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Effects of endothelin-1 on capsaicin-induced nociception in mice

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Abstract

The influence of endothelin-1 on nociception induced by capsaicin was assessed in the mouse hindpaw. Local endothelin-1 injection (1 to 20 pmol/paw) 30 min prior to ipsilateral injection of capsaicin (0.1 μg/paw) increased, in a graded fashion, the time spent licking the injected paw. Maximal hyperalgesia was obtained with 10 pmol/paw of endothelin-1 (capsaicin-induced hindpaw licking time increased from 43 ± 3 s to 114 ± 7 s, $n = 6$), but no hyperalgesia was evident following 30 pmol/paw of endothelin-1. The selective endothelin ET_B receptor agonists sarafotoxin S6c (≤ 30 pmol/paw) and IRL 1620 (i.e., Suc[Glu^9,Ala^{11,15}]endothelin-1-(10–21); ≤ 100 pmol/paw) failed to induce hyperalgesia. Local treatment with BQ-123 (i.e., cyclo[DTrp-DAsp-Pro-DVal-Leu] 1 nmol/paw selective endothelin ET_A receptor antagonist), 10 min before endothelin-1 (10 pmol/paw), fully blocked the hyperalgesic response, whereas similar treatment with the selective endothelin ET_B receptor antagonist BQ-788 (i.e., *N-cis*-2,6-dimethylpiperidinocarbonyl-L-γ-methylleucyl-D-1-methoxycarboyl-D-norleucine) was ineffective. Intravenous injection of bosentan (17 and 52 μmol/kg a non-peptidic mixed endothelin ET_A/ET_B receptor antagonist) or BMS 182874 (i.e., 5-[dimethylamino]-*N*-[3,4-dimethyl-5-isoxazolyl]-1-naphthalenesulphonamide; 10 and 30 μmol/kg; a non-peptidic selective endothelin ET_A receptor antagonist), 1 h before endothelin-1, inhibited its hyperalgesic effect in a graded fashion and abolished the response at the higher doses. None of the antagonists modified nociception induced by capsaicin alone or the hyperalgesia induced by local injection of 5-hydroxytryptamine (5-HT; 2 nmol/paw, 30 min before capsaicin). Hyperalgesia induced by 5-HT was abolished by simultaneous injection of endothelin-1 or the endothelin ET_B receptor agonist IRL 1620 (each at 30 pmol/paw). Therefore, local endothelin-1 exerts a dual influence in this model: at low doses it causes endothelin ET_A receptor-mediated hyperalgesia (i.e., it potentiates capsaicin-induced nociception), whereas at higher doses it induces an anti-hyperalgesic effect against 5-HT which seems to be mediated via distinct endothelin ET (possibly ET_B) receptors.

Keywords: Endothelin; Endothelin receptor antagonist; Inflammation; Pain; Hyperalgesia (mouse)

1. Introduction

Hyperalgesia is a key feature of the inflammatory response whereby the pain elicited by noxious stimuli applied to the inflamed site is enhanced (for review, see Carstens, 1995). The complex mechanisms underlying hyperalgesia involve the concerted action of various mediators, including cytokines generated by resident cells and/or recruited leucocytes, on the peripheral branches of primary sensory neurons and centrally to enhance the sensitivity of pain-signalling neurons. Tumour necrosis factor-α (TNF-α), interleukin-1 and interleukin-6, which are important effectors of the hyperalgesia induced by Gram-negative bacterial lipopolysaccharide, carrageenan or bradykinin (Stein et al., 1988; Cunha et al., 1991; Ferreira, 1993; Ferreira et al., 1993; Fukuoka et al., 1994), can each trigger endothelin-1 release from various cultured cells and raise plasma levels of the peptide in vivo (for review, see Rae and Henriques, 1998).

Peptides of the endothelin family act through stimulation of at least two specific receptors: (a) the endothelin ET_A receptor, which displays higher affinity for endothelin-1 than for endothelin-3 and is selectively blocked by antagonists such as the peptide BQ-123 (cyclo[DTrp-DAsp-Pro-DVal-Leu]); and (b) the endothelin ET_B receptor, which shows equal affinity for both peptides, is selectively stimulated by sarafotoxin S6c and IRL 1620 (Suc[Glu^9, Ala^{11,15}]endothelin-1-(10–21)) and is blocked by antagonists such as the peptide BQ-788 (*N-cis*-2,6-dimethylpiperidinocarbonyl-L-γ-methylleucyl-D-1-methoxycarboyl-

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D-norleucine)(Arai et al., 1990; Sakurai et al., 1990; for reviews, see Masaki et al., 1994; Webb and Meek, 1997).

