Perifollicular and Perivascular Mouse Skin Mast Cells Express Corticotropin-Releasing Hormone Receptor

Journal of Investigative Dermatology (2006) **126,** 932–935. doi:10.1038/sj.jid.5700153; published online 26 January 2006

TO THE EDITOR

Mast cells are necessary for allergic reactions, but they are also important in inflammatory conditions that are worsened by stress (Theoharides and Cochrane, 2004). Dermatoses, such as atopic dermatitis and psoriasis, are aggravated by stress (Panconesi and Hautmann, 1996; Katsarou-Katsari *et al.*, 1999). Acute stress or intradermal administration of corticotropin-releasing hormone (CRH) can activate skin mast cells (Theoharides *et al.*, 1998; Singh *et al.*, 1999) that are located perivascularly close to nerve endings. CRH and CRH receptor (CRH-R) mRNA are expressed in human and rodent skin (Slominski *et al.*, 1995, 1996), in association with the hair cycle (Roloff *et al.*, 1998; Pisarchik and Slominski, 2001). Increased CRH-R1 gene expression was documented in the affected skin from patients with chronic urticaria (Papadopoulou *et al.*, 2005). Moreover, CRH-R1 is involved in the stress-induced exacerbation of chronic contact dermatitis in rats (Kaneko *et al.*, 2003). Mast cells could be unique targets of CRH and related peptides, contributing to neurogenic inflammation (Theoharides *et al.*, 2004).

Here, *in situ* hybridization and immunohistochemistry (IHC) were used to localize CRH-R on mouse skin mast cells. Mouse skin sections hybridized with ^{33}P-labeled antisense CRH-R1 riboprobe displayed silver grains in the outer root sheath of hair follicles (Figure 1a). Clusters of signal were also associated with certain perifollicular cells (Figure 1b) with morphological characteristics of mast cells: fairly large size, irregular shape, and perivascular localization. A skin section hybridized with ^{33}P-labeled sense CRH-R1 probe displayed no specific labeling (Figure 1c). To confirm whether mast cells express CRH-R protein, IHC was performed, and the sections were subsequently stained with toluidine blue, a histological stain for mast cells. The distribution and morphology of the perifollicular cells expressing CRH-R1 mRNA (Figure 1b) determined by *in situ* hybridization, was virtually identical to the CRH-R-like immunoreactivity detected using IHC (Figure 2a); these perifollicular cells stained with toluidine blue (Figure 2b). CRH-R-like immunoreactivity (Figure 2c) was also present on toluidine blue positive cells found adjacent to blood vessels, (Figure 2d). The present results suggest that a small population of perifollicular and perivascular skin mast cells express CRH-R. The findings were confirmed recently with FACS analysis of a population of disaggregated skin cells double positive for CRH-R and the mast cell growth factor receptor c-kit (Donelan *et al.*, 2006). Previous reports have shown CRH-R1 expression in the outer root sheath, hair matrix, and dermal papilla of mouse skin (Slominski *et al.*, 1996, 2001; Roloff *et al.*, 1998). CRH-R2 expression in the epidermis, hair follicle, and sebaceous glands of rodents had been reported by molecular and immunological detection (Slominski *et al.*, 2004).

The expression of CRH-R1 in and around the hair follicle may help explain stress-induced hair loss. Expression of different CRH-R1 isoforms is hair cycle dependent; whereas CRH-R1α is absent in telogen, it is present in anagen (Pisarchik and Slominski, 2001), which may explain differential responsiveness of mouse skin to CRH ligands. During the cyclic remodeling of the hair follicle, the perifollicular innervation undergoes significant changes in sensory skin innervation and in mast cell–nerve contacts (Peters *et al.*, 2001); perifollicular mast cells also modulate hair follicle cycling (Paus *et al.*, 1994). Moreover, the hair follicle was recently identified as a major source of skin mast cell progenitors (Kumamoto *et al.*, 2003).

