Original Research

Molecular signaling of pruritus induced by endothelin-1 in mice

Jiexian Liang[1], Tomoyuki Kawamata[2] and Wenjin Ji[3]

[1]Division of Anesthesiology, Department of Cardiovascular Surgery, Guangdong General Hospital, Guangdong Academy of Medical Sciences, Guangzhou 510080, China; [2]Department of Anesthesiology and Resuscitology, Shinshu University School of Medicine, Matsumoto 390-8621, Japan; [3]Department of Anesthesiology, Guangdong General Hospital, Guangdong Academy of Medical Sciences, Guangzhou 510080, China

Corresponding author: Dr Wenjin Ji, Department of Anesthesiology, Guangdong General Hospital, Guangdong Academy of Medical Sciences, 96 DongChun Road, Guangzhou 510080, P.R. China. Email: jiwenjin@msn.com

Abstract

Endothelin-1 (ET-1) has recently been identified to evoke pruritus/itching sensation in both humans and animals. It is most likely that the signaling is through the specific G-protein-coupled ET_A and ET_B receptors, but the downstream signaling mediators for ET-1 remain elusive. In the present study, we examined the potential involvement of several distinct signaling molecules in ET-1-induced pruritus in a murine model. We applied an *in vivo* pruritus model in C57BL/6J mice by injecting ET-1 intradermally into the scruff, and recording the number of scratching bouts within 30 min after injection. Then specific antagonists/inhibitors for distinct signaling molecules, including cell-surface ET_A and ET_B receptors, histamine receptor type 1 (H1 receptor), protein kinases A (PKA) and C (PKC), phospholipase C (PLC) or adenylyl cyclase (AC), were co-injected with ET-1. The results showed that ET-1 induced a vigorous scratching response in mice in a dose-dependent manner. This response was further enhanced by a specific antagonist for ET_B receptor, BQ-788, reduced by a specific antagonist for ET_A receptor, BQ-123, and not affected by mepyramine, the specific inhibitor for H1 receptor. In addition, the scratching response was significantly reduced by inhibitors for PKC and AC, but was significantly enhanced by PLC inhibitor, while PKA inhibitors showed no effects in the ET-1-induced scratching response. Our data suggested that ET-1 may signal through the ET_A receptor, AC and PKC pathway to induce pruritus sensation, while ET_B receptor and PLC may antagonize the pruritus evoked by ET-1. These results may provide a basis for the future development of antipruritic therapy.

Keywords: endothelin-1, pruritus, protein kinase C, phospholipase C, adenylyl cyclase

Experimental Biology and Medicine 2010; **235**: 1300–1305. DOI: 10.1258/ebm.2010.010121

Introduction

Pruritus/itching is an unpleasant sensory experience associated with the desire to scratch.[1] An 'intensity theory' proposed by Dr v Frey about 90 years ago suggests that both itching and pain result from the activation of nociceptors, but to a different extent, with a weak one leading to itching and a stronger one to pain.[2] This theory holds true considering the great overlap in the mediators, processing mechanisms and therapeutic approaches for both sensations.[1] However, accumulative evidence also points to their distinct differences.[3] First, they induce different behavioral responses; pain elicits a withdrawal reflex while itching leads to a scratch reflex. Second, they seem to antagonize each other; pain induced by a scratch can inhibit itching, and many analgesics can generate itching. Third, different neuronal pathways are utilized to preferentially conduct one sensation over the other.

The nociceptors are suggested in itching development and progression. In tissue damage subsequent to inflammation, ischemia, infection, injury or tumor invasion, various chemical mediators activate or sensitize nociceptor terminals to elicit or exacerbate pain. Among these mediators, endothelin-1 (ET-1) is generated by a variety of cell types, including endothelial cells, vascular smooth muscle cells, leukocytes, cardiomyocytes and mesangial cells, and some tumor cell lines.[4,5] Local administration of ET-1 induces pain-related behavior in both humans and animals.[6–8]

