British Journal of Pharmacology (2007) 151, 278–284
 0007–1188/07 $30.00
www.brjpharmacol.org

RESEARCH PAPER

Endothelin-1 activates ETA receptors to cause reflex scratching in BALB/c mice

DS McQueen, MAH Noble and SM Bond

Division of Neuroscience, School of Biomedical Sciences, University of Edinburgh College of Medicine & Veterinary Medicine, University of Edinburgh, Edinburgh, UK

Background and purpose: Endothelin-1 (ET-1) is present in murine and human skin and causes itch (pruritus) when injected in humans. This behavioural study examined the scratch reflex evoked by ET-1 in mice.
Experimental approach: An automated detector was used to determine whether ET-1 causes reflex scratching, the behavioural correlate of itching, in BALB/c mice. Selective agonists and antagonists were used to probe the ET receptor(s) involved.
Key results: ET-1 evoked dose-related reflex scratching lasting up to 20 min following intradermal injection (0.1-100 ng; 0.04-40 pmol). The ED_{50} for ET-1 induced scratching was 2.1 ng and desensitization occurred with cumulative dosing. High doses of the ET_B receptor agonist IRL1620 (10 μg; 5.5 nmol), also caused scratching (ED_{50} 1.3 μg, 0.7 nmol). The ET_A receptor antagonist BQ123 significantly reduced scratching evoked by ET-1 and IRL 1620, suggesting that both agonists caused scratching via an ET_A receptor-dependent mechanism. The ET_B receptor antagonist BQ788 significantly reduced scratching evoked by IRL1620 but had no effect on scratching evoked by ET-1. This indicated that activation of ET_B receptors by high doses of ET_B agonist, but not ET-1, can trigger scratching.
Conclusion and implications: ET-1 is a potent endogenous activator of reflex scratching (itch). Mechanisms for ET-induced scratching are considered, including direct action of ET-1 on pruriceptive nerve endings and indirect actions via release of endogenous mediators such as histamine from mast cells. ET-1 and ET_A receptors, possibly also ET_B receptors, are potential targets for developing specific anti-pruritic drugs to treat pruritic skin disorders such as atopic dermatitis.
British Journal of Pharmacology (2007) **151**, 278–284. doi:10.1038/sj.bjp.0707216; published online 12 March 2007

Keywords: endothelin-1; ETA receptors; ET_B receptors; BQ123; BQ788; pruritus; itch; IRL1620; BALB/c mice; pruriceptors

Abbreviations: AD, atopic dermatitis; CGRP, calcitonin Gene-related peptide; CI, confidence interval; ET-1, endothelin-1; ET_A, endothelin A receptor; ET_B, endothelin B receptor; H_1, histamine H_1 receptor; PBS, phosphate-buffered saline

Introduction

Endothelin-1 (ET-1) is a 21 amino-acid peptide which is an endogenous agonist at Endothelin A (ET_A) and Endothelin B (ET_B) receptors (Masaki *et al*., 1994). ET-1 has been reported to cause itch (pruritus) when injected intradermally in man (Ferreira *et al*., 1989), but detailed study of this pruritogenic action has not been undertaken. The peptide is present in human skin (Bull and Dowd, 1993) and its synthesis and release has been demonstrated in cultures of human endothelial cells and keratinocytes (Terenghi *et al*., 1991; Yohn *et al*., 1993). ET-1 is upregulated in response to hypoxia, sheer stress and a range of inflammatory cytokines (Kurihara *et al*., 1989; Yoshizumi *et al*., 1990; Horio *et al*., 1991; Elton *et al*., 1992). It has potent vasoconstrictor actions (Yanagisawa and Masaki, 1989), and can evoke the triple response in skin (Brain *et al*., 1992). ET-1 is also involved in cell signalling (Simonson and Dunn, 1990) and inflammatory processes (Xu *et al*., 1998; Kuryliszyn-Moskal *et al*., 2005), which are important in chronic itchy skin disorders such as atopic dermatitis (Goligorsky *et al*., 1999; Stander and Steinhoff, 2002).

The present investigation was performed to establish whether ET-1, which is present in murine skin (Ahn *et al*., 1998), causes pruritus when injected into the outer layers of the skin in BALB/c mice. Selective endothelin receptor agonists and antagonists (Davenport, 2002) were used to characterize the pharmacological receptors involved in the response obtained. Pruritus was measured non-invasively using an automated scratch detector to record reflex scratching (Brash *et al*., 2005), which is the behavioural correlate of itch in animals. There is considerable clinical need for specific anti-itch drugs (Rees and Murray, 2005;

Correspondence: Professor DS McQueen, Division of Neuroscience, School of Biomedical Sciences, University of Edinburgh College of Medicine & Veterinary Medicine, University of Edinburgh, 1 George Square, Edinburgh EH8 9JZ, UK.
E-mail: d.s.mcqueen@ed.ac.uk
Received 27 November 2006; accepted 31 January 2007; published online 12 March 2007

Summey and Yosipovitch, 2005), and the information obtained from this functional study should be valuable for the development of new drugs.

Methods

Animals

All experiments were performed in accordance with Local Ethics Committee approval and UK Home Office regulations. Female BALB/c mice ($n=84$; Charles River, Margate, Kent, UK), 10-week-old adults, were used in the experiments. Mice weighed 16–21 g and were housed under controlled light (0700–1900 hours) and temperature (22°C) with food and water available *ad libitum*.

