Corticotropin-Releasing Hormone and Its Structurally Related Urocortin Are Synthesized and Secreted by Human Mast Cells

DURAISAMY KEMPURAJ, NIKOLETTA G. PAPADOPOULOU, MICHAEL LYTINAS, MAN HUANG, KRISTIANA KANDERE-GRZYBOWSKA, BHUVANESHWARI MADHAPPAN, WILLIAM BOUCHER, SPYRIDON CHRISTODOULOU, ACHILLES ATHANASSIOU, AND THEOHARIS C. THEOHARIDES

Departments of Pharmacology & Experimental Therapeutics (D.K., N.G.P., M.L., M.H., K.K.-G., B.M., W.B., S.C., T.C.T.), Biochemistry (K.K.-G., T.C.T.), Obstetrics and Gynecology (A.A.), and Internal Medicine (T.C.T.), Tufts University School of Medicine and Tufts-New England Medical Center, Boston, Massachusetts 02111

Stress activates the hypothalamic-pituitary-adrenal axis through CRH, leading to production of glucocorticoids that down-regulate immune responses. However, acute stress also has proinflammatory effects. We previously showed that restraint stress, as well as CRH and its structurally related urocortin (Ucn), could activate mast cells and trigger mast cell-dependent vascular permeability. Here we show for the first time that human cord blood-derived cultured mast cells (hCBMC) at 10 wk, but not at 2 wk, are immunocytochemically positive for CRH and Ucn; human leukemic mast cells are weakly positive for both peptides. The ability of these mast cells to synthesize CRH and Ucn was confirmed by showing mRNA expression with RT-PCR. hCBMC (8–14 wk) synthesize and store 1–10 ng/10^6 cells (10–20 μg/g) of both CRH and Ucn detected by ELISA of cell homogenates. Stimulation of IgE-sensitized hCBMC with anti-IgE results in secretion of most CRH and Ucn. These findings indicate that mast cells are not only the target, but also a potential source of CRH and Ucn that could have both autocrine and paracrine functions, especially in allergic inflammatory disorders exacerbated by stress. (*Endocrinology* 145: 43–48, 2004)

S TRESS ACTIVATES THE hypothalamic-pituitary-adrenal axis through the release of CRH leading to secretion of catecholamines and glucocorticoids; these, in turn, suppress the immune response (1). CRH is a 41-amino-acid peptide synthesized in the hypothalamus and mediates its effects through at least two types of receptors (R): CRHR-1, CRHR-2, of which a number of subtypes have been identified. These receptors are also present in other brain regions and outside the central nervous system including immune cells (1); therefore, CRH, or structurally related compounds such as urocortin (Ucn) (2), could have immunoregulatory actions (3). However, stress also worsens a number of neuroinflammatory disorders through proinflammatory effects of CRH (4) that are apparently mediated by mast cell activation (5). For instance, acute restraint stress induces intracranial rat mast cell activation (6) that is CRH-dependent as it is blocked by the nonpeptide CRHR-1 antagonist Antalarmin (7). Stress also increases blood-brain-barrier permeability (8) that is dependent on mast cells and CRH (9). Moreover, CRH (5) and Ucn (10) induce mast cell degranulation and vascular permeability in rodent skin, actions mimicked by acute restraint stress (11), but absent in W/Wᵛ mast cell-deficient

mice and also blocked by Antalarmin (5). Acute restraint stress also increases skin CRH levels (12); it also increases skin mast cells and exacerbates delayed hypersensitivity reactions through CRHR-1 (13).

Mast cells are ubiquitous in the body and are critical for allergic reactions, as they secrete numerous vasoactive molecules and cytokines (14, 15). Increasing evidence, however, indicates that mast cells also secrete proinflammatory cytokines (14, 15), such as IL-6 (16) and are involved in neuroinflammatory processes (17). These findings have prompted the speculation that mast cells may have a much more versatile role than previously suspected (18, 19). It has been difficult to obtain human mast cells, as they are usually isolated from clear margins of lung resections or from foreskin following circumcisions. Most of our current knowledge about mast cell-derived cytokines comes from human leukemic (HMC-1) mast cells, but these cells do not respond to physiologic triggers, such as stimulation by antigen or anti-IgE, because they do not express IgE binding protein (FcεRI) (20). A number of investigators have developed and used human umbilical cord blood-derived mast cells (hCBMC) grown in the presence of stem cell factor (SCF) and IL-6 (21) for about 16 wk. Sensitized hCBMC respond adequately to anti-IgE stimulation and secrete histamine, tryptase, and a variety of cytokines (21, 22).

Here, we show for the first time that human umbilical cord blood-derived cultured mast cells and HMC-1 cells synthesize both CRH and Ucn, which they can secrete in response to immunologic stimulation.

Abbreviations: CRHR-1 and -2, CHR receptors 1 and 2; hCBMC, human cord blood-derived cultured mast cells; HMC-1, human leukemic mast cells; RA, rheumatoid arthritis; SCF, stem cell factor; TBS, Tris buffer saline; Ucn, urocortin.