More recently, ET-1 has been demonstrated to elicit pruritus in mice[9,10] and humans.[11,12] ET-1-induced pruritus does not rely on the presence of either phospholipase C β3 (PLCβ3) or transient receptor potential vanilloid 1 (TRPV1), but requires TRPV1-expressing nociceptors, potentially through some alternative signal-transducing mechanisms independent of TRPV1.[13] Other studies

suggested that pruritus induced by ET-1 could be due to indirect actions of other endogenous mediators such as histamine,[9,10] which was supported by the observation that intradermal injection of ET-1 mainly induced mast cell degranulation and mast cell-dependent inflammation.[14] The signaling of ET-1 is mediated via two main membrane G-protein-coupled receptor (GPCR) subtypes, ET_A and ET_B receptors. The expression of ET_A receptor is largely confined to the vascular endothelium, as well as neurons and mast cells, while ET_B receptor is mainly expressed by keratinocytes, smooth muscle cells and vascular endothelium.[15–19] So far, little is known about the molecular mechanisms underlying the pruritogenic action of ET-1. By applying selective antagonists/inhibitors of key signaling molecules within intracellular signaling systems coupled to GPCRs including histamine receptor type 1 (H1 receptor), ET_A and ET_B receptors, we found that signals downstream of H1 receptor were not involved in the process of pruritus induced by ET-1; those through ET_A receptor, adenylyl cyclase (AC) and the protein kinase C (PKC) pathway induced pruritus sensation, while those through ET_B receptor and PLC antagonized the pruritus evoked by ET-1. The results may be helpful in further determining the molecular mechanisms and the development of novel therapeutic approaches targeting pruritus.

Materials and methods

Chemicals and reagents

Synthetic ET-1, BQ-123 (cyclo[D-Trp-D-Asp-Pro-D-Val-Leu]) and BQ-788 (*N*-cys-2,6-dimethylpiperidinocarbonyl-L-γ-methylleucyl-D-1-methoxycarboyl-D-norleucine), the latter two being selective antagonists for ET_A and ET_B receptors, respectively, were purchased from American Peptides (Sunnyvale, CA, USA). SQ22536 (9-(tetrahydro-2-furanyl)-9H-purin-6-amine, inhibitor for adenylyl cyclases), Bisindolylmaleimide I (BIM; a PKC inhibitor), U73122 (1-[6-[[(17b)-3-methoxyestra-1,3,5(10)-trien-17-yl]amino]hexyl]-1H-pyrrole-2,5-dione, a PLC inhibitor), H89 (N-[2-[[3-(4-bromophenyl)-2-propenyl]amino]ethyl]-5-isoq uinolinesulfonamide dihydrochloride, a protein kinase A [PKA] inhibitor) and mepyramine (a specific receptor for H1 receptor) were purchased from Sigma Chemical Co (Saint Louis, MO, USA). All chemicals were dissolved in phosphate-buffered saline (pH 7.4) for stock solutions, except for BIM and U73122, which were dissolved in dimethyl sulfoxide (DMSO), and the final DMSO concentration in working solution was $\leq 2\%$.

Animals

Male C57BL/6J mice, weighing 20–22 g, were obtained from the Center for Laboratory Animals, Sun Yat-sen University (Guangzhou, China). The animals were housed at a room temperature of $22 \pm 1^{\circ}C$ on a 12/12-h light/dark cycle, with access to food and water *ad libitum*. The animal use and care protocols were approved by the Committee on Ethical Use of Animals of Guangdong General Hospital (Guangzhou, China), following the National Institutes of Health animal use and care guidelines. All efforts were made to minimize animal suffering and to reduce the number of animals used.

Pruritus model and behavioral analysis

The pruritus model was established as previously described with minor modifications.[20] Briefly, one day after shaving the rostral part of the back of the neck, mice were placed into a small plastic chamber ($22 \times 12 \times 20\ cm^3$) 30 min before the experiment. For drug administration, mice were briefly removed from the chamber, and 100 μL of each test drug was intradermally injected with a 30-gauge needle. Then the mice were returned to the chamber, and the hind limb scratching directed towards the shaved area at the back of the neck was observed and recorded for 30 min. One scratch was defined as a lift of the hind limb towards the injection site and then a reposition of the limb back to the floor, regardless of the scratching strokes that took place between the two movements. Mepyramine (40 mg/kg body weight) was intraperitoneally injected 30 min before intradermal injection of ET-1. To reduce the injection times, other antagonists or inhibitors were co-injected with ET-1. The specific doses of BQ-123, BQ-788, mepyramine, H89 and U73122 used in this study were selected based on previous studies.[21–24]

Statistical analysis

All data are presented as mean ± SEM. Data were statistically evaluated by analysis of variance followed by Bonferroni's test or, when only two means were to be compared, unpaired Student's *t*-test, and a P value of <0.05 was considered statistically significant.

