

Potent Mast Cell Degranulation and Vascular Permeability Triggered by Urocortin Through Activation of Corticotropin-Releasing Hormone Receptors¹

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ABSTRACT

Urocortin (Ucn) is related to corticotropin-releasing hormone (CRH), and both are released in the brain under stress where they stimulate CRH 1 and 2 receptors (CRHR). Outside the brain, they may have proinflammatory actions through activation of mast cells, which are located perivascularly close to nerve endings and degranulate in response to acute psychological stress. Here, we report that a concentration of intradermal Ucn as low as 10 nM induced dose-dependent rat skin mast cell degranulation and increased vascular permeability. This effect appeared to be equipotent to that of calcitonin gene-related peptide and neurotensin. Ucn-induced skin vasodilation was inhibited by pretreatment with the mast cell stabilizer disodium cromoglycate (cromolyn) and was absent in the mast cell-deficient *W/W^v* mice. The selective nonpeptide CRH receptor 1 antagonist, antalarmin and the nonselective peptide

antagonist astressin both reduced vascular permeability triggered by Ucn but not that by Substance P or histamine. In contrast, the peptide antagonist α -helical CRH-(9–41) reduced the effect of all three. The vasodilatory effect of Ucn was largely inhibited by pretreatment with H₁ receptor antagonists, suggesting that histamine is the major mediator involved in vitro. Neuropeptide depletion of sensory neurons, treatment with the ganglionic blocker hexamethonium, or in situ skin infiltration with the local anesthetic lidocaine did not affect Ucn-induced vascular permeability, indicating that its in situ effect was not mediated through the peripheral nervous system. These results indicate that Ucn is one of the most potent triggers of rat mast cell degranulation and skin vascular permeability. This effect of Ucn may explain stress-induced disorders, such as atopic dermatitis or psoriasis, and may lead to new forms of treatment.

Urocortin (Ucn) is a peptide identified from rat midbrain with 45% sequence identity to corticotropin-releasing hormone (CRH) (Vaughan et al., 1995) and 95% amino acid identity with human Ucn (Donaldson et al., 1996). CRH and Ucn activate the hypothalamic-pituitary-adrenal axis in response to stress (Reichlin, 1993) by stimulating CRH receptor (R)₁, as well as CRHR₂, for which Ucn has higher affinity. Ucn has been identified outside the central nervous in the duodenum (Vaughan et al., 1995) and in human lymphocytes (Bamberger et al., 1998), whereas CRHR₂ mRNA is expressed in the heart and lungs (Lovenberg et al., 1995). CRH also has been localized in extracranial sites such as in dorsal root (Merchenthaler et al., 1983; Skofitsch et al., 1985) and sympathetic ganglia (Merchenthaler et al., 1983; Suda et al., 1984), from which it may be released and exert proinflammatory actions in rheumatoid arthritis, autoimmune thyroid-

itis, and ulcerative colitis (Chrousos, 1995). Moreover, systemic administration of anti-CRH serum reduced carrageenin-induced s.c. inflammation (Karalis et al., 1991). Ucn along with CRH may, therefore, participate in the pathophysiology of neuroinflammatory conditions precipitated by stress.

Intravenous Ucn administration reduces mean arterial blood pressure (Vaughan et al., 1995), an effect more potent than the hypotension caused by CRH, which is also accompanied by flushing and itching (Chrousos, 1995). This symptomatology may be due to release of vasoactive and neurosensitizing mediators, such as histamine and cytokines (Galli, 1993), from mast cells that are located close to nerves and vessels (Foreman, 1987; Williams et al., 1995), leading to neurogenic inflammation (Theoharides, 1990). For instance, acute psychological stress induced CRH-dependent rat dura mast cell degranulation (Theoharides et al., 1995). This effect could be explained through orthodromic stimulation of the trigeminal nerve (Dimitriadou et al., 1991), especially be-

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ABBREVIATIONS: ACTH, adrenocorticotrophic hormone; CRH, corticotropin-releasing hormone; BSA, bovine serum albumin; CRHR, corticotropin-releasing hormone receptor; Ucn, urocortin; C48/80, compound 48/80; CGRP, calcitonin gene-related peptide; ES, electrical stimulation; NT, neurotensin; SP, Substance P; VIP, vasoactive intestinal peptide.

cause CRHR mRNA was identified in the trigeminal nucleus (Rivest et al., 1995). A direct CRH effect could also be possible because CRH was recently shown to trigger skin mast cell degranulation, leading to increased vascular permeability (Theoharides et al., 1998a). A pathophysiological role for CRH and/or Ucn is supported by the recent findings of CRH (Roloff et al., 1998) and CRHR (Roloff et al., 1998) gene expression in rodent and human skin (Slominski et al., 1998).

