Interleukin-8 as a mediator of sympathetic pain

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1 The hyperalgesic effects of interleukin-8 (IL-8), interleukin-1 β (IL-1 β) and carrageenin were measured in a rat paw pressure test.

2 IL-8 evoked a dose-dependent hyperalgesia which was attenuated by a specific antiserum, the β -adrenoceptor antagonists atenolol and propranolol, the dopamine₁ receptor antagonist SCH 23390 and the adrenergic neurone-blocking agent guanethidine. The hyperalgesia was not attenuated by the cyclo-oxygenase inhibitor indomethacin or the IL-1 β analogue Lys-D-Pro-Thr.

3 IL-1 β -evoked hyperalgesia was attenuated by indomethacin and Lys-D-Pro-Thr but not by atenolol or SCH 23390.

4 Carrageenin-evoked hyperalgesia was attenuated by atenolol, indomethacin and anti-IL-8 serum. The effects of atenolol and anti-IL-8 serum were not additive. The effects of indomethacin and anti-IL-8 serum were additive: this combination abolished carrageenin-evoked hyperalgesia.

5 A new biological activity of IL-8 is described, namely the capacity to evoke hyperalgesia by a prostaglandin-independent mechanism. IL-8 is the first endogenous mediator to be identified as evoking hyperalgesia involving the sympathetic nervous system. Since IL-8 is released by activated macrophages and endothelial cells it may be a humoral link between tissue injury and sympathetic hyperalgesia.

Keywords: Interleukin 8; sympathetic pain; hyperalgesia; inflammatory pain

Introduction

Interleukin-1 (IL-1) and interleukin-8 (IL-8) are cytokines (inflammatory proteins) which in response to tissue injury are secreted by various cell types including those of the phagocyte mononuclear system (Westwick *et al.*, 1989). Recently we provided evidence for a role for IL-1 β in hyperalgesia, the hypersensitivity of pain receptors which is the common denominator of inflammatory and sympathetic pain (Ferreira *et al.*, 1988). IL-1 β evoked hyperalgesia, to which prostaglandins contributed, and this hyperalgesia was abolished by the IL-1 β analogue Lys-D-Pro-Thr (Ferreira *et al.*, 1988). Lys-D-Pro-Thr also antagonized hyperalgesia evoked by the inflammatory agent carrageenin, and this antagonism was additive with antagonism by the adrenergic neurone-blocking agent, guanethidine (Ferreira *et al.*, 1988). These results suggest that Lys-D-Pro-Thr was antagonizing the contribution of IL-1 β to the component of carrageenin-evoked hyperalgesia, known to be sensitive to aspirin-like drugs (Ferreira *et al.*, 1978).

The pharmacological blockade of sympathetic nerve fibres or the inhibition of the release of sympathetic mediators diminishes the hyperalgesia and pain associated with painful scars, post herpetic pain and post traumatic dystrophies (Hannington-Kliff, 1974; Loh *et al.*, 1981). Such pain is not amenable to treatment with aspirin-like drugs (Verrill, 1984). Also, the involvement of a sympathetic component in inflammatory nociception has been described in three distinct experimental models (Coderre *et al.*, 1984; Nakamura & Ferreira, 1987; Duarte *et al.*, 1988), in two of which the sympathetic component adds to a component mediated by prostaglandins: hyperalgesia evoked by carrageenin as measured in the rat paw pressure test (modification of the Randall-Selitto test, Nakamura & Ferreira, 1987) and abdominal contortions evoked by acetic acid in mice (Duarte *et al.*, 1988).

In the present study we tested IL-8 for its ability to cause sensitization of nociceptors and examined the possibility that this cytokine has a role in pain mediated by the sympathetic nervous system.

Methods

Hyperalgesia (nociception) was measured by a modification of the Randall-Selitto rat paw pressure test (Ferreira *et al.*, 1978). The development of hyperalgesia was evaluated by the application of a constant pressure of 20 mmHg to the hind paws of rats (Wistar strain, male, weight 150 $\pm$ 15 g, 5 per group) which was discontinued when animals presented a characteristic freezing reaction: reaction time). The intensity of hyperalgesia was quantified as the variation of the reaction time (delta (Δ) reaction time) obtained by subtracting the value measured at the specified time interval after injection of the hyperalgesic agent from the pre-injection control reaction time (zero time). The experimenter was unaware of the group treatments. Hyperalgesia was measured following intra-plantar (i.p.) injections of drugs into the hind paws. The drugs injected were IL-8, IL-1 β , antiserum to IL-8, Lys-D-Pro-Thr, the inflammatory agent carrageenin (Ferreira *et al.*, 1978), the cyclo-oxygenase inhibitor, indomethacin (Vane, 1971), the β -adrenoceptor antagonists, atenolol (Robertson *et al.*, 1983) and propranolol (Nakamura & Ferreira, 1987) and the dopamine₁ receptor antagonist, SCH 23390 (Kebabian & Calne, 1979; Kebabian *et al.*, 1986). In some experiments the adrenergic neurone-blocking agent, guanethidine (Chang *et al.*, 1965) was given s.c. daily for 3 days before the injection of IL-8.

