Endothelin-1 Causes Pruritus in Mice

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Endothelin (ET)-1 evokes a burning pruritus sensation when injected intradermally in humans and nocifensive behavior when injected into the hind paw of rodents. Because pain and pruritus are clearly distinct nociceptive sensory modalities in humans, the current study evaluates the potential of ET-1 to elicit scratching behavior in mice. Mice received an intradermal injection of 1–30 pmol ET-1; 10 μg of the mast cell degranulator compound, 48/80; 100 nmol histamine; or vehicle into the scruff, and the number of scratching bouts displayed during the first 40 mins was recorded. ET-1 caused dose-dependent scratching bouts, which, like the responses to histamine and compound 48/80, occurred mainly during the first 5 to 10 mins of injection, but fewer episodes were also seen up to 35 mins. The effect of ET-1 was maximal at 10 pmol (total 40 ± 7 bouts), a value similar to that caused by histamine (52 ± 5 bouts) and compound 48/80 (53 ± 6 bouts). The selective ET_B receptor agonist, IRL-1620 (10 pmol), was not pruritic *per se*, and actually inhibited responses to histamine and ET-1. Pruritus induced by ET-1 was inhibited by the ET_A receptor antagonists, 10 nmol BQ-123 (co-injected; net inhibition, 87%) and 10 mg/kg atrasentan (intraperitoneal administration; net inhibition, 83%), or the ET_B receptor antagonist, 20 mg/kg A-192621 (intraperitoneal administration; net inhibition, 64%), but the response was augmented by co-injection of the ET_B receptor antagonist, 3 nmol BQ-788 (net potentiation, 234%). Responses to compound 48/80 or responsiveness of vehicle-treated mice were unaffected by these antagonists. Thus, ET-1 displays potent pruritic actions in the mouse mediated to a substantial extent *via* local ET_A receptors. The findings with IRL-1620 and BQ-788 suggest that local ET_B receptors exert an antipruritic role, but, for reasons still unknown, the results obtained using systemic A-192621 injection are at variance with this view. Exp Biol Med 231:1146–1151, 2006

Key words: pruritus; endothelin-1; histamine; compound 48/80; scratching behavior; mice

Introduction

Itch and pain are unpleasant sensory experiences that can lead to serious life-quality impairment. Along with pain, itch can be an important symptom of systemic malfunctions, as well as skin diseases. However, the behavioral response patterns evoked by both sensations differ, that is, pain elicits a withdrawal reflex, whereas itch leads to a scratch reflex (1). Progress in understanding the neuropathophysiology of pruritus has been made with the elucidation of a distinct pathway that carries this kind of sensory information. These itch neurons consist of C-fibers that can be identified by their lasting response to histamine application and represent approximately 5% of C-fibers in human skin afferents (2, 3).

Histamine is one of the most outstanding chemical mediators causing pruritus and, indeed, the histamine H_1 receptor antagonists provide first-choice treatment for pruritus (4). However, other peripheral mediators can be involved in induction of itch alongside mast cell–derived histamine (5). One such candidate is endothelin (ET)-1, a peptide of the ET family produced and released by various cell types including mast cells (6, 7). The ETs exert physiopathologic actions through activation of specific G-protein–coupled ET_A and ET_B receptors (8). Injection of ET-1 into the rat or mouse hind paw evokes nociceptive behavior (i.e., overt pain) as well as hyperalgesia (i.e., sensitization to noxious stimuli; Refs. 9–12). In volunteers, the peptide causes deep pain when infused into the brachial artery, as well as intense burning pruritus, tactile allodynia, and vasodilatation if injected intradermally into the forearm (13–15). The ET receptors mediating such actions can differ considerably depending on the species and nociceptive model considered. Thus, ET_A and ET_B receptors subserve pronociceptive and/or prohyperalgesic roles in abdominal writhes induced by ET-3 (16) and mechanical hyperalgesia induced by ET-1 in mice (17), but only ET_B receptors seem

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to mediate the latter effect in rats (18). On the other hand, only ET_A receptors mediate the nociceptive and hyperalgesic responses to intraplantar ET-1 injection into the hind paw of mice or rats, whereas activation of ET_B receptors in this body region usually elicits antinociceptive and/or antihyperalgesic effects (9–12).

Although the participation of the ET system has been widely demonstrated in animal models of immune, inflammatory, neuropathic, and neoplasic pain (9, 19–23), to the best of our knowledge, no studies have yet investigated the capability of these peptides to induce pruritus in animals. Thus, the present study assesses the potential pruritic actions of ET-1 in mice and attempts to characterize the receptors implicated in these effects.