We investigated the effect of Ucn on local mast cell degranulation and vascular permeability in rodent skin.

Materials and Methods

Evans' Blue or Iodinated Bovine Serum Albumin Extravasation. Male Sprague-Dawley rats, each weighing approximately 350 g (Charles River, NY), or 8-week-old male W/W^v mast cell-deficient mice [WBBGF₁ (WB-W/+x^{C57BL/6-W^v})] and their +/+ normal counterparts (Jackson Laboratories, Bar Harbor, ME) were housed three per cage, provided with food and water ad libitum, and maintained on a diurnal lighting cycle. Each animal was handled one at a time to minimize stress. Rats were anesthetized with a single i.p. injection of 0.25 ml of ketamine and xylazine for rats (100 mg/ml each) or 0.01 ml for mice (10 and 80 mg/kg, respectively). They were then injected i.v. (0.6 ml for rats and 0.2 ml for mice) via the tail vein with 1% Evans' blue or ¹²⁵I-labeled bovine serum albumin (BSA) (4.3 μCi/μg; New England Nuclear, Boston, MA) 10 min before treatment. Experimental molecules were then tested by intradermal injection in 0.05 ml of normal saline (0.9% NaCl) using a tuberculin syringe. All peptides were obtained from Peninsula Laboratories (Belmont, CA). The pretreatment solution, when appropriate, was injected first and was allowed to remain in the skin for 5 min. The secretagogue was then injected with a different syringe. The animal was sacrificed 15 min later by asphyxiation over CO₂ vapor and decapitated; the skin was removed, turned over, and photographed. Identical circular skin areas (1 cm²) were then cut with a surgical blade, and the extravasated Evans' blue was extracted by incubating the skin samples in 99% *N,N*-dimethyl formamide (Sigma Chemical Co., St. Louis, MO) for 24 h at 55°C. The dye was measured fluorometrically (excitation wavelength, 620 nm; emission, 680 nm) (Markowitz et al., 1987). Results are presented as the mean ± S.D. of arbitrary fluorescence units for Evans' blue or cpm of ¹²⁵I-labeled BSA measured in a γ-counter and were evaluated by ANOVA.

Microscopy. For light microscopy, skin samples were rapidly removed as before and were fixed in 4% paraformaldehyde for 2 h at 24°C and then overnight at 4°C (Theoharides et al., 1998a). The tissue was then frozen, and thin sections (7 μm) were cut using a cryostat (Jung CM 3000; Leica, Deerfield, IL). The sections were stained with acidified (pH < 2.5) toluidine blue (Sigma), and all mast cells were counted by two different researchers who were blinded to the experimental conditions at 200× magnification using a Diaphot inverted Nikon microscope (Don Santo, MA). Degranulated mast cells were determined by the presence of extruded granule contents with or without the loss of >50% toluidine blue staining. For electron microscopy, samples were fixed in modified Kanovsky's fixative containing 0.2% paraformaldehyde, 3% glutaraldehyde, and 0.5% tannic acid in 0.1 mM Na-cacodylate buffer; they were processed and photographed using a Philips-300 transmission electron microscope as described previously (Theoharides et al., 1998).

