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Endothelin ET_B receptors inhibit articular nociception and priming induced by carrageenan in the rat knee-joint

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Abstract

The participation of the endothelin system on nociception and priming induced by carrageenan in the knee-joint was investigated. Intra-articular (i.a.) carrageenan (300 μg) caused long-lasting nociceptive effects (i.e., increases in paw elevation time [PET]), which were potentiated by endothelin-1 (dual endothelin ET_A/ET_B receptor agonist) and inhibited by sarafotoxin S6c (endothelin ET_B receptor agonist; both at 30 pmol, i.a., 24 h beforehand). Priming the naive joint with carrageenan augmented nociceptive responses to a second carrageenan challenge, 72 h later. Carrageenan-induced priming, but not nociception, was potentiated by local BQ-788 (10 nmol, i.a., 15 min before priming; endothelin ET_B receptor antagonist; *N-cis*-2,6-dimethylpiperidinocarbonyl-L-γ-methylleucyl-D-1-methoxycarbonyl-tryptophanil-D-norleucine), but BQ-123 (endothelin ET_A receptor antagonist; cyclo [D-Asp-Pro-D-Val-Leu]) was ineffective. Sarafotoxin S6c markedly suppressed carrageenan-induced priming to nociception triggered by carrageenan, endothelin-1 or sarafotoxin S6c, and BQ-788 prevented this action. Thus, selective endothelin ET_B receptor agonists inhibit carrageenan-induced nociception and priming in the naive joint. This priming effect of carrageenan to nociception evoked by subsequent inflammatory insults is limited by an endothelin ET_B receptor-operated mechanism.

Keywords: Articular incapacitation; Hyperalgesia; Analgesia; Arthritic pain; IRL 1620; Endothelin receptor

1. Introduction

It is now firmly established that endothelins, a family of 21-amino-acid residue peptides produced by many cell types, can exert multiple and important actions in many tissues and systems, including those implicated in nociceptive signalling functions (for reviews, see Goto et al., 1996; Rae and Henriques, 1998; Kedzierski and Yanagisawa, 2001). The potent and widespread actions of endothelin-1 and other isopeptides of the family (endothelin-2 and endothelin-3) are mediated by specific G protein-coupled endothelin ET_A and ET_B receptors (for a review, see Davenport, 2002). The endothelin ET_A receptor shows higher affinity for endothelin-1 and endothelin-2 than for endothelin-3 and is blocked by several selective antagonists such as BQ-123 (cyclo [D-Asp-Pro-D-Val-Leu]). The endothelin ET_B receptor displays equal affinities to all three isopeptides, can be selectively activated by agonists, such as sarafotoxin S6c and IRL 1620 {Suc-[Glu^9,$Ala^{11,15}$]-endothelin-1 (8–21)}, or blocked by antagonists such as BQ-788 (*N-cis*-2,6-dimethylpiperidinocarbonyl-L-γ-methylleucyl-D-1-methoxycarbonyl-tryptophanil-D-norleucine).

Humans report deep burning pain and tenderness sensations following endothelin-1 injection into the forearm (Ferreira et al., 1989; Dahlof et al., 1990). In rats and mice, the peptide elicits endothelin ET_A receptor-mediated hind-paw licking/flinching responses, allied to hyperalgesia (i.e., enhanced nociceptive responsiveness) to noxious chemical, mechanical and/or thermal stimuli when administered into the footpad (Piovezan et al., 1997, 1998, 2000; Gokin et al., 2001; Menéndez et al., 2003). Endothelin ET_B receptors in the rodent hind paw normally appear to display antihyperalgesic or antinociceptive functions (Piovezan et al., 1998,

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2000; Khodorova et al., 2002, 2003), yet, they can clearly contribute, alongside endothelin ET_A receptors, to nociception induced by antigen challenge (Piovezan et al., 2004) and mechanical hyperalgesia induced by carrageenan or complete Freund's adjuvanct (Baamonde et al., 2004). Likewise, both receptor types mediate abdominal writhes induced by endothelin-1 (Raffa et al., 1996), but similar responses caused by phenylbenzoquinone are virtually abolished by endothelin ET_B (but not ET_A) receptor antagonists (Griswold et al., 1999).

Endothelin-1 also causes articular nociception as well as hyperalgesia to prostaglandin E_2 in dogs (Ferreira et al., 1989) and to carrageenan in rats (De-Melo et al., 1998b), when injected into a naive knee-joint. In rats, this long-lasting nociceptive effect of endothelin-1 in the naive knee-joint is sensitive to inhibition by local endothelin ET_A receptor blockade and is not reproduced by the selective ET_B receptor agonist sarafotoxin S6c (De-Melo et al., 1998a). However, prior peripheral sensitisation of the joint with carrageenan (i.e., priming) markedly augments the nociceptive potency of endothelin-1 and confers similar activity to sarafotoxin S6c. In addition, nociceptive responses induced by endothelin-1 in the carrageenan-primed joint can be blocked substantially by local injection of selective antagonists to either endothelin ET_A or ET_B receptors. The current study attempts to gain further insight into the roles of endothelins in mechanisms of articular nociception and priming, by examining the contributions of endothelin ET_A and ET_B receptor-operated mechanisms in the acute nociceptive and long-lasting priming effects of carrageenan in the rat knee-joint.

