

# Interleukin-1 $\beta$ enhances capsaicin-induced neurogenic vasodilatation in the rat skin

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1 This study examined the effect of interleukin-1 $\beta$  (IL-1 $\beta$ ) on the capsaicin-induced increase in cutaneous blood flow of anaesthetized rats as measured by laser Doppler flowmetry.

2 The substances were administered by intraplantar subcutaneous injection of 10  $\mu$ l-volumes, saline being injected into one hindpaw and IL-1 $\beta$  into the other.

3 IL-1 $\beta$  (0.5–500 pg) was without effect on blood flow on its own but dose-dependently enhanced the hyperaemic response to intraplantar capsaicin (0.3  $\mu$ g) up to 180% ( $P < 0.05$ ) of the response seen in saline-treated paws.

4 IL-1 $\beta$ -(163–171), a fragment devoid of proinflammatory activity, failed to enhance capsaicin-induced hyperaemia when given at a dose of 50 pg.

5 Indomethacin (10 mg kg<sup>-1</sup>, i.p.) did not alter the capsaicin-induced vasodilatation but prevented IL-1 $\beta$  (50 pg) from augmenting the hyperaemic response to capsaicin.

6 The hyperaemia evoked by intraplantar calcitonin gene-related peptide (0.038–3.8 ng) was not altered by IL-1 $\beta$  (50 pg).

7 These data indicate that IL-1 $\beta$  enhances the cutaneous hyperaemic response to afferent nerve stimulation with capsaicin in a prostaglandin-dependent manner. This proinflammatory action of the cytokine appears to arise from sensitization of afferent nerve endings.

**Keywords:** Interleukin-1 $\beta$ ; capsaicin; afferent nerve stimulation; cutaneous hyperaemia; neurogenic inflammation; sensitization of afferent nerve endings; proinflammatory action; prostaglandins; calcitonin gene-related peptide (CGRP)

## Introduction

Intradermal injection of capsaicin leads to vasodilatation and increased vascular permeability (Hughes & Brain, 1991). These effects are elicited by activation of nociceptive afferent nerve fibres and release of vasoactive peptides from their peripheral endings. Among them, calcitonin gene-related peptide (CGRP) and substance P are the best studied candidate mediators (Holzer, 1992). The process of neurogenic inflammation is pathophysiologically relevant because it is potentially involved in a variety of diseases including inflammation of the skin, eye and respiratory tract, arthritis, migraine, and inflammatory bowel disease (Holzer, 1988; Maggi & Meli, 1988; Barnes, 1991; Basbaum & Levine, 1991; Moskowitz & Buzzi, 1991; Sharkey, 1992).

In addition to altering vascular functions, peptides released from afferent nerve endings can influence the activity of immunocompetent cells including mast cells, granulocytes, macrophages and lymphocytes (Donnerer *et al.*, 1990; McGillis *et al.*, 1990; Holzer, 1992). Mediators released from these cells, such as histamine and prostaglandins, can sensitize afferent nerve endings and in this way lead to hyperalgesia and prolong the inflammatory process (Handwerker, 1991). Sensitization is expected to facilitate the release of neuropeptides from afferent nerve endings and thus to reinforce the signs of neurogenic inflammation. Another putative link between the immune system and afferent nerve fibres is interleukin-1 $\beta$  (IL-1 $\beta$ ). This cytokine is not only a major immune mediator but is also able to initiate inflammatory reactions or to exacerbate existing inflammation (Dinarello, 1992).

The present study was designed to examine the effect of IL-1 $\beta$  on neurogenic inflammation as measured by the

cutaneous vasodilator response to the afferent nerve stimulant, capsaicin, in the rat hindpaw. As IL-1 $\beta$  was found to enhance the vasodilator action of capsaicin, further experiments were aimed at elucidating the mechanism of the sensitizing effect of this cytokine.