Murine hair growth *in vivo* and *in situ* is modulated by stress response mediators that can act as endogenous mast cell secretagogues (Peters *et al.*, 2004). Sound-induced psychological stress was shown to increase the number of apoptotic cells in the hair follicle and significantly increase the number of degranulated mast cells (Arck *et al.*, 2001); these effects could be mimicked by injection with the neuropeptide substance P. In the absence of functional mast cells or the substance P receptor NK-1R, stress failed to induce premature hair follicle regression (Arck *et al.*, 2005). In addition to substance P, neurotensin (Singh *et al.*, 1999) could activate skin mast cells and it was recently shown that the intradermal effect of CRH on vascular permeability was dependent on neurotensin (Donelan *et al.*, 2006).

Acute stress increases CRH in the skin of rodents (Lytinas *et al.*, 2003) and intradermal CRH administration in rats leads to activation of skin mast cells and increased vascular permeability (Theoharides *et al.*, 1998). Both CRH and its structurally related peptide urocortin are produced by human skin cells (Slominski *et al.*, 1998; Slominski *et al.*, 2000), whereas mice only produce urocortin (Slominski *et al.*, 2000). In mice, CRH is delivered to the skin by nerve endings (Slominski *et al.*, 1996, 1999). CRH administration in humans

Abbreviations: CRH, corticotropin-releasing hormone; CRH-R, corticotropin-releasing hormone receptor; IHC, immunohistochemistry

© 2006 The Society for Investigative Dermatology

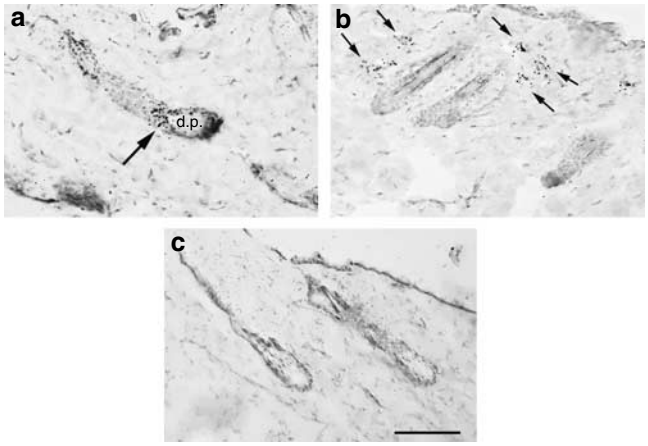

Figure 1. CRH-R1 mRNA is localized in the hair follicle and cells surrounding the hair follicle. (**a**) Photomicrograph of mouse skin section hybridized with ^{33}P-labeled antisense CRH-R1 probe. CRH-R1 autoradiographic signal (silver grains) in hair follicle (d.p. stands for dermal papillae); signal is localized to the outer root sheath cells (arrow). (**b**) Photomicrograph of mouse skin section hybridized with ^{33}P-labeled antisense CRH-R1 probe. CRH-R1 autoradiographic signal is localized in cells (arrow) surrounding the hair follicle with morphological characteristics of mast cells. (**c**) Negative control; photomicrograph of mouse skin section hybridized with ^{33}P-labeled sense CRH-R1 probe. (**a**–**c**) Bar = 200 μm.