Endothelin-1 displays several pro-inflammatory properties, including enhancement of vascular permeability (Filep et al., 1995), leucocyte activation (Ishida et al., 1990) and recruitment via upregulation of endothelial adhesion molecule expression (McCarron et al., 1993) as well as cytokine release (Helset et al., 1993; Stankova et al., 1996), and mast cell degranulation (Yamamura et al., 1994). Endothelins may also have a role in inflammatory nociception, because endothelin-1 elicits nociceptive behavior when injected intra-articularly in dogs (articular incapacitation test) or intraperitoneally in mice (writhing test) (Ferreira et al., 1989; Raffa and Jacoby, 1991). Intradermal injections of endothelin-1 also promote hyperalgesia to mechanical stimuli in the rat paw or in the human forearm (Ferreira et al., 1989; Dahlof et al., 1990), and to a chemical stimulus (formalin) in the mouse paw (Piovezan et al., 1997). The only study conducted to date to identify, by use of various endothelin receptor agonists and a selective endothelin ET_A receptor antagonist, the specific receptors mediating the nociceptive effects of endothelins has suggested, at least in the mouse abdominal constriction (writhing) test, that such actions are mediated by activation of both endothelin ET_A and ET_B receptors (Raffa et al., 1996).

The aim of the present study is to assess the influence of endothelin-1 on nociceptive responses induced by capsaicin in the hindpaw of the mouse and to identify the endothelin receptors involved in these effects. This model of nociception was chosen because, unlike many well known chemical nociceptive stimuli (e.g., formalin, acetic acid and phenylbenzoquinone), capsaicin selectively activates primary sensory neurons (i.e., nociceptors) in the periphery by stimulation of specific vaniloid receptors (Holzer, 1991; Szallasi, 1995). Indeed, this capsaicin-sensitive receptor (named the vaniloid VR1) has been cloned and is a rapidly desensitizing, somewhat calcium-selective ionotropic receptor which appears to be expressed exclusively by small diameter nociceptive sensory neurons (Caterina et al., 1997).

2. Materials and methods

2.1. Animals

Male Swiss mice (25–30 g), from our own colony, were housed in a room with controlled temperature (22 ± 2°C) and lighting (lights on from 0600 to 1800 h), with free access to laboratory chow and tap water. All experiments were conducted between 1000 and 1700 h and were in accordance with the ethical guidelines of the International Association for the Study of Pain (Zimmermann, 1983).

2.2. Capsaicin-induced nociception

Experiments were conducted essentially as described by Sakurada et al. (1992). Briefly, conscious animals received a 20 μl intraplantar (i.pl.) injection of capsaicin (0.06 to 3.2 μg) into the right hindpaw. Control animals were similarly injected with 20 μl of vehicle only (12.5% dimethylsulfoxide (DMSO) in saline). Immediately after the injection, each animal was placed in a separate glass jar over a mirror (set at an angle of about 60° relative to the table to enable full view of the paws at all times), and the amount of time a mouse spent licking the injected hindpaw (in seconds) was recorded cumulatively over the first 5 min (unless stated otherwise), using a stopwatch chronometer. All animals were killed by cervical dislocation immediately after completion of the observation period.

To establish the dose-response relationship for capsaicin-induced nociception, we initially assessed the responses of different groups of mice given i.pl. injections of either 0.06, 0.1, 0.4, 1.6, 2.4 or 3.2 μg/paw of the compound. From these experiments, we selected the dose of 0.1 μg/paw of capsaicin for the rest of the study. In one set of experiments, each animal received a 20 μl i.pl. injection of either endothelin-1 (1 to 30 pmol/paw), sarafotoxin S6c (3 to 30 pmol/paw), IRL 1620 (3 to 100 pmol/paw), 5-hydroxytryptamine (5-HT; 0.3 to 3 nmol/paw) or vehicle (phosphate-buffered saline, PBS) 30 min before capsaicin was injected into the ipsilateral paw (unless stated otherwise). Other experiments were performed to evaluate, by using endothelin receptor antagonists, the contribution of endothelin ET_A and ET_B receptors to the influence of endothelin-1 on the nociceptive reactivity of mice to capsaicin. In these studies, some mice were pretreated in situ with either BQ-123 or BQ-788 (1 nmol/paw in 20 μl of PBS, i.pl.), selective peptidic antagonists of endothelin ET_A and ET_B receptors, respectively, 10 min prior to ipsilateral i.pl. injection of endothelin-1 (10 pmol/paw) or 5-hydroxytryptamine (5-HT; 2 nmol/paw). Other mice received, 1 h before the endothelin-1 (or 5-HT) injection, an intravenous injection (into the caudal vein; i.v.) of non-peptidic antagonists bosentan (17 or 52 μmol/kg) or BMS 182874 (10 or 30 μmol/kg), which block both endothelin ET_A and ET_B receptors or solely ET_A receptors, respectively. Control animals were similarly pretreated in situ or i.v. with the appropriate vehicle. A final set of experiments examined the influence of endothelin-1 or IRL 1620 (each at 30 pmol/paw) on hyperalgesia triggered by 5-HT (2 nmol/paw). In these experiments each peptide (or vehicle only) was injected together with 5-HT (co-injection), 30 min prior to an ipsilateral injection of capsaicin.

