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Tumor necrosis factor-α prevents interleukin-1β from augmenting capsaicin-induced vasodilatation in the rat skin

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Abstract

The effect of tumor necrosis factor-α (TNFα) and tumor necrosis factor-β (TNFβ) on the capsaicin-induced increase in cutaneous blood flow was investigated in anaesthetized rats. Skin blood flow was measured by laser-Doppler flowmetry. Intraplantar subcutaneous injections of 5–500 pg TNFα and 50–5000 pg TNFβ had no effect on local blood flow, whereas 5000 pg TNFα induced a transient hyperaemia. However, neither the pretreatment with TNFα (5–5000 pg) nor that with TNFβ (50–5000 pg) enhanced the vasodilatator response to intraplantar capsaicin (0.03 μg; 0.1 μg), whereas 50 pg interleukin-1β augmented the capsaicin-induced hyperaemia ($P < 0.05$). This enhancement of the cutaneous hyperaemic response to capsaicin was absent when interleukin-1β (50 pg) was co-injected with TNFα (500 pg or 5000 pg). The vasodilatation caused by calcitonin gene-related peptide or bradykinin was not altered by 500 pg or 5000 pg TNFα. These data indicate that TNFs, in contrast to interleukin-1β, do not amplify the hyperaemic response to afferent nerve stimulation with capsaicin but reverse the augmentation mediated by interleukin-1β.

Keywords: TNF-α (tumor necrosis factor-α); Capsaicin; Cutaneous hyperemia; Afferent nerve stimulation; CGRP (calcitonin gene-related peptide); Bradykinin

1. Introduction

Neurogenic vasodilatation is part of the inflammatory response to noxious stimuli and is elicited by the release of vasoactive neuropeptides, such as calcitonin gene-related peptide (CGRP) and substance P, from peripheral afferent nerve endings (Holzer, 1992). This neurogenically mediated hyperaemia can be evoked by capsaicin (8-methyl-*N*-vanillyl-6-nonenamide), which activates nociceptive afferents and subsequently releases CGRP and substance P. Recently it has been shown that the capsaicin-induced vasodilatation is enhanced by the proinflammatory immune mediator interleukin-1β (Herbert and Holzer, 1994a). This augmentation depends on the sensitization of afferent nerve fibres by interleukin-1β rather than on sensitization of blood vessels to capsaicin, since the vasodilator effect of CGRP, the major neurogenic mediator of the capsaicin-induced vasodilatation (Hughes and Brain, 1991; Brain et al., 1993), was not altered by interleukin-1β (Herbert and Holzer, 1994a). As the neuropeptide-mediated hyperaemia is considered to be involved in the pathogenesis of various diseases including inflammation of the joints, gut, meninges and the respiratory tract (Basbaum and Levine, 1991; Barnes, 1991; Moskowitz and Buzzi, 1991; Sharkey, 1992), it can be hypothesized that elucidation of the proinflammatory interaction of immune mediators with the peripheral afferent nervous system will contribute to the understanding of the mechanisms of acute or chronic inflammatory states.

The work described here continues work reported from previous studies (Herbert and Holzer, 1994a,b) on the sensitizing action of cytokines on cutaneous capsaicin-induced vasodilatation. Tumor necrosis factors (TNFs) are major mediators of the host immune response to injury, infection, and sepsis (Beutler and Cerami, 1986; Tracey et al., 1988; Dal Nogare, 1991; Dinarello, 1992; Spooner et al., 1992). TNFs are secreted in two different molecular forms, as tumor necrosis factor-α (TNFα, also called cachectin) from activated macrophages (Matthews, 1978) and as tumor

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necrosis factor-β (TNFβ, also called lymphotoxin) from stimulated T cells (Kobayashi et al., 1986). TNFs are suggested key mediators in the pathogenesis of multiple inflammatory diseases and TNFα has been detected in exudate and plasma of patients suffering from arthritis (DiGiovine et al., 1988; Yocum et al., 1989; Borzi et al., 1993) or sepsis syndrome (Cerami, 1992; Spooner et al., 1992). Since TNFα shares many biological activities with interleukin-1β (for review see Dinarello, 1992), the aim of the present investigation was to elucidate the influence of TNFα on capsaicin-evoked cutaneous hyperaemia and to compare its effects with those of interleukin-1β (Herbert and Holzer, 1994a). In addition, the action of TNFα on the cutaneous vasodilatation mediated by bradykinin and CGRP was also examined.

2. Materials and methods

2.1. Animals

The experiments, approved by the Animal Care and Use Committee at the Regierung von Unterfranken, were carried out with female Sprague-Dawley rats (Charles River, Germany) weighing 180–260 g. The animals were housed in a temperature-controlled room (22–24°C), kept under artificial light (12 h cycle) and had free access to food and water until use.