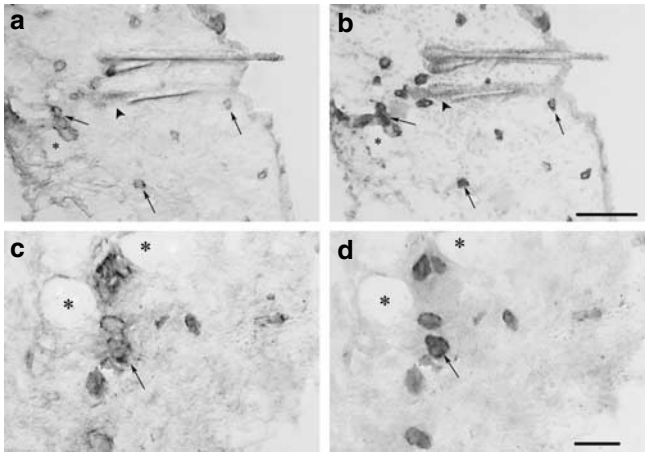

Figure 2. Skin mast cells express CRH-R. IHC was performed using a polyclonal rabbit CRH-RI/II antibody that was detected with a horseradish peroxidase-conjugated secondary antibody. Following IHC, skin sections were stained with toluidine blue to identify skin mast cells. (**a**) Low magnification (× 10) demonstrating CRH-R-like immunoreactivity localized in the hair follicle (arrowhead) and perifollicular and perivascular cells (arrow) (*denotes blood vessel lumen). (**b**) Low magnification (× 10) of a skin section stained with toluidine blue. Mast cells are localized both around the hair follicles and blood vessels (arrow). (**c**) Higher magnification (× 20) of a mouse skin section showing strong CRH-R-like immunoreactivity on cells (arrow) located adjacent to blood vessels (*denotes blood vessel lumen). (**d**) Skin mast cells (arrow) stained with toluidine blue are adjacent to blood vessels (*denotes blood vessel lumen). Bar: (**a**, **b**) = 100 μm; (**c**, **d**) = 50 μm.

causes peripheral skin vasodilation and flushing dependent on CRH-R1 and mast cells (Crompton *et al.*, 2003). Human mast cells express mRNA and protein for a number of CRH-R1 isoforms and CRH-R2 (Cao *et al.*, 2005), suggesting there could be autocrine effects. Human mast cells synthesize both CRH and urocortin that could be released in response to immunologic stimulation (Theoharides and Cochrane, 2004). Mast cells can secrete histamine and tryptase, which can stimulate proteinase-activated receptors on sensory neurons, which in turn enhance the inflammatory response via the release of additional substance P (Steinhoff *et al.*, 2000). Mast cells can also secrete vascular endothelial growth factor, an isoform of which is vasodilatory (Grutzkau *et al.*, 1998). In fact, CRH could stimulate selective release of vascular endothelial growth factor (Cao *et al.*, 2005), which is overexpressed in psoriatic skin and in delayed hypersensitivity reactions (Brown *et al.*, 1995); moreover, overexpression of vascular endothelial growth factor in mouse skin was shown to lead to skin inflammation resembling psoriasis (Xia *et al.*, 2003).

The skin has been shown to have a peripheral equivalent of the hypothalamic–pituitary–adrenal axis (Slominski and Mihm, 1996; Slominski and Wortsman, 2000; Ito *et al.*, 2005; Slominski *et al.*, 2005a, b). A ''brain–skin connection'' that involves the hair follicle, neuropeptides, and mast cells has been implicated in the pathogenesis of allergic and inflammatory skin diseases exacerbated by stress (Paus *et al.*, 2006). The present findings indicate that a subpopulation of skin mast cells are involved in these processes.

MATERIALS AND METHODS

In situ Hybridization

Male 25–30 g C57BL/6 mice (Charles River, NY) were anesthetized with intraperitoneal administration of ketamine/xylazine (100 and 20 mg/kg each) and killed by decapitation. The fur was removed and the skin was dissected, immediately frozen in Tissue-Tek embedding medium and stored at −80°C. Sections (7 μm thick) were cut on a cryostat, were adhered to slides at room temperature, and were stored at −80°C before *in situ* hybridization. These experiments were approved by the Tufts University Intstitutional Animal Care and Use Committee.

CRH-R1 Probes

A 700 bp fragment of the 5′ end of mouse CRH-R1 coding region was subcloned into the pcDNA-1 expression vector (Invitrogen, Eugene, OR) that contains the T7 and Sp6 RNA polymerase promoter sequence. T7 and Sp6 RNA polymerase were used to transcribe the cRNA for CRH-R1 from linearized plasmids. CRH-R1 cRNA transcripts were labeled using T3 polymerase and [α-^{33}P]UTP (10 μCi/ml) by a standard transcription reaction (Marchand, 1992, 1994). Isotopically labeled transcripts were run on an acrylamide denaturing

gel to confirm the correct size of the transcripts.