Results

ET-1 evokes scratching response in a dose-dependent manner

The dose-dependent itching-inducing effects of ET-1 were first examined. As shown in Figure 1, ET-1 increased the number of scratches in a dose-dependent manner, with a significant increase starting from 1 pmol ET-1 ($P < 0.05$). At 1000 pmol, ET-1 evoked an average of 360 bouts of scratches within 30 min. At 100 pmol, ET-1 induced a moderate increase in the scratching behavior (an average of 187 bouts for 30 min), within the linear range of ET-1 effects. Therefore, the dose of 100 pmol was selected for subsequent experiments.

ET_A-mediated signaling enhances, ET_B-mediated signaling reduces, while H1-mediated signaling does not affect the pruritogenic effect of ET-1

The selective antagonists for ET_A and ET_B receptors, BQ-123 and BQ-788, as well as inhibitors of the various intracellular signaling elements, when administered individually, did not cause scratching behavior of the mice (data not shown). BQ-123 at 100 nmol significantly reduced the number of scratches (from an average of 185 to 8 bouts),

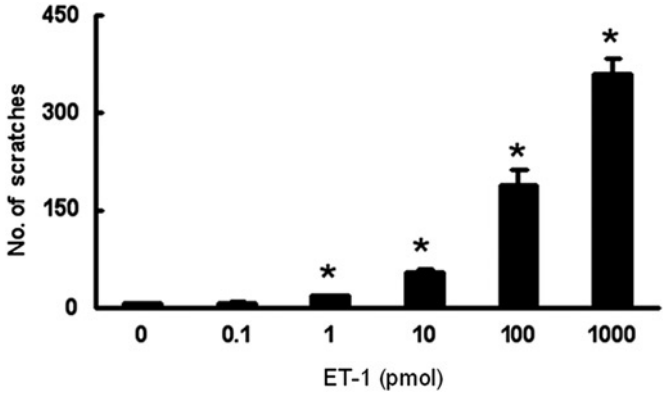

Figure 1 Pruritus induced by ET-1. ET-1 at indicated doses was injected intradermally into the scruff of mice. The bouts of scratches within 30 min after the injection were recorded. Values are presented as mean ± SEM (n = 10 in each group). $^*P < 0.05$, as compared with the control (phosphate-buffered saline-treated) group (ET-1 = 0). ET-1, endothelin-1

while BQ-788 at 30 nmol increased the number of scratches from an average of 185–337 bouts (all $P < 0.05$, Figure 2a). These results indicate that ET_A receptor is involved in ET-1-induced pruritus, but the ET_B receptor can modulate these pruritogenic effects. Besides, H1 receptor antagonist mepyramine (150 ± 23.6) did not statistically alter the scratching behavior of mice from that seen in vehicle-treated mice (165.1 ± 28.8) ($P > 0.05$, Figure 2b).

AC and PKC mediate but PLC antagonizes the ET-1-induced pruritus while PKA has no effect

When stimulated by ET-1, there is an increase in calcium influx and mobilization of intracellular calcium, which is associated with the activation of multiple signaling pathways, such as AC/PKA signaling and PLC/PKC signaling.[25] Therefore, we evaluated the role of PKC and PKA in the pruritus induced by ET-1. Inhibiting PKC activity by BIM reduced the scratching response at the doses of 2 μg and 10 μg ($P < 0.05$), with a stronger reduction achieved following a higher dose of BIM. On the other hand, the specific PKA inhibitor, H89, did not affect the scratching response ($P > 0.05$, Figure 3a). U73122, the specific PLC inhibitor, enhanced the scratching response by increasing the number of scratches from 173 to 320 bouts ($P < 0.05$, Figure 3b). As shown in Figure 3c, SQ22536, a selective inhibitor of AC, inhibited the scratching response in a dose-dependent manner. It reduced the average number of scratches from 178 to 154 bouts at 30 nmol and to 69 bouts at 300 nmol (all $P < 0.05$).