Materials and Methods

Animals. Experiments were conducted on male Swiss mice (30–35 g) from our own colony, housed at 22 ± 1°C on a 12:12-hr light:dark cycle (lights on at 0700 hrs) and had free access to food and water. The experimental procedures and protocols were approved by the Committee on Ethical Use of Animals of the Universidade Federal de Santa Catarina.

Behavioral Experiments. One day after trichotomy of the rostral part of the back and scruff (i.e., the back of the neck), mice were given an intradermal injection into the scruff of 1–30 pmol ET-1, 3–100 μg of the mast cell degranulator compound 48/80, 100 nmol histamine, or vehicle (20 μl of PBS solution). Each mouse was placed immediately and individually under inverted glass funnels, and the number of scratching bouts displayed during the first 40 mins was recorded, as described previously (24).

Two protocols were conducted with ET receptor antagonists to functionally identify the receptors implicated in the effects of ET-1. In the first, selective peptidic antagonists for the ET_A receptor (10 nmol BQ-123), ET_B receptor (3 and 10 nmol BQ-788), or vehicle were intradermally co-injected with 10 pmol ET-1 or 10 μg compound 48/80, in a final volume of 20 μl, in separate groups of animals. In the second protocol, mice were pretreated intraperitoneally with selective nonpeptidic antagonists of ET_A receptors (10 mg/kg atrasentan), ET_B receptors (20 mg/kg A-192621), or vehicle, 1 hr before injection of ET-1 or compound 48/80. The main rationale for testing a peptidic and a nonpeptidic antagonist for either ET receptor type, each *via* a distinct route of administration, was to compare the local versus systemic effects of ET_A or ET_B receptor blockade on the pruritic behavior. In additional experiments, the selective ET_B receptor agonist, IRL-1620 (10 pmol), or vehicle was co-injected with 10 pmol ET-1 or 100 nmol histamine. Doses of the various drugs used were selected based on doses shown to be effective in previous studies (19, 21, 22, 25).

Drugs. The following drugs were used: ET-1 and IRL-1620 (Suc-[Glu^9, $Ala^{11,15}$]-ET-1; Refs. 8–21) from American Peptide Co. (Sunnyvale, CA); compound 48/80 and histamine from Sigma Chemical Co. (St. Louis, MO); BQ-123 (cyclo[DTrp-DAsp-Pro-DVal-Leu]) and BQ-788 (*N-cis*-2,6-dimethylpiperidinocarbonyl-L-γ-methylleucil-D-L-methoxycarboyl-D-norleucine) from Research Biochemicals International (Natick, MA); and atrasentan and A-192621 ([2R-{2a,3b,4a}]-4-[1,3-benzodioxol-5-yl]-1-[2-{2,6-diethylphenyl}amino]-2-oxoethyl-2-[4-propoxyphenyl]-3 pyrrolidinecarboxylic acid), which were kindly provided by Abbott Laboratories (Abbott Park, IL). Atrasentan and A-192621 were dissolved in phosphate-buffered saline (PBS) containing 3% ethanol and 100 μl of 0.1 *N* NaOH. All of the other drugs were dissolved in PBS.

Statistical Analysis. All data are expressed as the mean ± SEM of six to eight animals. Statistical comparisons were made using one-way analysis of variance (ANOVA) followed by Bonferroni's test. In all statistical analyses, differences with $P < 0.05$ were considered significant.

Results

Intradermal injection of 1–30 pmol ET-1, but not of vehicle, evoked dose-dependent scratching bouts (Fig. 1A). The maximal response to ET-1 was obtained using 10 pmol of the peptide (40 ± 7 bouts) and was equivalent to the effects induced by 10 μg of compound 48/80 (53 ± 6 bouts) or 100 nmol of histamine (52 ± 5 bouts; Fig. 1B). Scratching bouts triggered by ET-1 followed a time course similar to that of responses to histamine and compound 48/80, with most of the bouts occurring during the first 5 to 10 mins after injection and fewer episodes during the remainder of the 40-min observation period (data not shown).

Pruritus induced by ET-1 was markedly inhibited when it was coadministered locally together with the selective ET_A receptor antagonist, BQ-123, or by systemic pretreatment with either the selective antagonist for ET_A receptors, atrasentan, or for ET_B receptors, A-192621, (net inhibitions of 87%, 83%, and 64%, respectively; Fig. 2). In contrast, local co-injection of 3 nmol of the selective ET_B receptor antagonist, BQ-788, substantially augmented the effect of ET-1 (net potentiation of 235%), whereas a higher dose of the antagonist (10 nmol) evoked significant pruritus responses *per se*. On the other hand, co-injection of the selective ET_B receptor agonist, IRL-1620, profoundly inhibited scratching behavior induced either by ET-1 or histamine (net inhibitions of 96% and 81%, respectively, Fig. 3). None of the antagonists nor IRL 1620 affected the responsiveness of vehicle-treated mice (Figs. 2 and 3). The antagonists also failed to affect responses to compound 48/80 (data not shown).