Procedure

The itch-inducing properties of ET-1 and the ET_B agonist IRL1620 (Davenport, 2002) were studied by injecting the drugs intra-dermally (i.d.; into the outer layer of skin; could also be termed subdermal or intra-cutaneous) in the nape of the unshaven neck via a 26 G needle and measuring the subsequent reflex scratching of the area by the hind paw during the 20 min post-injection period. Doses of ET-1 or IRL1620 were administered at 90 min intervals on a cumulative basis. Control values were taken from the results of phosphate-buffered saline (PBS) injections given during this study and in previous experiments using the same protocol (Bell *et al*., 2004). After dose–response experiments involving ET-1, 5-HT (10 μg, i.d.) was administered 60 min after the last ET-1 dose to check that mice still retained the ability to scratch in response to a standard pruritogen.

In experiments involving the antagonist, scratching was induced by a mid-range dose of ET-1 (3 ng; 1.2 pmol) or IRL1620 (10 μg; 5.5 nmol), with a repeat injection 90 min after the initial dose. These doses were chosen on the basis of results from the dose–response experiments (see Results). The ET_A antagonist, BQ123 (Davenport, 2002), was administered at a dose of 500 $\mu g\,kg^{-1}$ intra-peritoneally (i.p.), 30 min before the second injection of agonist. This dose was chosen on the basis of its effectiveness in reducing scratching in earlier protocols involving 10, 50 and 500 $\mu g\,kg^{-1}$ (i.p.) BQ123. The effect of the same dose of BQ123 on IRL1620-induced scratching was also assessed. The ET_B antagonist, BQ788 (Davenport, 2002), was administered cumulatively in doses of 5, 50 and 500 $\mu g\,kg^{-1}$ (i.p.), following the same protocol as used for studying BQ123.

To minimize any behavioural changes owing to the novel environment of the recording boxes, all mice were acclimatized to the boxes for 20–30 min before the experiment, with another 30 min in the holding cage in advance of any recording.

Measurement of scratching

Scratching was measured using the repetitive movement detector system designed, built and validated in our laboratory (Brash *et al*., 2005). Briefly, four mice were placed in individual lightweight Styrofoam boxes, which were placed on separate force platforms, positioned over sensitive strain gauges. Scratching evoked by i.d. injection in this strain of mice was mainly in the 11–15 Hz range, and the detector was set to record all repetitive motor movements of a frequency of 11–20 Hz; grooming behaviour occurs below this frequency. A 'bout' of scratching was recorded when three or more repetitive scratch movements or 'beats' made by the hindlimb were recorded and ended when the interval between successive movements was greater than 0.2 s. The total number of scratch movements in each bout was recorded, and on average there were five scratch movements per bout, a value that was independent of the pruritogen and the dose injected.

Data were recorded and processed using a MacLab Recorder. To verify that only scratching events were being automatically processed, the mice were filmed using a video camera (Vista, NCD 132) positioned above the recording boxes, and the image was recorded on a VCR (Panasonic, NV-HD640). This arrangement enabled the observer remotely to ensure the animals' welfare, observe behaviour, and note any 'non-scratch' events for checking against the automated data record. The scratch score from the automated system correlated very well with the very time-consuming operator-derived manual scoring of activity recorded on video tape, as reported previously (Brash *et al*., 2005).

Scratching was recorded over a 20 min period immediately following the i.d. injection, on the basis of previous studies using histamine as a pruritogen in man and animals (Keele and Armstrong, 1964; Laidlaw *et al*., 2002; Bell *et al*., 2004), and during pilot experiments in this study.

Statistical analysis

Data are shown as mean ± s.e.m. for n values or mean with 95% confidence intervals (CI), unless otherwise stated. Log dose–response curves were plotted using the nonlinear regression (sigmoidal log dose–response) function in GraphPad Prism v4.0. The mean 'apparent' ED_{50}, the estimated dose to elicit half the apparent maximal response under non-steady conditions *in vivo*, was calculated from the pooled data. Non-parametric statistical tests used are given in the text, and the null hypothesis was rejected at $P<0.05$.

Materials

ET-1 (MW 2492; Tocris, Avonmouth, UK), BQ123 (ET_A antagonist, MW 611; Tocris), BQ788 (ET_B antagonist MW 664; Tocris), histamine diphosphate (MW 307; Sigma, Poole, UK) and 5-HT (serotonin creatinine sulphate, MW 387; Sigma) were all dissolved in PBS, (pH = 7.4). IRL1620 (ET_B agonist, MW 1820; Tocris), was dissolved in PBS (pH = 7.4; stock solution: saline with 4% hydrochloric acid). ET-1 and IRL1620 were injected in a volume of 100 μl. The antagonists BQ123 and BQ788 were injected i.p. in a volume of 100 μl.

Results

Scratching induced by ET-1

ET-1 caused dose-dependent scratching when injected i.d. in doses ranging from 0.1 to 1000 ng (0.04–400 pmol), as

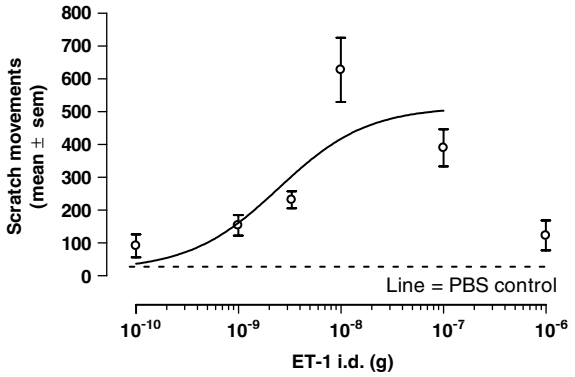

Figure 1 Pooled data for ET-1-induced scratching in female BALB/c mice during the 20-min post-injection period ($n=8$–40). ET-1 induced dose-dependent scratching. Dashed line represents the mean level of scratching induced by injection of 100 μl PBS i.d., which served as a control (31 ± 8, scratch movements in 20 min, $n=18$). The solid line shows a sigmoidal curve fitted to scratching evoked by ET-1 over the range 10^{-10}–10^{-7} g.

