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Endothelin-1 induces itch and pain in the mouse cheek model

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ABSTRACT

Aims: To date, suggestions that endothelin-1 (ET-1) causes nociception and pruritus are based on results in preclinical models in which responses to pruritic and nociceptive stimuli cannot be distinguished. This study reexamines these sensory effects of ET-1 in the new mouse cheek model, in which pruritogens and algogens evoke distinct behavioral responses.

Main methods: Mice received intradermal (i.d.) injections of test substances into the left cheek and bouts of hind limb scratches or forepaw wipes, directed to the injection site, were considered indicative of pruritus and nociception, respectively.

Key findings: Histamine and capsaicin selectively evoked scratching and wipes, respectively, whereas ET-1 (3–60 pmol) promoted dose-dependent bouts of both behaviors. While scratching and wipe responses to ET-1 (30 pmol) were potentiated by BQ-788 (an ET_B receptor antagonist) and reduced by co-injection of BQ-788 plus BQ-123 (an ET_A receptor antagonist), BQ-123 alone inhibited scratching responses only. CTOP (μ-opioid receptor selective antagonist) only augmented scratching responses to ET-1, whereas DAMGO (μ-opioid receptor selective agonist) reduced both behaviors. Loratadine (histamine H_1 receptor antagonist) marginally reduced scratching, but markedly suppressed wipes.

Significance: These results demonstrate that ET-1 evokes pruritic and nociceptive behaviors in the mouse cheek model. Both responses to ET-1 appear to be mediated via ET_A receptors and subjected to limitation by simultaneous ET_B receptor activation. Local endogenous opioids acting on μ-opioid receptors selectively modulate the pruritic response to ET-1, whereas histamine, possibly derived from mast cells and acting on H_1 receptors, contributes importantly to the nociceptive effect of ET-1 in this model.

Introduction

Itch (i.e. pruritus) is a cutaneous sensory modality different from pain and a common symptom in dermatology and general medicine. Recent elucidation of distinct pathways that convey sensory pruriceptive information to the brain has enhanced our understanding of its basis (Davidson and Giesler, 2010). Pruritus requires the activation by pruritogenic stimuli of distinct subgroups of dedicated primary afferent C-fibers, one of mechano-insensitive/histamine-sensitive fibers (Schmelz et al., 1997) and another of histamine-insensitive non-nociceptive polymodal fibers (Imamachi et al., 2009; Sun et al., 2009), but nociceptive polymodal fibers are also involved.

Keratinocytes, leukocytes, mast cells, fibroblasts, endothelial cells and nerves in the skin produce several endogenous pruritogens, including histamine, kinins, proteases, neurotrophins, some opioids and cytokines (Ikoma et al., 2006). Many of them are also nociceptive and complex functional interactions among them can exacerbate and perpetuate itch sensation to promote chronic pruritic diseases (Steinhoff et al., 2006; Metz and Ständer, 2010). Another potentially relevant endogenous pruritogen produced by mast cells, endothelial cells and keratinocytes in the skin is endothelin-1 (ET-1), a peptide member of the endothelin family which activates specific G protein-coupled ET_A and ET_B receptors to promote potent and diversified effects (Kedzierski and Yanagisawa, 2001).

In mice, ET-1 injection into the nape of the neck elicits ET_A receptor-mediated bouts of hind paw scratching directed to the injected area (Trentin et al., 2006; McQueen et al., 2007). This effect of ET-1 is signaled by ET_A receptors possibly located on neurons expressing transient receptor potential vanilloid subfamily 1 (TRPV1) receptors and coupled to adenylyl cyclase and protein kinase C pathways, but not to TRPV1 receptors themselves or phospholipase $C\beta_3$ (Imamachi et al., 2009; Liang et al., 2010a). Scratching behavior induced by ET-1 can be limited locally by activation of ET_B receptors (Trentin et al., 2006; Liang et al., 2010a) or transient receptor potential ankyrin subfamily member 1 (TRPA1) receptors (Liang et al., 2011). However, if injected into a mouse's hind paw foot pad, ET-1 induces licking behavior directed to the injected limb. This behavior is interpreted as indicative of nociception, and is accompanied by sensitization (i.e. hyperalgesia) to nociceptive effects of mechanical, thermal and chemical stimuli (Piovezan et al., 2000; Baamonde et al., 2004). ET-1-induced paw licking is also mediated by ET_A receptors

