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Endothelin-1 inhibits PAF-induced paw oedema and pleurisy in the mouse

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1 The current study analyses the effects of endothelin-1 (ET-1) on paw oedema and pleurisy induced by platelet activating factor (PAF) and other inflammatory agents in the mouse.

2 Combined subplantar injection of ET-1 (0.5 pmol/paw) did not modify oedema caused by histamine (1 to 100 μmol/paw), 5-hydroxytryptamine (1 to 100 μmol/paw) or bradykinin (1 to 100 nmol/paw) but markedly inhibited the response to PAF (0.95 to 3.8 nmol/paw). The selective action of ET-1 against PAF-induced (1.9 nmol/paw) oedema was dose-dependent, reaching a maximum at 0.5 pmol/paw and lasted up to 2 h.

3 ET-1 (0.5 pmol/paw) also inhibited paw oedema (3–4 h) caused by zymosan (500 μg/paw). In contrast, it did not modify either the early (1–4 h) or late (48–72 h) phases of the oedematogenic response to carrageenin (300 μg/paw), when given either together with or 24 h after the carrageenin.

4 Intrathoracic injection of PAF (1.9 nmol/cavity) induced pleurisy characterized by an increase in pleural exudate volume, and in accumulation of Evans Blue which was maximal at 30 min and lasted up to 4 h. When injected together with PAF, ET-1 (0.5 pmol/cavity) virtually abolished PAF-induced pleurisy.

5 It is concluded that ET-1 is a potent inhibitor of PAF-induced inflammation in the mouse. Its mechanism of anti-inflammatory action in this species, in contrast to what has been found in other species, does not appear to derive from its potent vasoconstrictor properties as ET-1, at the doses used, failed to affect oedematogenic responses to other inflammatory mediators.

Keywords: Endothelin-1; mouse, paw oedema; pleurisy; inflammation; zymosan; carrageenin; histamine; 5-hydroxytryptamine; bradykinin

Introduction

The vascular endothelium can exert an important modulatory role on blood vessel tone by releasing vasoactive substances, such as prostacyclin (Moncada & Vane, 1979) and endothelium-derived relaxing factor (Furchgott & Zawadski, 1980), the latter recently identified as nitric oxide (Palmer *et al.*, 1987). Production of endothelium-derived contracting factors (EDCFs) has also been detected in response to certain stimuli (Rubanyi, 1988). One such EDCF, endothelin-1 (ET-1), has been isolated from the culture supernatant of porcine aortic endothelial cells and characterized as a 21-residue peptide with potent and sustained pressor and vasoconstrictor activities (Yanagisawa *et al.*, 1988). On a molar basis, ET-1 is at least 10 fold more potent than other known vasoconstrictors in constricting isolated rings of porcine coronary artery. It is now clear that ET-1 belongs to a family of peptides which also includes ET-2, ET-3 (Inoue *et al.*, 1989), 'vasoactive intestinal contractor' (Ishida *et al.*, 1989) and the sarafotoxins present in venom of *Atractaspis engaddensis* (Kloog *et al.*, 1988).

Besides its potent vasoconstrictor action on both arterial and venous conductance vessels (Yanagisawa *et al.*, 1988; De Nucci *et al.*, 1988), ET-1 can also affect smooth muscle tone in the microvasculature. It is a powerful constrictor of rat mesenteric arterioles and venules *in vitro* (Warner, 1990) and *in vivo* (Fortes *et al.*, 1989a) and of arterioles of the hamster cheek pouch (Öhlén *et al.*, 1989), rabbit tenuissimus muscle (Öhlén *et al.*, 1989) and skin (Brain *et al.*, 1988), porcine pia matter (Armstead *et al.*, 1989) and human skin (Brain *et al.*, 1989) *in vivo*. Moreover, ET-1 was found to constrict mesenteric lymphatic vessels in the anaesthetized rats (Fortes *et al.*, 1989b). Hence, ET-1 may be an important regulator of systemic blood pressure and of local haemodynamics.