2. Materials and methods

2.1. Animals

Experiments were performed on male Wistar rats (150–180 g) housed under controlled ambient temperature (22 ± 2 °C) and light/dark cycle (lights on between 07:00 and 19:00 h) with free access to food chow and tap water. The experimental procedures and protocols were approved by the committee on ethical use of laboratory animals of the Universidade Federal de Santa Catarina, and are in accordance with the ethical guidelines for the use of experimental animals of the European Community and International Association for the Study of Pain (Zimmermann, 1983) and with Brazilian national legislation. Each animal was used only once and was killed by CO_2 overdose immediately after completion of the experiment.

2.2. Algesimetric test

The rat knee-joint incapacitation test was conducted as described in detail elsewhere (Tonussi and Ferreira, 1992; De-Melo et al., 1998a). In this test, rats are placed on a revolving cylinder (30 cm diameter; 3 rpm) for 1-min periods and a computer-assisted device measures the time that a specific hind paw fails to touch its metallic surface (i.e., paw elevation time [PET]). Normally, control animals display a PET of approximately 10 s, whereas procedures which cause nociception in the knee-joint increase this value only in the affected limb.

2.3. Experimental procedures

To minimise variations in PET estimations, all naive animals were introduced to the experimental environment and trained on the apparatus on the day preceding the start of the experiments. On the day of the experiment proper, PET values were assessed prior to treatment (basal values) and then again, repeatedly, at 1-h intervals up to 8 h after intra-articular (i.a.) injection of carrageenan (300 μg) or vehicle (40 μl of sterile phosphate-buffered solution [PBS]). Animals were returned to their home cages in between measurements. All treatments were given to gently-restrained conscious animals via intra-articular injections into the same (right) knee joint, using a 27-gauge needle. These injections did not elicit signs of major distress, such as audible vocalisations or attempts to bite the experimenter.

2.3.1. Influence of endothelin-1, sarafotoxin S6c and IRL 1620 on carrageenan-induced nociception

Twenty-four hours before injecting carrageenan (300 μg, i.a., or PBS), the rats received an i.a. injection of either endothelin-1 (dual endothelin ET_A/ET_B receptor agonist; 15, 30 or 60 pmol), the selective endothelin ET_B receptor agonists sarafotoxin S6c (15, 30 or 60 pmol) and IRL 1620 (0.6, 1.2 or 2.4 nmol) or PBS. In these experiments, some animals also received BQ-123 or BQ-788 (each at 10 nmol; or PBS) 15 min before the injection of endothelin-1 or sarafotoxin S6c (each at 30 pmol). Articular nociception was assessed in all groups over the first 8 h following carrageenan injection.

2.3.2. Influence of BQ-123 and BQ-788 on carrageenan-induced nociception and priming

Rats initially received an i.a. injection of BQ-123, BQ-788 (selective antagonists of endothelin ET_A and ET_B receptors, respectively; each at 10 nmol) or vehicle (40 μl of l of sterile PBS), 15 min prior to an ipsilateral injection of either carrageenan (300 μg) or PBS. These same animals also received a second injection of carrageenan, given 72 h after the first, to assess the influence of the antagonists on the priming (i.e., sensitising) effects of carrageenan to articular nociception induced by this same algogen. In these experiments, PET was evaluated on two occasions: (1) following the first carrageenan injection, to evaluate articular nociception elicited in naive joints; and (2) after the second challenge with carrageenan, to test the influence of the treatments on priming.