Discussion

The current study demonstrates, to our knowledge for the first time, that ET-1 induces pruritus when injected intradermally in mice. This response was inhibited by local

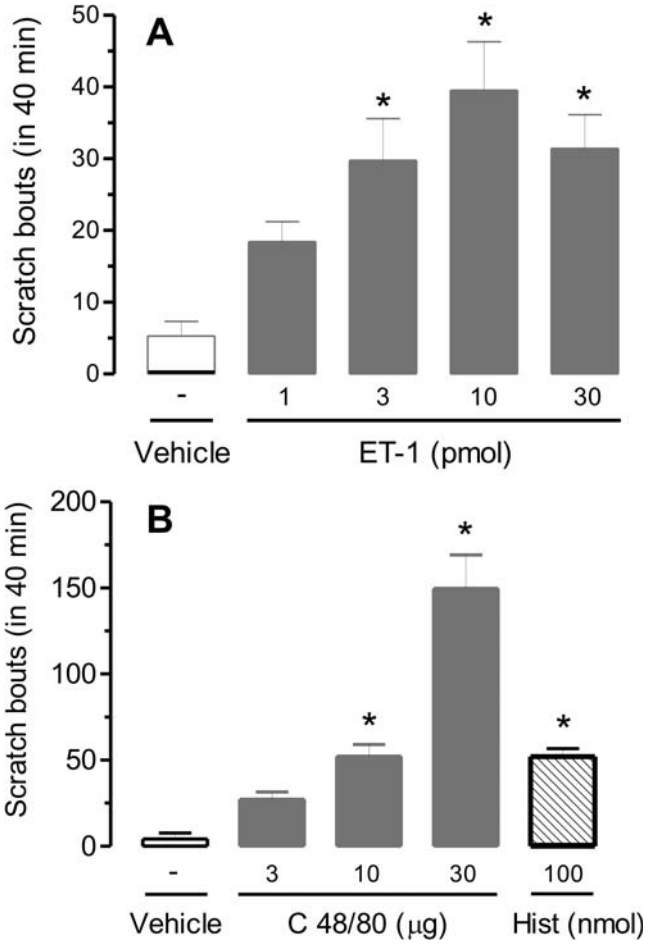

Figure 1. Pruritus induced by ET-1 (A), and compound 48/80 and histamine (B) in mice. The drugs were injected intradermally at the doses indicated; controls were similarly treated with 20 μl of PBS. Values represent the mean ± SEM of the number of scratching bouts recorded during 40 mins ($n = 6$–8 animals per group). Asterisks denote $P < 0.05$ relative to PBS-treated controls (one-way ANOVA followed by Bonferroni's test).