2.2. Experimental protocol

As in the experimental protocol described by Herbert and Holzer (1994a,b) the rats were anaesthetized with phenobarbitone (250 mg/kg) injected intraperitoneally, additional doses of phenobarbitone (25–50 mg/kg) being given as required to achieve and maintain a deep level of anaesthesia throughout the experiments (Herbert and Holzer, 1994a). In all animals the trachea was cannulated and blood pressure was monitored continuously via a catheter in the right carotid artery. Spontaneous breathing was assisted by blowing a gentle jet of oxygen towards the opening of the tracheal cannula. Rectal temperature was maintained at a physiological level of 37.5–38°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. The probe holders were mounted to a frame keeping the paws in a vertical position. The experimental procedure started after a 1 h resting period for stabilization of blood pressure, body temperature and plantar skin blood flow.

Intraplantar subcutaneous injections of 10 μl volumes were performed with a 10 μl Hamilton syringe with a 26 gauge needle which was inserted from the lateral aspect of the hindpaw as described previously (Herbert and Holzer, 1994a). The tip of the needle was directed subcutaneously into the centre of the hole of the laser probe holder. In all experiments the first injection was given into the right hindpaw and the second injection into the left hindpaw after an interval of 45 s. This procedure provided enough time to record the cutaneous blood flow in the right paw and then to place the laser probe on the left paw.

2.3. Experimental groups

Experimental groups consisted of 6 rats unless otherwise stated. Doses of 50–5000 pg TNFα or 50–5000 pg TNFβ in 10 μl volumes were injected subcutaneously into the left hindpaws (time 0). The right hindpaws served as controls and received 10 μl of the vehicle. Forty minutes later 0.03 μg/10 μl capsaicin was injected into those areas which had been pretreated with TNFs or vehicle. In another series of experiments 0.03 μg/10 μl capsaicin was injected into both paws 10 min, 20 min or 40 min after local pretreatment with TNFα or vehicle. These groups consisted of 5 rats each. In separate groups of animals both hindpaws were injected with a higher dose of 0.1 μg/10 μl capsaicin, 40 min after pretreatment with 5–5000 pg TNFα to the left and 10 μl vehicle to the right hindpaws. Preliminary experiments showed that 0.03 μg capsaicin is just suprathreshold to elicit a reproducible increase of cutaneous blood flow, whereas 0.1 μg capsaicin provides a more pronounced vasodilator response. To examine whether TNFα augments or interferes with the sensitizing effect of interleukin-1β to subsequent capsaicin injection (0.03 μg), the left hindpaws were treated with either 50 pg/10 μl interleukin-1β or with a combination of 50 pg interleukin-1β plus 500 pg TNFα or 50 pg interleukin-1β plus 5000 pg TNFα in a 10 μl volume. The right paws received vehicle (10 μl) only. To study the effect of TNFα on the cutaneous vasodilatation induced by CGRP or bradykinin, the left hindpaws were pretreated with (i) 500 μg TNFα followed by 0.38 or 3.8 ng/10 μl CGRP, or (ii) 500 or 5000 pg TNFα followed by 1.1 μg/10 μl bradykinin. The left paws served as controls and were pretreated with 10 μl vehicle.

2.4. Drugs

Human recombinant TNFα, human recombinant TNFβ, rat CGRP-α (Bachem, Heidelberg, Germany), human recombinant interleukin-1β, and bradykinin (Sigma, Deisenhofen, Germany) were dissolved in a modified, phosphate-buffered Tyrode solution (adjusted to pH = 7.3) containing 0.1% bovine serum albumin (Sigma). Solutions of TNFα and TNFβ were

stored as small aliquots at −70°C, vehicle and interleukin-1β at −40°C. According to the Analytical Data Sheets of Bachem the biological activity (corresponding to 1 U) of purchased recombinant human TNFα and TNFβ (expressed as ED_{50} for inducing cytolysis of murine L929 cells) was 0.2 ng/ml for TNFα and in the range of 0.06–0.122 ng/ml for TNFβ.

Capsaicin (Serva, Heidelberg, Germany) was dissolved in absolute ethanol (30 mg ml^{-1}) and diluted with saline (0.15 M NaCl) before use. Final concentrations of ethanol for intraplantar injection did not exceed 0.1% (w/w). The vascular effect of ethanol at this concentration is negligible (Herbert and Holzer, 1994b). All other chemicals were from commercial sources and of the highest purity available. The vehicle consisted of the modified, phosphate-buffered Tyrode solution with 0.1% bovine serum albumin.

2.5. Measurement of cutaneous blood flow

Cutaneous blood flow, measured with a laser-Doppler flowmeter (Periflux PF3, Perimed, Sweden) as described in detail previously (Herbert and Holzer, 1994a), was expressed in arbitrary blood flow values (perfusion units, PU).