Possibly because of its vasoconstrictor properties, ET-1 also inhibits plasma extravasation induced in rat skin by intradermal injection of the inflammatory mediators 5-hydroxytryptamine (5-HT), histamine, bradykinin (BK), and platelet activating factor (PAF) and of the vasodilatation induced by nitric oxide and nitroprusside (Chander *et al.*, 1988). When given intradermally together with BK or the chemotactic peptide N-formyl-methionyl-leucyl-phenylalanine (FMLP) into rabbit dorsal skin, ET-1 dose-dependently reverses the increased extravascular accumulation of radiolabelled albumin potentiated by calcitonin gene-related peptide (CGRP; Brain *et al.*, 1989). The current study reassesses the potential anti-inflammatory properties of ET-1 in another species, the mouse, by analysing the effects of the peptide on paw oedema and pleurisy induced by several mediators and phlogistic agents. We have found that, in contrast to the results obtained in other species, ET-1 exhibits a selective action against PAF-induced vascular leakage in the mouse.

Methods

Mice (20–25 g) of either sex from our own colony of the Swiss 44 strain were used.

Production of paw oedema

The subplantar surface of one hind paw of mice was injected with 50 μl NaCl solutions containing one of the following substances bradykinin (1–100 nmol/paw), 5-HT (1–100 μmol/paw), histamine (1–100 μmol/paw), PAF (0.9–3.8 nmol/paw), and zymosan (500 μg/paw) or carrageenin (300 μg/paw). The

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contralateral paw received the same volume of saline (50 μl) and was used as control.

To assess the anti-oedematogenic activity of ET-1, the peptide (0.1, 0.25, 0.5 pmol/paw) was given together with each inflammatory stimulus. The volumes of both hindpaws were measured with a plethysmograph and oedema was calculated as the volume difference (μl) between control and mediator-injected paws. Oedema induced by the mediators was evaluated at the time of peak responses (30 min for PAF; 1 h for 5-HT, BK or histamine), whereas that caused by the phlogistic agents was determined at several times after injection (1, 2, 3 and 4 h for zymosan; 2, 4, 24, 48 and 72 h for carrageenin). Another set of experiments was performed to determine the time course of the anti-inflammatory effect of ET-1 against PAF-induced paw oedema. In such experiments, ET-1 (0.5 pmol/paw) was injected into the footpad at various times before PAF (1.9 nmol/paw) injection. The contralateral paws were treated simultaneously with an equal volume of saline (50 μl) to serve as controls.

Induction of pleurisy

Pleurisy was induced by the technique of Spector (1956) as modified for mice by Henriques *et al.* (1990). Briefly, an adapted needle (13 × 5 gauge) was inserted carefully 2 mm through the parietal pleura into the right side of the thoracic cavity of mice to enable injection of PAF (1.9 nmol/cavity), either alone or in combination with ET-1 (0.5 pmol/cavity). Control animals received an equal volume (50 μl) of sterile saline only.

Exudate quantification

Mice were injected intravenously with Evans blue 25 mg kg^{-1} solution 24 h before receiving the intrathoracic injection of saline, PAF or PAF plus ET-1. The animals were killed at different times (15–240 min) after injection and their thoracic cavities were washed with 1 ml saline containing heparin (10 iu ml^{-1}). The fluid was collected, its volume was measured and the lipids were extracted by the addition of 1 ml chloroform. After removal of the dye-free pleural wash, absorbance was read at 600 nm with a Beckman DU-8 spectrophotometer. The results on exudate accumulation are expressed either as the volume of pleural wash or as total Evans blue (μg) recovered per cavity.

Materials

The following substances were used: ET-1 (porcine endothelin, Peptide Institute Inc., Japan), heparin (Liquemine Roche, Brazil), Evans blue dye (Merck, Germany), 5-hydroxytryptamine, histamine hydrochloride, zymosan, bradykinin (all from Sigma, St. Louis, U.S.A.) and hexadecyl PAF (Bachen, Switzerland).

Statistical analysis

All results are presented as the mean ± s.e.mean. The data were anlaysed statistically by means of Student's t test for unpaired samples (Snedecor, 1953) with $P \leq 0.05$ considered significant.