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and limited by ET_B receptors (Piovezan et al., 2000), but the signaling pathways (unlike scratching) involve phospholipase C activation, but not protein kinase C (Liang et al., 2010b).

Many substances elicit back scratching bouts and hind paw licking in animals. This usually is taken as evidence that they cause pruritus at one locus and nociception at another. However, this interpretation could be biased by the impossibility of mice to lick their backs or scratch their hind paws. Recently, Shimada and LaMotte (2008) proposed a new mouse cheek model which enables a clear-cut behavior-based separation of pruritoceptive and/or nociceptive effects of substances. The present study sought to use this model to discriminate the potential of ET-1 to induce pruritoceptive and nociceptive behaviors, and investigate some of the mechanisms underlying these effects.

Materials and methods

Animals

Experiments were conducted on male CD-1 mice (25–35 g), from our own colony, lodged in a room with controlled temperature (22 ± 1 °C) and lighting (lights on from 06:00 to 18:00 h), with free access to lab chow and tap water. All experimental procedures and protocols were previously approved by the UFSC's committee on ethical use of laboratory animals and are in accordance with Brazilian Legislation and European Union's Directive 2010/63/EU.

Behavioral experiments

Pruritoceptive and nociceptive behaviors were evaluated in the cheek model described by Shimada and LaMotte (2008), with minor methodological modifications. Two days before the experimental procedure, animals were briefly anesthetized with isoflurane (2% in 100% oxygen) and the fur on both cheeks was shaved. On the day of the experiment, each mouse was placed in an individual clear plastic container (9 cm × 9 cm × 13 cm), fitted with four angled mirrors to enable complete unobstructed view of the subject at all times, and left to habituate for 1 h before intradermal (i.d.) injection of a test substance into the left cheek only.

As originally proposed by Shimada and LaMotte (2008), bouts of single or repetitive back-and-forth scratching movements of the ipsilateral hind limb directed to the injected cheek, followed by licking or biting of the toes and/or placement of hind paw on the floor, were counted and considered indicative of pruritoceptive behavior. Conversely, bouts of unilateral wipes of the ipsilateral forelimb directed initially to the caudal portion of the injected cheek and proceeding in a rostral direction, with the paw closed, were counted and considered indicative of nociceptive behavior.

Substances tested for their potential pruritoceptive/nociceptive effects included: histamine (5, 10, 20 or 50 μg/site), capsaicin (1, 10 or 40 μg/site) or ET-1 (3, 10, 30 or 60 pmol/site), or an equal volume of the vehicle (phosphate-buffered saline, PBS). These substances were always injected intradermally (i.d.) in a volume of either 20 μl (when tested alone) or 10 μl (when preceded by a given i.d. pretreatment injection of similar volume, see cases below). The animals were filmed, using a camcorder (Intelbras, model 200VM, Brazil) positioned in front of the container and connected to a computer, and their pruritoceptive/nociceptive behaviors recorded continuously over the first 40 min after treatment, in 5-min bins.