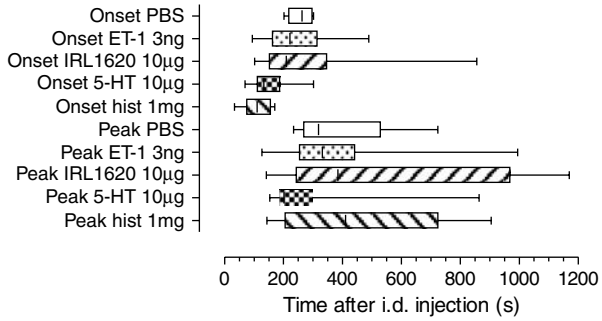

Figure 2 Box and whisker plot showing the range, median, upper and lower quartiles for the delay to onset of scratching and the time at which the maximal response occurred following i.d. injection of PBS (100 μl; $n=4$), ET-1 (3 ng; $n=29$), IRL1620 (10 μg; $n=23$), 5-HT (10 μg; $n=15$) and histamine (1000 μg; $n=10$). Scratching evoked by ET-1 or IRL1620 began significantly later than that caused by 5-HT or histamine ($P<0.05$; Kruskal–Wallis, with Dunn's *post hoc* test) and this test also showed that the onset for scratching following injection of ET-1 was not significantly different from that for IRL1620. There was no significant difference between 5-HT and histamine in the delay to onset of scratching. The lower part of the figure shows the time at which scratching was most intense following an i.d. injection, which varied considerably between individual animals within treatment groups, and between groups. There was no significant difference between the group median values, apart from for the peak response to IRL1620 which occurred significantly later than that to 5-HT ($P<0.05$; Kruskal–Wallis, with Dunn's *post hoc* test; the other combinations did not differ significantly; $P>0.05$).

summarized in Figure 1. The scratch response tended to diminish following doses of ET-1 greater than 10 ng, and the overall log dose–response plot for cumulative dosing was bell-shaped. Statistical analysis showed that ET-1 induced scratching which, apart from the lowest (0.1 ng) and highest (1000 ng) cumulative doses of ET-1 was significantly greater than that caused by a control injection of the same volume of PBS ($P<0.05$; Kruskal–Wallis test with Dunn's *post hoc* test versus PBS).

The mean apparent ED_{50} for scratching induced by ET-1 i.d. was calculated as 2.5 ng (1.0 pmol; 95% CI 0.6–9.5 ng) from a sigmoidal plot fitted to the pooled data for doses in the range 10^{-10}–10^{-7} g (Figure 1).

Desensitization. Injection of ET-1 (100 ng) as the first, rather than the last dose of an increasing sequence, evoked 662 ± 66 scratches ($n=5$), which was significantly more than observed during cumulative dose–response experiments (Figure 1; $P<0.05$; Mann–Whitney test), and was not significantly different from the response to 10 ng ET-1 during cumulative dosing (Figure 1; $P>0.05$, Mann–Whitney test).

The time to onset of scratching following ET-1 or IRL1620 was somewhat variable and not dose-dependent. Most of the scratching occurred during the first 15 min following injection, and had normally ended by 20 min. Scratching induced by a mid-range dose of ET-1 (3 ng) started significantly later than that evoked by a standard dose of histamine (data by Bell *et al.*, 2004), as can be seen from the summary in Figure 2.

A single injection of 5-HT 10 μg (25 nmol) was made 60 min after the last dose of ET-1, to determine whether mice retained the ability to respond to a pruritogenic compound despite the apparent decline in responsiveness following cumulative high doses of ET-1. The mean response to 5-HT was 325 ± 49 scratches ($n=16$), which was not significantly different from control responses to 5-HT 10 μg given before any other substance (369 ± 45 scratch movements, $n=7$; $P>0.05$ Mann–Whitney test versus 5-HT after preceding doses of ET-1).

Subjectively, no abnormal behaviour was observed in the mice after administration of ET-1. However, following the highest doses (100 and 1000 ng) the mice generally were less active and spent more time in the corner of the recording box.

Role of ET_A receptors in scratching induced by ET-1

The ET_A antagonist, BQ123, reduced scratching induced by the 3 ng (1.2 pmol) mid-range dose of ET-1, as shown in Figure 3. Lower doses of BQ123 (10 and 50 μg kg^{-1} i.p.) reduced scratching, but with some variability such that group mean values before and after the antagonist were not significantly different. A higher dose of antagonist (500 μg kg^{-1}) was required to achieve a statistically significant reduction in ET-1-induced scratching. The scratch response to ET-1 returned to normal approximately 3 h after BQ123 500 μg kg^{-1}. Subjectively, no alteration in behaviour was observed in mice given BQ123.