## Methods

### Animal preparation

The experiments of this study were approved by the Austrian Ministry of Science and Research. Female Sprague-Dawley rats (Versuchstierzuchtanstalt Himberg, Austria) weighing 180–245 g were anaesthetized with phenobarbitone, 250 mg kg<sup>-1</sup> i.p. (Lippe & Holzer, 1992). Additional doses of phenobarbitone (25–50 mg kg<sup>-1</sup>) were given as required to achieve a deep level of anaesthesia, which was checked by the absence of any reaction to pinching the forepaw in the interdigital web with a surgical clamp. In all animals the trachea was cannulated and blood pressure was monitored continuously via a catheter in the right carotid artery. Rectal temperature was monitored and maintained at a physiological level of 37.5 to 38°C with the aid of a heating pad.

A transparent probe holder for laser Doppler flowmetry (see below) was fixed rigidly with double-sided adhesive tape to the plantar skin of each paw. Then the probe holders were mounted to a frame keeping the paws in a vertical position. To ensure optimal paw perfusion, the rats were positioned supine and with their rostral-caudal axis inclined such that the level of the hindpaws was identical with that of the heart. The experimental procedure started after a 1 h resting period for stabilization of blood pressure, body temperature and plantar skin blood flow.

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### Measurement of cutaneous blood flow

Cutaneous blood flow was measured with a laser Doppler flowmeter (Periflux PF3, Perimed, Sweden) which operates on the principle that a virtually monochromatic laser beam (2 mW helium-neon laser) scattered by moving red blood cells undergoes a frequency shift according to the Doppler effect, while light scattered in static tissues remains unshifted in frequency. The laser light is guided to the skin surface by an optical fibre and permeates the skin up to a depth of 1 mm. Part of the reflected light is collected and transmitted by 2 optical fibres to 2 photodetectors. The output signal of the Periflux PF3 is expressed in dimensionless blood flow values (perfusion units, PU) and represents the red blood cell flow (determined by the number and velocity of these cells) in a semisphere with a radius of about 1 mm (Oberg *et al.*, 1984).

For the determination of the PU zero level, the laser probe was placed on a white static surface and the value of 250 PU was calibrated against the physical motility standard solution PF100 (Perimed) under the conditions recommended by Perimed. The band width of the instrument was set at 12 kHz and the time constant of the damping filter was set at 3 s in order to smooth out fluctuations due to the cardiac cycle.

For measurement of skin blood flow, the laser probe was inserted into the probe holder attached to the plantar skin and thus applied perpendicularly to the skin surface. After a 20–30 s period of stabilization, skin blood flow was determined as the average PU recorded during a period of 15–20 s.

### Subcutaneous injection protocol

Intraplantar subcutaneous injections of 10  $\mu$ l volumes were performed with a 50  $\mu$ l Hamilton syringe and a 27 gauge needle (0.4  $\times$  12 mm) which was inserted from the lateral aspect of the hindpaw. The tip of the needle was directed subcutaneously into the centre of the hole of the laser probe holder. With the tip of the needle in this position, the 10  $\mu$ l volume was injected carefully under direct view. This small injection volume had the advantage of being readily absorbed by the tissue within a few minutes, as judged by the disappearance of the local blanching seen immediately after the injection.

The injection protocol was identical in all experiments inasmuch as the first injection was given into the right paw, while the second injection was given into the left paw after an interval of 45–60 s. This interval provided enough time to record the cutaneous blood flow in the right paw and then to place the laser probe on the left paw.

### Experimental groups

All experimental groups consisted of 6 rats: treatment was as follows:

**Groups 1–4** Doses of 0.5–500 pg IL-1 $\beta$ , dissolved in sterile saline, were injected into the left paw. The right paws served as controls and received an equal volume (10  $\mu$ l) of saline. Forty min later 0.3  $\mu$ g/10  $\mu$ l capsaicin was injected into those areas of both paws which had been pretreated with IL-1 $\beta$  or saline (see Figure 1). The dose of 0.3  $\mu$ g capsaicin was chosen after preliminary experiments had shown this dose to be suprathreshold in causing a reproducible increase in skin blood flow. Since desensitization to capsaicin is a characteristic consequence of topical application of the drug to the rat skin (Lynn *et al.*, 1992), capsaicin was administered only once to each paw.