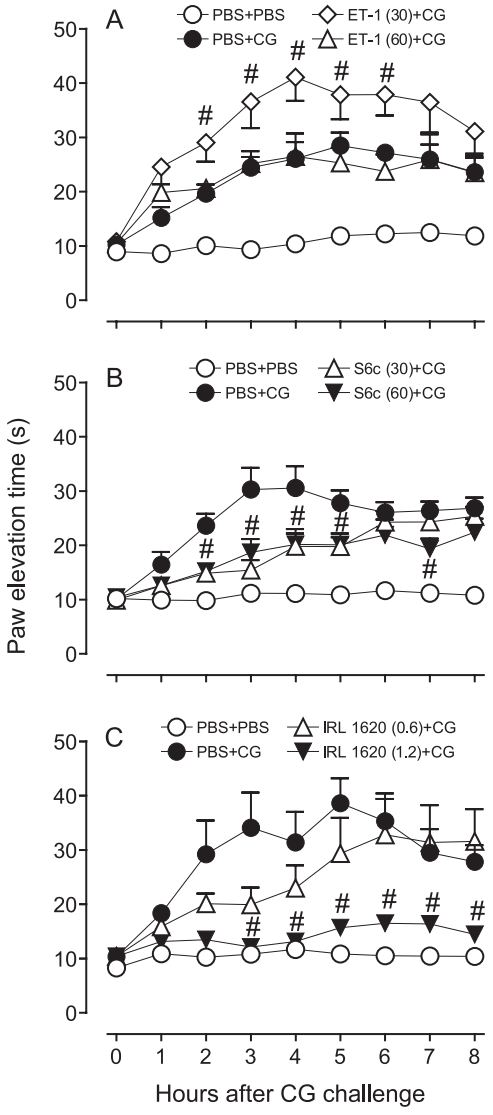

Fig. 1. Effects of endothelin-1 (dual endothelin ET_A/ET_B receptor agonist), sarafotoxin S6c and IRL 1620 (selective endothelin ET_B receptor agonists) on nociceptive responses induced by carrageenan (CG) challenge in the rat naive knee-joint. CG (300 μg, i.a.) was injected 24 h after i.a. treatment with endothelin-1 (ET-1; 30 or 60 pmol; panel A), sarafotoxin S6c (S6c; 30 or 60 pmol; panel B) or IRL 1620 (0.6 and 1.2 nmol; panel C). Nociceptive responses (paw elevation times) were recorded over 1-min periods immediately before (time 0) and then each hour up to 8 h after CG challenge. Values are means ± S.E.M. of six experiments. Fences denote $P<0.05$ when compared to the corresponding value of the PBS-treated/CG-challenged group (ANOVA followed by Bonferroni's test). Indications of the highly significant differences between PBS-treated groups challenged with either CG or PBS were omitted for sake of clarity.

2.3.3. Influence of sarafotoxin S6c on carrageenan-induced priming to subsequent algogen challenges

To determine the influence of sarafotoxin S6c on the priming effect of carrageenan, rats were treated with this selective endothelin ET_B receptor agonist (at 30 pmol), either alone or following 10 nmol BQ-788 (or PBS), given i.a. 15 min beforehand. The same joint was challenged 24 h later with carrageenan. Seventy-two hours after this first (priming) carrageenan injection, the same joint was challenged once again, either with carrageenan (300 μg), endothelin-1, sarafotoxin S6c (each at 30 pmol) or PBS, and PET values were estimated as before over the next 8 h.

2.4. Statistical analysis

Results are expressed either as the absolute PET values at the various time points, or as the areas under the time-course PET curves between 0 and 8 h after algogen injection (AUC 0–8 h; in arbitrary units). In either case, values are shown as mean ± S.E.M. of six to eight animals. In the figures in which the error bars of some values do not appear, they are smaller than the symbols. To calculate net percentages of inhibition or potentiation of nociceptive responsiveness induced by drug treatments or conditions, the mean AUC 0–8 h value(s) of the responses recorded in the corresponding PBS-treated control group(s) was subtracted from those displayed by the groups being compared. Statistical analyses were carried out using INSTAT Graphpad®. Comparisons between groups were made using

Table 1
Effects of endothelin-1, sarafotoxin S6c or IRL 1620 on nociceptive responses induced by carrageenan (300 μg, i.a.) challenge in the rat naive knee-joint

Treatment		Challenge	AUC[a] (0–8 h)	% Net change[b]
Agonist	pmol			
PBS	–	PBS	85 ± 4	–
		Carrageenan	184 ± 8	–
Endothelin-1	15	PBS	80 ± 5	–
		Carrageenan	254 ± 12	+70
	30	PBS	78 ± 3	–
		Carrageenan	264 ± 28[c]	+80
	60	PBS	83 ± 3	–
		Carrageenan	184 ± 14	+0
PBS	–	PBS	86 ± 6	–
		Carrageenan	199 ± 12	–
Sarafotoxin S6c	15	PBS	82 ± 3	–
		Carrageenan	164 ± 5	−35
	30	PBS	86 ± 4	–
		Carrageenan	149 ± 9[c]	−45
	60	PBS	81 ± 4	–
		Carrageenan	144 ± 9[c]	−49
PBS	–	PBS	85 ± 2	–
		Carrageenan	236 ± 32	–
IRL 1620	600	PBS	86 ± 4	–
		Carrageenan	193 ± 29[c]	−40
	1200	PBS	83 ± 4	–
		Carrageenan	113 ± 6[c]	−85
	2400	PBS	79 ± 5	–
		Carrageenan	138 ± 8[c]	−68

The treatment was given i.a. 24 h before the challenge.

[a] Mean ± S.E.M. values of AUC 0–8 h calculated (in arbitrary units) from the variations in paw elevation time values measured consecutively each hour up to 8 h after challenge (six experiments in each group).

[b] Percent change in AUC 0–8 h relative to corresponding group treated with PBS and challenged with carrageenan, after subtracting the AUC 0–8 h of the group treated and challenged with PBS from both groups.

[c] $P<0.05$ when compared to corresponding group treated with PBS and subjected to same challenge (ANOVA followed by Bonferroni's test).

analysis of variance (ANOVA) followed by the Bonferroni test, or two-tailed paired Student's *t* test, whenever appropriate. The level of significance was set at $P < 0.05$.