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, cutaneous blood flow was determined as the average PU recorded during a period of 15–20s at the following times: A control recording was made on each paw 5 min before the first injection. At time 0 the first injection (TNF or vehicle) was performed. A second series of recordings was made 5, 20 and 35 min after this injection. Forty minutes after the first injection, the second injection (capsaicin, bradykinin or CGRP) was carried out. 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. In the experiments where capsaicin was injected 10 or 20 min after 500 pg TNFα, the recordings were taken 5 and 9 min or 5 and 19 min after TNF injection, respectively. The third series of recordings were performed 5, 20 and 35 min after the capsaicin injection as described above.

2.6. Statistical analysis

All results are expressed as the means ± S.E.M for $n = 6$ rats per group. For statistical evaluation the PU values of the right and left paw, recorded 5 min after the injection of either capsaicin, bradykinin, CGRP, 5000 pg TNFα or 5000 pg TNFβ were analyzed with the Wilcoxon matched-pairs signed rank test (two-tailed). Differences at the level of $P < 0.05$ were considered to be significant.

3. Results

3.1. Effect of TNFα and TNFβ on cutaneous blood flow and capsaicin-induced hyperaemia

Intraplantar injections of 5–500 pg TNFα or 50–5000 pg TNFβ were without consistent effect on local cutaneous blood flow in the rat hindpaw. The small hyperaemia seen after 50 pg or 500 pg TNFα did not differ from that evoked by the injection of 10 μl vehicle (Fig. 1). The increase in cutaneous blood flow due to vehicle or TNFα declined to pre-injection values 15 min later (Fig. 1). In contrast, 5000 pg TNFα evoked a significant rise of cutaneous blood flow by 150.7 ± 23.2 PU ($n = 6$) compared to intraplantar injection of vehicle (increase of cutaneous blood flow by 45.8 ± 11.1, $n = 6$). Blood flow returned to the pre-TNFα level within 15 min. Pretreatment with TNFα or TNFβ, in all doses tested (50–5000 pg), failed to augment the hyperaemia induced by 0.03 μg/10 μl capsaicin 40 min after TNF injection (Table 1). Even the

Table 1
Cutaneous blood flow (PU) evoked by capsaicin in paws pretreated with tumor necrosis factors or vehicle

Capsaicin [a]	Pretreatment [b]	TNF 5 pg	Vehicle	TNF 50 pg	Vehicle	TNF 500 pg	Vehicle	TNF 5000 pg	Vehicle
0.03 μg	TNFα			151.2 ± 16.8	144.2 ± 8.2	129.5 ± 13.7	105.7 ± 11.8	120.5 ± 17.8	121.5 ± 11.9
				(55.7 ± 8.8)	(59.6 ± 7.3)	(39.0 ± 4.5)	(42.8 ± 6.8)	(52.5 ± 10.4)	(49.8 ± 10.5)
0.1 μg	TNFα	157.0 ± 23.7	148.5 ± 26.8	192.3 ± 13.2	197.2 ± 16.4	220.3 ± 28.0	179.5 ± 30.9	165.3 ± 25.9	156.0 ± 18.7
		(61.3 ± 8.0)	(52.8 ± 5.3)	(70.6 ± 11.5)	(57.0 ± 4.4)	(75.8 ± 13.1)	(57.2 ± 9.8)	(49.5 ± 7.9)	(60.6 ± 14.1)
0.03 μg	TNFβ			157.8 ± 15.0	124.3 ± 27.6	166.7 ± 19.1	160.0 ± 26.9	183.3 ± 23.6	177.7 ± 16.9
				(46.7 ± 9.1)	(45.8 ± 8.1)	(68.0 ± 12.5)	(51.3 ± 8.9)	(48.6 ± 13.0)	(41.6 ± 4.7)

PU, perfusion units; TNFα, tumor necrosis factor-α; TNFβ, tumor necrosis factor-β. Values are expressed as the means ± S.E.M. for six rats. Cutaneous hyperaemia was evoked by [a] capsaicin (0.03 and 0.1 μg/10 μl) in paws [b] preteated with vehicle (left paws) or TNFα or TNFβ (right paws). The preinjection values for cutaneous blood flow are given in parentheses. At time 0 of the experiment, 10 μl vehicle was injected into the plantar side of the right paw whilst the left paw was treated with TNFα or TNFβ (doses as indicated). Forty minutes later each paw was injected with capsaicin. Cutaneous blood flow was recorded by laser-Doppler flowmetry and expressed in perfusion units (PU).