To identify the receptors implicated in the effects of ET-1, mice were given an i.d. injection of either BQ-123 (selective ET_A receptor antagonist), BQ-788 (selective ET_B receptor antagonist; each at 10 nmol/site and in 10 μl) or a combination of both, 5 min prior to i.d. injection of ET-1 (30 pmol/site) into the same cheek. To verify if local mechanisms operated by μ-opioid receptors influenced the effects of ET-1, the peptide was injected (at 30 pmol/site) either alone or in combination (i.e. co-injected) with the μ-opioid receptor selective antagonist CTOP (20 nmol/site) or agonist DAMGO (100 nmol/site). Another set of experiments assessed the influence of intraperitoneal pretreatment (1 h beforehand) with the histamine H_1 receptor antagonist loratadine (10 mg/kg) on the effects of i.d. ET-1 (30 pmol/site), in order to evaluate the contribution of endogenous histamine. In all cases, control mice were always treated accordingly with identical volumes of the vehicle (PBS). The doses of all drugs used were chosen on the basis of previous studies (Seike et al., 2005; Trentin et al., 2006; Khodorova et al., 2009; Sun et al., 2009). Mice were temporally removed from their containers for treatments and pretreatments. Each animal was used only once and promptly sacrificed by CO_2 asphyxia in an acrylic chamber soon after termination of the experiment.

Drugs

The following drugs were used: ET-1, 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 American Peptide Co. (Sunnyvale, CA, USA); capsaicin, histamine dichloride, CTOP (D-phenylalanyl-L-cysteinyl-L-tyrosyl-D-tryptophyl-L-ornithyl-L-threonyl-3-mercapto-L-valyl-,cyclic(2–7)-disulfide L-threoninamide), DAMGO ([D-Ala^2, N-Me-Phe^4, Gly^5-ol]-Enkephalin acetate) and loratadine from Sigma Chemical Co. (St Louis, MO, USA). All drugs were dissolved in PBS.

Statistical analysis

All behavioral data are expressed as the mean ± S.E.M. of six to eight animals, and are presented either as the total number of scratching or wiping bouts during the first 40 min following injection of the nociceptive/pruritoceptive compound, or in 5-min bins throughout the observation period. Statistical comparisons were made using one-way analysis of variance (ANOVA) followed by Newman–Keuls post-hoc test. Differences with $P<0.05$ were considered to be significant.

Results

Histamine (5 to 50 μg/site) induced dose-dependent scratching bouts (total events in 40 min: PBS 6.2 ± 1.2 vs. histamine 50 μg/site 46.0 ± 5.1, $P<0.05$), but no wiping bouts (total events in 40 min: PBS 5.0 ± 2.1 vs. histamine 50 μg/site 12.9 ± 3.0, $P>0.05$, $N=6–8$), whereas capsaicin (1 to 40 μg/site) promoted dose-dependent wiping bouts (total events in 40 min: PBS 11.1 ± 2.5 vs. capsaicin 40 μg/site 60.2 ± 10.1, $P<0.05$), but no scratching bouts (total events in 40 min: PBS 9.0 ± 3.6 vs. capsaicin 40 μg/site 5.3 ± 2.1, $P>0.05$, $N=6–8$). These results fully corroborate the original findings of Shimada and LaMotte (2008) in the model.

Intradermal ET-1 injections (3 to 60 pmol/site) induced dose-dependent bouts of scratching (Fig. 1A) and wiping (Fig. 1B). Both pruritoceptive and nociceptive behaviors displayed rapid onsets and occurred over the first 20 min after injection (panel insets show time-courses of the effects of ET-1 30 pmol/site, in 5-min bins). As shown in Fig. 2, pretreatment with BQ-123 (10 nmol/site) markedly reduced the incidence of scratching bouts (pruritus; 89% inhibition) induced by ET-1 (30 pmol/site), but not that of wipes (nociception). In contrast, BQ-788 augmented both effects of ET-1 substantially (scratching and wiping bouts were potentiated by 72% and 108%, respectively). Co-injection of both ET_A and ET_B receptor antagonists resulted in significant attenuation of both responses when compared to those induced by ET-1 in cheeks pretreated with BQ-788 alone (Fig. 2). Importantly, neither BQ-123 nor BQ-788 affected pruritic or nociceptive responses of PBS-treated control mice.