Hybridization procedure. Tissue sections were thawed and fixed in 4% paraformaldehyde for 15 minutes at room temperature. After fixation, skin sections from the same experiment were hybridized together for single-labeled autoradiographic *in situ* hybridization as described previously (Cain *et al*., 2003).

IHC for CRH-R

IHC was performed on mouse skin sections using a CRH-RI/II rabbit polyclonal antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) together with the DAKO EnVision + System-HRP (DAB) (DakoCytomation, Carpinteria, CA). Cryostat sections (7 μm) were prepared from mouse skin and fixed with Bouin's fixative for 5 minutes and incubated with endogenous peroxidase blocking reagent for an additional 5 minutes. The sections were incubated with primary antibody (1:75–1:200 dilution) overnight at 4°C. The following day, the sections were incubated in goat anti-rabbit Ig conjugated to horseradish peroxidase for 45 minutes followed by addition of the substrate chromogen DAB for 10 minutes. The sections were rinsed in Tris-buffered saline for 5 minutes between incubations. Negative controls were performed by the omission of the primary antibody or its substitution by a naive isotypic antibody. A blocking/neutralizing peptide was not available from Santa Cruz because the CRH-R antibody used was raised against a recombinant peptide and not a peptide antigen. The presence of brown-colored end product at the site of the target antigen indicated positive CRH-RI/II staining. The slides were then stained with toluidine blue to label the mast cells.

ACKNOWLEDGMENTS

This work was supported in part by grant AR 47652 from NIH, USA to TCT. The authors thank Ms Jessica Christian for her word processing skills.

Jill Donelan[1], James E. Marchand[2], Duraisamy Kempuraj[1], Nikoletta Papadopoulou[1], Dean Papaliodis[1] and Theoharis C. Theoharides[1,3,4]

[1]Department of Pharmacology & Experimental Therapeutics, Tufts University School of Medicine and Tufts-New England Medical Center, Boston, Massachussets, USA; [2]Department of Anatomy & Cell Biology, Tufts University School of Medicine and Tufts-New England Medical Center, Boston, Massachussets, USA; [3]Department of Biochemistry, Tufts University School of Medicine, Boston, Massachussets, USA and [4]Department of Internal Medicine, Tufts University School of Medicine and Tufts-New England Medical Center, Boston, Massachussets, USA. E-mail: theoharis.theoharides@tufts.edu

REFERENCES

Arck PC, Handjiski B, Hagen E, Joachim R, Klapp BF, Paus R (2001) Indications for a ''brain–hair follicle axis (BHA)'': inhibition of keratinocyte proliferation and up-regulation of keratinocyte apoptosis in telogen hair follicles by stress and substance P. *FASEB J* 15:2536–8

Arck PC, Handjiski B, Kuhlmei A, Peters EM, Knackstedt M, Peter A *et al.* (2005) Mast cell deficient and neurokinin-1 receptor knockout mice are protected from stress-induced hair growth inhibition. *J Mol Med* 83:386–96

Brown LF, Olbricht SM, Berse B, Jackman RW, Matsueda G, Tognazzi KA *et al.* (1995) Overexpression of vascular permeability factor (VPF/VEGF) and its endothelial cell receptors in delayed hypersensitivity skin reactions. *J Immunol* 154:2801–7

Cain BM, Connolly K, Blum A, Vishnuvardhan D, Marchand JE, Beinfeld MC (2003) Distribution and colocalization of cholecystokinin with the prohormone convertase enzymes PC1, PC2, and PC5 in rat brain. *J Comp Neurol* 467:307–25

Cao J, Papadopoulou N, Kempuraj D, Boucher WS, Sugimoto K, Cetrulo CL *et al.* (2005) Human mast cells express corticotropin-releasing hormone (CRH) receptors and CRH leads to selective secretion of vascular endothelial growth factor (VEGF). *J Immunol* 174:7665–75

Crompton R, Clifton VL, Bisits AT, Read MA, Smith R, Wright IM (2003) Corticotropin-releasing hormone causes vasodilation in human skin via mast cell-dependent pathways. *J Clin Endocrinol Metab* 88: 5427–32