Discussion

ET-1 is secreted by multiple cell types and through GPCRs, ET_A and ET_B receptors, regulates vascular homeostasis and contributes to vessel pathologies, such as type II diabetes,[26] coronary artery disease[27] and pulmonary arterial hypertension.[28] Besides vascular regulation, ET-1 also evokes pruritus sensation when injected intradermally in humans,[11,12] and induces nociceptive responses in rodents.[7,29] Several studies have looked into the contributions of ET_A and ET_B receptors in ET-1-induced pruritus.[9,10] Using a similar mouse itching model, McQueen *et al.*[9] and Trentin *et al.*[10] demonstrated that ET-1 induces scratching responses in a dose-dependent manner. However, the effective dose range varies among different species. For instance, in male Swiss mice, ET-1 generated a maximal response at 10 pmol, with higher doses showing a diminished response.[10] In female BALB/c mice, the peak scratch response appeared at approximately 4 pmol.[9] In male C57BL/6J mice used for this study, ET-1-induced scratching responses in a near-linear fashion within the range of 1–1000 pmol, indicating the significance of genetic backgrounds in determining pruritogenic sensitivity to ET-1. The co-administration of selective antagonists for ET_A or ET_B receptor with ET-1 exhibited opposite effects on ET-1-induced pruritus, with blocking ET_A receptor

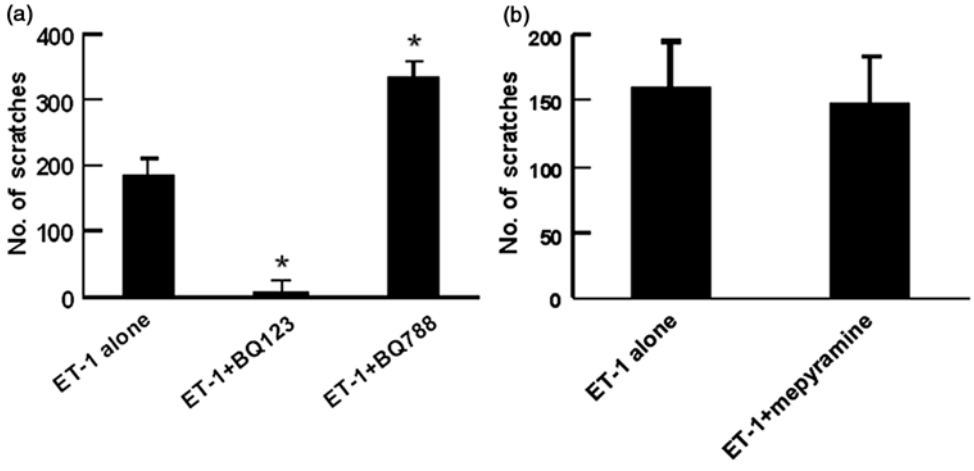

Figure 2 Effects of specific inhibitors for ET_A, ET_B and H1 receptor on the scratching response after ET-1 injection. (a) 100 pmol ET-1 alone or together with 100 nmol BQ-123 (ET_A antagonist) or 30 nmol BQ-788 (ET_B antagonist) were injected intradermally into the scruff of mice. The bouts of scratches within 30 min after the ET-1 injection were recorded. (b) 100 pmol ET-1 were injected intradermally into the scruff of mice following intraperitoneal injection with mepyramine (40 mg/kg) or vehicle (ET-1 alone). The bouts of scratches within 30 min after the ET-1 injection were recorded. Values are presented as mean ± SEM (N = 7 in each group). $^*P < 0.05$, as compared with the group treated with ET-1 only. ET-1, endothelin-1