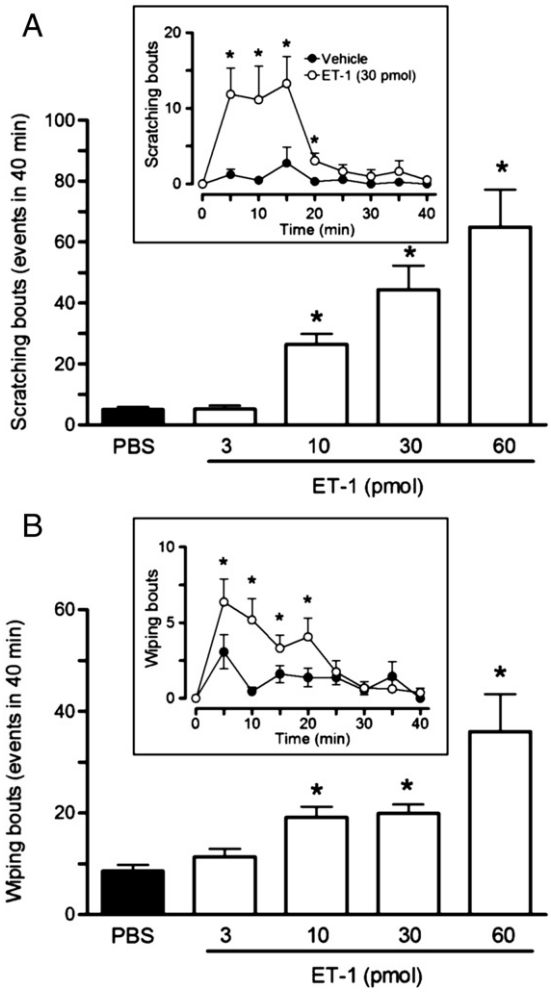

Fig. 1. Injection of ET-1 into the cheek evokes pruritic and nociceptive behaviors in mice. Histograms indicate the total number of scratching (A) and wiping (B) bouts observed over the first 40 min after i.d. injection of ET-1 at the doses indicated or PBS. The insets show the time-courses of each behavioral response to ET-1 (30 pmol), in 5-min bins. Values represent the mean ± SEM of 6–8 animals. Asterisks denote $P<0.05$ when compared to the corresponding value of PBS-treated animals (ANOVA followed by Newman–Keuls test).

