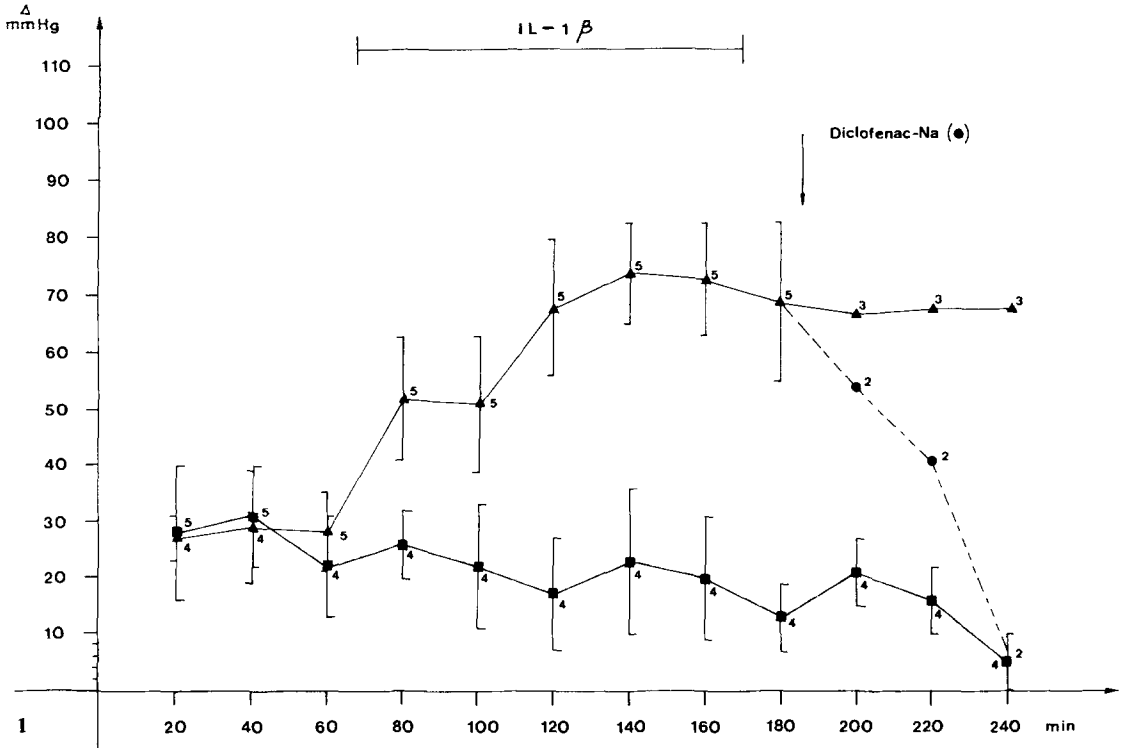
Concentration of PGE<sub>2</sub> in the eluate of the isolated rabbit ear after perfusion with IL-1 $\beta$ . For symbols see figure 1.