**Group 5** Rats were pretreated by injection of 50 pg IL-1 $\beta$  into both hindpaws. After 40 min 0.3  $\mu$ g/10  $\mu$ l capsaicin was injected into the left hindpaw while the right hindpaw received 10  $\mu$ l saline.

**Group 6** A dose of 50 pg of IL-1 $\beta$ -(163–171), a fragment of human IL-1 $\beta$  devoid of inflammatory activity (Antoni *et al.*, 1986), was injected into the left hindpaw, the contralateral paw receiving saline alone. Forty min later 0.3  $\mu$ g/10  $\mu$ l capsaicin was injected into both hindpaws (see Figure 2).

**Groups 7–10** To examine the possible involvement of prostaglandins in the sensitizing effect of IL-1 $\beta$ , rats were pretreated with indomethacin (10 mg kg<sup>-1</sup> body weight) or its vehicle (1 ml kg<sup>-1</sup>), which were injected intraperitoneally 60 min prior to the subcutaneous injection of 50 pg IL-1 $\beta$  or saline. Forty min later 0.3  $\mu$ g/10  $\mu$ l capsaicin was injected into both paws (see Figure 3).

**Groups 11–13** To study the effect of IL-1 $\beta$  on the cutaneous vasodilatation induced by calcitonin gene-related peptide (CGRP) this peptide (0.038, 0.38 and 3.8 ng in 10  $\mu$ l saline) was injected into both hindpaws 40 min after IL-1 $\beta$  (50 pg) had been injected into the left paw and saline (10  $\mu$ l) into the right paw (see Figure 4).

**Group 14** In a separate experiment the change in the paw volume after intraplantar injection of saline (50  $\mu$ l) into the right hindpaw and of capsaicin (1.5  $\mu$ g in 50  $\mu$ l saline) into the left hindpaw was examined with a plethysmometer (model 7150, Ugo Basile, Comerio-Varese, Italy). The paw volume was measured 5, 20 and 35 min post-injection.

### Recording protocol

Cutaneous blood flow was measured three times at 15 min intervals before the first injection. In this way control recordings were made on each paw at -35, -20 and -5 min. At time 0 the first injection (usually IL-1 $\beta$  or saline) was performed. A second series of recordings was made 5, 20 and 35 min after this injection. Forty min after the first injection, the second injection (usually capsaicin or CGRP) was carried out. The interval of 40 min was chosen because the effect of IL-1 $\beta$  in sensitizing nociceptors is fully developed within 1 h (Ferreira *et al.*, 1988) and because the time of the experiment was to be kept short as skin blood flow tended to fall slightly with time (Figure 1). The third series of recordings started 5 min after the second injection, i.e. 45 min after the first injection, and was continued at 60 and 75 min.

### Statistics

The results are expressed as means  $\pm$  s.e.mean. For statistical evaluation the PU values of the right and left paw, recorded 5 min after the capsaicin injection, were analysed with the Wilcoxon matched-pairs signed rank test (two-tailed). This test was also used to compare peak changes of blood flow induced by saline with those induced evoked by IL-1 $\beta$  or capsaicin. A value of  $P < 0.05$  was regarded as significant.

### Substances

Human recombinant IL-1 $\beta$  and IL-1 $\beta$ -fragment-(163–171) were purchased from Sigma (Deisenhofen, Germany), calcitonin gene-related peptide (rat CGRP- $\alpha$ ) from Bachem (Bubendorf, Switzerland), capsaicin from Fluka (Buchs, Switzerland), and indomethacin from Merck, Sharp and Dohme (Munich, Germany). All other chemicals were from commercial sources and were of analytical purity. Capsaicin was dissolved in absolute ethanol and dissolved with saline such that the ethanol concentration in the injection solution was 0.1% (weight/weight). Indomethacin was dissolved in 2% (wt/wt) Na<sub>2</sub>CO<sub>3</sub> at a concentration of 30 mg ml<sup>-1</sup> and further diluted with saline to a concentration of 10 mg ml<sup>-1</sup>. CGRP was dissolved (100  $\mu$ M) in, and diluted with, saline.