2.5. Drugs

The study was conducted using: lambda carrageenan (Marine Colloids, USA), endothelin-1, sarafotoxin S6c, IRL 1620 and BQ-123 (all from American Peptide, Sunnyvale, CA, USA) and BQ-788 (Bachem, Torrance, CA, USA). Stock solutions (10–100 μM in sterile PBS) of endothelin-1, sarafotoxin S6c, BQ-123 and BQ-788 were stored at − 18 °C and diluted to the desired concentration in PBS just before use. Carrageenan was prepared daily in PBS. The doses of carrageenan, endothelin-1, sarafotoxin S6c, BQ-123 and BQ-788 employed in all the experiments were chosen on the basis of previous studies conducted in this same model of articular nociception in the rat (Tonussi and Ferreira, 1992; De-Melo et al., 1998a,b), whereas those of IRL-1620 were selected considering that it is a less potent (albeit selective) agonist of endothelin ET_B receptors than endothelin-1 (Takai et al., 1992).

3. Results

3.1. Influence of endothelin-1, sarafotoxin S6c and IRL 1620 on carrageenan-induced nociception in the naive joint

Endothelin-1, sarafotoxin S6c (each at 15, 30 or 60 pmol) or IRL 1620 (0.6, 1.2 or 2.4 nmol), injected i.a. into a naive joint, did not modify per se PET values up to 8 h after treatment (data not shown). However, the nociceptive responses evoked by carrageenan 24 h later were differentially affected by treatment with these endothelin receptor agonists. Endothelin-1, at 15 or 30 (but not 60) pmol, significantly potentiated the nociceptive effect of carrageenan, enhancing the PET values recorded 2–6 h after challenge (Fig. 1A shows values for 30 and 60 pmol only) as well as the AUC 0–8 h (with 15 and 30 pmol causing net potentiations of AUC 0–8 h of 70% and 80%, respectively; Table 1). Conversely, both the selective endothelin ET_B receptor agonists diminished nociceptive responses evoked by carrageenan. These inhibitory actions only achieved significance following the two highest doses of both sarafotoxin S6c and IRL 1620.

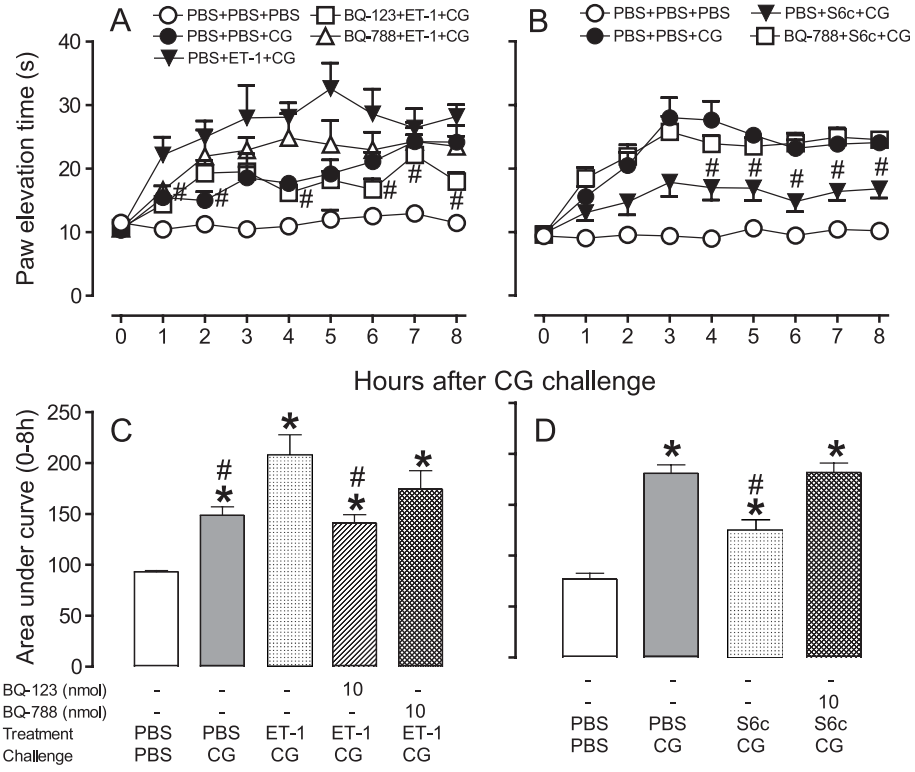

Fig. 2. Influence of BQ-123 or BQ-788 (selective endothelin ET_A and ET_B receptor antagonists, respectively) on changes of nociceptive responses to carrageenan (CG) challenge in the rat knee-joint induced by endothelin-1 or sarafotoxin S6c. Endothelin-1 (ET-1; 30 pmol; panels A and C), sarafotoxin S6c (S6c; 30 pmol; panels B and D) or PBS was injected 15 min after treatment with BQ-123, BQ-788 (each at 10 nmol, i.a.) or PBS, and the joint was challenged with CG (300 μg, i.a.) 24 h later. Nociceptive responses (paw elevation times) were recorded over 1-min periods immediately before (time 0) and then each hour up to 8 h after CG challenge (panels A and B). Panels C and D display the areas under each of the curves depicted in A and B (calculated in arbitrary units), respectively. Values are means ± S.E.M. of six to eight experiments. Significant differences ($P < 0.05$; ANOVA followed by Bonferroni's test) from corresponding values of the PBS-challenged group (treated only with PBS) are denoted by asterisks (in C and D only, for sake of clarity), whereas those that differ from the agonist-treated (antagonist-free) group challenged with CG are shown by fences.