2.3. Vasoconstrictor effects of endothelin receptor agonists

Since endothelin-1 is a potent vasoconstrictor, we tested if this action could underlie its influence on capsaicin-in-

duced nociceptive responses, i.e., via pharmacokinetic alterations of local capsaicin disposal. To this effect, two experiments were performed. In the first one, mice were pretreated with either endothelin-1 (10 pmol/paw) or PBS (control) 30 min before injection of capsaicin, as before, but the time each animal spent licking the injected paw was recorded in 1-min periods for 10 min, rather than for the usual 5-min period described above. In the second experiment, after removal of dorsal hair by trichotomy followed by 3-min exposure of the surface to Nudit® cream (Helena Rubenstein, Rio de Janeiro, Brazil), mice received an i.v. injection of Evans blue dye (30 mg/kg, i.v.). One hour later, endothelin-1 (0.3 to 10 pmol/site), sarafotoxin S6c (10 or 30 pmol/site), IRL 1620 (10 or 30 pmol/site) or vehicle (PBS) was injected intradermally into the dorsum (4 sites per animal), and the greatest diameter of the contrasting halo formed at the border of each injection site (which, immediately following injection, is typically paler than the surrounding skin) was measured with a paquimeter every 15 min for 1 h.

2.4. Statistical analysis

In all experiments, the responses of drug-treated animals were always assessed in parallel to those of vehicle-treated mice, to minimize interference of possible fluctuations in responsiveness. Thus, the responses of each drug-treated group were compared to those of the corresponding day-matched control group. Results are presented as mean ± S.E.M. of either the absolute values of hindpaw licking time or of the differences between the responses elicited by capsaicin alone and those elicited by the compound following treatment with endothelin-1, sarafotoxin S6c, IRL 1620 or 5-HT (i.e., Δ licking time). Data were statistically evaluated by analysis of variance followed by Bonferroni's test or, when only two means were to be compared, unpaired Student's *t*-test. The significance level was set at $P < 0.05$.

2.5. Drugs

Stock solutions (0.1 to 1 mM) of endothelin-1, sarafotoxin S6c and IRL 1620 (all from American Peptide, Sunnyvale, USA), BQ-123 (Bachem, Torrance, USA), 5-HT hydrochloride and BQ-788 (both from Research Biochemicals International, Natick, USA) were prepared in PBS, whereas the stock solution of capsaicin (from Sigma, St. Louis, USA) was prepared in 12.5% dimethylsulfoxide in saline at a concentration of 240 μg/ml. All stock solutions were kept at −18°C as 50 to 100 μl aliquots and diluted to the desired concentration in the same vehicles just prior to use. Bosentan (kind gift from F. Hoffmann La-Roche, Basel, Switzerland) and BMS 182874 (5-[dimethylamino]-*N*-[3,4-dimethyl-5-isoxazolyl]-1-naphthalene-sulphonamide; kind gift from Bristol-Myers Squibb, Princeton, USA) were dissolved daily in heated (50–60°C) distilled water or 5% $NaHCO_3$, respectively.

3. Results

3.1. Characterization of capsaicin-induced nociception

Capsaicin injection evoked short-lasting dose-dependent nociceptive responses, which occurred largely within the first 5 min, whereas a similar injection of the vehicle (dimethylsulfoxide 12.5%) was without effect (Fig. 1A). Because the licking response induced by 0.1 μg/paw of capsaicin was submaximal, and to minimize the possible occurrence of non-specific actions of this agent (Holzer, 1991), this dose was selected for all other experiments.

3.2. Effects of endothelin receptor agonists and 5-HT on capsaicin-induced nociception

Intraplantar injection of endothelin-1 (10 pmol/paw) significantly potentiated nociception when given either