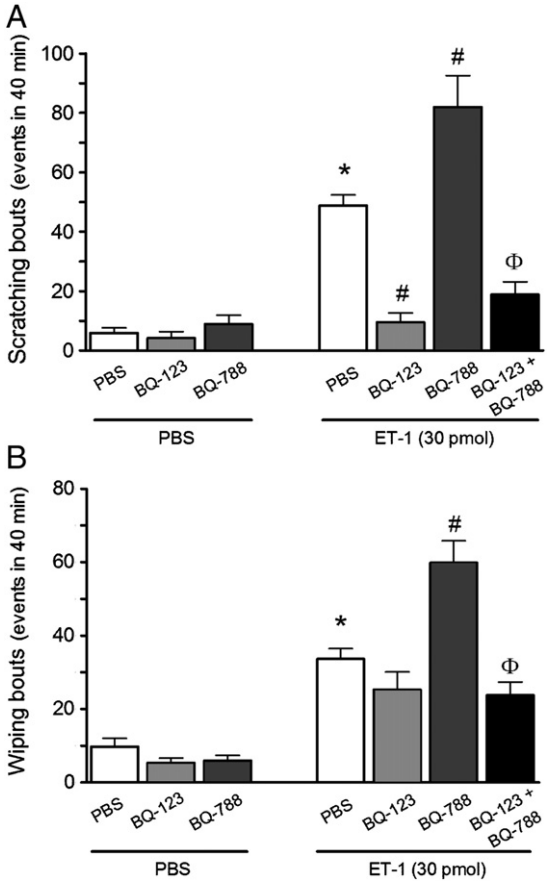

Fig. 2. Influence of ET_A or ET_B receptor antagonists on scratching (A) and wiping (B) behaviors induced by ET-1 in the cheek. BQ-123 (ET_A receptor antagonist), BQ-788 (ET_B receptor antagonist), both antagonists (BQ-123 + BQ-788; each at 10 nmol) or PBS were injected into the left cheek 5 min before i.d. injection of ET-1 (30 pmol) or PBS into the same cheek. Values represent the mean ± SEM of 6–8 animals. Asterisks, fences and φ denote $P<0.05$ relative to groups treated with PBS + PBS, ET-1 + PBS or ET-1 + BQ-788, respectively (ANOVA followed by Newman–Keuls test).

Co-administration of CTOP (20 nmol/site) potentiated the pruritoceptive effect of ET-1 (10 pmol/site) by 60%, but the nociceptive effects of the peptide were unaffected by this selective μ-opioid receptor antagonist (Fig. 3). Nonetheless, the selective agonist of μ-opioid receptors DAMGO (100 nmol/site) reduced both behavioral responses to ET-1, inhibiting scratching and wiping bouts by 50% and 87%, respectively. Basal responses of vehicle-treated controls were not modified by co-administration of CTPO or DAMGO.

Systemic pretreatment with the histamine H_1 receptor antagonist loratadine (10 mg/kg) significantly reduced both effects of ET-1, but scratching bouts were inhibited to a lesser extent than wiping bouts (22% and 61% inhibition, respectively; Fig. 4). Loratadine did not modify the responses of PBS-treated control mice.

Discussion

Humans can accurately discriminate itch from pain and report on the relative intensities at which many stimuli evoke these sensations. In contrast, preclinical behavior-based models to characterize pruritic and/or nociceptive effects of substances or procedures are subjected to the potential bias of assuming that the behaviors measured are specific responses to pruritus or nociception. For example, humans report that i.d. injections of histamine and capsaicin cause itch and pain, respectively, but when these substances are injected into the nape of the neck of mice, they both evoke bouts of hind limb scratching directed to the injection site, a behavior interpreted as indicative of pruritoception (Shimada and LaMotte, 2008). However, by changing the site of injection of these substances to the cheek, the authors could clearly distinguish the bouts of hind limb scratching (pruritus) evoked by histamine from the bouts of forepaw wipes (nociception) induced by capsaicin.

Previous studies concluded that ET-1 displays pruritoceptive and nociceptive properties, based on its ability to induce back scratching and paw licking behaviors, respectively. The main contribution of the current study is that it demonstrates, to our knowledge for the first time, that ET-1 indeed evokes unbiased dose-dependent behavioral responses suggestive of both pruritus and nociception in the mouse cheek model, alongside results which fully confirm the clear-cut distinct behavioral effects of histamine and capsaicin originally reported by Shimada and LaMotte (2008).

Using this same cheek model, Akiyama et al. (2010a,b) found that agonists of protease-activated receptor subtypes PAR-2 and PAR-4 and the anti-malarial drug chloroquine elicit mainly bouts of hind limb scratching, whereas bradykinin and the TRPA1 receptor agonist allyl isothiocyanate selectively evoke bouts of forelimb wipes. Interestingly, those studies also reported that serotonin, cowhage spicules and formalin promote approximately equal amounts of both types of behavior. Thus, ET-1 appears to belong to a category of substances