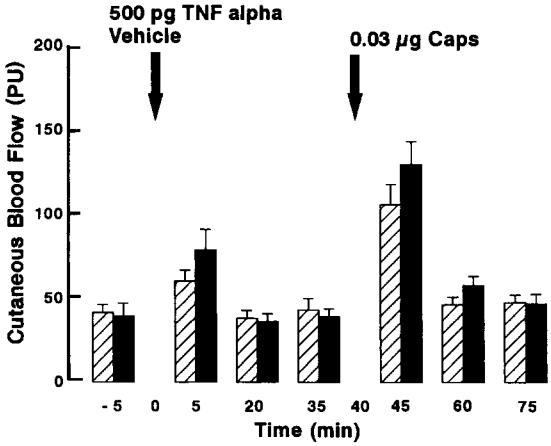

Fig. 1. Graph illustrating the time course of the experiment and the effect of TNFα (TNF alpha) on the cutaneous hyperaemia evoked by 0.03 μg capsaicin (Caps). At time 0 of the experiment, 10 μl vehicle was injected into the plantar side of the right paw (hatched columns) whilst the left paw (filled columns) was treated with 500 pg TNFα. Forty minutes later 0.03 μg capsaicin was injected into each paw. Cutaneous blood flow was recorded by laser-Doppler flowmetry and expressed in perfusion units (PU). The bars represent means ± S.E.M. for 6 rats.

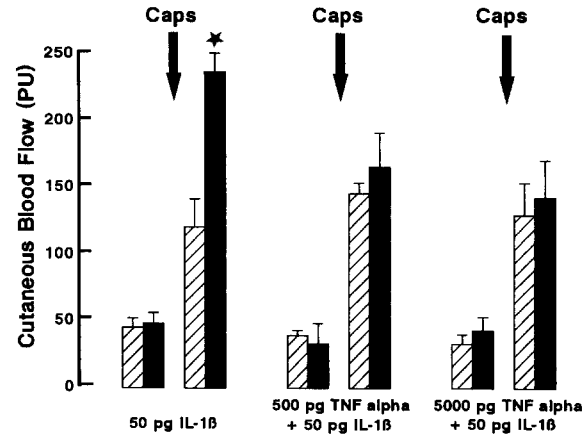

Fig. 2. Effect of interleukin-1β (IL-1β) or the combination of IL-1β plus TNFα (TNF alpha) to enhance the cutaneous hyperaemia evoked by 0.03 μg capsaicin (Caps). At time 0 of the experiment (not shown), 10 μl vehicle was injected into the plantar side of the right paw (hatched columns) whilst the left paw (filled columns) was treated with 50 pg/10 μl IL-1β, the combination of 500 pg TNFα plus 50 pg IL-1β, or the combination of 5000 pg TNFα plus 50 pg IL-1β, in 10 μl volumes. Forty minutes later 0.03 μg capsaicin was injected into each paw. Cutaneous blood flow was recorded by laser-Doppler flowmetry and expressed in perfusion units (PU). The left-hand bars (pre-capsaicin) represent the recordings of cutaneous blood flow 5 min before and the right-hand bars (post-capsaicin) the values 5 min after the injection of capsaicin. The bars represent means ± S.E.M. for 6 rats. * $P < 0.05$ versus respective values measured after injection of vehicle into the contralateral paw (two-tailed Wilcoxon matched-pairs signed rank test).

vasoactive dose of 5000 pg TNFα was ineffective to enhance the vasodilatation due to 0.03 μg capsaicin. The stronger hyperaemia evoked by 0.1 μg/10 μl capsaicin was likewise left unchanged by pretreatment with 5–5000 pg TNFα when compared to that seen in the vehicle-treated paws (Table 1). Injections of capsaicin (0.03 μg/10 μl) given at shorter intervals (10 min, 20 min) after 500 pg TNFα also failed to induce an augmentation, when compared to vehicle treatment or to the effect after the 40 min interval (Table 2).

In a series of control experiments, 50 pg/10 μl interleukin-1β significantly enhanced the capsaicin-induced hyperaemia by 186.8 ± 17.7 PU (Fig. 2), which agrees with the results obtained by Herbert and Holzer (1994a). TNFα (500 or 5000 pg) given together with interleukin-1β (50 pg) prevented the ability of interleukin-1β to augment the vasodilatation caused by capsaicin (0.03 μg) (Fig. 2).

3.2. Effect of TNFα on CGRP- or bradykinin-induced cutaneous hyperaemia

Subcutaneously injected CGRP (0.38–3.8 ng) induced a dose-dependent increase of local cutaneous blood flow, which did not differ between the paws pretreated with 500 pg TNFα and those pretreated with 10 μl vehicle only (Fig. 3). Similarly, in another series of experiments, the local hyperaemia evoked by 1.1 μg bradykinin remained unaffected by the preceding injection of 500 pg or 5000 pg TNFα (Fig. 3). Preliminary experiments had established that 1.1 μg bradykinin was suprathreshold to elicit a reproducible local hyperaemia.