Drug Treatment. For sensory nerve neuropeptide depletion (Dimitriadou et al., 1991), neonatal rat littermates were injected s.c. within 48 h of birth with 50 mg/kg capsaicin (Sigma) diluted in a vehicle containing 0.9% NaCl/100% ethanol/Tween 80 (8:1:1); control littermates received the same volume of the vehicle solution alone. The male rats were used 8 weeks later. For ganglionic blockade, rats were injected i.p. 10 min before Ucn with either hexamethonium (15 mg/kg) obtained from Sigma or normal saline as control. Nerve conductance was blocked locally with tissue infiltration around the

site of experimental injection by 1% lidocaine obtained from Sterling Labs (New York, NY) or saline 10 min before intradermal injection of Ucn. Animals were also pretreated with various CRHR antagonists. The selective nonpeptide CRHR₁ antagonist antalarmin (*N*-butyl-*N*-ethyl-2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrolo [2,3-*d*] pyrimidin-4-yl] amine) (National Institutes of Health, Bethesda, MD) was used i.v. (10 mg/kg b.wt.), dissolved in absolute ethanol 6 h before treatment because it had previously been shown to require that long to exert its maximal effect (Theoharides et al., 1998). The nonspecific, peptide CRHR antagonist astressin ([cyclo (30–33) {D-Phe¹²,Nle^{21,38},Glu³⁰,Lys³³} hCRF (12–41)] (Neurocrine, La Jolla, CA) was used intradermally at 10⁻⁷ M 5 min before stimulation. The nonspecific peptide CRHR antagonist α-helical CRH-(9–41) was used intradermally (10⁻⁴ M) 30 min before intradermal injection of the test agents. Diphenhydramine and cyproheptadine, like the peptide antagonists, were dissolved in normal saline and were used intradermally as indicated in *Results*.

Presentation of Results. The results are presented in the text as the mean ± S.D. values of Evans' blue or ¹²⁵I-labeled BSA extravasation under different experimental conditions. These results were evaluated by ANOVA and Student-Newman-Keuls tests, whereas the mast cell degranulation results were evaluated with nonparametric analysis using Mann-Whitney *U* test. The number of animals tested is denoted (*n*), and significance is indicated by *p* < .05.

Results

Intradermally injected Ucn (10⁻⁷ M) induced a marked increase in skin vascular permeability as shown by tissue extravasation of i.v. injected Evans' blue (Fig. 1A). This effect was equivalent to that of equimolar (10⁻⁷ M) CGRP and neurotensin (NT) but was more pronounced than that of CRH, ACTH, β-endorphin, Substance P (SP), somatostatin, and vasoactive intestinal peptide (VIP) (Fig. 1A). These results make Ucn one of the most potent rat skin vasodilators known. The vasodilatory effect of Ucn was first assessed by extraction of extravasated Evans' blue it was dose dependent from 10⁻⁴ to 10⁻⁸ M and was statistically significant when compared with that from control sites (*p* < .05) injected with saline (Fig. 1B). Vascular permeability was also quantified by ¹²⁵I-labeled BSA extravasation (Fig. 1C) and was statistically significant from 10⁻⁵ to 10⁻⁷ M (*p* < .05), comparable to that seen with CRH (Fig. 1D). The effect of Ucn was greater when quantified by Evans' blue than with ¹²⁵I-labeled BSA, possibly because Evans' blue is smaller and also may not be completely bound to BSA, thus being able to leave the circulation easier.

It was hypothesized that the increased vascular permeability may be secondary to the release of vasodilatory molecules from skin mast cells. The effect of Ucn (10⁻⁶ M) on mast cell degranulation was documented morphologically with light microscopy by identifying the mast cells in the skin samples from the injection site that showed extrusion of their granule contents (Fig. 2, A and B). Degranulation was present in 52.3 ± 8.0% of the mast cells at skin sites treated with 10⁻⁵ M Ucn (*n* = 5 rats, 2116 mast cells counted), 45.1 ± 5.95% with 10⁻⁶ M (*n* = 52,096 mast cells counted), and 31.3 ± 6.7% with 10⁻⁷ M (*n* = 5 rats, 3071 mast cells counted); all results were statistically significant (*p* < .05) compared with the 19.7 ± 5.2% degranulated mast cells from control sites (*n* = 3 rats, 1983 mast cells counted). Ultrastructural observations of mast cells from control sites showed intact mast cells with homogeneous electron dense granules (Fig. 3 A and B). On the contrary, mast cells from sites injected with Ucn