To ascertain which endothelin receptor types signalled the potentiation and inhibition of carrageenan-induced nociception afforded by endothelin-1 and sarafotoxin S6c, respectively, we tested the influences of BQ-123 and BQ-788 (each at 10 nmol). The selective endothelin ET_A receptor antagonist BQ-123, given 15 min before endothelin-1 (30 pmol), fully prevented the augmentation of carrageenan-induced nociceptive PET responses caused by the agonist (Fig. 2A and C). However, similar treatment with the selective ET_B receptor antagonist BQ-788 failed to modify this effect of endothelin-1 significantly. On the other hand, the inhibition of carrageenan-induced nociceptive effects by sarafotoxin S6c (30 pmol) was abolished by prior BQ-788 treatment (Fig. 2B and D). The influence of BQ-123 on this effect of sarafotoxin S6c was not tested.

3.2. Influence of BQ-123 and BQ-788 on carrageenan-induced nociception and priming

Intra-articular injection of carrageenan (300 μg) into the naive joint induced sustained increases in PET values. This nociceptive effect attained significance 2 h after injection and remained elevated at least up to 8 h (Fig. 3A). When injected once again into a carrageenan-primed joint, 72 h after the first challenge (at a time when PET responses had already fully returned to control PBS-treated values), the nociceptive responses to the algogen were significantly increased over those evoked in a naive joint (Fig. 3B). In these particular experiments, the sensitisation to carrageenan afforded by priming the joint with this same algogen (but not PBS) is more evident when the AUC 0–8 h of both groups are compared (in arbitrary units: naive 162 ± 8 vs. primed 217 ± 9, amounting to a net potentiation of 60%; $P < 0.001$, paired two-tailed Student's t test; Fig. 3C and D).

Prior treatment of the naive joint with BQ-123 or BQ-788 (selective endothelin ET_A and ET_B receptor antagonists, respectively; each at 10 nmol) did not affect nociceptive responses to the first carrageenan challenge. However, when the same (carrageenan-primed) animals were re-challenged with carrageenan, those that had originally received BQ-788 (but not BQ-123) showed significantly greater nociceptive responses (i.e., PET values) than their PBS-pretreated carrageenan-primed counterparts (Fig. 3B), amounting to a 62% net potentiation of the AUC 0–8 h (Fig. 3D).

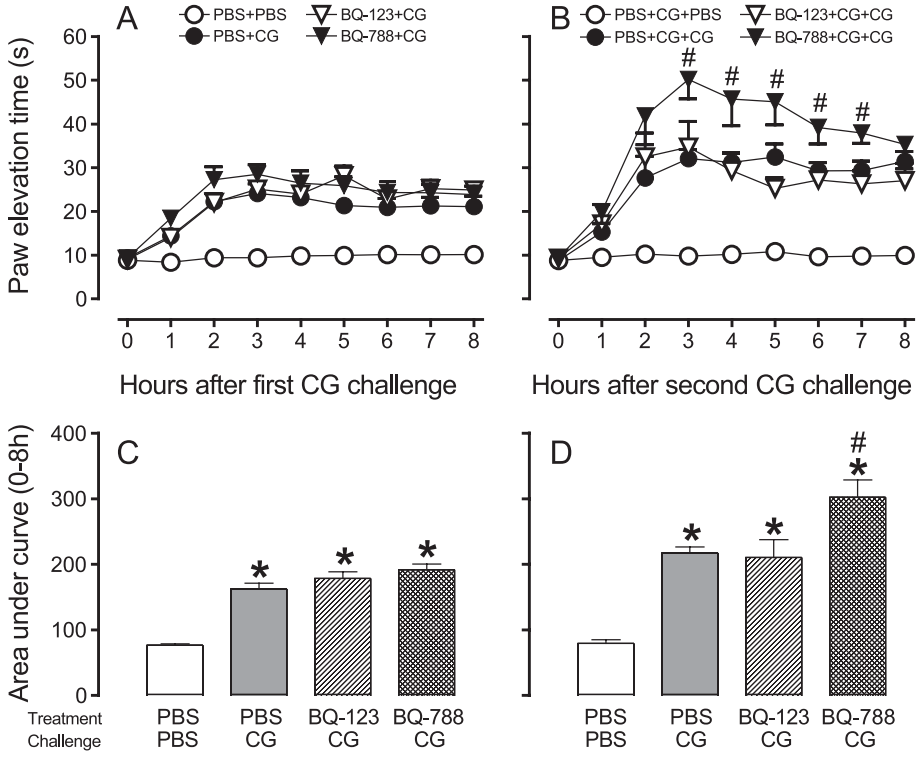

Fig. 3. Influence of BQ-123 or BQ-788 (selective endothelin ET_A and ET_B receptor antagonists, respectively) on nociceptive responses induced by two consecutive carrageenan (CG) challenges in the rat knee-joint. CG (300 μg, i.a.) was injected 15 min (first challenge; panels A and C) and 72 h (second challenge; panels B and D) after treatment with BQ-123, BQ-788 (each at 10 nmol, i.a.) or PBS. Nociceptive responses (paw elevation times) were recorded over 1-min periods immediately before (time 0) and then each hour up to 8 h after each CG (or PBS) challenge (panels A and B). Panels C and D display the areas under each of the curves depicted in A and B (calculated in arbitrary units), respectively. Values are means ± S.E.M. of six to eight experiments. Asterisks (in C and D only, for sake of clarity) and fences denote $P < 0.05$ when compared to the corresponding value of the PBS-treated/PBS-challenged control or the PBS-treated/CG-challenged groups, respectively (ANOVA followed by Bonferroni's test).