Effect of the ET_B receptor agonist IRL1620 on scratching behaviour

IRL1620 evoked dose-dependent scratching, as summarized in Figure 4a. The mean apparent ED_{50} for the agonist determined from the pooled data was 1.3 μg (0.7 nmol) with 95% CI 0.08–21 μg. On average, the scratching induced by IRL1620 started 266 ± 48 s ($n=12$) after i.d. injection, which was not significantly different from the mean onset of response to ET-1 ($P>0.05$; Mann–Whitney test). Scratching

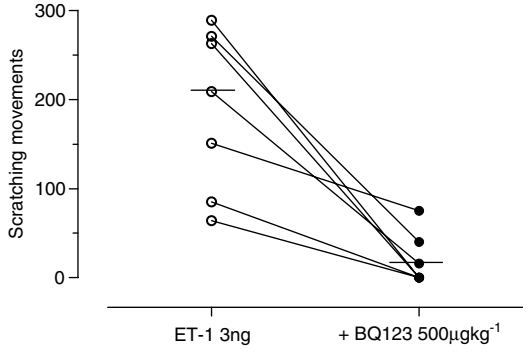

Figure 3 The ET_A receptor antagonist, BQ123, (500 μg kg^{-1} i.p.), significantly reduced ET-1-induced scratching in BALB/c mice ($P<0.05$; Wilcoxon signed rank test). Open symbols show response before, filled symbols after the antagonist, and lines connect responses from individual animals ($n=7$). Horizontal bar shows the median value.

evoked by IRL1620 was only significantly different from that caused by PBS at the highest dose (10 μg; 5.5 nmol; Kruskal–Wallis test with Dunn's *post hoc* test versus PBS). The number of scratch movements caused by 10 μg IRL1620 was not significantly different from that evoked by ET-1, 3 ng, ($P>0.05$; Mann–Whitney test, Figures 1 and 4a).

Effect of the ET_A receptor antagonist BQ123 on scratching induced by IRL1620

The ET_A antagonist, BQ123 (500 μg kg^{-1} i.p.), significantly reduced scratching induced by the 10 μg dose of IRL1620 (Figure 4b; $P<0.05$; Wilcoxon paired rank test). The antagonism lasted for several hours before responses to the agonist recovered. Subjectively, no change in behaviour was observed after the ET_A antagonist.

Effect of the ET_B receptor antagonist BQ788 on scratching induced by ET-1 and IRL 1620

The ET_B antagonist, BQ788 (5–500 μg kg^{-1} i.p.), had no significant effect on scratching induced by the 3 ng mid-range dose of ET-1 ($P>0.05$; Kruskal–Wallis test with Dunn's *post hoc* test versus control), as shown in Figure 5a. However, scratching evoked by the 10 μg dose of IRL1620 was significantly reduced by the 50 and 500 μg kg^{-1} doses of BQ788 (Figure 5b; $P<0.01$; Kruskal–Wallis test with Dunn's *post hoc* test versus control).

Discussion

The results of this study show that ET-1 is a very potent pruritogen in mice, causing dose-dependent scratching, the behavioural correlate of itch, with a mean apparent ED_{50} of 1 pmol (2.5 ng). ET-1 is therefore about six million times more potent as a pruritogen than histamine, which has an apparent ED_{50} of 5.8 μmol in this strain of mice (Bell *et al*., 2004). The log dose–response plot for ET-1-induced scratching was bell-shaped and cumulative dosing with the peptide led to desensitization of the scratch reflex.

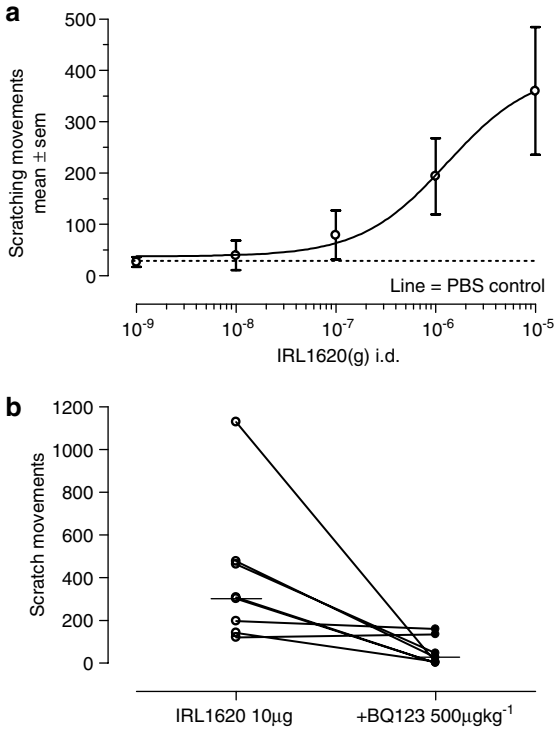

Figure 4 (**a**) Pooled data for scratching evoked by the ET_B receptor agonist, IRL-1620 during the 20 min post-injection period ($n=4$–8). Sigmoidal curve fitted to data points. Dashed line represents the mean level of scratching induced by PBS, which served as a control for the injection. (**b**) The effect of ET_A receptor antagonist, BQ123 (500 μg kg^{-1} i.p.), on scratching induced by the 10 μg dose of IRL1620 ($n=8$). Responses before (open symbols) were significantly reduced after (closed symbols) the antagonist ($P<0.05$; Wilcoxon paired rank test). The lines join paired responses from individual animals, and the horizontal bars represent the median values.

Two previous studies found that ET-1 causes pruritus when administered to human skin. Both Ferreira *et al*. (1989) and Katugampola *et al*. (2000) described the development of a burning pruritus in human subjects following intradermal injection of ET-1. The present experiments in mice used an automated repetitive movement detector (Brash *et al*., 2005) to measure accurately the scratching induced by injecting ET-1, and we thought this was the first report of the pruritogenic action of ET-1 in BALB/c mice. However, after the work was submitted for publication, a paper was published which demonstrated that ET-1 causes pruritus in mice via similarly potent actions involving ET_A receptors in murine skin (Trentin *et al*., 2006).