Materials and Methods

Human mast cell culture

Human umbilical cord blood was obtained from healthy mothers during normal deliveries, with approval by the Hospital's Human Investigation Review Board and without any identifying information or other medical or social history of the mother. The hCBMC were generated as described previously with minor modifications (21). Briefly, mononuclear cells were isolated by centrifuging heparin-treated cord blood onto lymphocyte separation medium (INC Biomedical, Aurora, OH) at $350 \times g$ for 30 min at room temperature and the buffy coat was removed. Mast cell progenitors were then isolated by positive selection of AC133 expressing cells (CD133$^+$) by an immunomagnetic cell sorting method (Miltenyi Biotec, Auburn, CA). The CD133 is a surface antigen with a molecular mass of 177 kDa, which is selectively expressed on a CD34^{bright} (CD34$^+$) subset of human hemopoietic stem and progenitor cells. The purified CD34$^+$ cells were cultured in Iscove's modified Dulbecco's medium (GIBCO BRL, Life Technologies, Grand Island, NY) supplemented with 100 ng/ml recombinant human SCF (a generous gift from Amgen, Thousand Oaks, CA), 50 ng/ml IL-6 (Chemicon International, Inc., Temecula, CA), 10% fetal bovine serum (BioWhittaker, Walkersville, MD), 50 μM 2-mercaptoethanol (GIBCO BRL), 1% penicillin-streptomycin (GIBCO BRL) at 37 C in a 5% CO_2 incubator. Both SCF and IL-6 are required for normal growth and maturation of hCBMC (21). Cultured hCBMC respond adequately to immunologic stimulation for up to about 16 wk. The hCBMC used in this study were 10 wk old for the positive immunocytochemistry and RNA extraction, but were from 8–14 wk for the ELISA studies because more cells were required. The purity of mast cells reached greater than 99% by May-Gruenwald and Giemsa Staining and were 100% tryptase positive by 8 wk of culture.

HMC-1 cell culture

HMC-1 cells were kindly supplied by Dr. J. H. Butterfield (Mayo Clinic, Rochester, MN) and were cultured as previously described (23).

Immunocytochemistry for CRH and Ucn

Cytospin smears of cells (hCBMC and HMC-1) were prepared using Cytospin 3 (Shandon, Pittsburgh, PA) and were stained immunohistochemically for CRH and Ucn by peroxidase using the Dako EnVision System, Peroxidase Kit (Dako Corp., Carpinteria, CA) as per the directions of the manufacturer. Cytospin smears were fixed with Carnoy's solution (60% ethanol, 30% chloroform, and 10% glacial acetic acid) for 3 min. Slides were then incubated with peroxidase blocking reagent (0.03% hydrogen peroxide containing sodium azide) for 5 min and placed in Tris-buffered saline (TBS) for 5 min. The primary antibodies used were polyclonal rabbit antihuman CRH (Phoenix Pharmaceuticals, Inc., Belmont, CA) and polyclonal rabbit antihuman Ucn (Phoenix) at 1:60 and 1:500 dilutions for 30 min. Slides were placed in TBS for 5 min and incubated with peroxidase-labeled polymer conjugated to goat antirabbit and antimouse Igs for 30 min. Slides were then rinsed with TBS and incubated with substrate 3,3'-diamino-benzidine chromogen solution for 5 min. All the incubations were carried out at room temperature. Presence of a brown-colored end product at the site of the target antigens indicates positive reactivity. Negative controls contained samples in which the primary antibody was omitted or replaced by normal rabbit serum, as well as immature hCBMC of 2-wk culture. HMC-1 were also included for comparison.