3.3. Influence of sarafotoxin S6c on nociception induced in the carrageenan-primed joint

The results depicted in Fig. 4 again confirm that priming the joint with carrageenan 72 h beforehand markedly augments subsequent nociceptive PET responses evoked by this algogen (105% net potentiation of AUC 0–8 h). Nonetheless, prior treatment of the naive joint with sarafotoxin S6c (i.e., given before priming; 30 pmol) fully prevented the increase in PET responses caused by the priming procedure. Likewise, early treatment with the selective ET_B receptor agonist before priming the joint markedly reduced the nociceptive responses evoked by either endothelin-1 or sarafotoxin S6c (each at 30 pmol) 72 h after the challenge with carrageenan (Fig. 5). Furthermore, the depressant actions of sarafotoxin S6c on nociceptive PET responses evoked by all three algogens (carrageenan, endothelin-1 and sarafotoxin S6c) in the primed joint were completely blocked by pretreatment of the naive joint with BQ-788 (10 nmol).

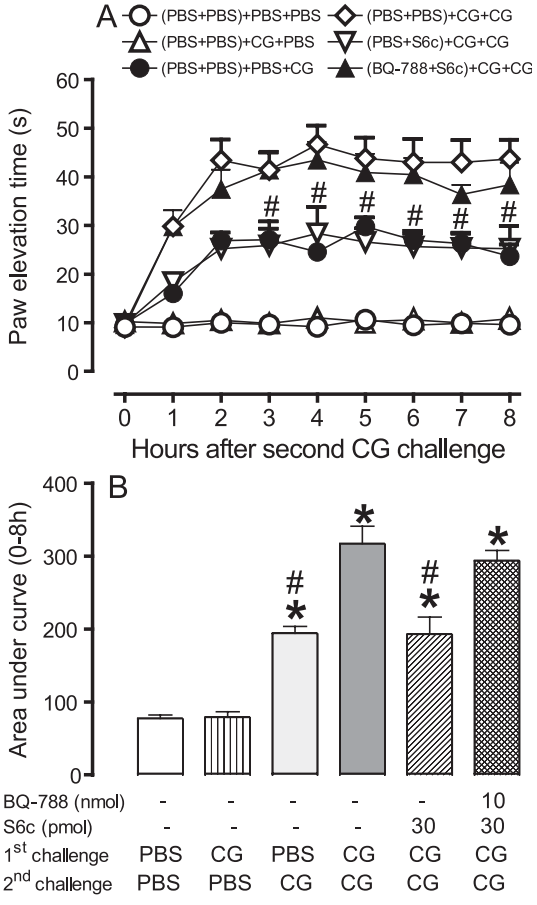

Fig. 4. The selective endothelin ET_B receptor antagonist BQ-788 prevents sarafotoxin S6c from inhibiting carrageenan (CG)-induced priming of the rat knee-joint to nociceptive responses evoked subsequently by CG itself. BQ-788 (10 nmol, i.a.) or PBS was injected 15 min before i.a. sarafotoxin S6c (S6c; 30 pmol) or PBS and 24 h later the joint was primed with CG (300 μg, i.a.). Nociceptive responses (paw elevation times) to a second CG challenge (given 72 h after priming) were recorded over 1-min periods immediately before (time 0) and then each hour up to 8 h after this challenge (panel A). Panel B displays the areas under each of the curves depicted in A (calculated in arbitrary units). Values are means ± S.E.M. of six to eight experiments. Asterisks (in B only, for sake of clarity), and fences denote $P<0.05$ when compared to the corresponding value of the PBS-treated/PBS-primed control or the PBS-treated/CG-primed groups, respectively (ANOVA followed by Bonferroni's test).

4. Discussion

The current study demonstrates that the nociceptive effects of carrageenan in the naive knee-joint can be modulated in opposing ways by activation of local endothelin ET_A and ET_B receptors. Though neither of these signalling mechanisms seem to be activated by carrageenan challenge in the naive joint to influence acute nociceptive responsiveness, the algogen clearly triggers an endothelin-dependent ET_B receptor-mediated mechanism which can limit its capacity to prime (or sensitise) the joint to nociceptive effects evoked by subsequent inflammatory insults.