## Results

### General observations

Cutaneous blood flow during the control period ( $-35$ ,  $-20$ ,  $-5$  min) was similar in both paws of all groups and tended to fall slightly during the time of the experiment (Figure 1). The injection of  $10 \mu\text{l}$  saline increased blood flow from  $56.8 \pm 6.5$  to  $79.5 \pm 8.5$  PU ( $n = 6$ ) as measured 5 min post-injection while injection of  $50 \text{ pg}$  IL-1 $\beta$  enhanced skin blood flow from  $59.3 \pm 9.1$  to  $91.8 \pm 12.2$  PU ( $n = 6$ ). The effect of IL-1 $\beta$  was not significantly different from that caused by saline and was not related to the dose of IL-1 $\beta$  injected (not shown). Twenty and 35 min post-injection skin blood flow had always returned to the level measured before the first injection.

Subcutaneous injection of  $0.3 \mu\text{g}$  capsaicin into the saline-treated paw augmented plantar blood flow by  $144.1 \pm 61.0\%$  ( $n = 6$ ) (Figure 1), this increase being significantly ( $P < 0.05$ ) higher than that caused by saline alone ( $26.7 \pm 21.0\%$ ,  $n = 6$ ). In the paw treated with  $50 \text{ pg}$  IL-1 $\beta$ , capsaicin enhanced blood flow by  $223.0 \pm 56.8\%$  ( $n = 6$ ). This hyperaemic reaction to capsaicin was significantly larger than that seen in the saline-treated paw (Figure 1). Blood flow returned to basal values within 20 min from the injection of capsaicin into the saline-treated paw, whereas blood flow in the paw treated with IL-1 $\beta$  took longer to return to the pre-capsaicin level (Figure 1).

In a control experiment both paws were pretreated with  $50 \text{ pg}$  IL-1 $\beta$ . Injection of  $0.3 \mu\text{g}/10 \mu\text{l}$  capsaicin into the left paw increased cutaneous blood flow from  $38.2 \pm 7.8$  PU to  $106.8 \pm 21.0$  PU ( $n = 6$ ). The effect of capsaicin was significantly ( $P < 0.05$ ) higher than the minor effect of saline which after injection into the right paw increased blood flow from  $36.8 \pm 6.8$  PU to  $52.5 \pm 10.2$  PU ( $n = 6$ ). This observation corroborates that the vasodilator reaction to capsaicin represents primarily a reaction to this algescic chemical and not a reaction to the needle insertion or injection of a  $10 \mu\text{l}$  volume of fluid.

In another experiment it was observed that, at the doses used here, capsaicin failed to augment vascular permeability

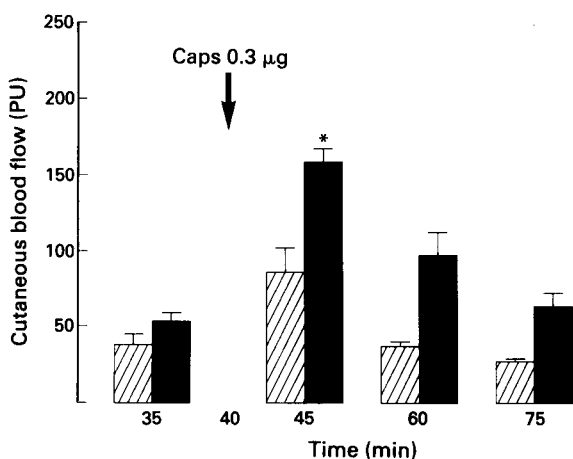
in the rat hindpaw as determined by plethysmometry. The maximal rise of the paw volume as seen 5 to 20 min after intraplantar injection of  $1.5 \mu\text{g}$  capsaicin in  $50 \mu\text{l}$  saline amounted to  $14 \pm 3\%$  ( $n = 6$ ), which did not differ from the rise evoked by  $50 \mu\text{l}$  saline alone ( $13 \pm 2\%$ ,  $n = 6$ ).