Total RNA extraction and RT-PCR for CRH and Ucn mRNA expression in mast cells

Total RNA was prepared from 10-wk-old hCBMC and HMC-1 cells using the TRI Reagent (Sigma, St. Louis, MO). Cells (10^7) were collected by centrifugation and lysed in 1 ml TRI reagent according to the manufacturer's recommendations for RNA isolation from suspension cells. First-strand cDNA synthesis was performed from 2 μg total RNA (heated for 3 min at 65 C) using 200 U murine Moloney virus reverse transcriptase, 500 μg/ml oligo deoxythymidine primer, 40 U/μl RNaseOUT recombinant ribonuclease inhibitor (Invitrogen Life Technologies, Carlsbad, CA) and 5 mM deoxynucleotide triphosphates (Roche Diagnostics, Mannheim, Germany). The reaction (20 μl) was run for 50 min
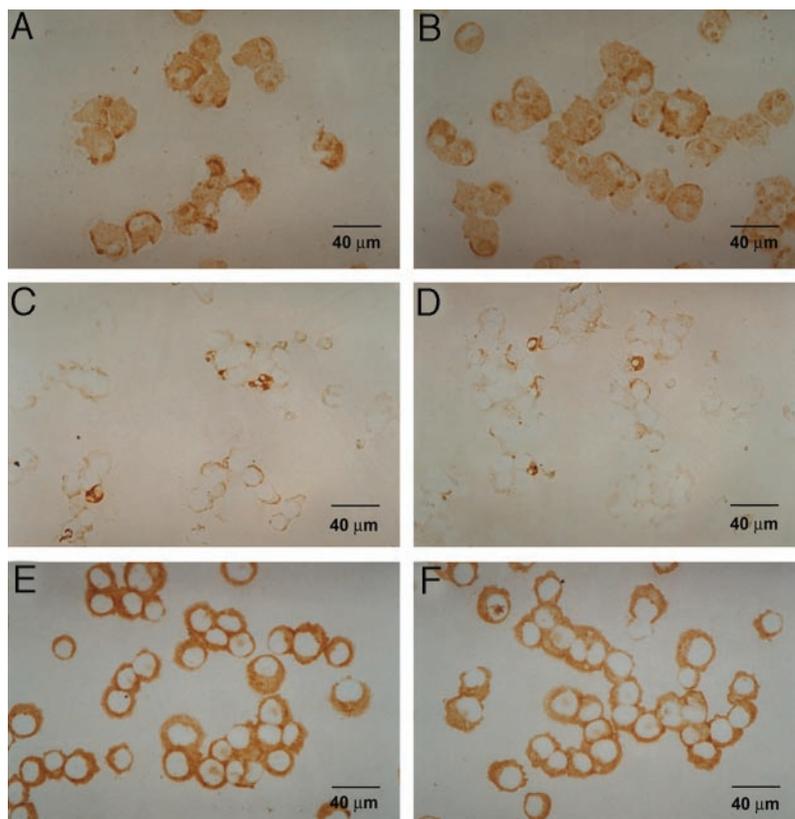

FIG. 1. Photomicrographs of hCBMC and HMC-1 cells immunostained for CRH and Ucn. Ten-week-old hCBMC (A and B) and 2-wk-old hCBMC (C and D) cells showing immunocytochemical staining with anti-CRH (A and C) and anti-Ucn (B and D) antibodies; note that 10-wk-old hCBMC stain positively for both peptides (*brown color*), whereas 2-wk-old hCBMC are almost negative; HMC-1 cells (E and F) show moderate staining for both CRH (E) and Ucn (F) peptides.

at 37 C, and inactivated by heating at 72 C for 15 min. A portion of cDNA (10%) was used for amplification in PCR for 30 cycles. Two specific oligonucleotide sets of primers, previously published for CRH (sense: 5′-TTCCGAGGAGCCTCCCATC-3′ and antisense: 5′-AATCTCCAT-GAGTTTCCTGTTGC-3′ (24) and Ucn (sense: 5′ CAGGCGAGCGGC-CGCG-3′ and antisense: 5′-CTTGCCCACCGAGTCGAAT-3′ (25) mRNA detection, were employed in this study using the same amplification conditions with the following modifications: all PCRs (50 μl volume) were carried out using 1 U *Taq* DNA Polymerase (Invitrogen Life Technologies), 0.2 mM deoxynucleotide triphosphates, 6 pmol of each primer, 4.5 mM $MgCl_2$ (Invitrogen Life Technologies) for CRH and 3.5 mM $MgCl_2$ for Ucn, in a DNA Engine-Peltier Thermal Cycler (MJ Research, Watertown, MA). Placental and whole uterus cDNAs (CLON-TECH, Palo Alto, CA) were used as positive controls for CRH and Ucn PCR amplification respectively; cDNA was replaced with sterile distilled water as negative control for all PCRs. The amplified product was electrophoresed on a 2% agarose gel and visualized by ethidium bromide, using 100-bp DNA ladder (Invitrogen Life Technologies) to estimate the band sizes, which were 122 bp for CRH and 145 bp for Ucn.

Activation of hCBMC with anti-IgE

For anti-IgE stimulation experiments, hCBMC (10^6 cells/ml) were washed with Iscove's modified Dulbecco's medium and Tyrode's buffer, once in each, and passively sensitized by incubation with human myeloma IgE (2 μg/ml/10^6 cells; Chemicon Inc.) for 48 h in culture medium at 37 C. Cells were then washed two times and resuspended in fresh culture medium. Cells were stimulated with anti-IgE (Dako) at 15 μg/ml in 96-well round bottom culture plates (2×10^5 cells in 200 μl medium/ sample) for 6 h at 37 C in 5% CO_2 as we previously reported (22). Tryptase was measured in the supernatants and cell pellets by fluoroenzyme-immunoassay (Pharmacia, Uppsala, Sweden) as reported previously (26). CRH, Ucn, and tryptase results were expressed as pg/ml and as percent of total = [supernatant/(supernatant + pellet)] $\times$ 100%. This presentation allowed normalization of any baseline differences between cultures. These peptides were measured in the supernatant and pellet to calculate percentage of release.