Confirming our previous study in this articular incapacitation model (De-Melo et al., 1998b), nociceptive responses evoked by carrageenan in the naive joint were markedly potentiated by i.a. endothelin-1 (dual endothelin ET_A/ET_B receptor agonist), at doses much lower than those required to cause nociceptive effects per se (15–30 pmol vs. ≥120 pmol). In sharp contrast, two highly selective endothelin ET_B receptor agonists, sarafotoxin S6c and IRL 1620 (Davenport, 2002), clearly inhibited carrageenan-induced nociceptive behaviour. This finding was somewhat surprising, as our original study failed to detect any antinociceptive effects of sarafotoxin S6c (De-Melo et al., 1998b). However, in that study the dose of carrageenan chosen to induce nociceptive responses (150 μg, rather than the 300 μg dose used in the present study) may simply have been too modest to detect any significant suppressive actions of sarafotoxin S6c, yet sufficient to demonstrate the potentiating influence of endothelin-1. On the other hand, although the best interval between treatments to demonstrate the modulatory infuences of endothelin-1 and sarafotoxin S6c on nociceptive responses to carrageenan was 24 h, both peptides were also effective, albeit slightly less so, when given only 30 min (but not 48 h) prior to the algogen (data not shown; IRL 1620 was not tested).

As potentiation of nociceptive responses to carrageenan by endothelin-1 (30 pmol) was fully prevented by i.a. BQ-123, a selective endothelin ET_A receptor antagonist (Ihara et al., 1992), this hyperalgesic response seems to rely exclusively on receptors of this type, located in the close vicinity of or in the joint itself. In this regard, endothelin-1-induced hyperalgesia to noxious chemical (capsaicin) or thermal (heat) stimulation of the mouse hind-paw also depends solely on endothelin ET_A receptors (Piovezan et al., 1998, 2000; Menéndez et al., 2003), even though both receptor types are implicated in promoting mechanical hyperalgesia (Baamonde et al., 2004). Conversely, local injection of BQ-

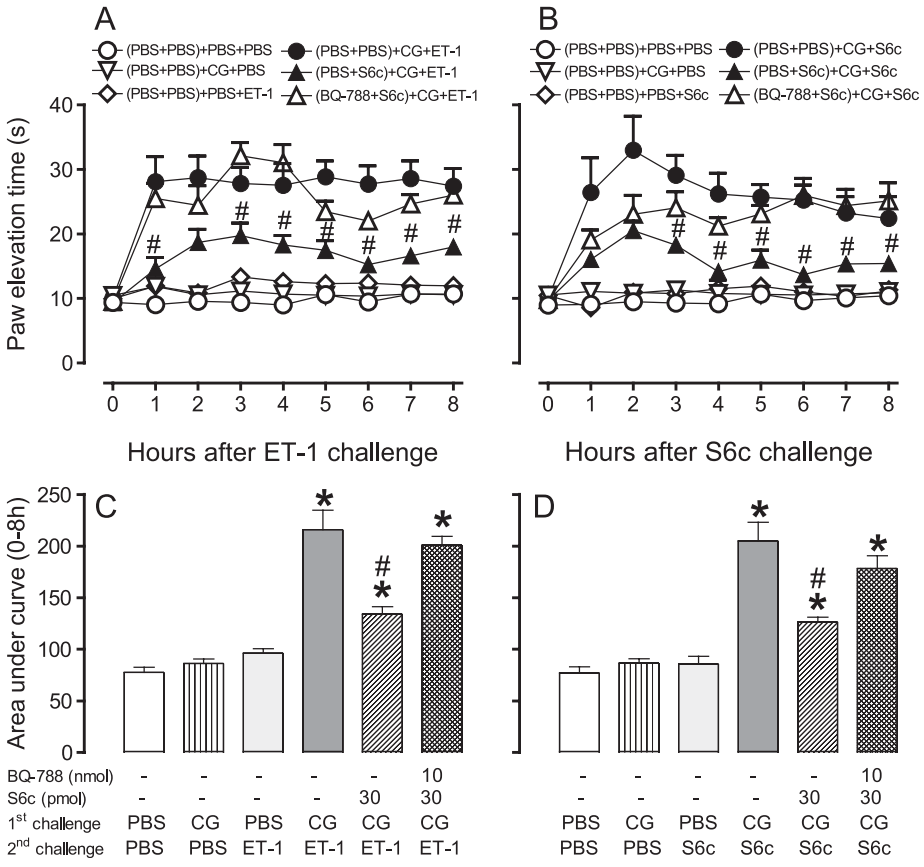

Fig. 5. The selective endothelin ET_B receptor antagonist BQ-788 prevents sarafotoxin S6c from inhibiting carrageenan (CG)-induced priming of the rat knee-joint to nociceptive responses evoked subsequently by endothelin-1 (ET-1) or sarafotoxin S6c (S6c). BQ-788 (10 nmol, i.a.) or PBS was injected 15 min before i.a. ET-1, S6c (each at 30 pmol) or PBS and 24 h later the joint was primed with CG (300 μg, i.a.). Nociceptive responses (paw elevation times) to ET-1 or S6c (given 72 h after CG-priming) were recorded over 1-min periods immediately before (time 0) and then each hour up to 8 h after challenge (panels A and B, respectively). Panels C and D display the areas under each of the curves depicted in A and B (calculated in arbitrary units), respectively. Values are means ± S.E.M. of six to eight experiments. Asterisks (in C and D only, for sake of clarity) and fences denote $P < 0.05$ when compared to the corresponding value of the control group primed and challenged with PBS or of the PBS-treated group primed with CG and challenged with the agonist (ET-1 or S6c), respectively (ANOVA followed by Bonferroni's test).