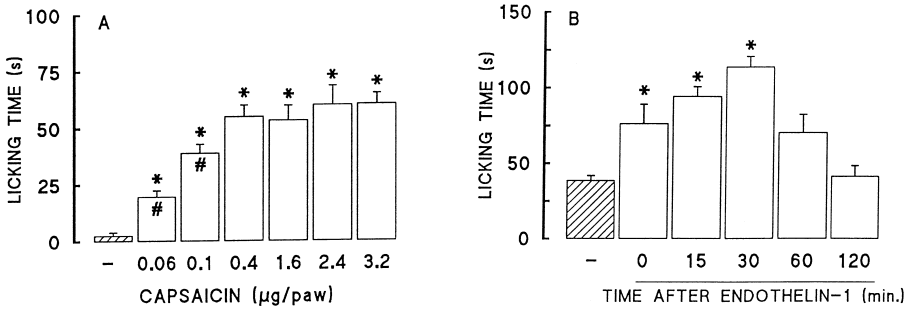

Fig. 1. Effects of i.pl. capsaicin injection on hindpaw licking time in the mouse. In Panel A, capsaicin (open bars) or vehicle (control; 20 μl of 12.5% DMSO; hatched bar) was injected at the doses indicated. In Panel B, capsaicin (0.1 μg/paw) was injected either alone (control; hatched bar) or at various time intervals following ipsilateral injection of endothelin-1 (ET-1, 10 pmol/paw; open bars). The value for time 'O' was obtained after simultaneous injection of endothelin-1 together with capsaicin. Each value represents the mean ± S.E.M. for 6 animals. Asterisks and fences (#) denote $P < 0.05$ when compared to the control value or response to 3.2 μg/paw of capsaicin, respectively (ANOVA followed by Bonferroni's test).

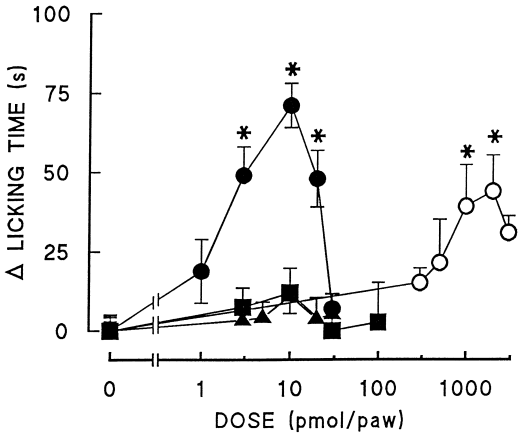

Fig. 2. Increase in capsaicin-induced (0.1 μg/paw) hindpaw licking time induced by ipsilateral injection of endothelin-1 (solid circles), sarafotoxin S6c (solid triangles), IRL 1620 (solid squares) or 5-hydroxytryptamine (open circles), at the doses indicated. Each agonist was injected intraplantarly 30 min prior to capsaicin. Results are presented as the difference between the responses of agonist-treated and parallel vehicle-treated (20 μl) control mice (i.e., Δ licking time), and represent the mean ± S.E.M. for 6–10 animals per dose. Asterisks denote $P < 0.05$ when compared to respective control value (ANOVA followed by Bonferroni's test).

together (co-injection) or 15 and 30 min before ipsilateral capsaicin administration (Fig. 1B). As this hyperalgesic effect was greatest at the later time point (194 ± 18% over basal responses to capsaicin alone), the 30-min interval was chosen to assess the dose-response relationships of the various agonists on capsaicin-induced nociception.

Given 30 min prior to capsaicin, endothelin-1 was found to cause dose-dependent potentiation of nociception (Fig. 2). The dose-response curve for this effect of endothelin-1 was bell-shaped, with the maximum response being attained with the dose of 10 pmol/paw, whereas a slightly higher dose (30 pmol/paw) failed to potentiate nociception altogether. In contrast, the selective endothelin ET_B receptor agonists sarafotoxin S6c and IRL 1620 did not significantly modify the responses to capsaicin, when injected under similar conditions (up to 30 and 100 pmol/paw, respectively; Fig. 2). Though less potent than endothelin-1, 5-HT at doses in excess of 500 pmol/paw also potentiated the nociceptive responses to capsaicin (Fig. 2).

No significant potentiation of capsaicin-induced nociception was detected when endothelin-1 (10 pmol/paw) was injected into the contralateral paw 30 min prior to the noxious stimulus (licking times: contralateral endothelin-1 53 ± 11 s, contralateral vehicle 47 ± 4 s; $n = 6$ in each group; $P > 0.05$).

3.3. Influence of endothelin receptor antagonists on hyperalgesia induced by endothelin-1

In situ pretreatment with the selective peptidic endothelin ET_A receptor antagonist BQ-123 (1 nmol/paw) abolished the potentiation of capsaicin-induced nociception caused by endothelin-1 (10 pmol/paw) (Fig. 3A). Similar pretreatment with BQ-788 (1 nmol/paw), a selective endothelin ET_B receptor antagonist, did not modify the effect of endothelin-1 (Fig. 3B). The potentiation of capsaicin-induced nociception by endothelin-1 was also inhibited, in a graded fashion, by prior i.v. administration of the non-peptidic selective endothelin ET_A receptor antagonist BMS 182874 (10 and 30 μmol/kg; Fig. 4A) or by the non-peptidic mixed endothelin ET_A/ET_B receptor antagonist bosentan (17 and 52 μmol/kg; Fig. 4B).