Assay for CRH and Ucn by ELISA

CRH and Ucn were measured in the supernatant or the cell pellet after cells were lysed in lysis buffer (0.5% Triton X-100 containing Aprotinin and 100 mM PMSF in PBS) with sonication three times for 15 sec each at full power (Dimco Gray Co., Dayton, OH; model no.161) at 4 C. CRH and Ucn were assayed by ELISA (Phoenix Pharmaceuticals, Belmont, CA).

Statistics

Results are expressed as mean $\pm$ SD and similar groups were compared using Student's *t* test. Results from different cultures were compared using ANOVA. Significance is denoted by $P < 0.05$.

Results

Immunocytochemistry for CRH and Ucn in hCBMC and HMC-1 cells

Cultured hCBMC respond adequately to immunologic stimulation from 8–16 wk of culture. For immunocytochemistry, we compared 2 and 10-wk-old cells. hCBMC and HMC-1 cells were immunostained with specific antibodies to CRH (Fig. 1, A, C, and E) or Ucn (Fig. 1, B, D, and F). The cytoplasm of 10-wk-old hCBMC (Fig. 1, A and B) was uniformly stained brown with antibodies to both CRH and Ucn, indicating that hCBMC contain both peptides. Negative controls did not show any immunoreactivity for either peptide (not shown) and included 2-wk-old hCBMC that were almost negative (Fig. 1, C and D). HMC-1 cells were moderately stained for both peptides (Fig. 1, E and F). hCBMC and HMC-1 cells without primary antibody or by using normal

rabbit serum showed no positive staining for either CRH or Ucn (results not shown).

Expression of CRH and Ucn mRNA in HMC-1 and hCBMC

RT-PCR using total RNA extracted from HMC-1 and hCBMC was used for detection of CRH and Ucn expression as described in *Materials and Methods*. Oligonucleotide primers of previously published sequences for CRH (24) and Ucn (25) were used and yielded the expected bands of 122 bp for CRH (Fig. 2A) and 145 bp for Ucn (Fig. 2B). Positive controls included placental cDNA and whole uterus cDNA for CRH and Ucn, respectively; they were previously shown to express these PCR products using the same sets of primers. No amplification product was detected in the negative control as shown in Fig. 2 (blank) in which cDNA was substituted with water. PCR amplification product using primers for β-actin
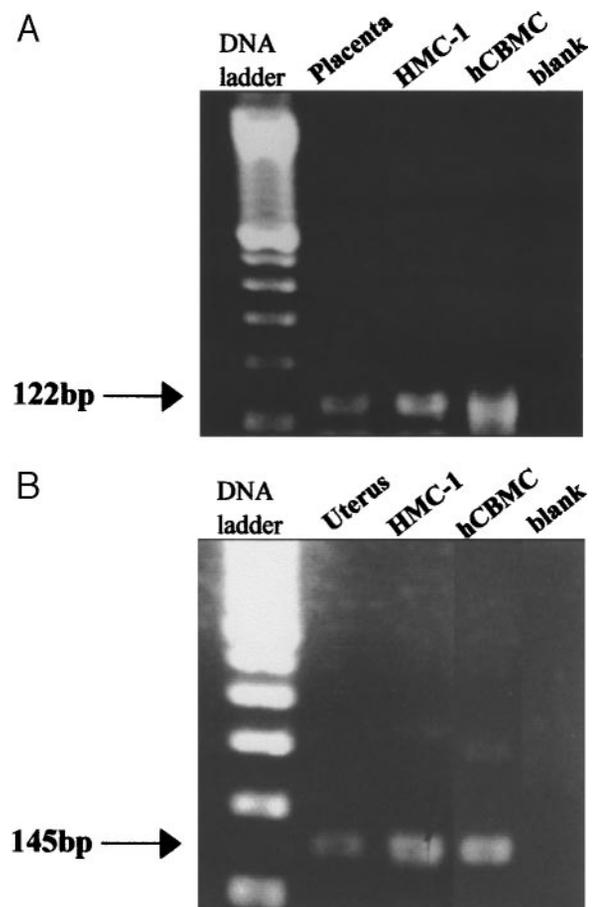

FIG. 2. CRH and Ucn mRNA expression in HMC-1 and hCBMC cells. RT-PCR using total RNA extracted from HMC-1 and 10-wk-old hCBMC was used for detection of CRH and Ucn expression as described in *Materials and Methods*. A, HMC-1 and hCBMC cDNAs express a 122-bp band (*arrow*) that corresponds to CRH mRNA amplification product. Placental cDNA that was used as positive control confirms that the 122-bp band is the expected PCR product specific for CRH. B, HMC-1 and hCBMC cDNAs express a 145-bp band (*arrow*) that corresponds to Ucn mRNA amplification product. Whole uterus cDNA that was used as positive control confirms that the 145-bp band is the expected PCR product specific for Ucn. No amplification product was detected in the blank, as expected, in which the cDNA has been substituted with water serving as negative control.

as a housekeeping gene did not show any variation in the cDNA content among these samples (results not shown).