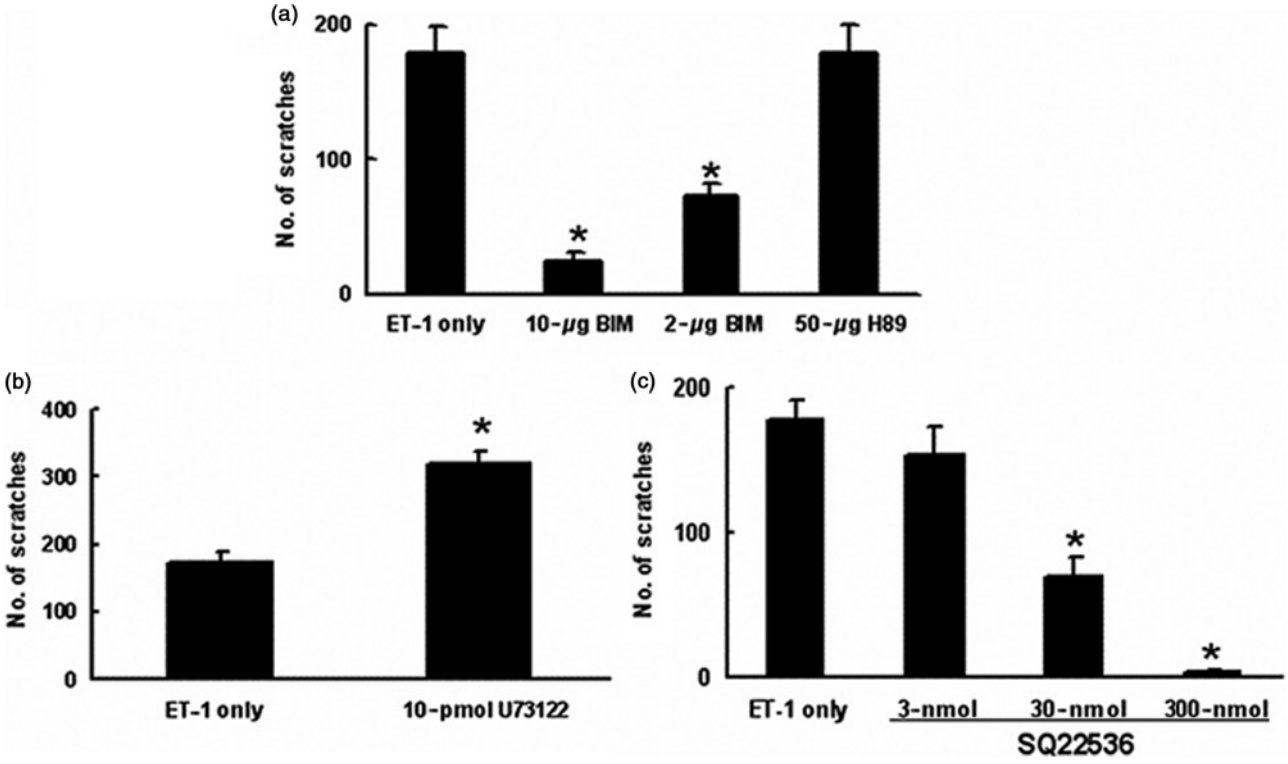

Figure 3 Effects of selective inhibitors for protein kinase C (PKC), protein kinase A (PKA), phospholipase C (PLC) and adenyl cyclase (AC) on the scratching responses after endothelin-1 (ET-1) injection. 100 pmol ET-1 only or together with BIM (PKC inhibitor, a) at indicated doses, or 50 μg H89 (PKA inhibitor, A) or 100 pmol U73122 (PLC inhibitor, b) or SQ22536 (AC inhibitor, c) at indicated doses were injected intradermally into the scruff of mice. The bouts of scratches within 30 min after the injection were recorded. Values are presented as mean $\pm$ SEM ($n = 7$ in each group). $^{*}P < 0.05$, as compared with the group treated with ET-1 only

dramatically reducing the scratching response and blocking ET_B receptor significantly enhancing it. These results suggest that, similar to the actions of ET-1 on vascular regulation, the activations of ET_A and ET_B receptors also contradict each other in mediating pruritus, with the former being pruritogenic and the latter antagonizing it.

ET-1 has been demonstrated as a potent pro-inflammatory agent. Since many inflammatory mediators, such as histamine, bradykinin, serotonin and prostaglandins, are also known to evoke itch sensation, it was suggested that in addition to directly acting on nerve endings, ET-1 may induce pruritus through indirect actions via release of endogenous mediators such as histamine from mast cells, which was supported by the observation that skin inflammation following intradermal injection of ET-1 depends on mast cells.[9,14] To test whether histamine-related inflammation may contribute to ET-1-induced pruritus, we co-injected specific inhibitor for H1 receptor and observed no significant behavioral change, suggesting that ET-1 may directly act on pruriceptive nerve endings to induce itching response.

So far, little is known regarding downstream signaling from ET_A and ET_B receptors to the intracellular signaling pathways to transduce the pruritogenic response of ET-1. We found that AC and PKC were among the molecules to stimulate the itching sensation by ET-1, that PLC antagonized the pruritus evoked by ET-1, and that PKA had no effects on the pruritogenic activity of ET-1.