At the highest doses used, none of the peptidic or non-peptidic antagonists affected the potentiation of capsaicin-induced nociception by 5-HT (2 nmol/paw) (Figs. 3 and 4) or the nociceptive responses triggered by capsaicin alone ($P > 0.05$; $n = 6$ in each group; results not shown).

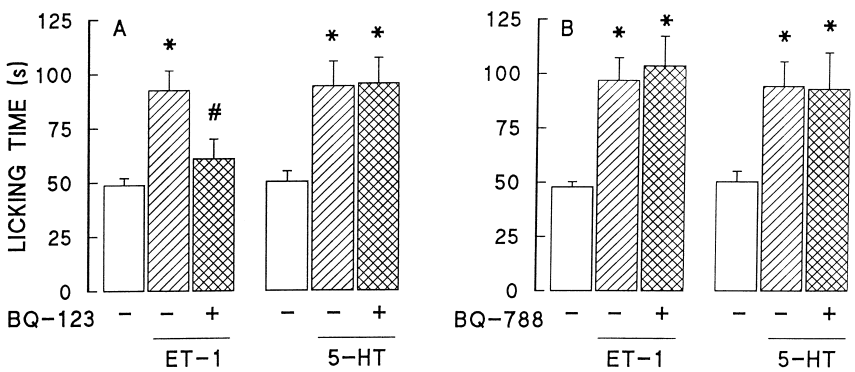

Fig. 3. Influence of local treatment with the endothelin ET_A receptor antagonist BQ-123 (1 nmol/paw; Panel A) or the endothelin ET_B receptor antagonist BQ-788 (1 nmol/paw; Panel B) on the potentiation of capsaicin-induced (0.1 μg/paw) nociception by endothelin-1 (ET-1; 10 pmol/paw) or 5-hydroxytryptamine (5-HT; 2 nmol/paw) in the mouse hindpaw. These peptidic antagonists were injected 10 and 30 min prior to agonist and capsaicin injections, respectively (cross-hatched bars). Each value represents the mean ± S.E.M. of 6–8 observations. Asterisks and fences (#) indicate $P < 0.05$ when compared to value of mice treated with capsaicin alone (open bars) or with capsaicin plus endothelin-1 or 5-HT (hatched bars in both cases), respectively (ANOVA followed by Bonferroni's test).

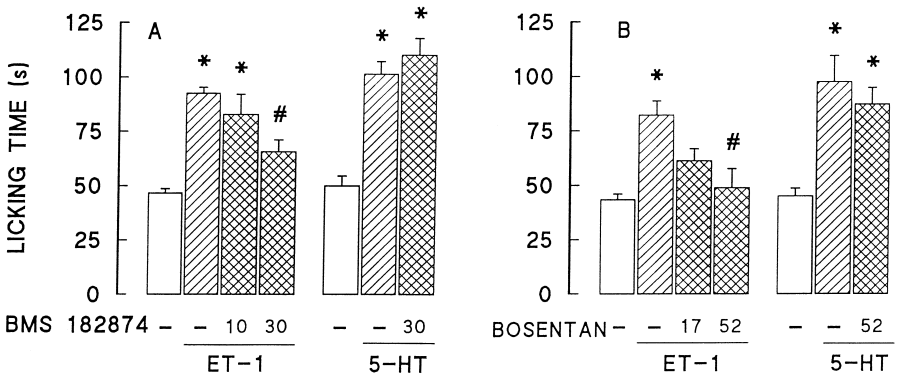

Fig. 4. Influence of systemic treatment with the endothelin ET_A receptor antagonist BMS 182874 (10 or 30 μmol/kg; Panel A) or the mixed endothelin ET_A/ET_B receptor antagonist bosentan (17 or 52 μmol/kg; Panel B) on the potentiation of capsaicin-induced (0.1 μg/paw) nociception by endothelin-1 (ET-1; 10 pmol/paw) or 5-hydroxytryptamine (5-HT; 2 nmol/paw) in the mouse hindpaw. These non-peptidic antagonists were injected i.v. 60 and 90 min prior to agonist and capsaicin injections, respectively (cross-hatched bars). Each value represents the mean ± S.E.M. of 6 observations. Asterisks and fences (#) indicate $P < 0.05$ when compared to the value for mice treated with capsaicin alone (open bars) or with capsaicin plus endothelin-1 or 5-HT (hatched bars in both cases), respectively (ANOVA followed by Bonferroni's test).