CRH and Ucn levels in hCBMC and HMC-1 cells by ELISA

The presence of CRH and Ucn was also confirmed by ELISA in lysed hCBMC and HMC-1 cells. hCBMC and HMC-1 cell samples were assayed in quantuplicate and contained 1.1 ± 0.3 ng/10^6 cells and 0.8 ± 0.2 ng/10^6 cells of CRH, respectively (Table 1). The corresponding values for Ucn were 1.2 ± 0.6 ng/10^6 hCBMC, and 0.9 ± 0.3 ng/10^6 HMC-1 cells, respectively.

Secretion of CRH, Ucn, and tryptase from hCBMC

Stimulation of IgE-sensitized hCBMC with anti-IgE for 6 h increased tryptase release (Table 2) from a spontaneous level of 63.7 ± 45.3 ng/5×10^5 cells (3.2%) to 360.3 ± 144.1 ng/5×10^5 cells (27.8%) (n = 6, $P < 0.05$). Similar stimulation induced considerable secretion of CRH (Table 1) from spontaneous release of 0.06 ± 0.01 ng/10^6 cells (5.4%) to 0.8 ± 0.2 ng/10^6 cells (41.9%). Ucn secretion was also increased from spontaneous release of 0.05 ± 0.02 ng/10^6 cells (4.1%) to 0.8 ± 0.3 ng/10^6 cells (69.2%, $P < 0.05$).

Discussion

This is the first time that CRH and Ucn are shown to be expressed, synthesized, stored, and secreted from human umbilical cord-blood-derived mast cells. The amount of CRH and Ucn in hCBMC was 1–10 ng/10^6 cells and varied almost 10-fold among the different primary cultures. The reason for the observed variability on the levels of CRH/Ucn in this study might be in part due to a wide variation among the individual batches of hCBMC, as previously reported for the difference in mediator secretion from hCBMC (27, 28), or the duration of culture of different batches of cells. This variability may be due to genetic factors, as well as the allergic or stress history of the mother/infant that were not available to us during umbilical cord blood collection. Using the average weight of one mast cell as 500 pg (see Table 1), the maximal CRH and Ucn content is calculated to be approximately 10–20 μg/g. This amount of CRH is approximately 1000 times more than what has been reported for human placenta at term (1 ng/g) (29), more than 100 times

higher than what was reported for the hypothalamus (106 ng/g) (30), and more than 10 times higher than what has been reported for carcinoid tumors (1000 ng/g) (31) (Table 1). It appears that these cultured mast cells may be one of the richest sources of CRH and Ucn reported to date; however, the recent advances in assay sensitivity makes difficult to make direct comparisons with older reports and such advances may account for the different levels reported. Moreover, our conclusion that mast cells may be one of the richest sources may not necessarily reflect tissue mast cell levels because hCBMC may: 1) lack any inhibitory signals otherwise provided by the tissue microenvironment and 2) have CRH/Ucn up-regulated by the IL-6 required for hCBMC culture. The mast cell content of Ucn is almost 100 times more than that reported in human peripheral lymphocytes (300 pg/10^8 cells) (32) that, unlike mast cells, produce only Ucn and not CRH (33). These results, along with the previous finding that HMC-1 (Ref. 5 and unpublished observations) and articular mast cells also express CRHR-1 (34), indicate that CRH and Ucn may also have autocrine effects on mast cells.

Mast cells are found everywhere in the body and are necessary for allergic and late phase reactions because they secrete many vasoactive molecules and cytokines (14, 15). Recent evidence indicates that mast cells may also be involved in neuroinflammatory conditions (17) and may play a much more versatile role than previously reported (18). A case in point is rheumatoid arthritis (RA), where mast cells are increased in the joints (35, 36), and express CRH receptors (34). Both CRH and Ucn have been reported to be elevated in the joints of patients with RA (37), a condition known to be exacerbated by stress (38–40). For instance, CRH was elevated in the synovium of patients with RA (104 ± 33 pg/ml) and was significantly higher than in osteoarthritis (25 ± 4 pg/ml) (41). We recently also showed that neither vascular permeability (42) nor carrageenan-induced arthritis (43) could develop in mast cell-deficient mice. Inflammatory arthritis was also greatly reduced in CRH knockout mice (43), whereas autoimmune arthritis was reduced by Antalarmin (44). Our results are also supported by the recent finding that autoimmune arthritis could not develop in mast cell-deficient mice (45). Mast cells could, therefore, participate in RA both by being the targets of CRH/Ucn secreted from local

TABLE 1. Comparison of the highest CRH and Ucn levels in representative human tissues

Tissues	CRH	Ucn	Ref.
hCBMC	10–20 μg/g[a]	10–20 μg/g[a]	Present results
Placenta/decidua (early or late pregnancy)	1 ng/g	Not reported	Saijonmaa *et al.*, *Placenta* 9:373–385, 1988 (29)
Hypothalamus	106 ng/g	Not reported	Wakabayashi *et al.*, *Cancer* 55:995–1000, 1985 (30)
Carcinoid tumors	1000 ng/g	Not reported	Tsuchihashi *et al.*, *Jpn J Clin Oncol* 22:232–237, 1992 (31)

[a] Average weight of one mast cell = 500 pg (52).