Intracellular cAMP increases upon activation of AC; the major effects of cAMP in mammalian cells were mediated intracellularly by PKA. We have proven that PKA had no effects on the pruritogenic activity of ET-1. Our study also showed that the effects of blocking PKC or AC on ET-1-induced pruritus were similar to that exerted by blocking the ET_A receptor. The cAMP-to-PKC signaling might be involved in the process. Other studies suggest an association between cAMP and PKC signaling. Cunha *et al.*[30] demonstrated that the ET-1-induced mechanical hypernociception in rat hindpaw is through ET_B/cAMP/PKC, but not PKA pathway. Another study on the mechanical hyperalgesia induced by ET-1 revealed that this effect is mediated via ET_B receptor coupled with PKC, as well as PLC, but not PKA.[31] In addition, Hucho *et al.*[32] demonstrated that, for inflammatory pain, the cAMP-to-PKC signaling is mediated through the cAMP-activated guanine exchange factor Epac, which signals downstream to PLC and PLD, both of which are necessary for translocation and activation of PKC. However, the PKC signaling did not seem to descend from the PLC pathway in our study, since blocking PLC by U73122 produced an opposite effect from blocking PKC. Our results reveal a new pathway for the cAMP-to-PKC signaling that is PLC-independent, which could be due to the distinct isoforms of PKC that were activated or the cell-type specificity where the activation occurs (Figure 4).

ET-1 induced pain, itching and thermal hyperalgesia in an ET_A receptor-dependent manner. We found that both ET-1-induced pain-like behavior and thermal hyperalgesia are reduced in TRPV1 gene knockout mice.[33,34] While

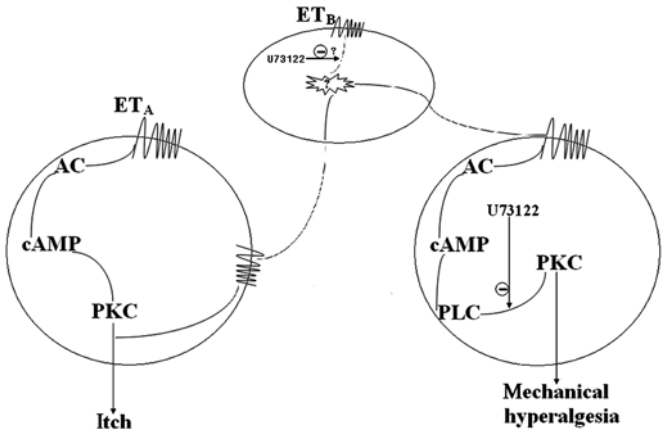

Figure 4 Schematic representations of the differential involvement of AC/cAMP/PKC pathways in ET-1-mediated pruritus and mechanical hyperalgesia in a cell-dependent manner, with cells represented as circles. In neurons or nerve endings, ET-1 could signal through ET_A/AC/cAMP/PKC pathway to induce pruritus. In non-neuronal cells, such as keratinocytes, ET-1 signals through the ET_B receptor to release chemicals such as beta-endorphine or inflammatory mediators. Some of the chemicals inhibit ET-1-induced pruritus through unknown mechanisms. The release of these antipruritic chemicals could be inhibited by PLC inhibitor, U73122, which presents pro-pruritic function. Some chemicals may act on neurons and induce mechanical hyperalgesia via AC/cAMP//PLC/PKC signaling pathway. ET-1, endothelin-1; PKC, protein kinase C; PKA, protein kinase A; PLC, phospholipase C; AC, adenyl cyclase

thermal hyperalgesia could be inhibited by a PKC inhibitor,[33] ET-1-induced pain-like behavior could not be inhibited by a PKC inhibitor.[21,34] For ET-1-induced pruritus, however, TRPV1 itself is not required, but the TRPV1-expressing neurons are essential, implying the involvement of other signaling molecules within the TRPV1-expressing neurons.[13] Pain induced by ET-1 is not related to TRPV1 in rat; recently we found that ET-1-induced pain in mice is TRPA1-dependent,[21] and the pain was inhibited by U73122.

The data from the current study imply that, for the pruritogenic activity of ET-1, AC and PKC may act downstream from the ET_A receptor, while PLC may act from the ET_B receptor. However, several questions arose from this study. First, the search for signaling mediators of ET-1 is definitely not complete from the current work. Although our study revealed, for the first time, a partial list of the functionally important molecules, there may be more molecules involved in ET-1-induced pruritus. Second, since the study was carried out on living mice, it is difficult to conclude the functions of specific signaling molecules in different cell types, and how they contribute to the overall functional readout. Based on these data, more detailed *in vitro* studies should be carried out in the future. Third, it is not clear how those signaling pathways are regulated, whether directly by ET-1 through the ET_A, ET_B, or other receptors, or indirectly through other pruritogenic factors. As suggested by several studies, there might be a crosstalk between ET-1 and other nociceptors.[22,35]

In summary, we investigated the potential involvement of several signaling molecules in ET-1-induced pruritus in mice. ET_A receptor, AC and PKC were found to promote the itching sensation, and ET_B receptor and PLC antagonized it, while signaling via the H1 receptor presented no effect. Given that ET-1 can be secreted by multiple cell types in response to a variety of stress stimuli, such as inflammation and tissue injury, this study may provide a basis for searching novel antipruritic targets for various clinical situations.