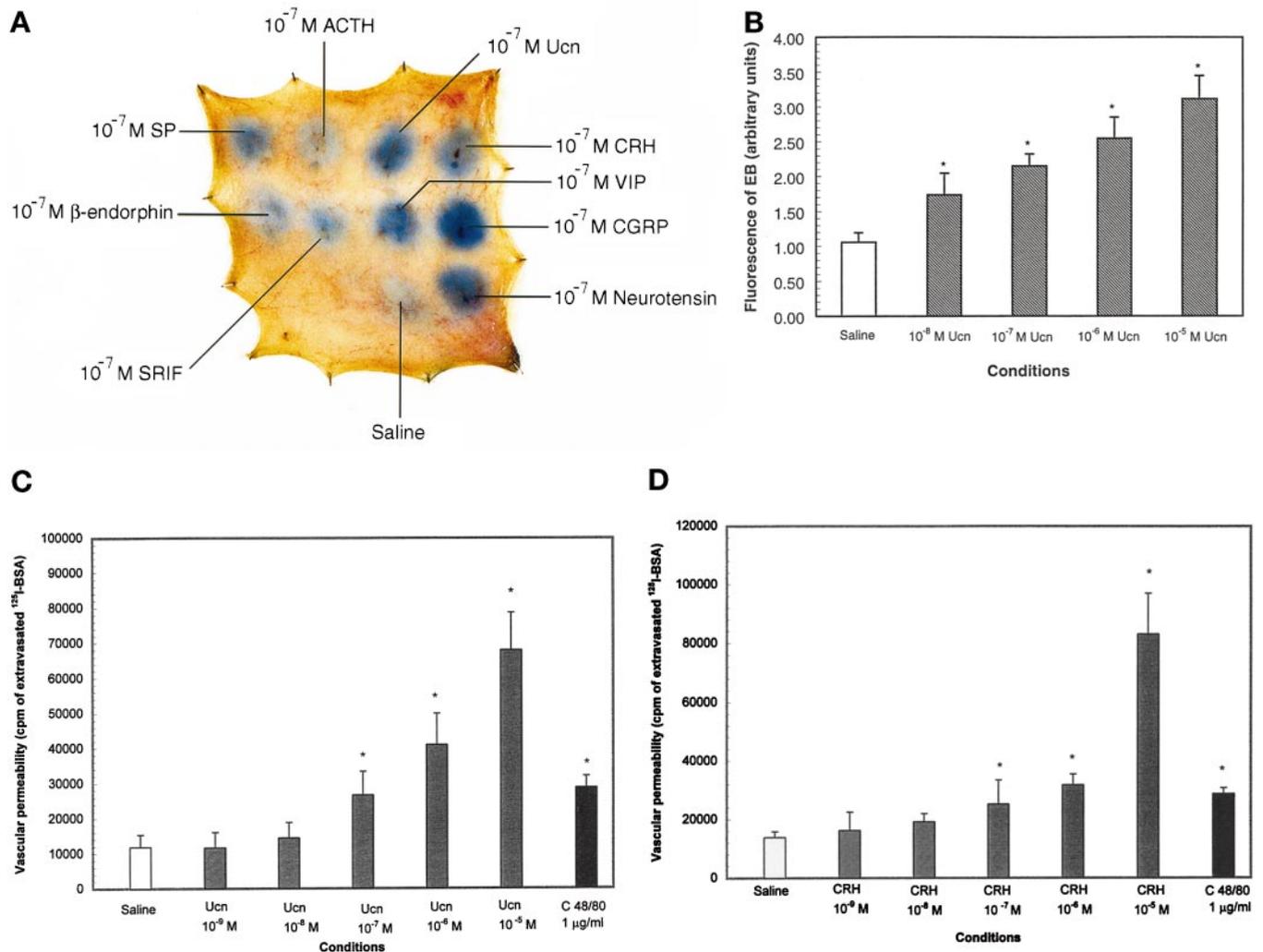


Fig. 1. Ucn is more potent than other neuropeptides in inducing vascular permeability ($n = 8$). A, photographs of rat skin showing vasodilatory effect of equimolar concentrations (10^{-7} M) of Ucn and CRH (Peninsula), as well as ACTH, SP, CGRP, VIP, somatostatin, β -endorphin, and NT (Sigma). Each neuropeptide or normal saline is shown connected to corresponding injection site by a solid line. B, dose-response curve showing effect of Ucn on Evans' blue extravasation extracted by formamide ($n = 4$). Dose-response curves showing effect of (C) Ucn and (D) CRH on ^{125}I -labeled BSA extravasation ($n = 5$). * $p < .05$ using ANOVA.