Prostaglandin E₂ (PGE₂) in media conditioned by human peripheral blood monocytes (5 $\times$ 10⁶ cells per ml) incubated for 16 h with endotoxin (ET), IL-1 β , and IL-8 was quantified by radioimmunoassay (NEN Research Products, Boston, U.S.A.). The cells were isolated from the buffy coats of heparinized human peripheral blood by density gradient centrifugation (Poole *et al.*, 1989).

Results are presented as means with s.e.mean. Formal statistical tests are not reported since for all of the differences discussed means differed by more than three times the larger s.e.mean.

Materials

IL-1 β and IL-8 (72 amino acids) were recombinant proteins obtained from NIBSC. The sheep anti-IL-8 serum was a gift

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from Dr Robin Thorpe, Division of Immunobiology, NIBSC. Indomethacin was a gift from Merck, Sharpe & Dohme Ltd (Hoddesdon, Herts.). Carrageenin was a gift from FMC Corporation (Philadelphia, U.S.A.), SCH 23390 (R-(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepine-7-ol) was a gift from Schering (New Jersey, U.S.A.). Guanethidine monosulphate was a gift from Ciba-Geigy (Basle, Switzerland). Atenolol and propranolol were purchased from Sigma (St. Louis, U.S.A.). Lys-D-Pro-Thr was (custom) synthesized by Cambridge Research Biochemicals (Cambridge, England) and purified by preparative high performance liquid chromatography (h.p.l.c.). Identity and purity were established by fast atom bombardment mass spectrometry, amino acid analysis and analytical reverse phase h.p.l.c.

Results

Injection of IL-8 into one hind paw (intraplantar, i.pl.) evoked a dose-dependent hyperalgesia in both hind paws although the effects of smaller doses were greatest in the injected paws (Figure 1). The intensity of hyperalgesia reached a plateau within 60 min of injection of IL-8, started declining 6 h after injection and returned to pre-injection values within 24 h. Local pretreatment (i.pl.) with the cyclo-oxygenase inhibitor indomethacin or with an antagonist of IL-1 β evoked hyperalgesia, Lys-D-Pro-Thr, had no effect on IL-8-evoked hyperalgesia (Figure 2b). The lack of effect of these drugs contrasts with their attenuation of IL-1 β -evoked hyperalgesia (Figure 2a). The hyperalgesic effect of IL-8 was abolished when it was incubated and injected with a neutralizing sheep anti-IL-8 serum but not by incubation and injection with preimmune sheep serum (Figure 2b). Carrageenin (Cg)-evoked hyperalgesia was partially inhibited by local pretreatment with the sympatholytic atenolol, indomethacin or anti-IL-8 serum. Treatment with atenolol together with anti-IL-8 serum had an inhibitory effect upon carrageenin-evoked hyperalgesia similar to that obtained with either agent given alone. Treatment with both indomethacin and the anti-IL-8 serum abolished carrageenin-evoked hyperalgesia (Figure 2c).

Direct evidence for the involvement of the sympathetic nervous system in IL-8 evoked hyperalgesia was obtained by measuring IL-8 evoked hyperalgesia in the presence of the β -adrenoceptor antagonists propranolol and atenolol, the dopamine₁ receptor antagonist SCH 23390 and the sympathetic neurone-blocking agent guanethidine. All four drugs

Figure 1 Time course of the development of hyperalgesia to interleukin-8 (IL-8) injected into rat paws: (a) shows the intensity of hyperalgesia in injected paws 15–180 min after injection of IL-8 (0.1 (●); 1 (▼); 10 (▲) and 100 (●) pg in 0.1 ml i.pl.); (b) shows the intensity of hyperalgesia in injected paws (I, cross-hatched columns) and in contra-lateral paws (CL, open columns) 3 h after injection of IL-8 (100 pg i.pl.). The vertical bars are standard errors of the means of values obtained in groups of 5 rats.

Figure 2 Inhibition of the hyperalgesic effects of (a) interleukin-1 β (IL-1 β), (b) IL-8 and (c) carrageenin. Responses were measured 3 h after injection (in 0.1 ml, i.pl.) of (a) IL-1 β (0.5 pg), (b) IL-8 (100 pg) and (c) carrageenin (Cg, 100 μ g). Pretreatments were given (0.1 ml, i.pl.) 30 min before cytokine or carrageenin: C-saline; Ind = indomethacin, 100 μ g; P = Lys-D-Pro-Thr, 200 μ g; Ab = anti-IL-8 serum, 80 μ l, incubated for 15 min with IL-8 before injection; PS = preimmune serum, 80 μ l, incubated for 15 min with IL-8 before injection; At = atenolol, 25 μ g. Vertical bars are s.e.mean in groups of 5 rats.

antagonized IL-8-evoked hyperalgesia (Figure 3a). The dose of IL-8 injected (100 pg in 0.1 ml) evoked hyperalgesia in injected paws and in contralateral paws. Propranolol, atenolol and SCH 23390, given i.pl., antagonized IL-8 evoked hyperalgesia in injected but not in contralateral paws; the systemic (s.c.) pretreatment of the animals with guanethidine antagonized hyperalgesia in both paws. Hyperalgesia evoked by IL-1 β was not attenuated by either atenolol or SCH 23390 (Figure 3b).