Author contributions: All authors participated in the design, interpretation of the studies, analysis of the data and review of the manuscript. JL and WJ conducted the experiments and wrote the manuscript. TK revised the manuscript.

ACKNOWLEDGEMENTS

This study was supported by the National Natural Science Foundation (grant number 30872437 to WJ) and the Natural Science Foundation of Guangdong Province, China (grant number 8151008004000017 to WJ). There is no conflict of interest to be declared for this study.

REFERENCES

1 Ikoma A, Steinhoff M, Stander S, Yosipovitch G, Schmelz M. The neurobiology of itch. *Nat Rev Neurosci* 2006;**7**:535–47
2 v.Frey M. Zur Physiologie der Juckempfindung. *Arch Neerl Physiol* 1922;**7**:142–5
3 Schmelz M. Itch and pain. *Neurosci Biobehav Rev* 2010;**34**:171–6
4 Gandhi CR, Berkowitz DE, Watkins WD. Endothelins. Biochemistry and pathophysiologic actions. *Anesthesiology* 1994;**80**:892–905
5 Hans G, Deseure K, Adriaensen H. Endothelin-1-induced pain and hyperalgesia: a review of pathophysiology, clinical manifestations and future therapeutic options. *Neuropeptides* 2008;**42**:119–32
6 Hans G, Deseure K, Robert D, De Hert S. Neurosensory changes in a human model of endothelin-1 induced pain: a behavioral study. *Neurosci Lett* 2007;**418**:117–21
7 Piovezan AP, D'Orleans-Juste P, Souza GE, Rae GA. Endothelin-1-induced ET(A) receptor-mediated nociception, hyperalgesia and oedema in the mouse hind-paw: modulation by simultaneous ET(B) receptor activation. *Br J Pharmacol* 2000;**129**:961–8
8 Gokin AP, Fareed MU, Pan HL, Hans G, Strichartz GR, Davar G. Local injection of endothelin-1 produces pain-like behavior and excitation of nociceptors in rats. *J Neurosci* 2001;**21**:5358–66
9 McQueen DS, Noble MA, Bond SM. Endothelin-1 activates ETA receptors to cause reflex scratching in BALB/c mice. *Br J Pharmacol* 2007;**151**:278–84
10 Trentin PG, Fernandes MB, D'Orleans-Juste P, Rae GA. Endothelin-1 causes pruritus in mice. *Exp Biol Med (Maywood)* 2006;**231**:1146–51
11 Katugampola R, Church MK, Clough GF. The neurogenic vasodilator response to endothelin-1: a study in human skin *in vivo*. *Exp Physiol* 2000;**85**:839–46
12 Ferreira SH, Romitelli M, de Nucci G. Endothelin-1 participation in overt and inflammatory pain. *J Cardiovasc Pharmacol* 1989;**13** (Suppl. 5):S220–2
13 Imamachi N, Park GH, Lee H, Anderson DJ, Simon MI, Basbaum AI, Han SK. TRPV1-expressing primary afferents generate behavioral responses to pruritogens via multiple mechanisms. *Proc Natl Acad Sci U S A* 2009;**106**:11330–5
14 Metz M, Lammel V, Gibbs BF, Maurer M. Inflammatory murine skin responses to UV-B light are partially dependent on endothelin-1 and mast cells. *Am J Pathol* 2006;**169**:815–22
15 Khodorova A, Navarro B, Jouaville LS, Murphy JE, Rice FL, Mazurkiewicz JE, Long-Woodward D, Stoffel M, Strichartz GR, Yukhananov R, Davar G. Endothelin-B receptor activation triggers an endogenous analgesic cascade at sites of peripheral injury. *Nat Med* 2003;**9**:1055–61
16 Matsushima H, Yamada N, Matsue H, Shimada S. The effects of endothelin-1 on degranulation, cytokine, and growth factor production by skin-derived mast cells. *Eur J Immunol* 2004;**34**:1910–19