Table 2
Cutaneous blood flow (PU) evoked by capsaicin 10 min, 20 min or 40 min after pretreatment with TNFα or vehicle

	10 min after		20 min after		40 min after	
	500 pg TNFα	Vehicle	500 pg TNFα	Vehicle	500 pg TNFα	Vehicle
Capsaicin	167.8 ± 16.1 (87.6 ± 12.6)	155.2 ± 28.3 (78.4 ± 28.3)	134.0 ± 20.7 (78.0 ± 19.7)	140.2 ± 22.8 (94.0 ± 24.8)	138.6 ± 16.7 (68.4 ± 9.4)	130.6 ± 16.3 (62.8 ± 11.2)

PU, perfusion units; TNFα, tumor necrosis factor-α. Values are expressed as the means ± S.E.M. for five rats. Cutaneous hyperaemia was evoked by capsaicin (0.03 μg/10 μl) 10 min, 20 min or 40 min after the pretreatment with 10 μl vehicle (left paws) or 500 pg/10 μl TNFα (right paws). The preinjection values for cutaneous blood flow (measured at 9 min, 19 min or 35 min after TNFα or vehicle) are given in parentheses. Cutaneous blood flow was recorded by laser-Doppler flowmetry and expressed in perfusion units (PU).

3.3. Effect on blood pressure

Mean arterial blood pressure was stable during the experiments. In the rats ($n = 42$) treated subcutaneously with TNFα and injected with 0.03 or 0.1 μg capsaicin, the blood pressure was 115.3 ± 1.8 mm Hg at the time before TNF injection (−5 min) and 110.5 ± 2.1 mm Hg at the end of the experiment (75 min post-injection). The blood pressure did not vary between the groups treated with different TNFα doses (5–5000 pg). Subcutaneously applied TNFβ (50–5000 pg) also had no effect on blood pressure (−5 min: 114.4 ± 3.7 mm Hg; 75 min post-injection: 113.5 ± 3.3 mm Hg; $n = 18$). The pressor responses to intraplantar injection of capsaicin (about 10–25 mmHg) were consistently higher than those to vehicle or TNFα (about 5–10 mm Hg) and usually lasted 1–4 min.

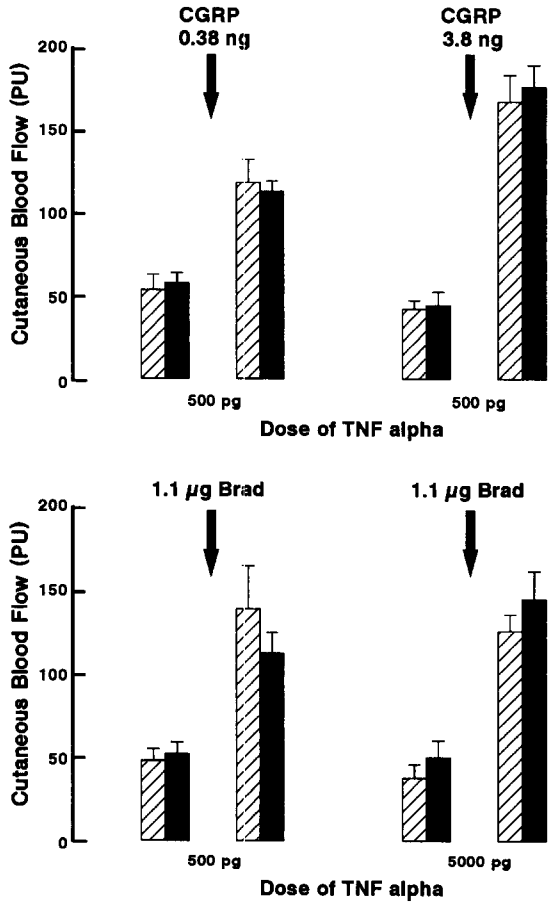

Fig. 3. Effect of TNFα (TNF alpha) on the cutaneous hyperaemia evoked by calcitonin gene-related peptide (CGRP) or bradykinin (Brad). Vehicle (10 μl) was injected into the plantar side of the right hindpaw (hatched columns) whilst the left hindpaw (filled columns) was treated with 500 pg/10 μl TNFα. Forty minutes later CGRP (doses as indicated) or 1.1 μg/10 μl bradykinin was injected into both hindpaws. Cutaneous blood flow was recorded by laser-Doppler flowmetry and expressed in perfusion units (PU). The left-hand bars (pre-injection) represent the recordings of cutaneous blood flow 5 min before and the right-hand bars (post-injection) the values 5 min after the injection of CGRP or bradykinin. The bars represent means ± S.E.M. for 6 rats.