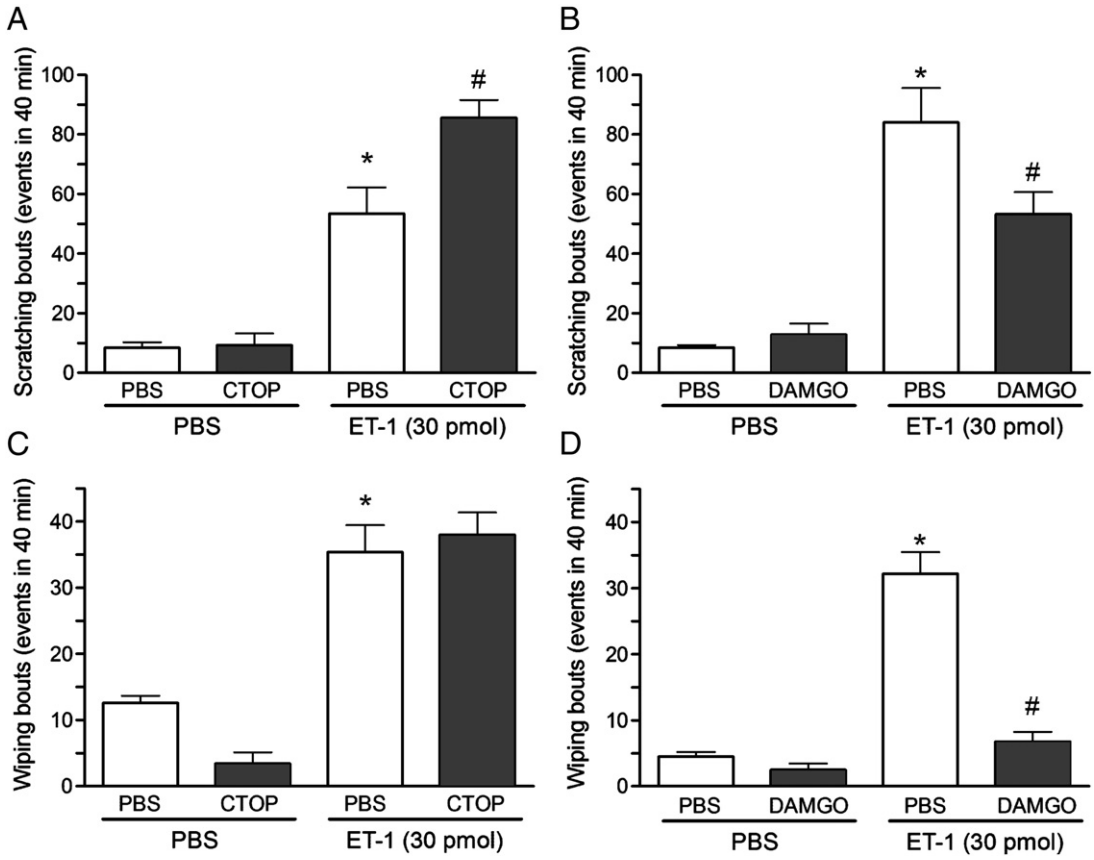

Fig. 3. Influence of μ-opioid receptor-mediated mechanisms on scratching (A, B) and wiping (C, D) behaviors induced by ET-1 in the cheek. The selective μ-receptor antagonist CTOP (20 nmol; in A and C) or agonist DAMGO (100 nmol; in B and D) were co-injected together with either ET-1 (30 pmol, i.d.) or PBS. Values represent the mean ± SEM of 6–8 animals. Asterisks and fences denote $P<0.05$ relative to groups treated with PBS + PBS or to ET-1 + PBS, respectively (ANOVA followed by Newman–Keuls test).

with dual pruritoceptive and nociceptive properties. In addition, the fact that volunteers receiving i.d. injections of ET-1 into the forearm report a sensation of burning pruritus (Ferreira et al., 1989) further substantiates the validity of the cheek model in behaviorally discriminating both components and its potential in reproducing the sensory effects the peptide promotes in humans.