### Effect of IL-1 $\beta$ on capsaicin-induced hyperaemia

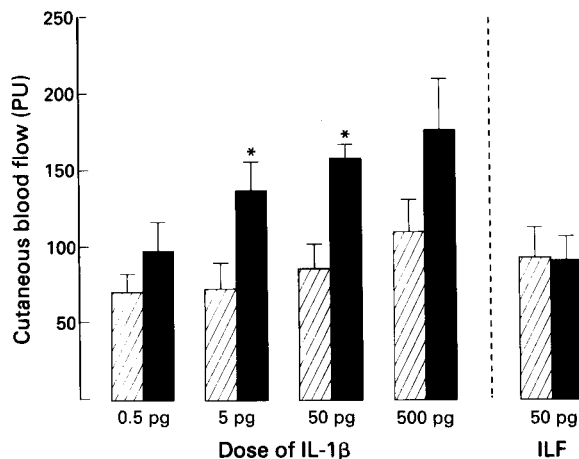
The effect of intraplantar IL-1 $\beta$  in enhancing the capsaicin-induced increase in cutaneous blood flow, for the sake of brevity referred to as 'sensitizing effect', depended on the dose of the cytokine (Figure 2). Doses as low as  $0.5 \text{ pg}$  IL-1 $\beta$  (Figure 2) tended to increase the capsaicin-evoked vasodilator effect but the response to this dose of the cytokine did not reach statistical significance. With  $5 \text{ pg}$  IL-1 $\beta$ , however, the capsaicin-induced cutaneous hyperaemia was significantly enhanced (Figure 2). A further increase in the dose of IL-1 $\beta$  to  $50 \text{ pg}$  did not further enhance the magnitude of the sensitizing effect (Figure 2) but caused a longer-lasting augmentation of the capsaicin-evoked hyperaemia than the dose of  $5 \text{ pg}$  IL-1 $\beta$  (not shown). Pretreatment of the hindpaw with  $500 \text{ pg}$  IL-1 $\beta$  also tended to enhance the hyperaemic response to capsaicin but the sensitizing effect of this cytokine dose did not reach statistical significance (Figure 2). It remains to be determined whether the failure of the highest dose of IL-1 $\beta$  was due to absorption of the cytokine into the circulation and augmentation of the capsaicin-induced hyperaemia in the contralateral paw. Mean arterial blood pressure was not affected by this dose of the cytokine ( $n = 6$ ).

### Effect of IL-1 $\beta$ (163–171) on capsaicin-induced hyperaemia

The fragment IL-1 $\beta$ (163–171) injected at a dose of  $50 \text{ pg}$  failed to exert any sensitizing effect on capsaicin-induced cutaneous vasodilatation. Five min post-injection the hyperaemic responses to capsaicin were identical in the IL-1 $\beta$ (163–171)-treated and saline-treated paws (Figure 2), and this was also true for the recordings taken 60 and 75 min post-injection.



**Figure 1** Time course of the effect of interleukin-1 $\beta$  in enhancing the cutaneous hyperaemia evoked by capsaicin (Caps). At time 0, saline ( $10 \mu\text{l}$ ) was injected into the plantar side of the right paw whilst the left paw was treated with  $50 \text{ pg}$  interleukin-1 $\beta$ . Forty min later capsaicin (Caps,  $0.3 \mu\text{g}$ ) was injected into each paw. Cutaneous blood flow in the right saline-treated (hatched columns) and left IL-1 $\beta$ -treated (solid columns) hindpaw was recorded by laser Doppler flowmetry and expressed in perfusion units (PU). Results are mean with s.e.mean;  $n = 6$ . \* $P < 0.05$  versus respective values measured in the saline-treated paw (two-tailed Wilcoxon matched-pairs signed rank test).