4. Discussion

The present data reveal that TNFs do not augment capsaicin-induced cutaneous vasodilatation in the rat hindpaw skin, although TNFs possess potent proinflammatory activity in various respects (Tracey et al., 1988; Cerami, 1992). The absence of an augmentation of neurogenic vasodilatation by TNFα or TNFβ is in contrast to results obtained with interleukin-1β in this and two previous studies (Herbert and Holzer, 1994a,b). The pleiotropic cytokine, interleukin-1β, which shares many biological activities with TNFs (Dinarello, 1992), enhances dose dependently the capsaicin-induced cutaneous vasodilatation (Herbert and Holzer, 1994a). This effect of interleukin-1β depends on the sensitization of neuronal rather than vascular structures and involves prostaglandins and nitric oxide as essential intermediaries (Herbert and Holzer, 1994a,b). TNFα and TNFβ failed to elicit any hyperaemia after plantar injection by themselves, except for the highest dose of TNFα (5000 pg) which induced a reproducible transient vasodilatation. The mechanism behind the vasodilatator response to this high dose of TNFα is unknown, and its elucidation was beyond the scope of the present study. It should be considered that the increased blood flow due to 5000 pg TNFα might be due to, at least in part, contaminating endotoxins which could be present in recombinant TNFs.

Nevertheless, none of the TNFα and TNFβ doses injected subcutaneously enhanced the vasodilatation due to capsaicin, irrespective of whether capsaicin was applied 10 min or 40 min after TNFα injection. In addition, our experiments showed that the failure of TNFα to enhance hyperaemia is not restricted to the vasodilatation induced by capsaicin, but extends to the hyperaemia due to the endogenous vasorelaxants, bradykinin and CGRP. The latter neuropeptide is the main, if not exclusive, mediator of capsaicin-induced vasodilatation (Hughes and Brain, 1991, 1994) and exerts its vasorelaxant effect independently of nitric oxide (Ralevic et al., 1992; Hughes and Brain, 1994; Holzer and Jocic, 1994). The control experiments of this study confirmed previously published results that interleukin-1β enhances capsaicin-induced vasodilatation (Herbert and Holzer, 1994a,b). In view of the synergism between TNF and IL-1β in several inflammatory events, with a more than additive effect e.g. on sustained hypotension (Okusawa et al., 1988; Tredget et al., 1988) or on proteolysis and muscle catabolism (Flores et al., 1989), interleukin-1β at a dose which augments capsaicin-induced hyperaemia was co-in-

jected with TNFα. The surprising result of these experiments was that, unlike interleukin-1β alone, the combination of interleukin-1β and TNFα failed to augment the capsaicin-induced hyperaemia. While most physiological and clinical studies have generally focused on the role of TNFα as a potent mediator in the progressive development of inflammation and hypotension, our results lead to the assumption that TNFα opposes the augmentation of capsaicin-induced vasodilatation by interleukin-1β. This conclusion is in line with observations of a vasoconstrictor effect of TNFα on pial arterioles (Megyeri et al., 1992) and an inhibitory action of TNFα on acetylcholine-induced endothelium-dependent relaxation in vitro in several arteries of various species (Aoki et al., 1989; Wheeler et al., 1990; Johnson and Ferro, 1992; Liu et al., 1992; Greenberg et al., 1993; Xie et al., 1993; Wang et al., 1994). Furthermore, TNFα might counteract the provasodilatator action of interleukin-1β by release of an endothelium-dependent vasoconstrictor from polymorphonuclear leucocytes (Sobey et al., 1992). Likewise it seems possible that the attenuation of interleukin-1β-induced sensitization by TNFα might be mediated by substances such as tryptase or histamine released from mast cells by TNFα (Van Overveld et al., 1991). Mast cell-derived histamine might also account for the vasodilatation due to high doses of TNFα. Conflicting data on TNFα are not confined to its action on vasodilatation, but have also been reported with regard to its effect on vascular permeability and nociception. Whereas the development of inflammatory oedema due to carrageenan is suggested to depend to a great extent on TNFα, Chen et al. (1994) failed to see marked paw oedema even when high doses of recombinant TNFα were injected subcutaneously (up to 10 000 U per paw). Interleukin-1β induces mechanical hyperalgesia in the rat paw (Cunha et al., 1992) and in the rat knee joint (Davis and Perkins, 1994), whilst TNFα exerts a hyperalgesic effect only after intraplantar injection (Cunha et al., 1992). Both cytokines activate the prostaglandin hyperalgesic pathway (Ferreira et al., 1988; Cunha et al., 1992), which is said to mediate, at least in part, mechanical hyperalgesia.