(10^{-6} M) showed extensive degranulation (Fig. 3, C and D). Unlike all other peptides tested, which carry a net positive charge thought to be important for triggering mast cell secretion, Ucn and CRH have one net negative charge, which sets them uniquely apart and supports a specific action.

To investigate whether the increased vascular permeability induced by Ucn was mast cell dependent, we used W/W^v mast cell-deficient mice and their +/+ controls. Ucn induced Evans' blue extravasation in the +/+ controls at 10^{-5} , 10^{-6} , and 10^{-7} M ($n = 4$; Fig. 4A). However, this effect was entirely absent in W/W^v mice (Fig. 4B). Moreover, the lack of vasodilation was not due to some vascular defect because histamine (10^{-4} M), used as a positive control, induced a strong vasodilatory effect in the W/W^v mice (Fig. 4C). One must, therefore, conclude that the vasculature of the W/W^v mice was intact and could respond to a direct vasodilator. Mast cell dependence was also confirmed with pretreatment of the injection site with the "mast cell stabilizer" cromolyn (10^{-4} M) for 5 min before the injection of Ucn (10^{-6} M), which inhibited Evans' blue extravasation (Fig. 4D). Comparison of the inhibitory effect of cromolyn on CRH and Ucn-induced

Evans' blue extravasation extracted with formamide showed that cromolyn inhibited the effect of both by more than 60%, even at 10^{-6} M (Fig. 4E). Dye extravasation induced by Ucn (10^{-6} M) was reduced ($p < .05$) by $78.8 \pm 7.9\%$ ($n = 6$), $72.5 \pm 8.4\%$ ($n = 5$), and $57.8 \pm 16.5\%$ ($n = 5$) by pretreating injection sites with 10^{-4} , 10^{-5} , and 10^{-6} M cromolyn, respectively.

We also investigated the possible contribution of the peripheral nervous system by administering Ucn intradermally to animals treated so as to block different components of the peripheral nervous system. First, animals were treated neonatally with capsaicin to prevent the accumulation of neuropeptides in sensory nerves. The absence of sensory neuropeptides was confirmed by the lack of vascular permeability in response to intradermal injection of 10^{-6} M capsaicin in neonatally treated animals, whereas it did induce permeability acutely in controls (results not shown). Capsaicin treatment did not affect the increase in vascular permeability induced by 10^{-5} M Ucn (results not shown). Similarly, ring-like infiltration of the test site with 1% lidocaine for 10 min before intradermal administration of Ucn or