Donelan J, Papadopoulou N, Marchand J, Kempuraj D, Lytinas M, Boucher W *et al.* (2006) Corticotropin-releasing hormone (CRH) induces skin vascular permeability through a neurotensin (NT)-dependent process. *Proc Natl Acad Sci USA*. (in press)

Grutzkau A, Kruger-Krasagakes S, Baumeister H, Schwarz C, Kogel H, Welker P *et al.* (1998) Synthesis, storage and release of vascular endothelial growth factor/vascular permeability factor (VEGF/VPF) by human mast cells: Implications for the biological significance of $VEGF_{206}$. *Mol Biol Cell* 9:875–84

Ito N, Ito T, Kromminga A, Bettermann A, Takigawa M, Kees F *et al.* (2005) Human hair follicles display a functional equivalent of the hypothalamic–pituitary–adrenal axis and synthesize cortisol. *FASEB J* 19:1332–4

Kaneko K, Kawana S, Arai K, Shibasaki T (2003) Corticotropin-releasing factor receptor type 1 is involved in the stress-induced exacerbation of chronic contact dermatitis in rats. *Exp Dermatol* 12:47–52

Katsarou-Katsari A, Filippou A, Theoharides TC (1999) Effect of stress and other psychological factors on the pathophysiology and treatment of dermatoses. *Int J Immunopathol Pharmacol* 12:7–11

Kumamoto T, Shalhevet D, Matsue H, Mummert ME, Ward BR, Jester JV *et al.* (2003) Hair follicles serve as local reservoirs of skin mast cell precursors. *Blood* 102:1654–60

Lytinas M, Kempuraj D, Huang M, Boucher W, Esposito P, Theoharides TC (2003) Acute stress results in skin corticotropin-releasing hormone secretion, mast cell activation and vascular permeability, an effect mimicked by intradermal corticotropin-releasing hormone and inhibited by histamine-1 receptor antagonists. *Int Arch Allergy Immunol* 130:224–31

Marchand JE, Wurm WH, Kato T, Kream RM (1994) Alterec tachykinin expression by dorsal root gangalion neurons a rat model of neuropathic pain. *Pain* 58:219–31

Marchand JE, Zaccheo TS, Connelly CS, Kream RM (1992) Selective *in situ* hybridization histochemical analyses of alternatively spliced mRNAs encoding preprotachykinins in rat central nervous system. *Mol Brain Res* 17:83–94

Panconesi E, Hautmann G (1996) Psychophysiology of stress in dermatology. The psychobiologic pattern of psychosomatics. *Dermatol Clin* 14:399–421

Papadopoulou N, Kalogeromitros D, Staurianeas NG, Tiblalexi D, Theoharides TC (2005) Corticotropin-releasing hormone receptor-1 and histidine decarboxylase expression in chronic urticaria. *J Investig Dermatol* 125:952–5

Paus R, Maurer M, Slominski A, Czarnetzki BM (1994) Mast cell involvement in murine hair growth. *Dev Biol* 163:230–40

Paus R, Theoharides TC, Arck P (2006) Neuroimmunoendocrine circuitry of the ''brain-skin connection''. *Trends Immunol*. (in press)

Peters EM, Botchkarev VA, Botchkareva NV, Tobin DJ, Paus R (2001) Hair-cycle-associated remodeling of the peptidergic innervation of murine skin, and hair growth modulation by neuropeptides. *J Investig Dermatol* 116:236–45

Peters EM, Handjiski B, Kuhlmei A, Hagen E, Bielas H, Braun A *et al.* (2004) Neurogenic inflammation in stress-induced termination of murine hair growth is promoted by nerve growth factor. *Am J Pathol* 165:259–71

Pisarchik A, Slominski AT (2001) Alternative splicing of CRH-R1 receptors in human and mouse skin: identification of new variants and their differential expression. *FASEB J* 15:2754–6