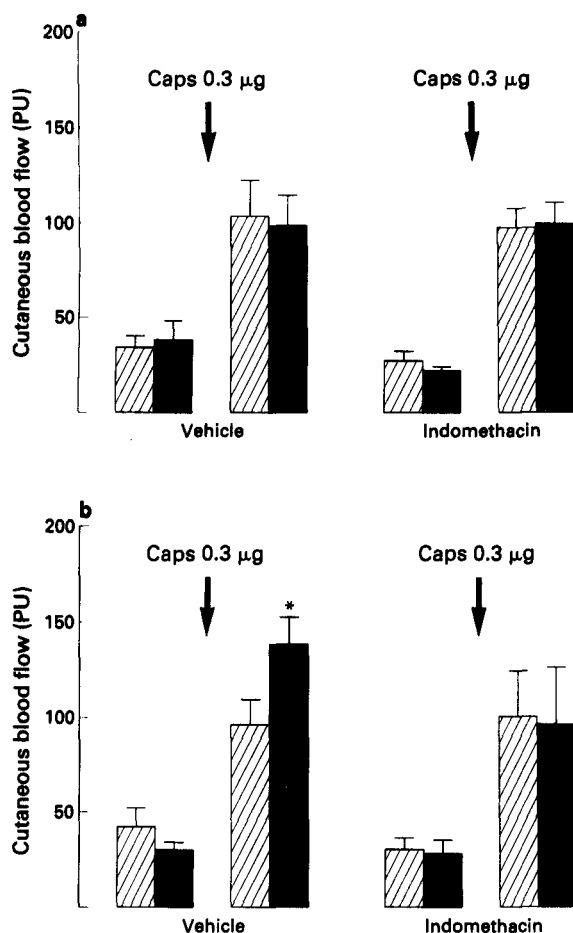
**Figure 2** Dose-response relationship for the interleukin-1 $\beta$  (IL-1 $\beta$ )-induced enhancement of the cutaneous hyperaemia evoked by capsaicin and the lack of effect of the fragment IL-1 $\beta$ (163–171) (ILF). Saline ( $10 \mu\text{l}$ ) was injected into the plantar side of the right paw (hatched columns) whilst the left paw (solid columns) was treated with IL-1 $\beta$  or ILF. Forty min later capsaicin ( $0.3 \mu\text{g}$ ) was injected into each paw. Cutaneous blood flow was recorded by laser Doppler flowmetry and expressed in perfusion units (PU). Results are mean with s.e.mean;  $n = 6$ . \* $P < 0.05$  versus respective values measured in the saline-treated paw (two-tailed Wilcoxon matched-pairs signed rank test).

### Effects of indomethacin

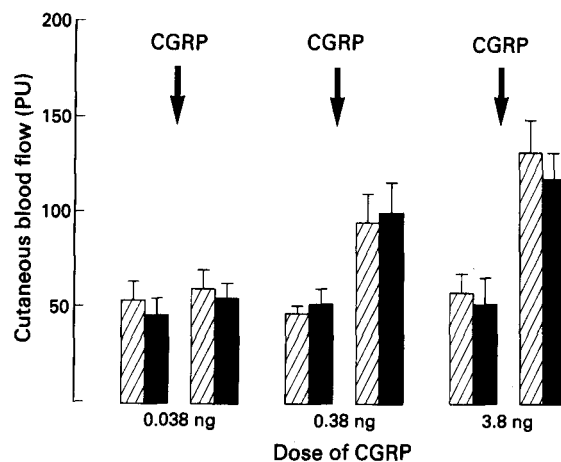
Neither basal blood flow in the hindpaw nor the vasodilator response to capsaicin was altered by intraperitoneal injection of indomethacin ( $10 \text{ mg kg}^{-1}$ ) when compared with the respective blood flow values measured after intraperitoneal injection of vehicle (Figure 3a). These experiments also showed that in both vehicle- and indomethacin-pretreated rats the hyperaemic reaction to intraplantar capsaicin in the left hindpaw was identical with that in the right paw when saline had been injected into either paw 40 min before capsaicin (Figure 3a). The injection of  $50 \text{ pg}/10 \mu\text{l}$  IL-1 $\beta$  to the left hindpaw of vehicle pretreated rats enhanced the vasodilator effect of capsaicin (Figure 3b) to an extent which was similar to that seen in untreated rats (Figures 1 and 2). This sensitizing effect of IL-1 $\beta$  was absent in rats treated with indomethacin (Figure 3b).