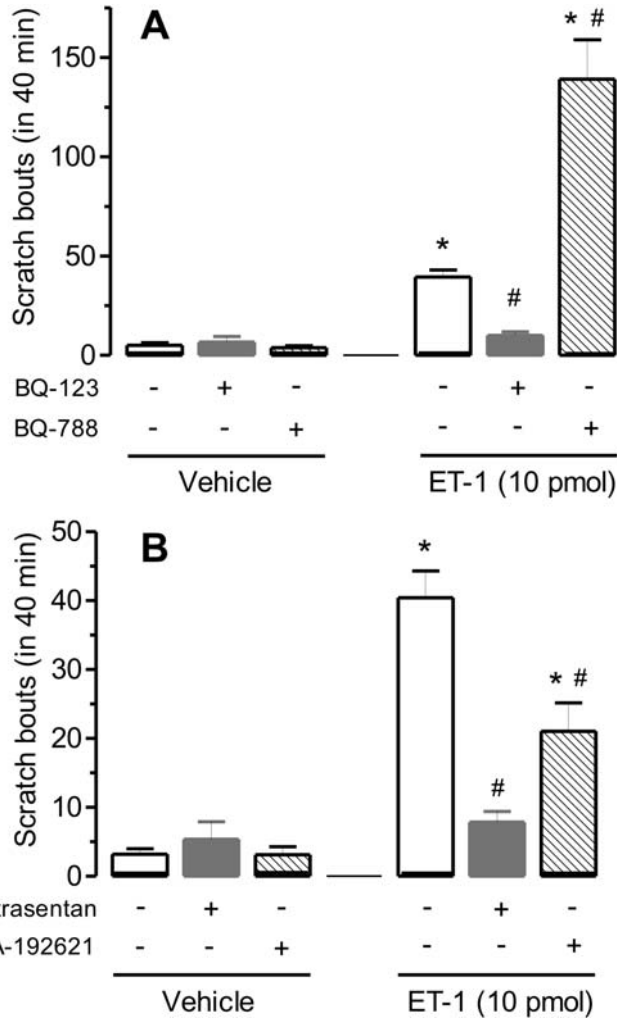

Figure 2. Influence of ET_A or ET_B receptor antagonists on scratching behavior induced by ET-1. In (A), mice were given an intradermal co-injection, into the scruff, of 10 nmol BQ-123 or 3 nmol BQ-788 together with 10 pmol ET-1 or vehicle (PBS). Mice in (B) received an intraperitoneal injection of 10 mg/kg atrasentan or 20 mg/kg A-192621 or the corresponding vehicle, 1 hr before intradermal injection of 10 pmol ET-1 or its vehicle into the scruff. Values represent the mean ± SEM of the number of scratching bouts during 40 mins ($n = 6$–8 animals per group). Asterisks and fences denote $P < 0.05$ relative to vehicle-treated and to ET-1–injected mice, respectively (one-way ANOVA followed by Bonferroni's test).

coadministration of an ET_A receptor antagonist or of an ET_B receptor agonist, but was potentiated by local co-injection of an ET_B receptor antagonist.

There is evidence that ET-1 can degranulate mast cells (26–29), can be produced by these cells (29–32), and that some mast cell subtypes display augmented levels of messenger RNA for ET-1 in certain physiopathologic conditions (33, 34). In the mouse hind paw, ET-1 injection elicits nociceptive licking behavior that is fully prevented by local ET_A receptor blockade (9) or previous desensitization of resident mast cells induced by multiple injections of the degranulating synthetic cationic polyamine, compound 48/80[2]. The current study shows that ET-1 elicited scratching bouts when injected into the scruff of mice; these scratching bouts were indistinguishable from and followed a similar time course to those induced by histamine and compound 48/80, with a high proportion of the responses occurring over the first 5 to 10 mins after injection. Although it is possible that the pruritic effect of ET-1 may have derived from mast cell degranulation, this remains to be adequately demonstrated. Nonetheless, the data are sufficient to state that ET-1 can stimulate intradermal sensory fibers, directly or indirectly, to evoke itch in mice. In humans, itch-selective neurons comprise only 5% of C-fibers present in the skin (2), but their proportion in mouse skin is unknown.

Considering that ET-1–induced scratching behavior was fully prevented by local coadministration of BQ-123 or previous systemic treatment with atrasentan (both selective ET_A receptor antagonists), ET_A receptors seem to play a

[2] Frighetto M, Trentin PG, Rae GA. Unpublished observations.