TABLE 2. Amount of CRH, Ucn, and tryptase secreted from hCBMC in response to anti-IgE

Conditions	Tryptase		CRH		Ucn	
	ng/5×10^5 Cells	% Release	ng/10^6 Cells	% Release	ng/10^6 Cells	% Release
Control	63.7 ± 45.3	3.23	0.06 ± 0.01	5.4	0.05 ± 0.01	4.1
Anti-IgE	360.3 ± 144.1	27.8[a]	0.8 ± 0.2	41.9[a]	0.8 ± 0.3	69.2[a]

[a] $P < 0.05$ (n = 6).

nerve endings upon stress and by secreting CRH/Ucn themselves. Secretion of CRH/Ucn from mast cells could also be triggered by proinflammatory molecules that are released during the initial phases of inflammation.

Acute restraint-stress led to mast cell-dependent increase in vascular permeability in rodent skin (11), an effect also induced by local administration of CRH (5) and its structurally related Ucn (10); these effects were blocked by the nonpeptide CRHR-1 antagonist Antalarmin. Acute stress also increased the CRH content of skin blister fluid (12), and CRH iontophoresis was recently shown to induce skin vasodilation in humans (46). Moreover, stress-induced alopecia was associated with CRH receptor up-regulation in affected sites (47).

Acute stress also increased the number of skin mast cells and worsened delayed hypersensitivity, effects blocked by pretreatment with a CRH receptor antagonist (13). Acute restraint stress also induced intracranial rat mast cell activation, a CRH-dependent action, as it was blocked by Antalarmin (6) and increased dura vascular permeability that was absent in W/W^v mast cell-deficient mice (48). Moreover, acute stress triggered brain mast cell activation and increased brain vascular permeability (8), an effect dependent on CRH and mast cells as it was absent in mast cell-deficient mice. These findings are relevant in view of the fact that hypothalamic mast cells could stimulate the hypothalamic-pituitary-adrenal axis (49–51).

In conclusion, the present demonstration that mast cells synthesize and secrete CRH/Ucn reinforces a bidirectional relationship of CRH/Ucn and mast cells in inflammatory conditions (19), especially those exacerbated by stress (52).

References

1. **Chrousos GP** 1995 The hypothalamic-pituitary-adrenal axis and immune-mediated inflammation. N Engl J Med 332:1351–1362
2. **Vaughan J, Donaldson C, Bittencourt J, Perrin MH, Lewis K, Sutton S, Chan R, Turnbull AV, Lovejoy D, Rivier C, Rivier J, Sawchenko PE, Vale W** 1995 Urocortin, a mammalian neuropeptide related to fish urotensin I and to corticotropin-releasing factor. Nature 378:287–292
3. **Karalis K, Louis JM, Bae D, Hilderbrand H, Majzoub JA** 1997 CRH and the immune system. J Neuroimmunol 72:131–136
4. **Karalis K, Sano H, Redwine J, Listwak S, Wilder RL, Chrousos GP** 1991 Autocrine or paracrine inflammatory actions of corticotropin-releasing hormone *in vivo*. Science 254:421–423
5. **Theoharides TC, Singh LK, Boucher W, Pang X, Letourneau R, Webster E, Chrousos G** 1998 Corticotropin-releasing hormone induces skin mast cell degranulation and increased vascular permeability, a possible explanation for its pro-inflammatory effects. Endocrinology 139:403–413
6. **Theoharides TC, Spanos CP, Pang X, Alferes L, Ligris K, Letourneau R, Rozniecki JJ, Webster E, Chrousos G** 1995 Stress-induced intracranial mast