Roloff B, Fechner K, Slominski A, Furkert J, Botchkarev VA, Bulfone-Paus S *et al.* (1998) Hair cycle-dependent expression of corticotropin-releasing factor (CRF) and CRF receptors in murine skin. *FASEB J* 12:287–97

Singh LK, Pang X, Alexacos N, Letourneau R, Theoharides TC (1999) Acute immobilization stress triggers skin mast cell degranulation via

corticotropin releasing hormone, neurotensin and substance P: A link to neurogenic skin disorders. *Brain Behav Immunity* 13:225–39

Slominski A, Ermak G, Hwang J, Chakraborty A, Mazurkiewicz JE, Mihm M (1995) Proopiomelanocortin, corticotropin releasing hormone and corticotropin releasing hormone receptor genes are expressed in human skin. *FEBS Lett* 374:113–6

Slominski A, Ermak G, Hwang J, Mazurkiewicz J, Corliss D, Eastman A (1996) The expression of proopiomelanocortin (POMC) and of corticotropin releasing hormone receptor (CRH-R) genes in mouse skin. *Biochim Biophys Acta* 1289:247–51

Slominski A, Ermak G, Mazurkiewicz JE, Baker J, Wortsman J (1998) Characterization of corticotropin-releasing hormone (CRH) in human skin. *J Clin Endocrinol Metab* 83:1020–4

Slominski A, Mihm MC (1996) Potential mechanism of skin response to stress. *Int J Dermatol* 35:849–51

Slominski A, Pisarchik A, Tobin DJ, Mazurkiewicz J, Wortsman J (2004) Differential expression of a cutaneous corticotropin-releasing hormone system. *Endocrinology* 145:941–50

Slominski A, Roloff B, Curry J, Dahiya M, Szczesniewski A, Wortsman J (2000) The skin produces urocortin. *J Clin Endocrinol Metab* 85:815–23

Slominski A, Wortsman J (2000) Neuroendocrinology of the skin. *Endocr Rev* 21:457–87

Slominski A, Wortsman J, Pisarchik A, Zbytek B, Linton EA, Mazurkiewicz JE *et al.* (2001) Cutaneous expression of corticotropin-releasing hormone (CRH), urocortin, and CRH receptors. *FASEB J* 15:1678–93

Slominski A, Zbytek B, Semak I, Sweatman T, Wortsman J (2005a) CRH stimulates POMC activity and corticosterone production in dermal fibroblasts. *J Neuroimmunol* 162: 97–102

Slominski A, Zbytek B, Szczesniewski A, Semak I, Kaminski J, Sweatman T *et al.* (2005b) CRH stimulation of corticosteroids production in melanocytes is mediated by ACTH. *Am J Physiol Endocrinol Metab* 288:E701–6

Slominski AT, Botchkarev V, Choudhry M, Fazal N, Fechner K, Furkert J *et al.* (1999) Cutaneous expression of CRH and CRH-R. Is there a ''skin stress response system?''. *Ann NY Acad Sci* 885:287–311

Steinhoff M, Vergnolle N, Young SH, Tognetto M, Amadesi S, Ennes HS *et al.* (2000) Agonists of proteinase-activated receptor 2 induce inflammation by a neurogenic mechanism. *Nat Med* 6:151–8

Theoharides TC, Cochrane DE (2004) Critical role of mast cells in inflammatory diseases and the effect of acute stress. *J Neuroimmunol* 146:1–12

Theoharides TC, Donelan JM, Papadopoulou N, Cao J, Kempuraj D, Conti P (2004) Mast cells as targets of corticotropin-releasing factor and related peptides. *Trends Pharmacol Sci* 25:563–8

Theoharides TC, Singh LK, Boucher W, Pang X, Letourneau R, Webster E *et al.* (1998) Corticotropin-releasing hormone induces skin mast cell degranulation and increased vascular permeability, a possible explanation for its pro-inflammatory effects. *Endocrinology* 139:403–13

Xia YP, Li B, Hylton D, Detmar M, Yancopoulos GD, Rudge JS (2003) Transgenic delivery of VEGF to mouse skin leads to an inflammatory condition resembling human psoriasis. *Blood* 102:161–8