In conclusion, the present data indicate that TNFs have no vasodilatator effect over a wide dose range and, in contrast to interleukin-1β, TNFs lack any augmenting effect on capsaicin-induced hyperaemia or on the vasodilatation due to bradykinin or CGRP. Importantly, the interleukin-1β-mediated increase in the capsaicin-induced hyperaemia is abolished after co-injection with TNFα. Thus, TNFα counteracts the effect of another cytokine (interleukin-1β) to facilitate neurogenic vasodilatation in the skin. This action of TNFα may be an important factor in the immunological control of the neurogenic component of inflammatory processes.

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References

Aoki, N., M. Siegfried and A.M. Lefer, 1989, Anti-EDRF effect of tumor necrosis factor in isolated, perfused cat carotid arteries, Am. J. Physiol. 256, H1509.

Barnes, P.J., 1991, Neurogenic inflammation in airways, Int. Arch. Allergy Appl. Immunol. 94, 303.

Basbaum, A.I. and J.D. Levine, 1991, The contribution of the nervous system to inflammation and inflammatory disease, Can. J. Physiol. Pharmacol. 69, 647.

Beutler, B. and A. Cerami, 1986, Cachectin and tumour necrosis factor as two sides of the same biological coin, Nature 320, 584.

Borzi, R.M., L. Arfilli, M.C. Focherini, L. Pulsatelli and R. Meliconi, 1993, Circulating tumor necrosis factor alpha in rheumatoid arthritis, Boll. Soc. Ital. Biol. Sper. 69, 39.

Brain, S.D., S.R. Hughes, H. Cambridge and G. O'Driscoll, 1993, The contribution of calcitonin gene-related peptide (CGRP) to neurogenic vasodilator responses, Agents Actions 38 (Special Issue), C19.

Cerami, A., 1992, Inflammatory cytokines, Clin. Immunol. Immunopathol. 62, S3.

Chen, Y.L., V.L. Vraux, J.P. Giroud and L. Chauvelot-Moachon, 1994, Anti-tumor necrosis factor properties of non-peptide drugs in acute-phase responses, Eur. J. Pharmacol. 271, 319.

Cunha, F.Q., S. Poole, B.B. Lorenzetti and S.H. Ferreira, 1992, The pivotal role of tumour necrosis factor alpha in the development of inflammatory hyperalgesia, Br. J. Pharmacol. 107, 660.

Dal Nogare, A.R., 1991, Septic shock, Am. J. Med. Sci. 302, 50.

Davis, A.J. and M.N. Perkins, 1994, The involvement of bradykinin B_1 and B_2 receptor mechanisms in cytokine-induced mechanical hyperalgesia in the rat, Br. J. Pharmacol. 113, 63.

DiGiovine, F.S., G. Nuki and G.W. Duff, 1988, Tumour necrosis factor in synovial exudates, Ann. Rheum. Dis. 47, 768.

Dinarello, C.A., 1992, Role of interleukin-1 in infectious diseases, Immunol. Rev. 127, 119.

Ferreira, S.H., B.B. Lorenzetti, A.F. Bristow and S. Poole, 1988, Interleukin-1 beta as a potent hyperalgesic agent antagonized by a tripeptide analogue, Nature 334, 698.

Flores, E.A., B.R. Bistrian, J.J. Pomposelli, C.A. Dinarello, G.L. Blackburn and N.W. Istfan, 1989, Infusion of tumor necrosis factor/cachectin promotes muscle catabolism in the rat. A synergistic effect with interleukin 1, J. Clin. Invest. 83, 1614.

Greenberg, S., J. Xie, Y. Wang, B. Cai, J. Kolls, S. Nelson, A. Hyman, W.R. Summer and H. Lippton, 1993, Tumor necrosis factor-alpha inhibits endothelium-dependent relaxation, J. Appl. Physiol. 74, 2394.

Herbert, M.K. and P. Holzer, 1994a, Interleukin-1β enhances capsaicin-induced neurogenic vasodilatation in the rat skin, Br. J. Pharmacol. 111, 681.

Herbert, M.K. and P. Holzer, 1994b, Nitric oxide mediates the amplification by interleukin-1β of neurogenic vasodilatation in the rat skin, Eur. J. Pharmacol. 260, 89.

Holzer, P., 1992, Peptidergic sensory neurons in the control of

vascular functions: mechanisms and significance in the cutaneous and splanchnic vascular beds, Rev. Physiol. Biochem. Pharmacol. 121, 49.

Holzer, P. and M. Jocic, 1994, Cutaneous vasodilatation induced by nitric oxide-evoked stimulation of afferent nerves in the rat, Br. J. Pharmacol. 112, 1182.

Hughes, S.R. and S.D. Brain, 1991, A calcitonin gene-related peptide (CGRP) antagonist (CGRP8–37) inhibits microvascular responses induced by CGRP and capsaicin in skin, Br. J Pharmacol. 104, 738.