### Effect of IL-1 $\beta$ on CGRP-induced hyperaemia

Intraplantar injection of CGRP (0.038–3.8 ng) caused a dose-dependent increase in cutaneous blood flow (Figure 4).



**Figure 3** Effect of intraplantar capsaicin on cutaneous blood flow in rats pretreated with indomethacin or its vehicle. Indomethacin ( $10 \text{ mg kg}^{-1}$ ) or its vehicle was administered intraperitoneally 60 min before saline or interleukin-1 $\beta$  was injected into the plantar side of the hindpaw. In (a) saline ( $10 \mu\text{l}$ ) was given to either paw whereas in (b) saline ( $10 \mu\text{l}$ ) was injected into the right paw and interleukin-1 $\beta$  ( $50 \text{ pg}$ ) into the left paw. Forty min later capsaicin (Caps,  $0.3 \mu\text{g}$ ) was injected into each paw. Cutaneous blood flow in the right (hatched columns) and left (solid columns) hindpaw was recorded by laser Doppler flowmetry and expressed in perfusion units (PU). Results are mean with s.e.mean;  $n = 6$ . \* $P < 0.05$  versus respective values measured in the saline-treated paw (two-tailed Wilcoxon matched-pairs signed rank test).



**Figure 4** Effect of interleukin-1 $\beta$  on the cutaneous hyperaemia evoked by calcitonin gene-related peptide (CGRP). Saline ( $10 \mu\text{l}$ ) was injected into the plantar side of the right hindpaw (hatched columns) whilst the left paw (solid columns) was treated with interleukin-1 $\beta$  ( $50 \text{ pg}$ ). Forty min later CGRP (doses as indicated) was injected into each paw. Cutaneous blood flow was recorded by laser Doppler flowmetry and expressed in perfusion units (PU). Results are mean with s.e.mean;  $n = 6$ .

The magnitude of the CGRP-evoked hyperaemia was the same when saline ( $10 \mu\text{l}$ ) or IL-1 $\beta$  ( $50 \text{ pg}$ ) had been injected into the hindpaw 40 min before the administration of CGRP (Figure 4).

### Effects on blood pressure

At the time of the first injection into the hindpaws mean arterial blood pressure was  $104 \pm 3 \text{ mmHg}$  ( $n = 78$ ). During the time of the experiment, blood pressure decreased on average by  $10\text{--}20 \text{ mmHg}$ . Rats were excluded from the study if blood pressure dropped below  $80 \text{ mmHg}$  during the experiment. In most of the rats, blood pressure rose transiently during/after insertion of the needle into the subcutaneous space of the hindpaw and the intraplantar injection of substances. Whilst pressor responses to intraplantar injection of saline or IL-1 $\beta$  did not differ from each other, the pressor responses to intraplantar capsaicin ( $0.3 \mu\text{g}/10 \mu\text{l}$ ) were consistently higher than those to saline or IL-1 $\beta$ . The increase in blood pressure following capsaicin injection was about  $20 \text{ mmHg}$  and lasted usually less than 4 min.

### Discussion

The major finding of the present study was the discovery that IL-1 $\beta$  enhanced the cutaneous vasodilator action of capsaicin. The sensitizing effect of IL-1 $\beta$  depended on the dose injected and was seen with picogram amounts of the cytokine. This high potency of IL-1 $\beta$  in augmenting capsaicin-evoked hyperaemia is comparable with the high activity of the cytokine in sensitizing the rat hindpaw to noxious pressure stimuli (Ferreira *et al.*, 1988; Follenfant *et al.*, 1989). The vasodilator action of capsaicin in the skin is due to stimulation of afferent nerve endings and subsequent release of vasodilator transmitters (Holzer, 1992), among which CGRP plays a major role, at least in the rabbit skin (Hughes & Brain, 1991). Since IL-1 $\beta$  failed to enhance the vasodilator response to CGRP it is inferred that this cytokine augmented the hyperaemic response to capsaicin by a sensitizing action on afferent nerve fibres but did not alter the sensitivity of blood vessels to the released vasodilator peptide. Together with the findings on nociception (Ferreira *et al.*, 1988; Follenfant *et al.*, 1989) the present data indicate

that IL-1 $\beta$  can sensitize afferent nerve fibres to both chemical and mechanical noxious stimuli, which results, on the one hand, in enhanced release of vasodilator transmitters from the peripheral nerve endings and, on the other hand, in hyperalgesia.