Results

Paw oedema induced by inflammatory mediators

As shown in Figure 1, histamine, 5-HT, BK and PAF each produced significant dose-related oedema when injected into the mouse hindpaw. The oedematogenic responses to these agonists peaked either at 30 (PAF) or at 60 min (5-HT, BK and histamine) after administration. Simultaneous subplantar injection of ET-1 (0.5 pmol/paw, which corresponds to 1.25 ng/paw) failed to affect the development of oedema in response to histamine, 5-HT or BK (Figure 1a, b and c, respectively). In marked contrast, the same dose of ET-1 caused substantial inhibition of PAF-induced oedema (Figure 1d). To ensure that these findings were reproducible, the effects of ET-1 against paw oedema induced by each of the four mediators were reassessed in two other experiments ($n = 6$ for each dose of mediator) and comparable data were obtained (results not shown).

This selective action of ET-1 against PAF was dose-dependent as, in another set of mice, ET-1 at 0.1 pmol/paw was ineffective and at 0.25 and 0.5 pmol/paw the peptide diminished the oedematogenic response to 1.9 nmol/paw of PAF from 55.6 ± 5.0 μl to 42.6 ± 7.8 μl (23.4% of inhibition; $P < 0.05$) and 22.5 ± 6.2 μl (60.4% of inhibition; $P \leq 0.05$), respectively ($n = 7$ in each group). Responses to PAF measured 1 h after its injection were also effectively suppressed when the lipid was mixed with ET-1 (results not shown).

Figure 2 illustrates the long-lasting effect of ET-1 against PAF-induced paw oedema. It is clear that, when injected 2 h or less before PAF, ET-1 caused significant interval-dependent attenuation of oedema. However, no detectable anti-inflammatory effect was seen in mice given ET-1 4 h or 8 h before PAF.

Paw oedema induced by zymosan and carrageenin

Zymosan (500 μg/paw) produced an oedematogenic response which reached a maximum 4 h after injection. Given simultaneously with the phlogistic agent, ET-1 (0.5 pmol/paw) did not change the magnitude of oedema measured 1 or 2 h later. However, the inflammatory reaction observed at 3 and 4 h after the stimulus was markedly inhibited in ET-1-treated mice ($P \leq 0.05$) as compared to control animals (Figure 3).

As described previously (Henriques *et al.*, 1987), subplantar injection of carrageenin (300 μg/paw) caused a typical biphasic oedematogenic response in mice, characterized by a initial peak of low magnitude within 4 h followed by a more substantial raise in paw volume at 48 and 72 h after administration. The response to carrageenin was not influenced by treatment with ET-1 (0.5 pmol/paw) either together with or 24 h after injecting carrageenin ($n = 7$; results not shown).

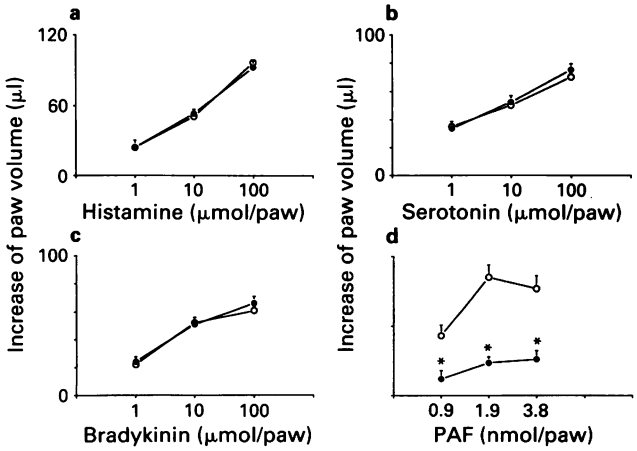

Figure 1 Paw oedema produced in mice by subplantar injection of histamine (a), 5-hydroxytryptamine (b), bradykinin (c) or PAF (d), either alone (○) or mixed with 0.5 pmol/paw of endothelin-1 (●). Only peak oedematogenic responses are shown, which occurred either 1 h (a, b and c) or 30 min (d) after injection. Oedema is expressed as the difference between mediator-injected and saline-injected (control) paws. Each value represents the mean of 7 mice and vertical lines indicate the s.e.means. *$P \leq 0.05$ when compared to PAF alone (Student's t test).