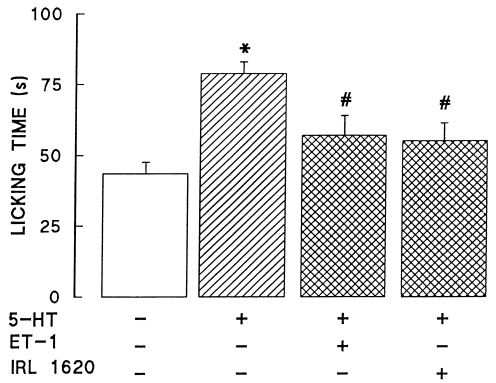

Fig. 5. Influence of endothelin-1 (ET-1; 30 pmol/paw) or IRL 1620 (30 pmol/paw) on the potentiation of capsaicin-induced (0.1 μg/paw) nociception by 5-hydroxytryptamine (5-HT; 2 nmol/paw) in the mouse hindpaw. Endothelin-1 or IRL 1620 was injected together with 5-HT 30 min prior to capsaicin injection (cross-hatched bars). Each value represents the mean ± S.E.M. of 6–10 observations. Asterisks and fences (#) indicate $P < 0.05$ when compared to the value for mice treated with capsaicin alone (open bar) or with capsaicin plus 5-HT (hatched bar), respectively (ANOVA followed by Bonferroni's test).

3.4. Influence of endothelin-1 and IRL 1620 on 5-HT-induced hyperalgesia

When given together with 5-HT (2 nmol/paw) 30 min prior to capsaicin injection, endothelin-1 (30 pmol/paw) and IRL 1620 (30 pmol/paw) were each found to fully block the hyperalgesic response to the amine. The results illustrating the anti-hyperalgesic effects of endothelin-1 and IRL 1620 are displayed in Fig. 5.

3.5. Vasoconstrictor effects of endothelin receptor agonists

When licking responses to capsaicin were recorded in 1 min periods for 10 min, rather than simply for a 5-min period (the usual procedure), the time course of the licking responses was found to be slightly faster in mice pretreated with PBS than with endothelin-1. Accumulated licking times measured at 1, 2, 3, 4, and 5 min (expressed as

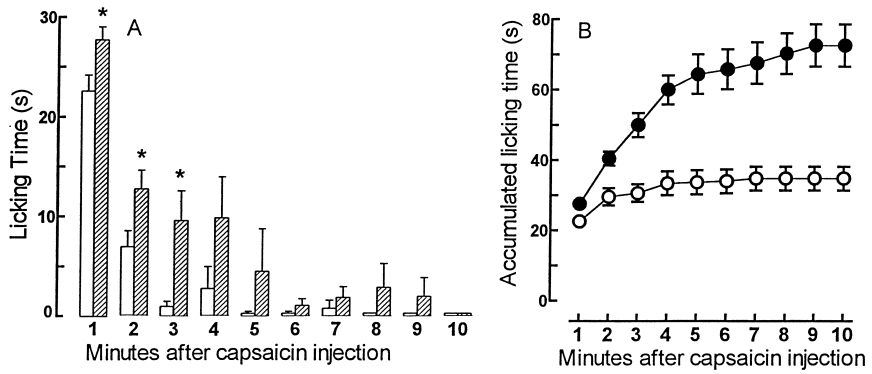

Fig. 6. Influence of endothelin-1 on the time course of capsaicin-induced hindpaw licking time. Mice were treated with either endothelin-1 (10 pmol/paw, hatched bars or closed circles) or vehicle (open bars and open circles) 30 min prior to ipsilateral injection of capsaicin (0.1 μg/paw). Non-cumulative licking times (in seconds) displayed as 10 consecutive 1 min periods following capsaicin injection (Panel A) or accumulated licking times recorded throughout the same session (Panel B). Results represent the mean ± S.E.M. for 12 animals. Asterisks in Panel A denote $P < 0.05$ when compared to respective control (vehicle-treated) value (ANOVA followed Student's *t*-test). All values for endothelin-1-treated mice in Panel B differ from corresponding control values ($P < 0.05$).

percentages of the total licking time displayed by each animal) were 69.2 ± 5.5, 87.9 ± 4.5, 90.5 ± 4.2, 96.2 ± 2.2, and 96.9 ± 2.2% in PBS-pretreated mice (total licking time 34.8 ± 3.4 s in 10 min), and 41.5 ± 4.0, 60.5 ± 6.0, 73.9 ± 6.7, 85.5 ± 4.9, and 90.3 ± 4.6% in endothelin-1-pretreated mice ($P < 0.05$ for first 3 values only, Student's *t*-test; total licking time 72.6 ± 6.0 s) ($n = 12$ in each group). Ten out of twelve PBS-pretreated mice had displayed their full responses to capsaicin within 5 min of injection, whereas 4 out of 12 endothelin-1-pretreated mice still responded slightly to the compound up to 8–9 min after injection (Fig. 6A). As also shown in Fig. 6, the actual licking times displayed by the endothelin-1-pretreated group in each period were significantly higher than those of the controls throughout the first 3 min following capsaicin, whereas the accumulated licking times measured in the former group were increased in all periods ($P < 0.05$).