cell degranulation. A corticotropin releasing hormone-mediated effect. Endocrinology 136:5745–5750
7. **Rozniecki JJ, Dimitriadou V, Lambracht-Hall M, Pang X, Theoharides TC** 1999 Morphological and functional demonstration of rat dura mast cell-neuron interactions *in vitro* and *in vivo*. Brain Res 849:1–15
8. **Esposito P, Gheorghe D, Kandere K, Pang X, Conally R, Jacobson S, Theoharides TC** 2001 Acute stress increases permeability of the blood-brain-barrier through activation of brain mast cells. Brain Res 888:117–127
9. **Esposito P, Chandler N, Kandere-Grzybowska K, Basu S, Jacobson S, Connolly R, Tutor D, Theoharides TC** 2002 Corticotropin-releasing hormone (CRH) and brain mast cells regulate blood-brain-barrier permeability induced by acute stress. J Pharmacol Exp Ther 303:1061–1066
10. **Singh LK, Boucher W, Pang X, Letourneau R, Seretakis D, Green M, Theoharides TC** 1999 Potent mast cell degranulation and vascular permeability triggered by urocortin through activation of CRH receptors. J Pharmacol Exp Ther 288:1349–1356
11. **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 Immunol 13:225–239
12. **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 intramural corticotropin-releasing hormone and inhibited by histamine-1 receptor antagonists. Int Arch Allergy Immunol 130:224–231
13. **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
14. **Kobayashi H, Ishizuka T, Okayama Y** 2000 Human mast cells and basophils as sources of cytokines. Clin Exp Allergy 30:1205–1212
15. **Marone G, Galli SJ, Kitamura Y** 2002 Probing the roles of mast cells and basophils in natural and acquired immunity, physiology and disease. Trends Immunol 23:425–427
16. **Huang M, Pang X, Karalis K, Theoharides TC** 2003 Stress-induced interleukin-6 release in mice is mast cell-dependent and more pronounced in apolipoprotein E knockout mice. Cardiovasc Res 59:241–249
17. **Theoharides TC** 1996 The mast cell: a neuroimmunoendocrine master player. Int J Tissue React 18:1–21
18. **Gurish MF, Austen KF** 2001 The diverse roles of mast cells. J Exp Med 194:1–6
19. **Theoharides TC, Cochrane D,** Critical role of mast cells in inflammatory diseases and the effect of acute stress. J Neuroimmunol, in press
20. **Nilsson G, Blom T, Kusche-Gullberg M, Kjellen L, Butterfield JH, Sundstrom C, Nilsson K, Hellman L** 1994 Phenotypic characterization of the human mast-cell line HMC-1. Scand J Immunol 39:489–498
21. **Kempuraj D, Saito H, Kaneko A, Fukagawa K, Nakayama M, Toru H, Tomikawa M, Tachimoto H, Ebisawa M, Akasawa A, Miyagi T, Kimura H, Nakajima T, Tsuji K, Nakahata T** 1999 Characterization of mast cell-committed progenitors present in human umbilical cord blood. Blood 93:3338–3346
22. **Kempuraj D, Huang M, Kandere K, Boucher W, Leutourneau R, Jeudy S, Fitzgerald K, Spear K, Athanasiou A, Theoharides TC** 2002 Azelastine is more potent than olopatadine in inhibiting interleukin-6 and tryptase release from human umbilical cord blood-derived cultured mast cells. Ann Allergy Asthma Immunol 88:501–506
23. **Butterfield JH, Weiler DA, Hunt LW, Wynn SR, Roche PC** 1990 Purification of tryptase from a human mast cell line. J Leukoc Biol 47:409–419
24. **Simoncini T, Apa R, Reism FM, Miceli F, Stomati M, Driul L, Lanzone A, Genazzani AR, Petraglia F** 1999 Human umbilical cord vein endothelial cells: a new source and potential target for corticotropin-releasing factor. J Clin Endocrinol Metab 84:2802–2806
25. **Florio P, Arcuri F, Ciarmela P, Runci Y, Romagnoli R, Cintorino M, Di Blasio AM, Petraglia F** 2002 Identification of human urocortin mRNA and peptide in the human endometrium. J Endocrinol 173:R9–R14
26. **Schwartz LB, Bradford RH, Rouse C, Irani A-M, Rasp G, Van der Zwan JK, Van der Linden P-WG** 1994 Development of a new, more sensitive immunoassay for human tryptase: use in systematic anaphylaxis. J Clin Immunol 14:190–204
27. **Yamaguchi M, Sayama K, Yano K, Lantz CS, Noben-Trauth N, Ra C, Costa JJ, Galli SJ** 1999 IgE enhances FcεRI expression and IgE-dependent release of histamine and lipid mediators from human umbilical cord blood-derived mast cells: synergistic effect of IL-4 and IgE on human mast cell FcεRI expression and mediator release. J Immunol 162:5455–5465
28. **Tachimoto H, Ebisawa M, Hasegawa T, Kashiwabara T, Ra C, Bochner B, Miura K, Saito H** 2000 Reciprocal regulation of cultured human mast cell cytokine production by IL-4 and IFN-γ. J Allergy Clin Immunol 106:141–149
29. **Saijonmaa O, Laatikainen T, Wahlström T** 1988 Corticotrophin-releasing factor in human placenta: localization, concentration and release in vitro. Placenta 9:373–385
30. **Wakabayashi I, Ihara T, Hattori M, Tonegawa Y, Shibasaki T, Hashimoto K** 1985 Presence of corticotropin-releasing factor-like immunoreactivity in human tumors. Cancer 55:995–1000
31. **Tsuchihashi T, Yamaguchi K, Abe K, Yanaihara N, Saito S** 1992 Production

of immunoreactive corticotropin-releasing hormone in various neuroendocrine tumors. Jpn J Clin Oncol 22:232–237