The current study shows that local treatment of the cheek with the ET_A receptor antagonist BQ-123 markedly reduced the pruritic effects of ET-1, without modifying significantly its nociceptive effects. Indeed, scratching induced by ET-1 was also found to be mediated by ET_A receptors in the nape of the neck model (Trentin et al., 2006; McQueen et al., 2007; Liang et al., 2010a). The failure of ET_A receptor blockade to inhibit the nociceptive effect of ET-1 in the cheek was rather unexpected, as previous studies had shown that BQ-123 reduces ET-1-induced hind paw licking in mice (Piovezan et al., 2000; Menéndez et al., 2003) or hind paw flinches in rats (Khodorova et al., 2003). However, contrasting the reported lack of effect of the ET_B receptor antagonist BQ-788 against ET-1-induced hind paw licking in mice (Piovezan et al., 2000; Menéndez et al., 2003), we observed that both nociceptive and pruritoceptive responses to injection of ET-1 in the cheek were increased following local treatment with this antagonist. Back scratching induced in mice by ET-1 injection into the nape of the neck was also found to be potentiated by BQ-788 and reduced by co-injection with an ET_B receptor agonist (Trentin et al., 2006; Liang et al., 2010a), but another study (using a different strain of mice) failed to confirm an anti-pruritic role for ET_B receptors in that model (McQueen et al., 2007). On the other hand, it is noteworthy that co-injection of BQ-123 together with BQ-788 significantly reduced both the pruritoceptive and nociceptive effects of ET-1 in the cheek model, relative to the (augmented) responses the peptide induced following BQ-788 treatment alone. We interpret these results as evidence that ET_A receptors mediate both effects of ET-1 in the cheek model, even if the nociceptive response to ET-1 following combined antagonist (BQ-123 plus BQ-788) treatment was not significantly different from the one it induced on its own. This view is strengthened by reports that ET_B receptors exert an anti-hyperalgesic effect in the mouse hind paw against nociception induced by capsaicin, heat or cancerous cells (Piovezan et al., 2000; Baamonde et al., 2004; Quang and Schmidt, 2010) and also limit ET-1-induced ET_A receptor-mediated hind paw flinching in rats (Khodorova et al., 2003).

Itch may be inversely related to pain because it is a common side-effect of centrally-administered opioids and is reduced by nociceptive counter-stimuli such as vigorous scratching (Szarvas et al., 2003). On the other hand, the nociceptive effect of ET-1 in the rat hind paw is limited by activation of ET_B receptors on skin keratinocytes coupled to release of β-endorphin, which in turn activates antinociceptive μ-opioid receptors on the nociceptive sensory fibers (Khodorova et al., 2003). Moreover, i.d. injection into the nape of the neck of loperamide, morphine or the selective μ-opioid receptor agonists fentanyl and DAMGO can each cause scratching behavior in mice (Yamamoto and Sugimoto, 2010; Yamamoto et al., 2010). In contrast, i.d. injection of morphine into the cheek fails to promote scratching behavior in mice (Kuraishi et al., 2000). We thus tested the effects of i.d. injections of the selective μ-opioid receptor antagonist CTOP and agonist DAMGO, at doses not affecting responses of PBS-treated control mice, on the pruritic and nociceptive behaviors evoked by ET-1. CTOP increased the number of scratching bouts induced by ET-1, but not of wiping bouts. This could suggest that μ-opioid receptors activated locally by endogenous opioids only inhibit the pruritic effects of ET-1 in the cheek. However, this does not appear to be the case, as we observed that DAMGO reduced both types of behavior evoked by