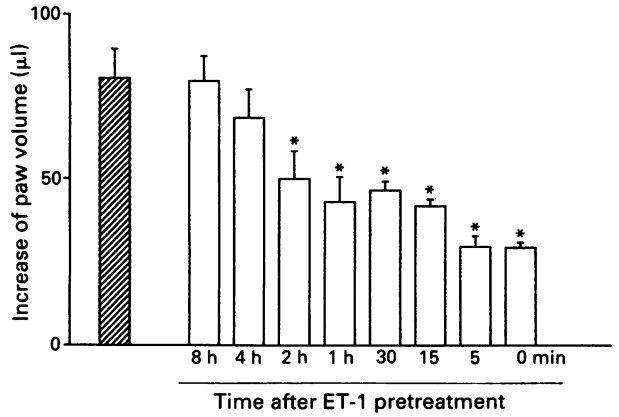

Figure 2 Time course of the inhibitory effect of endothelin-1 (ET-1) against PAF-induced mouse paw oedema. PAF (1.9 nmol/paw) was injected 5 min to 8 h after pretreatment of paws with either saline (hatched column) or ET-1 (0.5 pmol/paw; open columns). The amount of oedema caused by simultaneous treatment of PAF plus ET-1 (0 min) is also shown. Oedema is expressed as the difference between ET-1-injected and saline-injected paw volumes 30 min after PAF. Each value represents the mean of 7 mice and vertical lines indicate the s.e.means. $*P \leqslant 0.05$ when compared to PAF plus saline (Student's t test).

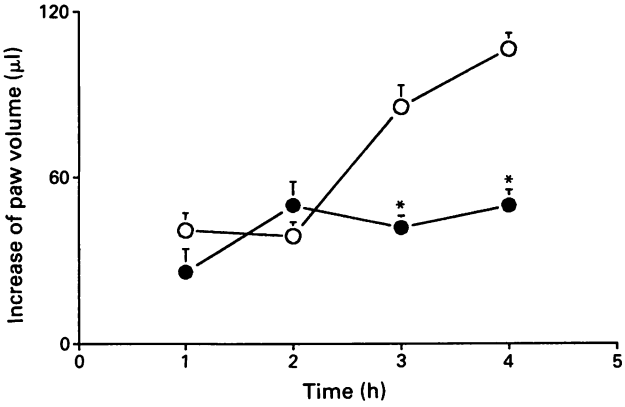

Figure 3 Paw oedema induced in mice by subplantar injection of zymosan (500 µg/paw) either alone (○) or together with (0.5 pmol/cavity) endothelin-1 (●). Oedema is expressed as the difference between volumes of drug-injected and saline-injected paws. Each value represents the mean of 7 mice and vertical lines indicate the s.e.means. $*P \leqslant 0.05$ when compared to corresponding value obtained with zymosan alone (Student's t test).

Pleural exudation induced by PAF

Intrathoracic injection of PAF (1.9 nmol/cavity) caused a pronounced increase in pleural exudate volume and in Evans blue pleural accumulation. This response remained relatively constant between 15 and 60 min after PAF administration and decreased thereafter (Figure 4). Mice receiving combined treatment with PAF plus ET-1 (0.5 pmol/cavity) exhibited significantly less pleural exudation and Evans blue accumulation at 15, 30 and 60 min than animals injected only with PAF ($P \leqslant 0.05$, Figure 4).

Discussion

The main finding of this study was that, in the mouse, ET-1 caused a prolonged dose-dependent and reproducible inhibition of paw oedema induced by PAF, without affecting that induced by 5-HT, BK or histamine. This selective anti-inflammatory action of ET-1 contrasts markedly with the reported nonselective inhibition by the peptide of vascular leakage induced by all four inflammatory mediators in the