Hughes, S.R. and S.D. Brain, 1994, Nitric oxide-dependent release of vasodilator quantities of calcitonin gene-related peptide from capsaicin-sensitive nerves in rabbit skin, Br. J. Pharmacol. 111, 425.

Johnson, A. and T.J. Ferro, 1992, TNF-alpha augments pulmonary vasoconstriction via the inhibition of nitrovasodilator activity, J. Appl. Physiol. 73, 2483.

Kobayashi, M., J.M. Plunkett, I.K. Masunaka, R.S. Yamamoto and G.A. Granger, 1986, The human LT system. XII. Purification and functional studies of LT and 'TNF-like' LT forms from a continuous human T cell line, J. Immunol. 137, 1885.

Liu, S.F., A. Dewar, D.E. Crawley, P.J. Barnes and T.W. Evans, 1992, Effect of tumor necrosis factor on hypoxic pulmonary vasoconstriction, J. Appl. Physiol. 72, 1044.

Matthews, N., 1978, Tumour necrosis factor from the rabbit. II. Production by monocytes, Br. J. Cancer 38, 310.

Megyeri, P., C.S. Abraham, P. Temesvari, J. Kovacs, T. Vas and C.P. Speer, 1992, Recombinant human tumor necrosis factor alpha constricts pial arterioles and increases blood-brain barrier permeability in newborn piglets, Neurosci. Lett. 148, 137.

Moskowitz, M.A. and M.G. Buzzi, 1991, Neuroeffector functions of sensory fibres: implications for headache mechanisms and drug actions, J. Neurol. 238 (Suppl. 1), S18.

Okusawa, S., J.A. Gelfand, T. Ikejima, R.J. Connolly and C.A. Dinarello, 1988, Interleukin 1 induces a shock-like state in rabbits. Synergism with tumor necrosis factor and the effect of cyclooxygenase inhibition, J. Clin. Invest. 81, 1162.

Ralevic, V., Z. Khalil, G.J. Dusting and R.D. Helme, 1992, Nitric oxide and sensory nerves are involved in the vasodilator response to acetylcholine but not calcitonin gene-related peptide in rat skin microvasculature, Br. J. Pharmacol. 106, 650.

Sharkey, K.A., 1992, Substance P and calcitonin gene-related peptide (CGRP) in gastrointestinal inflammation, Ann. NY Acad. Sci. 664, 425.

Sobey, C.G., G.J. Dusting and A.G. Stewart, 1992, Tumour necrosis factor alpha augments the release of an endothelium-dependent vasoconstrictor from human polymorphonuclear leukocytes, J. Cardiovasc. Pharmacol. 20, 813.

Spooner, C.E., N.P. Markowitz and L.D. Saravolatz, 1992, The role of tumor necrosis factor in sepsis, Clin. Immunol. Immunopathol. 62, S11.

Tracey, K.J., H. Wei, K.R. Manogue, Y. Fong, D.G. Hesse, H.T. Nguyen, G.C. Kuo, B. Beutler, R.S. Cotran, A. Cerami et al., 1988, Cachectin/tumor necrosis factor induces cachexia, anemia, and inflammation, J. Exp. Med. 167, 1211.

Tredget, E.E., Y.M. Yu, S. Zhong, R. Burini, S. Okusawa, J.A. Gelfand, C.A. Dinarello, V.R. Young and J.F. Burke, 1988, Role of interleukin 1 and tumor necrosis factor on energy metabolism in rabbits, Am. J. Physiol. 255, E760.

Van Overveld, F.J., P.G. Jorens, M. Rampart, W. De Backer and P.A. Vermeire, 1991, Tumour necrosis factor stimulates human skin mast cells to release histamine and tryptase, Clin. Exp. Allergy 21, 711.

Wang, P., Z.F. Ba and I.H. Chaudry, 1994, Administration of tumor necrosis factor-α in vivo depresses endothelium-dependent relaxation, Am. J. Physiol. 266, H2535.

Wheeler, A.P., G. Jesmok and K.L. Brigham, 1990, Tumor necrosis factor's effects on lung mechanics, gas exchange, and airway reactivity in sheep, J. Appl. Physiol. 68, 2542.

Xie, J., Y. Wang, H. Lippton, B. Cai, S. Nelson, J. Kolls, W.R. Summer and S.S. Greenberg, 1993, Tumor necrosis factor inhibits stimulated but not basal release of nitric oxide, Am. Rev. Respir. Dis. 148, 627.

Yocum, D.E., L. Esparza, S. Dubry, J.B. Benjamin, R. Volz and P. Scuderi, 1989, Characteristics of tumor necrosis factor production in rheumatoid arthritis, Cell Immunol. 122, 131.