Stimulation of peripheral blood mononuclear cells with bacterial endotoxin or IL-1 β evoked the secretion of immunoreactive PGE₂ (Figure 4); the response to IL-1 β was dose-dependent. Similar doses of IL-8 did not evoke secretion of immunoreactive PGE₂ above control levels.

Discussion

The hyperalgesia evoked in both hind paws by injection of IL-8 into one hind paw may be interpreted as indicating a systemic distribution of the injected IL-8, a notion supported by the slower onset of hyperalgesia in contralateral paws (data not shown).

The lack of effect of indomethacin and Lys-D-Pro-Thr on IL-8-evoked hyperalgesia contrasts with their attenuation of IL-1 β -evoked hyperalgesia and suggests that local release by

Figure 3 Effects of sympatholytic drugs on the hyperalgesic effects of (a) interleukin-8 (IL-8) and (b) IL-1 β . Responses were measured 3 h after injection (in 0.1 ml, i.pl.) of (a) IL-8 (100 pg) and (b) IL-1 β (0.5 pg) into one paw. Injected paws = I = cross hatched columns, contra-lateral paws = CL = open columns. Pretreatments had been given (0.1 ml, i.pl.) into the same paw 30 min before IL-8 or IL-1 β : C-saline; Prop = propranolol, 0.25 μ g; At = atenolol, 25 μ g; SCH = SCH 23390, 0.25 μ g. Guan = guanethidine was given s.c. (30 mg kg⁻¹) daily for 3 days before the injection of IL-8. Vertical bars are s.e.mean in groups of 5 rats.

Figure 4 Immunoreactive prostaglandin E₂ (PGE₂) release by human peripheral blood mononuclear cells (5×10^6 cells per ml) incubated for 16 h with endotoxin (ET), interleukin-1 β (IL-1 β) and IL-8. C = saline control. Vertical bars are s.e.mean of triplicates.

IL-8 of cyclo-oxygenase products or IL-1 β did not contribute to the IL-8-evoked hyperalgesia. The involvement of the sympathetic nervous system in IL-8-evoked hyperalgesia is indicated by the marked attenuation of the response to IL-8 after treatment with propranolol, atenolol, SCH 23390 or guanethidine. The lack of effect of atenolol and SCH 23390 on IL-1 β -evoked hyperalgesia illustrates the different mechanisms underlying the hyperalgesic effects of IL-1 β and IL-8. This difference is also clear from experiments in which carrageenin-evoked hyperalgesia was antagonized. A role for IL-1 β in the (inflammatory) component of carrageenin-evoked hyperalgesia that is sensitive to aspirin-like drugs is indicated by the finding that Lys-D-Pro-Thr in combination with guanethidine

abolished carrageenin-evoked hyperalgesia (Ferreira *et al.*, 1988) whereas a role for IL-8 in the sympathetic component (that is resistant to aspirin-like drugs) is indicated by the finding that antibody to IL-8 in combination with indomethacin abolished carrageenin-evoked hyperalgesia.

The hyperalgesic responses to IL-8 and their sensitivity to sympathetic blockade but not to the cyclo-oxygenase inhibitor, indomethacin, suggest a pharmacological profile for IL-8 similar to that of the sympathomimetic tyramine (Nakamura & Ferreira, 1987), which specifically releases amines from sympathetic neurones (Burn & Rand, 1958). Further indirect evidence that IL-8-evoked hyperalgesia was not the result of interference with the synthesis and release of prostaglandins by stimulating cyclo-oxygenase was obtained in an *in vitro* test system: PGE₂ release by human peripheral blood mononuclear cells. In contrast to bacterial endotoxin and IL-1 β , IL-8 did not evoke release of PGE₂ by mononuclear cells.

Previously we showed that carrageenin-evoked, cyclo-oxygenase dependent, hyperalgesia was mediated in part by IL-1 β (Ferreira *et al.*, 1988). Here we report a new biological activity of IL-8: the capacity to evoke hyperalgesia by a prostaglandin-independent mechanism. IL-8 is the first endogenous mediator to be identified as evoking hyperalgesia involving the sympathetic nervous system. Since IL-8 is released by activated monocytes, macrophages and endothelial cells (Gimbrone *et al.*, 1989; Rampart *et al.*, 1989) it is a strong candidate as a humoral link between tissue injury of various origins and the sensitization of pain receptors in the development of sympathetic hyperalgesia.

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