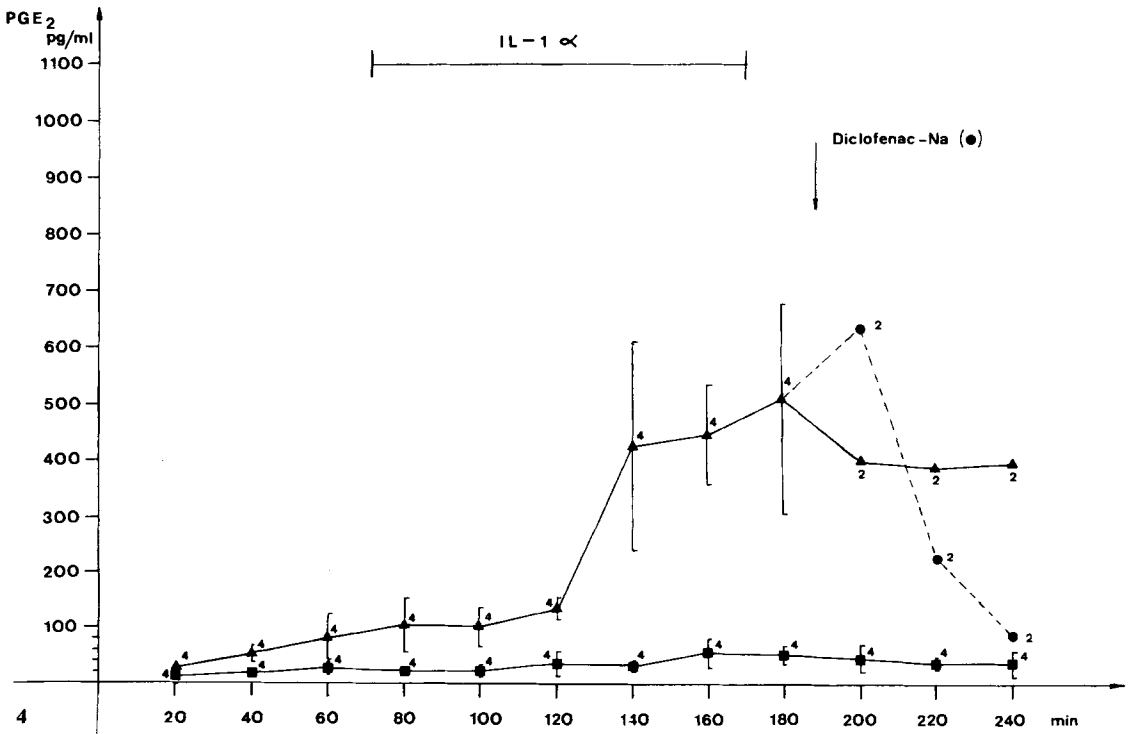
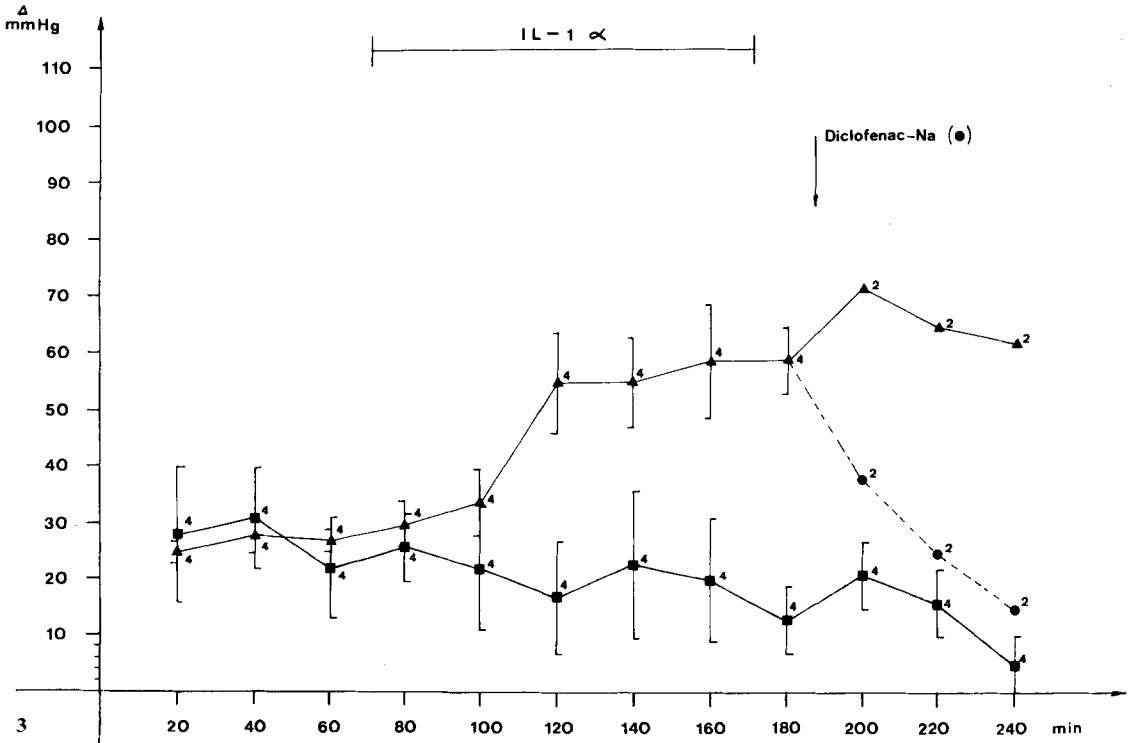
#### Figure 3

Pain reflex induced by acetylcholine after perfusion of IL-1 $\alpha$  into the isolated rabbit ear ■: Tyrode's solution, ▲: 200 ng/ml IL-1 $\alpha$ , ●: diclofenac-Na 10  $\mu\text{g}/\text{ml}$ .  $\bar{x} \pm \text{SE}$ , number of animals shown on each time-point.

#### Figure 4

Concentration of PGE<sub>2</sub> in the eluate of the isolated rabbit ear after perfusion with IL-1 $\alpha$ . For symbols see figure 3.





the eluate ranged between 30 and 100% of the concentration which had been infused. The enhancement of the pain reflex peaked at the same time as did the PGE<sub>2</sub>-concentration in the eluate. As indicated by a drop in blood pressure, 200 and 300 pg/ml of PGE<sub>2</sub> enhanced the pain reflex slightly by a factor of 1.5 and 2 respectively, whereas 1000 pg/ml PGE<sub>2</sub> enhanced this reflex at least 3-fold. Thus, the threshold concentration of PGE<sub>2</sub> for sensitizing the nociceptors to noxious stimuli was about 0.3 ng/ml. When PGE<sub>2</sub> perfusion was stopped, the enhancement of the pain reflex ceased within 30 minutes (data not shown).