Scratching in animals as a behavioural correlate of itch

It is impossible to determine what sensation any animal experiences, so behavioural correlates have to be used. Animals may scratch in response to painful stimuli as well as to those that are associated with different human sensations, such as itch and tickle (McMahon and Koltzenburg, 1992). However, it has been shown that whereas the pruritogenic agents compound 48/80 and substance P cause scratching when injected subcutaneously in mice, the

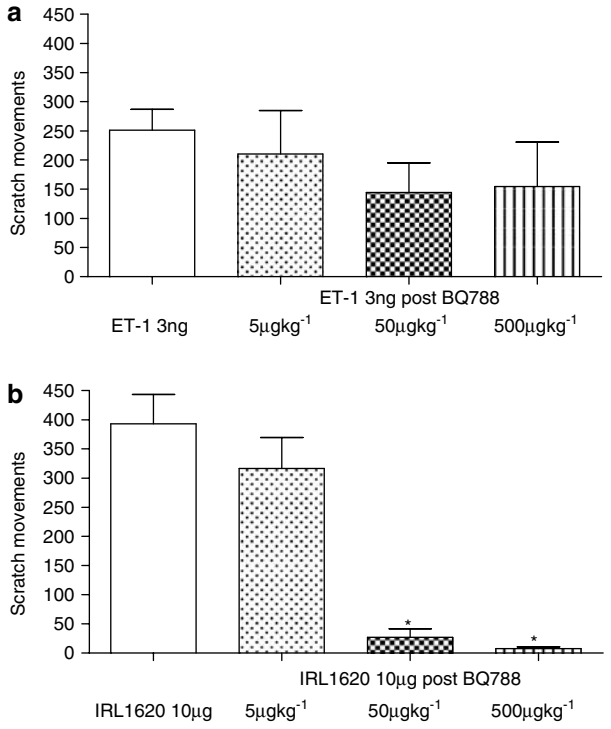

Figure 5 (**a**) The effect of the ET_B receptor antagonist BQ788 (5–500 μg kg^{-1} i.p.) on scratching induced by ET-1 (3 ng; $n=7$–11). Control responses to ET-1 (open column) did not differ significantly from those observed following increasing doses of antagonist (shaded columns; $P>0.05$; Kruskal–Wallis test with Dunn's *post hoc* test versus control). (**b**) The ET_B receptor antagonist, BQ788 (5–500 μg kg^{-1} i.p.), antagonized scratching induced by the ET_B agonist IRL1620 10 μg ($n=8$). Responses were significantly reduced by the two highest doses of the antagonist (shaded columns), in comparison with scratching in the pre-antagonist control (open column; *$P<0.01$; Kruskal–Wallis test with Dunn's *post hoc* test versus control).

algogenic (pain producing) agents capsaicin and formalin do not, but instead usually cause vocalization, biting and withdrawal (Kuraishi *et al*., 1995). We found that histamine or 5-HT caused dose-related scratching in mice, whereas injection of algogens evoked slight scratching which did not differ in intensity from that induced by PBS (Bell *et al*., 2004). Injection of histamine into human skin is invariably associated with the sensation of itch and reflex scratching (Keele and Armstrong, 1964), and even very high doses of ET-1 (up to 50 μg i.d.) only caused itch (Ferreira *et al*., 1989). We therefore consider it probable, by analogy with the results from man, that mice in the present study were scratching in response to 'itch' rather than 'pain' evoked by low doses of ET-1. It is the reflex scratching which causes damage to the skin in response to pruritogens both in man and mice, and that behaviour can be studied objectively in animals.

Involvement of ET_A and ET_B receptors in ET-1-induced scratching

The selective ET_A antagonist, BQ123 (Ihara *et al*., 1992), significantly reduced scratching evoked by a mid-range dose of ET-1 (Figure 3), which strongly suggests the involvement of ET_A receptors in ET-1-induced itch. The selective ET_B agonist, IRL-1620 (Takai *et al*., 1992), also induced scratching, which could be interpreted as evidence for involvement of ET_B receptors in the scratch reflex response to ET-1. However, the ED_{50} for the scratch response to IRL-1620 was 875 times greater than that of ET-1 (Figure 4a), and only scratching evoked by the highest dose of IRL-1620 was significantly greater than the vehicle-induced response. It therefore seemed probable that the high dose of IRL-1620 evoked scratching via weak agonist action at ET_A receptors (Takai *et al*., 1992). This was confirmed by showing that the ET_A antagonist, BQ123, significantly reduced scratching in response to IRL-1620 (Figure 4b).

The possibility existed that BQ123 might exert its antipruritic action through sedation, as occurs with sedating histamine H_1 receptor antagonists (Nicholson, 1983; Shuster, 1988). However, this was rejected because there is no evidence that ET_A antagonists such as BQ123 cause sedation, and we did not see any change in spontaneous exploration or grooming behaviour following BQ123 administration.

Taken together, this functional integrative behavioural evidence from intact animals suggested that ET-1 acts via ET_A receptors to evoke reflex scratching. Further studies were undertaken using the ET_B antagonist, BQ788, in the expectation that these would provide additional evidence that IRL 1620 evokes scratching via an ET_A receptor-dependent mechanism, that is, they would serve as a negative control. In fact, we found that scratching evoked by IRL1620 was antagonized by BQ788, whereas that caused by ET-1 was unaffected. This evidence therefore may mean that ET_B receptors are involved in scratching evoked by the ET_B agonist, IRL1620, but not in scratching caused by ET-1, which is an endogenous non-selective ET_A and ET_B receptor agonist.