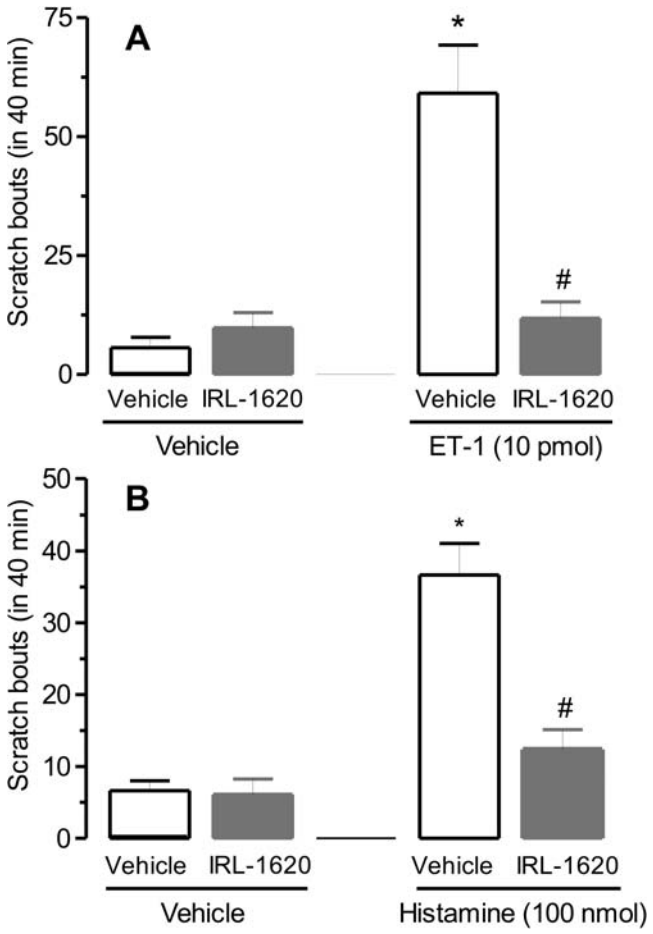

Figure 3. Influence of the selective ET_B receptor agonist, IRL 1620, on pruritus induced by ET-1 (A) or histamine (B). IRL-1620 (10 pmol) or vehicle were co-injected into the scruff together with ET-1, histamine (at doses indicated), or vehicle. Values represent the mean ± SEM of the number of scratching bouts during 40 mins ($n = 6$–8 animals per group). Asterisks and fences denote $P < 0.05$ relative to vehicle-treated and to (A) ET-1–injected or (B) histamine-injected groups, respectively (one-way ANOVA followed by Bonferroni's test).

major role in eliciting this response. This finding correlates very well with the central role played by ET_A receptors in mediating ET-1–induced nociceptive behavior and hyperalgesia to capsaicin or heat stimulation in the mouse hind paw (9, 10). Cultured mouse fetal skin mast cells express both ET receptors, but only ET_A receptors mediate ET-1–induced degranulation, whereas cultured (undifferentiated) bone marrow mast cells express fewer ET_A receptors and no ET_B receptors and are unresponsive to the peptide (29). Thus, if adult skin mast cells *in vivo* maintain the phenotype of fetal skin mast cells, ET_A receptor–mediated mast cell degranulation could account, at least in principle, for the pruritic action of ET-1. Another attractive possibility is direct activation of ET_A receptors on sensory neurons, because ET-1 sensitizes tetrodotoxin-resistant voltage-sensitive sodium channels to activation in acutely dissociated rat dorsal root ganglion sensory neurons *via* ET_A receptors (35).

Local coadministration of BQ-788 markedly augmented the pruritic effect of ET-1. This result, allied to the finding that ET-1–induced scratching behavior was substantially inhibited by local coadministration of the selective ET_B receptor agonist, IRL 1620, confirms the antipruritic role of local ET_B receptors. Moreover, because IRL 1620 also inhibited scratching elicited by histamine, it seems unlikely that this effect was mediated *via* mast cell ET_B receptors. Stimulation of local ET_B receptors in the hind paw of mice and rats suppresses hyperalgesia to capsaicin and nociception induced by intraplantar ET-1 administration, respectively (9, 12). Interestingly, the latter study also demonstrated that ET_B receptors on keratinocytes trigger β-endorphin release to limit the ET_A receptor–dependent nociceptive effect of ET-1. It seems reasonable to expect that such an ET_B receptor–operated mechanism might suppress pruritus, because intradermal injections of ET-1 used in the present study must deliver the peptide much closer to the keratinocytes in the scruff than intraplantar (subcutaneous) injections do in the hind paw.

Unexpectedly, however, systemic pretreatment with the selective ET_B receptor antagonist, A-192621, produced an effect opposite to that obtained with local BQ-788 administration (i.e., it diminished ET-1–induced scratching). This suggests that, somehow, systemic ET_B receptor blockade overrides this antipruritic modulatory influence of local ET_B receptors in the skin. At present, we cannot envisage a satisfactory explanation for this discrepancy. Nonetheless, because ET_B receptors on endothelial cells act as clearance receptors for ET-1 (36), their extensive blockade by systemic treatment with the ET_B receptor antagonist, A-192621, may well have increased the circulating levels of ET-1, thus enhancing vasoconstriction and reducing blood flow. Clearly, this hypothesis remains to be proven experimentally.

In addition to producing ET-1 and responding to the peptide by degranulating, serosal mast cells also secrete chymase, which can influence local ET-1 levels in two ways. First, chymase can generate ET-1 by converting extracellular big ET-1 (the inactive precursor of ET-1) into a 21-residue peptide [named ET-1 (1–31)], which is then cleaved to yield ET-1 by neutral endopeptidase and ET-converting enzyme *in vitro* (37) and *in vivo* (38, 39). Mast cell–derived chymase can also degrade ET-1, thus, exerting a protective role in situations in which high levels of ET-1 can be detrimental, such as peritonitis (40). We have shown that intradermal ET-1 injection causes ET_A receptor–mediated pruritus in the mouse, which can be limited locally by stimulation of ET_B receptors. It now seems important to assess whether this effect of ET-1 is indeed dependent on mast cells, and whether there is a link between mast cells and the ET system in pruritus associated with Type I hypersensitivity reactions and other pathophysiologic states.

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