17 Pomonis JD, Rogers SD, Peters CM, Ghilardi JR, Mantyh PW. Expression and localization of endothelin receptors: implications for the involvement of peripheral glia in nociception. *J Neurosci* 2001;**21**:999–1006
18 Shetty SS, Okada T, Webb RL, DelGrande D, Lappe RW. Functionally distinct endothelin B receptors in vascular endothelium and smooth muscle. *Biochem Biophys Res Commun* 1993;**191**:459–64
19 Perreault T, Baribeau J. Characterization of endothelin receptors in newborn piglet lung. *Am J Physiol* 1995;**268**(Pt 1):L607–14
20 Shimada SG, Shimada KA, Collins JG. Scratching behavior in mice induced by the proteinase-activated receptor-2 agonist, SLIGRL-NH2. *Eur J Pharmacol* 2006;**530**:281–3
21 Liang J, Bi H, Ji W. Involvement of TRPA1 in ET-1-induced pain-like behavior in mice. *Neuroreport* 2010;**21**:201–5
22 Xie C, Wang DH. Ablation of transient receptor potential vanilloid 1 abolishes endothelin-induced increases in afferent renal nerve activity. Mechanisms and Functional Significance. *Hypertension* 2009;**54**:1298–305
23 Shim WS, Tak MH, Lee MH, Kim M, Kim M, Koo JY, Lee CH, Kim M, Oh U. TRPV1 mediates histamine-induced itching via the activation of phospholipase A2 and 12-lipoxygenase. *J Neurosci* 2007;**27**:2331–7
24 Hu HJ, Bhave G, Gereau RWt. Prostaglandin and protein kinase A-dependent modulation of vanilloid receptor function by metabotropic glutamate receptor 5: potential mechanism for thermal hyperalgesia. *J Neurosci* 2002;**22**:7444–52
25 Nowycky MC, Thomas AP. Intracellular calcium signaling. *J Cell Sci* 2002;**115**(Pt 19):3715–16
26 Kalani M. The importance of endothelin-1 for microvascular dysfunction in diabetes. *Vasc Health Risk Manag* 2008;**4**:1061–8
27 Schiffrin EL. Role of endothelin-1 in hypertension and vascular disease. *Am J Hypertens* 2001;**14**(Pt 2):83S–9S
28 Davie NJ, Schermuly RT, Weissmann N, Grimminger F, Ghofrani HA. The science of endothelin-1 and endothelin receptor antagonists in the management of pulmonary arterial hypertension: current understanding and future studies. *Eur J Clin Invest* 2009;**39** (Suppl. 2):38–49
29 Menendez L, Lastra A, Hidalgo A, Baamonde A. Nociceptive reaction and thermal hyperalgesia induced by local ET-1 in mice: a behavioral and Fos study. *Naunyn Schmiedebergs Arch Pharmacol* 2003;**367**:28–34
30 da Cunha JM, Rae GA, Ferreira SH, Cunha Fde Q. Endothelins induce ETB receptor-mediated mechanical hypernociception in rat hindpaw: roles of cAMP and protein kinase C. *Eur J Pharmacol* 2004;**501**:87–94
31 Motta EM, Calixto JB, Rae GA. Mechanical hyperalgesia induced by endothelin-1 in rats is mediated via phospholipase C, protein kinase C, and MAP kinases. *Exp Biol Med (Maywood)* 2006;**231**:1141–5
32 Hucho TB, Dina OA, Levine JD. Epac mediates a cAMP-to-PKC signaling in inflammatory pain: an isolectin B4(+) neuron-specific mechanism. *J Neurosci* 2005;**25**:6119–26
33 Kawamata T, Ji W, Yamamoto J, Niiyama Y, Furuse S, Namiki A. Contribution of transient receptor potential vanilloid subfamily 1 to endothelin-1-induced thermal hyperalgesia. *Neuroscience* 2008;**154**:1067–76
34 Kawamata T, Ji W, Yamamoto J, Niiyama Y, Furuse S, Omote K, Namiki A. Involvement of transient receptor potential vanilloid subfamily 1 in endothelin-1-induced pain-like behavior. *Neuroreport* 2009;**20**:233–7
35 Motta EM, Chichorro JG, Rae GA. Role of ET(A) and ET(B) endothelin receptors on endothelin-1-induced potentiation of nociceptive and thermal hyperalgesic responses evoked by capsaicin in rats. *Neurosci Lett* 2009;**457**:146–50

(Received April 7, 2010, Accepted August 5, 2010)