Trentin *et al*. (2006) used male Swiss mice and measured bouts of scratching evoked by ET-1 (1–20 pmol). Scratching was antagonized by co-injecting the ET_A antagonist BQ123 into the skin, or by i.p. administration of a different ET_A receptor antagonist, atrasentan. Our results with ET-1 and BQ123 in female BALB/c mice confirm some of the findings of Trentin *et al*. (2006) and provide additional evidence that ET-1 is a potent pruritogen in mice, apparently acting via ET_A receptors in the skin. These authors also reported that IRL1620 (10 pmol) was not pruritic, and that accords with our findings using this dose (Figure 4a). However, we observed that higher doses of the ET_B agonist are pruritic, whereas they found that, when co-injected, it was anti-pruritic, inhibiting scratching evoked by ET-1 or histamine, an effect we did not investigate.

Our results showed that systemic administration of the ET_B antagonist, BQ788, did not affect the scratch response to the non-selective $ET_{A/B}$ receptor agonist ET-1 (Figure 5a), in contrast to the finding of Trentin *et al*. (2006) that co-injecting BQ788 enhanced scratching evoked by ET-1, suggesting that ET_B receptors inhibit scratching. Our results are thus discrepant, but Trentin *et al*. (2006) did comment on their unexpected finding that systemic administration of the ET_B antagonist A-1292621 diminished scratching induced by ET-1.

Attempting to characterize receptors on the basis of behavioural studies involving high doses of agonists and antagonists is complicated by the issue of the selectivity of

drugs for receptors in particular species. Thus, we found that only high doses of the ET_B agonist IRL1620 cause scratching, which was antagonized by the ET_A receptor antagonist BQ123 and also by the ET_B receptor antagonist BQ788. This suggests that ET_B receptors, as well as ET_A receptors are involved in evoking scratching, but that could be a misinterpretation if high doses of agonist and/or antagonist are acting non-selectively in mice to affect ET_A receptors. We did not co-inject drugs locally, and the situation is complicated by the possibility that systemic and local effects of a drug will differ *in vivo*. Also, as suggested by the delay to the start of scratching, ET-1 may be acting indirectly on pruriceptors via release of pruritogenic mediators (e.g. histamine from mast cells; Yamamura *et al*., 1994b). It seems probable that ET_A and ET_B receptors interact via receptor 'crosstalk' (Mickley *et al*., 1997), which makes it difficult to establish what contribution is made by the individual receptor subtypes in response to the endogenous ligand, ET-1. Further studies using *in vitro* techniques to establish the location and the type(s) of ET receptor involved in evoking the scratching reflex are clearly needed.

Mechanisms of ET-1-induced itch: possible indirect action via histamine release

The relatively long delay to onset of scratching following ET-1 injection into the skin is suggestive of an indirect action of the peptide. Several lines of evidence support the involvement of histamine in ET-1-induced pruritus. When injected into human skin ET-1 evokes pallor at the injection site, with flare in the surrounding tissue of similar character to that evoked by histamine (Brain *et al*., 1992). This flare response is markedly reduced by H_1 receptor antagonists (Crossman *et al*., 1991; Brain *et al*., 1992). ET-1-induced release of substance P from primary afferent neurons in human skin has been proposed (Bunker *et al*., 1992), and substance P induces itch via histamine-dependent and independent mechanisms in both humans (Hagermark *et al*., 1978; Hagermark, 1992) and mice (Andoh *et al*., 1998).

Research on murine foetal skin-derived mast cells shows the presence of ET_A receptors on their surface and ET-1 degranulates these cells in a dose- and ET_A receptor-dependent manner (Matsushima *et al*., 2004). Histamine release caused by ET-1 has also been demonstrated in both peritoneal and bone marrow-derived mast cells harvested from BALB/c mice (Yamamura *et al*., 1994a, b). It is thus possible that ET-1 degranulates mast cells in BALB/c mice and the delayed onset of itch is caused by liberated histamine. However, against this possibility, Brain *et al*. (1992) described the inability of ET-1 to activate human skin mast cells *in vitro,* and Katugampola *et al*. (2000) found only a minimal rise in histamine in the skin microvasculature after infusing ET-1, suggesting that histamine release is not important for the development of ET-1-induced pruritus in humans. A reciprocal interaction between ET-1 and mast cells has been reported (Hultner and Ehrenreich, 2005) and it will be of considerable interest to perform more detailed studies in normal and genetically modified mice, for example. mast cell deficient (Wershil and Galli, 1994), to establish whether or not ET-1 acts via ET_A and possibly ET_B receptors to trigger scratching indirectly, through release of histamine or other mediator(s) from neurones, glia, immune cells and blood vessels in the skin (Pomonis *et al*., 2001).

Direct activation of pruriceptive C-fibres

Although the overall onset of scratching following injection of ET-1 or IRL1620 was significantly slower in comparison with the pruritogens histamine and 5-HT, several mice began scratching quite rapidly after injection, and were close to the median delay for histamine or 5-HT (Figure 2). The high variance in time to onset of response may reflect the time taken for injected substances such as ET-1 and histamine to reach a threshold concentration for activating pruriceptive C-fibres in a particular receptive field, which may result from slight differences in the positioning of the needle in the skin. The fact that scratching started fairly soon after injection in some animals could be interpreted as direct action of ET-1 on ET_A receptors located on the small-diameter pruriceptive C-fibres described by Schmelz *et al*. (1997). ET-1 binding sites have been identified within the dorsal root ganglia (DRG) of the rat, rabbit and monkey, with ET_A receptors localized to small diameter DRG neuronal cell bodies (Kar *et al*., 1991; Pomonis *et al*., 2001), which may be involved in nociception or pruriception. Moreover, colocalization of ET_A receptors and the C-fibre marker, CGRP, has been demonstrated on these cell bodies. This evidence points to the presence of ET_A receptors on neurones in DRG which might be pruriceptive, but this is speculative. Detailed neuropharmacological investigation is required to identify mechanically insensitive pruriceptive C-fibres in murine skin which respond to low doses of ET-1 in an ET_A and possibly ET_B receptor-dependent manner.