IL-1 $\beta$  is released from activated macrophages and a large variety of other cell types including B-lymphocytes and endothelial cells (Libby *et al.*, 1986; Howells *et al.*, 1988; Dinarello, 1991) and is considered to be a key mediator of the immune and inflammatory reactions to infectious and foreign agents (Dinarello, 1992). This cytokine is not only a proinflammatory substance (Stimpson *et al.*, 1988; Hom *et al.*, 1992; van de Loo *et al.*, 1992) but can *per se* induce inflammation and tissue destruction (Petty *et al.*, 1986; Chandrasekhar *et al.*, 1990). The finding that intra-articular injection of IL-1 induces release of substance P, presumably from afferent nerve fibres, into the synovial fluid (O'Byrne *et al.*, 1990a,b) suggests that cytokines themselves could evoke signs of neurogenic inflammation. The present study, though, failed to furnish conclusive evidence for IL-1 $\beta$ -induced inflammatory reactions in the rat hindpaw skin.

The ability of IL-1 $\beta$  to augment the neurogenic vasodilator response to capsaicin needs to be seen in context with its ability to influence the activity and function of various immune and connective tissue cells (O'Byrne *et al.*, 1991; Dinarello, 1992). Specifically, IL-1 $\beta$  stimulates lymphocytes to release other cytokines (Lowenthal *et al.*, 1986) and causes fibroblasts and synovial cells to produce prostaglandin E<sub>2</sub> (Antoni *et al.*, 1986; Mochan *et al.*, 1986; O'Byrne *et al.*, 1990b). In the present study twofold evidence was obtained that IL-1 $\beta$  enhanced capsaicin-induced hyperaemia in the rat hindpaw via secondary mediators released from immunocompetent or other cells.

A first hint of such a mechanism of action came from the use of IL-1 $\beta$ -(163–171), a fragment of human IL-1 $\beta$  that is devoid of inflammatory activity, such as the induction of prostaglandin formation, but which retains the immunological activity of the parent molecule in activating T-lymphocytes (Antoni *et al.*, 1986). The finding that IL-1 $\beta$ -

(163–171) failed to enhance the capsaicin-evoked hyperaemia in the rat paw skin indicated, therefore, that immunological processes such as activation of, and cytokine release from, lymphocytes did not play a role in the sensitizing effect of IL-1 $\beta$ . The participation of inflammatory mediators such as prostaglandins was substantiated by the use of indomethacin. At a dose (10 mg kg<sup>-1</sup>) that is known to block prostaglandin synthesis by more than 90% (Whittle & Vane, 1987), this drug prevented the IL-1 $\beta$ -induced augmentation of the vasodilator response to capsaicin whereas basal blood flow and the capsaicin-induced hyperaemia measured in the absence of IL-1 $\beta$  remained unchanged. It follows that cyclooxygenase products generated by IL-1 $\beta$  in the vicinity of afferent nerve endings are responsible for the enhancement of the hyperaemic response to capsaicin. This inference is in keeping with the reported involvement of prostaglandins in the IL-1 $\beta$ -induced hyperalgesia in the rat paw (Ferreira *et al.*, 1988) and rabbit ear (Schweizer *et al.*, 1988) but does not negate the possibility that other mediators could also participate (Follenfant *et al.*, 1989).

In conclusion, IL-1 $\beta$  has been shown to augment cutaneous hyperaemic responses to capsaicin by a sensitizing action on afferent nerve fibres, an action which involves prostaglandins as secondary mediators. The current findings suggest that cytokines released in the course of infection or inflammation amplify the ability of nociceptive afferent nerves to increase blood flow in the affected tissue and in this way contribute to the reinforcement of neurogenic inflammatory processes.

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