32. **Baigent SM** 2001 Peripheral corticotropin-releasing hormone and urocortin in the control of the immune response. Peptides 22:809–820

33. **Bamberger CM, Wald M, Bamberger A-M, Ergun S, Beil FU, Schulte HM** 1998 Human lymphocytes produce urocortin, but not corticotropin-releasing hormone. J Clin Endocrinol Metab 83:708–711

34. **McEvoy AN, Bresnihan B, FitzGerald O, Murphy EP** 2001 Corticotropin-releasing hormone signaling in synovial tissue from patients with early inflammatory arthritis is mediated by the type 1α corticotropin-releasing hormone receptor. Arthritis Rheum 44:1761–1767

35. **Wasserman SI** 1984 The mast cell and synovial inflammation. Arthritis Rheum 27:841–844

36. **Tetlow LC, Woolley DE** 1995 Distribution, activation and tryptase/chymase phenotype of mast cells in the rheumatoid lesion. Ann Rheum Dis 54:549–555

37. **Kohno M, Kawahito Y, Tsubouchi Y, Hashiramoto A, Yamada R, Inoue KI, Kusaka Y, Kubo T, Elenkov IJ, Chrousos GP, Kondo M, Sano H** 2001 Urocortin expression in synovium of patients with rheumatoid arthritis and osteoarthritis: relation to inflammatory activity. J Clin Endocrinol Metab 86:4344–4352

38. **Thomason BT, Brantley PJ, Jones GN, Dyer HR, Morris JL** 1992 The relation between stress and disease activity in rheumatoid arthritis. J Behav Med 15:215–220

39. **Herrmann M, Scholmerich J, Straub RH** 2000 Stress and rheumatic diseases. Rheum Dis Clin North Am 26:737–763

40. **Johnson EO, Moutsopoulos M** 1992 Neuroimmunological axis and rheumatic diseases. Eur J Clin Invest 22:S2–S5

41. **Crofford LJ, Sano H, Karalis K, Friedman TC, Epps HR, Remmers EF, Mathern P, Chrousos GP, Wilder RL** 1993 Corticotropin-releasing hormone in synovial fluids and tissues of patients with rheumatoid arthritis and osteoarthritis. J Immunol 151:1–10

42. **Huang M, Berry J, Kandere K, Lytinas M, Karalis K, Theoharides TC** 2002 Mast cell deficient W/W^v mice lack stress-induced increase in serum IL-6 levels, as well as in peripheral CRH and vascular permeability, a model of rheumatoid arthritis. Int J Immunopathol Pharmacol 15:249–254

43. **Mattheos S, Christodoulou S, Kempuraj D, Kempuraj B, Karalis K, Theoharides TC** 2003 Mast cells and corticotropin-releasing hormone (CRH) are required for experimental inflammatory arthritis. FASEB J 17:C44 (Abstract 35.4)

44. **Webster EL, Barrientos RM, Contoreggi C, Isaac MG, Ligier S, Gabry KE, Chrousos GP, McCarthy EF, Rice KC, Gold PW, Sternberg EM** 2002 Corticotropin releasing hormone (CRH) antagonist attenuates adjuvant induced arthritis: role of CRH in peripheral inflammation. J Rheumatol 29:1252–1261

45. **Lee DM, Friend DS, Gurish MF, Benoist C, Mathis D, Brenner MB** 2002 Mast cells: a cellular link between autoantibodies and inflammatory arthritis. Science 297:1689–1692

46. **Clifton VL, Crompton R, Smith R, Wright IM** 2002 Microvascular effects of CRH in human skin vary in relation to gender. J Clin Endocrinol Metab 87:267–270

47. **Katsarou-Katsari A, Singh L, Theoharides TC** 2001 Alopecia areata and affected skin CRH receptor upregulation induced by acute emotional stress. Dermatology 203:157–161

48. **Kandere-Grzybowska K, Gheorghe D, Priller J, Esposito P, Huang M, Gerard N, Theoharides TC** 2003 Stress-induced dura vascular permeability does not develop in mast cell-deficient and neurokinin-1 receptor knockout mice. Brain Res 980:213–220

49. **Gadek-Michalska A, Chlap Z, Turon M, Bugajski J, Fogel WA** 1991 The intracerebroventicularly administered mast cells degranulator compound 48/80 increases the pituitary-adrenocortical activity in rats. Agents Actions 32:203–208

50. **Matsumoto I, Inoue Y, Shimada T, Aikawa T** 2001 Brain mast cells act as an immune gate to the hypothalamic-pituitary-adrenal axis in dogs. J Exp Med 194:71–78

51. **Theoharides TC** 2002 Mast cells and stress—a psychoneuroimmunological perspective. J Clin Psychopharmacol 22:103–108

52. **Bloom GD** 1974 Structural and biochemical characteristics of mast cells. In: Zweifach BW, Grant L, McCluskey RT, eds. The inflammatory process. New York: Academic Press; 545–599