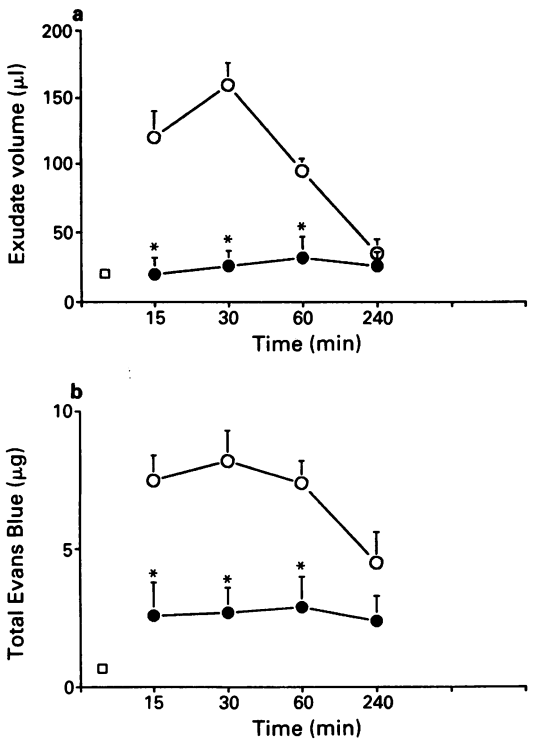

Figure 4 Pleural exudation stimulated by intrathoracic injection of PAF (1.9 nmol/paw) either alone (○) or together with (0.5 pmol/cavity) endothelin-1 (●) in mice. The volume of exudate was quantified either as µl of pleural wash (a) or by total Evans blue leakage (µg/cavity) into the pleura (b). The basal values found in control mice treated only with saline are also shown (□). Each value represents the mean of 7 mice and vertical lines indicate the s.e.means. $*P \leqslant 0.05$ when compared to corresponding value obtained with PAF alone (Student's t test).

rat skin (Chander *et al.*, 1988; 1990). It is also in contrast to the report of Brain *et al.* (1989) showing that, in rabbit skin, ET-1 suppressed plasma exudation induced by CGRP in combination with BK or FMLP. Thus, the selectivity of the anti-inflammatory actions of ET-1 appears to exhibit considerable species variation.

The mechanism(s) involved in the attenuation by ET-1 of PAF-induced mouse paw oedema is as yet unclear. However, it is possible to rule out that such an effect was mediated solely by microvascular vasoconstriction, as this should have also affected oedema caused by the other inflammatory mediators. Moreover, the substantial evidence accumulated from functional, binding and autoradiographic studies, showing that ET-1 interacts with specific ET-receptors or binding sites in many tissues (Yanagisawa *et al.*, 1988; Gu *et al.*, 1989; Davenport *et al.*, 1989) argues against a possible blockade of PAF receptors by the peptide.

Because PAF has been proposed as an important mediator of inflammation induced by zymosan in the rat (Martins *et al.*, 1989) we also tested whether ET-1 could affect paw oedema triggered by this agent in the mouse. Though the peptide did not modify the early stages of the oedematogenic response (up to 2 h), it significantly depressed the later stages of the inflammation (3 and 4 h). This result correlates well with the long-lasting effect of ET-1 against PAF-induced oedema detected in the time course experiments, and its sustained pressor action in rats (Yanagisawa *et al.*, 1988).

In contrast, ET-1 failed to affect both the early and late components of oedema caused by carrageenin. This was a rather unexpected finding, as paw oedema induced by this agent in mice has been shown to be sensitive to blockade by WEB 2170, a selective PAF receptor antagonist (Henriques *et al.*, 1990). Carrageenin-induced paw oedema in the rat, which develops much faster (peaking 3 to 4 h of injection) than in

mice and is mediated to a large extent by generation of eicosanoids and BK (Crunkhorn & Meacok, 1971; Hargreaves *et al.*, 1989) but not by PAF (Cordeiro *et al.*, 1986), has been shown to be inhibited, or at least postponed, by simultaneous injection of ET-1 (Chander *et al.*, 1990). The lack of effect of ET-1 against carrageenin-induced oedema in the mouse may reflect the fact that the biological effect of ET-1, given at 0 or 24 h, will have declined before full development of inflammation (48–72 h). Further experiments should help clarify this issue.

In line with its actions on mouse paw oedema, ET-1 also caused marked, sustained and dose-dependent suppression of PAF-induced pleural exudation. Though again the mechanism(s) underlying this effect remains to be established, the results indicate that the inhibitory effect of ET-1 against PAF-induced effects is not restricted to the paw oedema model of inflammation.

The results of the current study demonstrate that, in addition to its previously reported profound effects on vasomotor tone, ET-1 can modulate selectively microvascular leakage triggered by PAF in the mouse. The mechanism(s) involved in this selective anti-inflammatory effect of ET-1 is possibly unrelated to its vasoconstrictor action, but remains to be full characterized.

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