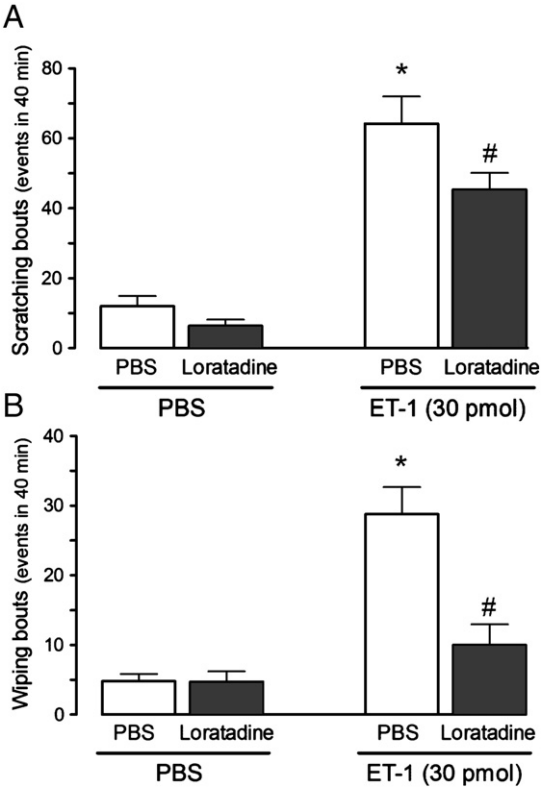

Fig. 4. Influence of the histamine H_1 receptor antagonist loratadine on scratching (A) and wiping (B) behaviors induced by ET-1 in the cheek. Mice were given an intraperitoneal injection of loratadine (10 mg/kg) or PBS 1 h before i.d. injection of ET-1 (30 pmol) or PBS into the left cheek. Values represent the mean ± SEM of 6–8 animals. Asterisks and fences denote $P<0.05$ relative to groups treated with PBS + PBS or ET-1 + PBS, respectively (ANOVA followed by Newman–Keuls test).

ET-1. This is at variance with a recent report that systemic (subcutaneous) injection of morphine does not modify scratching induced by ET-1 in the nape of the neck model (Liang et al., 2011). Thus, it may be that in the cheek, which is innervated by the trigeminal system, pruritus can suppress nociception, i.e. the opposite to what occurs in other body regions. Clearly further studies are needed to better understand the relations between both sensory modalities in this new model, as well as their modulation by mechanisms signaled locally by the endogenous opioids present and the receptor subtypes they activate.

Besides evoking a sensation of burning pruritus (Ferreira et al., 1989), injection of ET-1 into the forearm of volunteers also induces a wheal and flare response which is reduced by treatment with histamine H_1 receptors antagonists, but fails to release histamine from human acutely dispersed skin mast cells (Brain et al., 1992). Other studies, however, have shown that ET-1 can induce degranulation of peritoneal mast cells (Yamamura et al., 1994; Maurer et al., 2004). On the other hand, nociception induced by ET-1 in the mouse hind paw is markedly reduced by depletion of local resident mast cells (Rae et al., 2001), but its ability to evoke scratching behavior in the nape of the neck seems independent of histamine-mediated signaling mechanisms (Imamachi et al., 2009). The current study demonstrates that, in the cheek model, systemic treatment with the histamine H_1 receptor antagonist loratadine markedly suppressed bouts of forepaw wipes evoked by ET-1, while inhibiting only slightly (albeit significantly) the bouts of hind limb scratching induced by the peptide. These findings indicate that histamine, possibly released from mast cells and acting via H_1 receptors, contributes importantly to the nociceptive effects of ET-1, but plays a minor role in its pruritic effect.

The results of the current study show that ET-1 evokes distinct pruritic and nociceptive behaviors in the mouse cheek. These findings essentially confirm, in a single model, previous reports that the peptide elicited behaviors suggestive of either one or the other type of sensation, depending on the behavioral model used. Considering that ET-1 can be produced in the skin by mast cells, keratinocytes and endothelial cells, perhaps this experimental model could be useful to investigate the contribution of endogenous ET-1 to alterations in the pruritoceptive and nociceptive mechanisms associated with skin inflammation.

Conclusion

The results of the current study demonstrate that i.d. ET-1 injection into the cheek of the mouse evokes two distinct behavioral responses which are indicative of pruritus and nociception. Both effects of ET-1 are mediated via ET_A receptors and limited by simultaneous activation of ET_B receptors. Endogenous opioids acting on μ-opioid receptors inhibit only the pruritoceptive effect of ET-1. Conversely, histamine, acting on H_1 receptors, seems to contribute importantly to the nociceptive effect of ET-1, but less so to its pruritoceptive effect.

Conflict of interest statement

The authors declare that they have no conflicts of interest.

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