Conclusion

Our finding that ET-1 induces dose-dependent scratching in BALB/c mice via an ET_A receptor in skin confirms the work of Trentin *et al*. (2006) and complements the preliminary reports from studies in man in showing that this endogenous peptide is a potent activator of the scratch reflex (itch). The low ED_{50} for ET-1-induced scratching in mice implies that a small increase in ET-1 concentration within the skin could trigger pruritus. This may be very relevant in chronic skin disorders such as atopic dermatitis which are associated with distressing and damaging scratching. Our study of the acute effects of ET-1 needs to be extended to include animal models of chronic scratching, and a combination of selective drugs and suitable genetically modified strains of mice, a particular advantage of using this species, could be used to help establish how ET-1 acts to induce reflex scratching. ET-1 is clearly a putative mediator of itch, making the peptide and its receptors potential targets for developing much needed specific anti-itch drugs.

Conflict of interest

The authors state no conflict of interest.

References

Ahn GY, Butt KI, Jindo T, Yaguchi H, Tsuboi R, Ogawa H (1998). The expression of endothelin-1 and its binding sites in mouse skin increased after ultraviolet B irradiation or local injection of tumor necrosis factor alpha. *J Dermatol* **25**: 78–84.

Andoh T, Nagasawa T, Satoh M, Kuraishi Y (1998). Substance P induction of itch-associated response mediated by cutaneous NK1 tachykinin receptors in mice. *J Pharmacol Exp Ther* **286**: 1140–1145.

Bell JK, McQueen DS, Rees JL (2004). Involvement of histamine H_4 and H_1 receptors in scratching induced by histamine receptor agonists in BalbC mice. *Br J Pharmacol* **142**: 374–380.

Brain SD, Thomas G, Crossman DC, Fuller R, Church MK (1992). Endothelin-1 induces a histamine-dependent flare *in vivo*, but does not activate human skin mast cells *in vitro*. *Br J Clin Pharmacol* **33**: 117–120.

Brash HM, McQueen DS, Christie D, Bell JK, Bond SM, Rees JL (2005). A repetitive movement detector used for automatic monitoring and quantification of scratching in mice. *J Neurosci Methods* **142**: 107–114.

Bull HA, Dowd PM (1993). Edothelin-1 in human skin. *Dermatology* **187**: 1–5.

Bunker CB, Coulson ML, Hayes NA, Dowd PM, Foreman JC (1992). Further studies on the actions of endothelin-1 on blood flow in human skin. *Br J Dermatol* **127**: 85–90.

Crossman DC, Brain SD, Fuller RW (1991). Potent vasoactive properties of endothelin 1 in human skin. *J Appl Physiol* **70**: 260–266.

Davenport AP (2002). International Union of Pharmacology. XXIX. Update on endothelin receptor nomenclature. *Pharmacol Rev* **54**: 219–226.

Elton TS, Oparil S, Taylor GR, Hicks PH, Yang RH, Jin H *et al*. (1992). Normobaric hypoxia stimulates endothelin-1 gene expression in the rat. *Am J Physiol* **263**: R1260–R1264.

Ferreira SH, Romitelli M, de Nucci G (1989). Endothelin-1 participation in overt and inflammatory pain. *J Cardiovasc Pharmacol* **13** (Suppl 5): S220–S222.

Goligorsky MS, Budzikowski AS, Tsukahara H, Noiri E (1999). Co-operation between endothelin and nitric oxide in promoting endothelial cell migration and angiogenesis. *Clin Exp Pharmacol Physiol* **26**: 269–271.

Hagermark O (1992). Peripheral and central mediators of itch. *Skin Pharmacol* **5**: 1–8.

Hagermark O, Hokfelt T, Pernow B (1978). Flare and itch induced by substance P in human skin. *J Invest Dermatol* **71**: 233–235.

Horio T, Kohno M, Yokokawa K, Murakawa K, Yasunari K, Fujiwara H *et al*. (1991). Effect of hypoxia on plasma immunoreactive endothelin-1 concentration in anesthetized rats. *Metabolism* **40**: 999–1001.

Hultner L, Ehrenreich H (2005). Mast cells and endothelin-1: a life-saving biological liaison? *Trends Immunol* **26**: 235–238.

Ihara M, Noguchi K, Saeki T, Fukuroda T, Tsuchida S, Kimura S *et al*. (1992). Biological profiles of highly potent novel endothelin antagonists selective for the ET_A receptor. *Life Sci* **50**: 247–255.

Kar S, Chabot JG, Quirion R (1991). Quantitative autoradiographic localisation of [^{125}I]endothelin-1 binding sites in spinal cord and dorsal root ganglia of the rat. *Neurosci Lett* **133**: 117–120.

Katugampola R, Church MK, Clough GF (2000). The neurogenic vasodilator response to endothelin-1: a study in human skin *in vivo*. *Exp Physiol* **85**: 839–846.