Intradermal injection of PBS solution into the mouse dorsum induced a short-lasting halo which was minimal at 30 min (Table 1) and completely absent by 45 min (results not shown). In marked contrast, much wider halos were observed following similar injections of endothelin-1. At 30 min, this vasoconstrictor effect was clearly dose-dependent, reaching a maximum at 3 pmol/site whereafter it did not change further (Table 1). Moreover, although the large halos induced by 1, 3 or 10 pmol/site of endothelin-1 remained stable throughout the full length of the 1 h observation period, the effect caused by 30 pmol/site (which was similar at 15 and 30 min) diminished significantly and progressively at the 45- and 60-min timepoints (results not shown; $n = 11$; $P < 0.05$). Importantly, sarafotoxin S6c and IRL 1620 also displayed significant vasoconstrictor effects at 30 pmol/site (Table 1).

Table 1
Vasoconstrictor effects of endothelin receptor agonists in mouse dorsal skin

Agonist	Dose (pmol/site)	Halo diameter at 30 min (in mm)
Vehicle (control)	–	0.4 ± 0.3
Endothelin-1	0.3	3.6 ± 0.4[a]
	1.0	4.3 ± 0.4[a]
	3.0	5.3 ± 0.5[a]
	10.0	5.1 ± 0.2[a]
	30.0	5.4 ± 0.1[a]
Sarafotoxin S6c	10.0	0.4 ± 0.4
	30.0	3.3 ± 0.8[a]
IRL 1620	10.0	0.9 ± 0.6
	30.0	3.0 ± 0.7[a]

Vehicle (PBS solution) or agonists were injected intradermally 1 h after administration of Evans blue dye (30 mg/kg, i.v.). Values refer to the largest diameter (in mm) of the halo formed at the border of the paler injection site and the bluer surrounding skin 30 min after agonist injection and represent the mean ± S.E.M of 9–12 observations.
[a] Different from control. ANOVA followed by Bonferroni's test ($P < 0.05$).

4. Discussion

The present study clearly illustrates the peripheral hyperalgesic properties of endothelin-1 in the model of capsaicin-induced nociception in the mouse hindpaw. This effect is produced by low doses of the peptide (in the pmol range), peaks within 30 min of local administration, and appears to depend on a local action, rather than a systemic one, because endothelin-1 failed to enhance capsaicin-induced nociception when injected into the contralateral hindpaw.

Several lines of evidence collected in the current study indicate that the hyperalgesic effects of endothelin-1 on capsaicin-induced nociception are mediated via stimulation of endothelin ET_A receptors. Firstly, under the present conditions, two selective endothelin ET_B receptor agonists, namely sarafotoxin S6c and IRL 1620 (Masaki et al., 1994), failed to cause hyperalgesia at doses up to 30 or 100 pmol/paw, respectively. Secondly, prior local injection of the selective endothelin ET_A receptor antagonist BQ-123 (Masaki et al., 1994) fully inhibited the development of endothelin-1-induced hyperalgesia, whereas similar treatment with the selective endothelin ET_B receptor antagonist BQ-788 (Masaki et al., 1994) did not affect it. The efficacy of the sample of BQ-788 used was confirmed by its ability to block sarafotoxin S6c-induced contractions of the guinea pig isolated gall bladder (results not shown). Thirdly, systemic treatment with the non-peptidic selective endothelin ET_A receptor antagonist BMS 182874 (Stein et al., 1994) also prevented the development of endothelin-1-induced hyperalgesia, an action mimicked by similar treatment with bosentan, a mixed endothelin ET_A/ET_B receptor antagonist (Clozel et al., 1994). It is also important to mention that the anti-hyperalgesic actions of the endothelin receptor antagonists were specific against endothelin-1, since neither of them, nor BQ-788, modified the hyperalgesia elicited by 5-HT, which is most likely mediated via stimulation of $5\text{-}HT_{2A}$ receptors (Abbott et al., 1996).

Although peripheral hyperalgesic effects of endothelin-1 have already been reported, using different models such as the paw pressure test in the rat (Ferreira et al., 1989) or formalin-induced nociception in the mouse (Piovezan et al., 1997), this appears to be the first attempt to characterize the receptors involved in such an action by using selective receptor antagonists. Our finding that solely endothelin ET_A receptors mediate endothelin-1-induced hyperalgesia to a chemical stimulus in the mouse hindpaw differs from the report that the nociceptive effects of endothelins (and sarafotoxin S6c) in the abdominal constriction (writhing) test in this species are mediated by both endothelin ET_A and ET_B receptors (Raffa et al., 1996). The difference is instructive because nociception and hyperalgesia are distinct processes which could, conceivably, involve activation of distinct receptors and mechanisms, and nociceptive fibers (or neighbouring cells) present in distinct regions may well display different endothe-

lin receptors. The finding that the selective endothelin ET_B receptor agonists sarafotoxin S6c and IRL 1620 did not potentiate capsaicin-induced nociception agrees well with our report that sarafotoxin S6c failed to increase the first phase of nociception triggered by formalin in the mouse hindpaw (Piovezan et al., 1997), a response which, like that induced by capsaicin, is believed to represent neurogenic non-inflammatory pain (Hunskaar and Hole, 1987; Shibata et al., 1989).