788, a selective endothelin ET_B receptor antagonist (Ishikawa et al., 1994), abolished the antinociceptive effect of sarafotoxin S6c (30 pmol) against responses triggered by carrageenan in the naive joint. Other studies have disclosed similar antinociceptive or antihyperalgesic roles for endothelin ET_B receptors in the hind-paw of mice (Piovezan et al., 1998, 2000) and rats (Khodorova et al., 2002, 2003). One might have expected BQ-788 to also enhance the potentiation of carrageenan-induced responses afforded by the dual ET_A/ET_B receptor agonist endothelin-1, yet, this did not occur. As the curve to endothelin-1-induced hyperalgesia is bell-shaped, perhaps such an influence might have been evident if, instead of employing 30 pmol of the peptide (which is maximally effective in causing hyperalgesia), we had chosen to use a higher dose of the peptide in those experiments (e.g., 60 pmol, which does not potentiate carrageenan-induced nociception).

Injected into the hind-paw, carrageenan raises local endothelin-1 levels in rats (Bertelli et al., 1992) and evokes a hyperalgesia to noxious thermal and mechanical stimulation in mice which can be suppressed by endothelin ET_B and/or ET_A receptor blockade (Baamonde et al., 2004). In contrast, carrageenan-induced nociception in the rat naive joint is unaffected by the dual endothelin ET_A/ET_B receptor antagonist bosentan (De-Melo et al., 1998a). It could be argued that simultaneous blockade of both receptor types might be an unsuitable strategy to functionally probe the possible activation of the endothelin system by carrageenan in the naive joint, in light of their opposing (and hence, mutually counteracting) influences on articular nociception. However, this possibility seems to be unlikely in view of the current finding that neither BQ-123 nor BQ-788, selective antagonists of endothelin ET_A and ET_B receptors, respectively, were capable of modifying naive joint responsiveness to carrageenan.

Besides causing acute nociceptive effects, i.a. carrageenan also sensitises the naive knee-joint to noxious stimulation by additional challenges of carrageenan itself, *Escherichia coli* lipopolysaccharide, endothelin-1 or sarafotoxin S6c (De-Melo et al., 1998a; Tonussi and Ferreira, 1999). This

priming effect greatly outlasts (by up to several days) the duration of its acute nociceptive effect and mimics one of the hallmarks of arthritic pain in humans, the long-lasting sensitisation to subsequent noxious stimuli (Schaible et al., 2002). Carrageenan also causes a similar local priming effect in the rat hind-paw, which appears to depend importantly on prolonged activation of protein kinase C epsilon (Aley et al., 2000; Parada et al., 2003; Dina et al., 2003). The present study has now shown that priming-induced exacerbation of nociceptive responsiveness to carrageenan is unchanged by prior local treatment with BQ-123, but is markedly augmented by similar treatment with BQ-788. This finding strongly suggests that the priming effect of carrageenan in the naive joint is limited by activation of an endothelin-dependent ET_B receptor-mediated mechanism. The fact that i.a. sarafotoxin S6c, prior to the first priming challenge with carrageenan, completely abolished the enhancement of nociceptive responses to the second challenge, and that this influence was fully prevented by BQ-788, adds further strength to this view. Additionally, this endothelin ET_B receptor-mediated pre-emptive effect of sarafotoxin S6c on carrageenan-induced priming was also clearly evident when either endothelin-1 or sarafotoxin S6c were given as the second challenge, instead of carrageenan.

Endothelin-1 is known to be produced by several articular cell types, including chondrocytes (Khatib et al., 1997), macrophage-like type A synoviocytes (Yoshida et al., 1998) and synovial blood vessel endothelium (Wharton et al., 1992). In addition, periarticular tissues from rats expressing antigen-induced arthritis show marked increases endothelin-1 levels (Andersson et al., 1999), whereas polyarthritis induced by complete Freund's adjuvanct augments endothelin-1 activity in the circulation (Klemm et al., 1995). Likewise, increased endothelin-1 levels are seen in the synovial fluid and/or plasma of patients with rheumatoid arthritis, osteoarthritis and various other systemic rheumatic diseases (Miyasaka et al., 1992; Yoshida et al., 1998; Haq et al., 1999; Mayes, 2003). However, the cells and signalling mechanisms responsible for the respective pro- and antinociceptive influences of endothelin ET_A and ET_B receptors on carrageenan-induced nociception in the naive joint, as well as those underlying the ET_B receptor-dependent suppression of priming, remain to investigated.

In conclusion, the current study shows that stimulation of local endothelin ET_A and ET_B receptors with exogenous agonists exert pro- and antinociceptive influences, respectively, on nociceptive responsiveness to carrageenan in the naive knee-joint. Although the local endothelin system does not appear modulate the acute nociceptive responsiveness to carrageenan, this algogen clearly activates an endothelin-dependent ET_B receptor-operated mechanism which limits the extent of its priming (or sensitising) action towards nociceptive effects evoked by subsequent inflammatory insults. Nonetheless, if the model of articular nociception employed in this study bares any predictive value to the clinical situation, it appears most unlikely that selective ET_B receptor agonists would be useful for the treatment of human arthitic pain, because they evoke (rather than inhibit) nociceptive effects in a previously inflamed joint.

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