Keele CA, Armstrong D (1964). *Substances Producing Pain and Itch*. Edward Arnold: London, 399pp.

Kuraishi Y, Nagasawa T, Hayashi K, Satoh M (1995). Scratching behavior induced by pruritogenic but not algesiogenic agents in mice. *Eur J Pharmacol* **275**: 229–233.

Kurihara H, Yoshizumi M, Sugiyama T, Takaku F, Yanagisawa M, Masaki T *et al*. (1989). Transforming growth factor-beta stimulates the expression of endothelin mRNA by vascular endothelial cells. *Biochem Biophys Res Commun* **159**: 1435–1440.

Kuryliszyn-Moskal A, Klimiuk PA, Sierakowski S (2005). Soluble adhesion molecules (sVCAM-1, sE-selectin), vascular endothelial growth factor (VEGF) and endothelin-1 in patients with systemic sclerosis: relationship to organ systemic involvement. *Clin Rheumatol* **24**: 111–116.

Laidlaw A, Flecknell P, Rees JL (2002). Production of acute and chronic itch with histamine and contact sensitizers in the mouse and guinea pig. *Exp Dermatol* **11**: 285–291.

Masaki T, Vane JR, Vanhoutte PM (1994). International Union of Pharmacology nomenclature of endothelin receptors. *Pharmacol Rev* **46**: 137–142.

Matsushima H, Yamada N, Matsue H, Shimada S (2004). The effects of endothelin-1 on degranulation, cytokine, and growth factor production by skin-derived mast cells. *Eur J Immunol* **34**: 1910–1919.

McMahon SB, Koltzenburg M (1992). Itching for an explanation. *Trends Neurosci* **15**: 497–501.

Mickley EJ, Gray GA, Webb DJ (1997). Activation of endothelin ET_A receptors masks the constrictor role of endothelin ET_B receptors in rat isolated small mesenteric arteries. *Br J Pharmacol* **120**: 1376–1382.

Nicholson AN (1983). Antihistamines and sedation. *Lancet* **2**: 211–212.

Pomonis JD, Rogers SD, Peters CM, Ghilardi JR, Mantyh PW (2001). Expression and localization of endothelin receptors: implications for the involvement of peripheral glia in nociception. *J Neurosci* **21**: 999–1006.

Rees J, Murray CS (2005). Itching for progress. *Clin Exp Dermatol* **30**: 471–473.

Schmelz M, Schmidt R, Bickel A, Handwerker HO, Torebjork HE (1997). Specific C-receptors for itch in human skin. *J Neurosci* **17**: 8003–8008.

Shuster S (1988). Antihistamines in the treatment of urticarial disorders. *Cutis* **42**: 26–28.

Simonson MS, Dunn MJ (1990). Cellular signaling by peptides of the endothelin gene family. *FASEB J* **4**: 2989–3000.

Stander S, Steinhoff M (2002). Pathophysiology of pruritus in atopic dermatitis: an overview. *Exp Dermatol* **11**: 12–24.

Summey Jr BT, Yosipovitch G (2005). Pharmacologic advances in the systemic treatment of itch. *Dermatol Ther* **18**: 328–332.

Takai M, Umemura I, Yamasaki K, Watakabe T, Fujitani Y, Oda K *et al*. (1992). A potent and specific agonist, Suc-[Glu9,Ala11,15]-endothelin-1(8–21), IRL 1620, for the ET_B receptor. *Biochem Biophys Res Commun* **184**: 953–959.

Terenghi G, Bull HA, Bunker CB, Springall DR, Zhao Y, Wharton J *et al*. (1991). Endothelin-1 in human skin: immunohistochemical, receptor binding, and functional studies. *J Cardiovasc Pharmacol* **17** (Suppl 7): S467–S470.

Trentin PG, Fernandes MB, D'Orleans-Juste P, Rae GA (2006). Endothelin-1 causes pruritus in mice. *Exp Biol Med (Maywood)* **231**: 1146–1151.

Wershil BK, Galli SJ (1994). The analysis of mast cell function *in vivo* using mast cell-deficient mice. *Adv Exp Med Biol* **347**: 39–54.

Xu SW, Denton CP, Dashwood MR, Abraham DJ, Black CM (1998). Endothelin-1 regulation of intercellular adhesion molecule-1 expression in normal and sclerodermal fibroblasts. *J Cardiovasc Pharmacol* **31** (Suppl 1): S545–S547.

Yamamura H, Nabe T, Kohno S, Ohata K (1994a). Endothelin-1 induces release of histamine and leukotriene C4 from mouse bone marrow-derived mast cells. *Eur J Pharmacol* **257**: 235–242.

Yamamura H, Nabe T, Kohno S, Ohata K (1994b). Endothelin-1, one of the most potent histamine releasers in mouse peritoneal mast cells. *Eur J Pharmacol* **265**: 9–15.

Yanagisawa M, Masaki T (1989). Endothelin, a novel endothelium-derived peptide. Pharmacological activities, regulation and possible roles in cardiovascular control. *Biochem Pharmacol* **38**: 1877–1883.

Yohn JJ, Morelli JG, Walchak SJ, Rundell KB, Norris DA, Zamora MR (1993). Cultured human keratinocytes synthesize and secrete endothelin-1. *J Invest Dermatol* **100**: 23–26.

Yoshizumi M, Kurihara H, Morita T, Yamashita T, Oh-hashi Y, Sugiyama T *et al*. (1990). Interleukin 1 increases the production of endothelin-1 by cultured endothelial cells. *Biochem Biophys Res Commun* **166**: 324–329.