### 2. Effects of natural IL-1 from human monocytes and human glioblastoma cells

Initial experiments were performed with natural IL-1 preparations from human monocytes (3–10 units/ml) and an IL-1 like factor secreted by human glioblastoma cells (2–5 units/ml). Both preparations of IL-1 induced an increase in the amplitude of the acetylcholine-induced "head-flick" response as well as a drop in reflex blood pressure. After infusion of the IL-1 from human monocytes a rise in the concentration of PGE<sub>2</sub> in the eluate was observed.

### 3. Effects of recombinant IL-1 $\alpha$ and IL-1 $\beta$

Infusion of 20 or more ng/ml of IL-1 $\beta$  led to an enhancement of the pain reflex by 50% or more. The effect increased with higher concentrations of IL-1 $\beta$ . After cessation of the perfusion with IL-1 it persisted for at least 3 hours.

In a series of experiments the effect of perfusion at a concentration of 200 ng/ml IL-1 $\beta$  was compared with the effect obtained with Tyrode's solution alone. With IL-1 $\beta$  the pain reflex began to increase 20 minutes after the start of the infusion. It reached a plateau at 3-fold greater than the basal level after about 80 minutes (Fig. 1). This increase in the pain reflex was paralleled by an increase in the PGE<sub>2</sub> concentration in the eluate, which after 80 minutes also reached a peak level 30 times greater than the basal concentration in the Tyrode-perfused rabbit ears (Fig. 2). Both the enhanced pain reflex and the elevated PGE<sub>2</sub>-level persisted for more than 3 hours after the infusion of IL-1 was stopped.

Addition of the cyclooxygenase inhibitor diclofenac-Na (Voltaren®) 10  $\mu$ g/ml abolished the enhanced pain reflex as well as the PGE<sub>2</sub> production within 40–60 minutes (Figures 1+2). Both the rise in PGE<sub>2</sub> as well as the enhancement of the pain reflex was prevented when diclofenac-Na was concomitantly infused with IL-1 $\beta$  (data not shown). Using IL-1 $\alpha$  instead of IL-1 $\beta$ , qualitatively the same results were obtained (Figures 3–4).

## Discussion

In patients with bacterial infections, injury or chronic inflammatory disease, the multiple biological activities of IL-1 may account for the majority of the acute phase changes. However, in animal models it is difficult to investigate the pathogenesis of such subjective symptoms as headache, myalgia, arthralgia and lassitude. The high potency of IL-1 in inducing release of PGE<sub>2</sub> from fibroblasts, synovial and other cells suggests that these symptoms are mediated at least in part by increased levels of PGE<sub>2</sub>. In this context it has to be pointed out that fever and pain are ameliorated by cyclooxygenase inhibitors, demonstrating the role of PGE<sub>2</sub> in pain sensation and fever.

The present experiments support the concept that IL-1 increases the pain reflex, and that this effect is mediated by PGE<sub>2</sub>, particularly since there is a good correlation between the time-course of the IL-1 stimulated increase in PGE<sub>2</sub>-production and the pain reflex.

In some of the experiments a slight and transient elevation in the concentration of 6-Keto-PGF<sub>1 $\alpha$</sub>  was also measured. The leukotriene C<sub>4</sub> concentration was not found to be increased by IL-1 injection. This lack of effect on LTC<sub>4</sub> levels is of interest as previous studies using the same model have shown that the leukotrienes do not enhance the effects of acetylcholine but rather desensitize the nociceptors of the isolated rabbit ear to the painful stimuli of bradykinin [12].

It remains unclear which cells in the perfused rabbit ear are responsible for the IL-1-induced PGE<sub>2</sub> production. Vascular endothelial cells have been shown to produce PGI<sub>2</sub> and respond in various other ways to stimulation with IL-1 [13]. The IL-1-induced PGI<sub>2</sub> production (measured as 6-Keto PGF<sub>1 $\alpha$</sub> ) which was observed in our model was small and its time-course did not correlate with the

enhancement of the pain reflex. Fibroblasts and connective tissue cells respond with a marked PGE<sub>2</sub> synthesis upon IL-1 stimulation [2]. Therefore mesenchymal cells such as pericytes, mast cells, fibroblasts or perivascular macrophages in the vicinity of blood vessels are potentially a source for PGE<sub>2</sub> production.

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