Surprisingly, the range of endothelin-1 doses capable of eliciting hyperalgesic effects was quite narrow. The bell-shaped dose-response curve for endothelin-1 was so sharp that 30 pmol/paw of the peptide, a dose only 3-fold higher than that causing maximal hyperalgesia, did not influence capsaicin-induced nociception at all. In fact, at this higher dose (30 pmol/paw), endothelin-1 and IRL 1620 each significantly blocked the development of hyperalgesia triggered by 5-HT, but did not inhibit nociceptive responses to capsaicin alone. These preliminary results suggest that endothelin-1 can also trigger endothelin ET_B receptor-mediated anti-hyperalgesic (not analgesic) mechanisms in the mouse hindpaw, thus masking its hyperalgesic action on endothelin ET_A receptors, at higher doses. It will be interesting to confirm this view in the future in experiments with endothelin receptor antagonists.

Several factors could contribute to endothelin-1-induced hyperalgesia, including, among others, stimulation of prostanoid synthesis (for review, see Hyslop and De Nucci, 1992), cytokine release (Klemm et al., 1995), mast cell degranulation (Yamamura et al., 1994) and vasoconstriction (for review, see Webb, 1997). Only the latter possibility was addressed in the present study. It appears unlikely that the vasoconstriction induced by endothelin-1 may have markedly influenced the local disposal (washout) of capsaicin, because the peptide caused only a marginal delay (only in the first 3 min) in the time-course of the full nociceptive reaction to capsaicin, and actually significantly increased licking responses to capsaicin immediately after injection. Furthermore, like endothelin-1, the selective endothelin ET_B receptor agonists sarafotoxin S6c and IRL 1620 also displayed (albeit smaller) vasoconstrictor properties in the mouse dorsal skin. Also, doses of endothelin-1 which caused significant vasoconstriction (e.g., 0.3 and 1 pmol) did not cause hyperalgesia in the hindpaw, and 30 pmol (which did not cause hyperalgesia in the hindpaw) induced a vasoconstriction similar to that seen following 3 or 10 pmol. Therefore, the hyperalgesic effects of endothelin-1 cannot be ascribed merely to its vasoconstrictor effects, unless tissue blood flow needs to be reduced to a certain critical level. If this is the case, the hypoxia resulting from severe endothelin-1-induced vasoconstriction could lower local pH, which is known to sensitize nociceptors to capsaicin (Caterina et al., 1997). Though this possibility cannot be excluded on the basis of the present findings, other more direct actions of endothelin-1 on nociceptors can be envisaged, since this peptide depolarizes primary sensory neurons in vitro (Yoshizawa et al., 1989) and markedly potentiates the capsaicin-induced release of substance P and calcitonin gene-related peptide from cultured primary sensory neurons, while causing only minimal effects on neuropeptide release when added alone (Dymshitz and Vasko, 1994).

Concerning the putative anti-hyperalgesic mechanism of endothelin-1, it is tempting to speculate that the peptide may diffuse through the blood vessel walls and activate endothelin ET_B receptors on the endothelium to release of nitric oxide (Hyslop and De Nucci, 1992). This labile mediator has been suggested to mediate the peripheral anti-hyperalgesic effects of dipyrone, diclofenac and opioids (Duarte et al., 1992; Tonussi and Ferreira, 1994; Ferreira et al., 1995). The mechanisms underlying both the hyperalgesic and anti-hyperalgesic effects of endothelin-1 in the model of capsaicin-induced nociception remain to be fully determined.

In conclusion, we have shown that endothelin-1 can modulate the responsiveness of primary sensory neurons of the mouse hindpaw to capsaicin by activating either endothelin ET_A receptors to elicit hyperalgesia or distinct ET (possibly ET_B) receptors to cause an anti-hyperalgesic effect. As several inflammatory stimuli, such as carrageenan, lipopolysaccharide and various cytokines can trigger hyperalgesia (see Section 1 for references) as well as endothelin release (Bertelli et al., 1992; Sugiura et al., 1989; Vemulapalli et al., 1994; Klemm et al., 1995), endogenous endothelins may contribute significantly to the production of hyperalgesic states. The present finding that systemic administration of non-peptidic endothelin receptor antagonists (BMS 182874 and bosentan) can block endothelin-1-induced hyperalgesia suggests that such agents may constitute a new viable therapeutic strategy for the treatment of inflammatory pain.

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