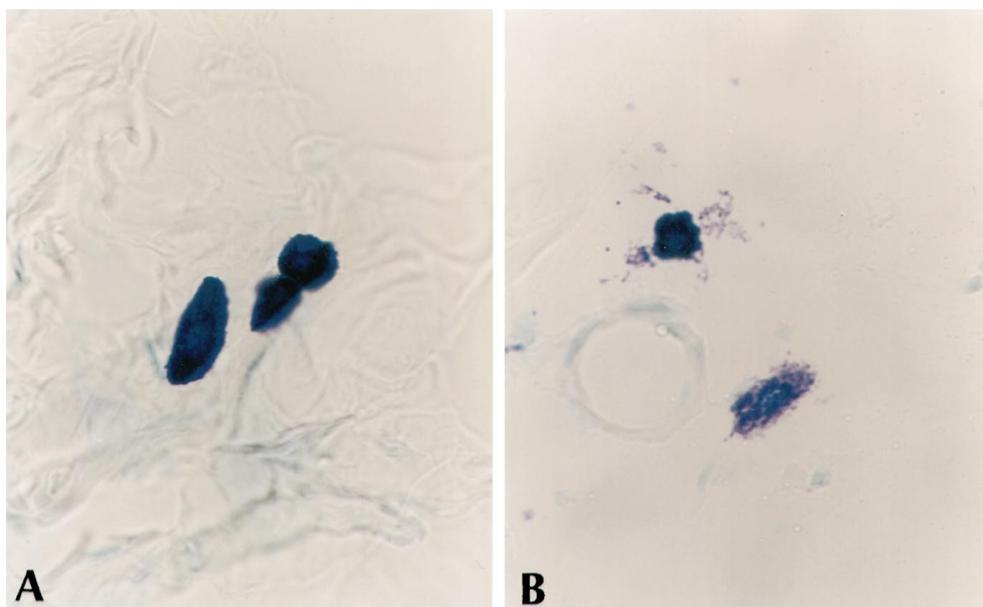


Fig. 2. Mast cell degranulation induced by Ucn. Photomicrographs of skin mast cells after intradermal administration of saline (A) or 10^{-6} M Ucn (B). Magnification, $400\times$.

CRH (10^{-5} M) or pretreatment i.p. with the ganglionic blocker hexamethonium did not affect the in situ effect of 10^{-5} M Ucn or CRH (Fig. 4). These results indicate that the action of CRH or Ucn in situ did not depend on the peripheral nervous system.

We then investigated whether the effect of Ucn was mediated by specific CRH receptors because the biologically inactive free acid form of Ucn had no effect (results not shown). Pretreatment of the injection site for 5 min with the nonspecific peptide CRHR antagonist astressin (10^{-7} M) inhibited the response to both Ucn and CRH by $32.5 \pm 1.9\%$ and $33.3 \pm 2.9\%$, respectively. We then studied the selective nonpeptide CRHR₁ antagonist antalarmin, which had previously been shown to reduce carrageenin inflammation (Webster et al., 1996) and CRH-induced skin vasodilation (Theoharides et al., 1998) by about 40% but only when given 6 h before the experimental conditions. Pretreatment i.v. with 10 mg/kg b.wt. for 6 h before intradermal Ucn or CRH (10^{-6} M) inhibited the response ($p < .05$) to both agents by $50.1 \pm 13.8\%$ ($n = 3$) and $51.0 \pm 21.8\%$ ($n = 3$), respectively. However, it had no effect on the response caused by SP, the mast cell secretagogue compound 48/80 (C48/80), or histamine (Fig. 5A). On the contrary, pretreatment intradermally with 10^{-4} M α -helical CRH-(9–41), a nonspecific peptide CRHR₁/CRHR₂ antagonist, inhibited ($p < .05$) vascular permeability induced by 10^{-6} M Ucn or CRH (Fig. 5B); this inhibition was $66.0 \pm 10.1\%$ ($n = 3$) and $43.8 \pm 13.2\%$ ($n = 4$), respectively. However, this CRHR antagonist ($p < .05$) also inhibited the effect of C48/80, SP, and histamine (Fig. 5C). One possible explanation for this unexpected finding could be that this peptide CRHR antagonist could have a partial agonist effect inducing systemic vasodilation, which could reduce the extent of the in situ response regardless of the stimulus. This possibility is supported by the previous findings that another peptide CRHR antagonist (D-Phe¹², Nie^{21,38}, Ala³²) rCRH (12–41) given intradermally not only did not block vascular permeability induced by CRH but also at 10^{-4} M acted as an agonist instead (Theoharides et al., 1998). This partial agonist activity of peptide CRHR antagonists suggested the presence of a unique type of receptor.

We then studied the effect of Ucn or CRH given i.v. on the respective in situ effects of each other. Pretreatment i.v. with 7.6 nmol/kg b.wt. CRH reduced vascular permeability induced by Ucn administered intradermally 5 min later (Fig. 5D); the same was true for Ucn (results not shown). However, such reduction was not apparent if there was no systemic effect. For instance, treatment of the test sites with CRH at a concentration (10^{-8} M) that has a minimal effect on its own did not reduce the increased vascular permeability induced by the subsequent intradermal injection of Ucn, CRH, or C48/80 (10^{-6} M); moreover, CRH (10^{-5} M) given in situ before intradermal injection of Ucn (10^{-5} M) resulted in vascular permeability that appeared to be additive (results not shown). It was therefore concluded that i.v. administration of vasodilatory molecules could inhibit their in situ effect nonspecifically by peripheral blood pooling.

To examine whether the mast cell-derived vasodilatory molecule histamine mediated the vasodilatory effect of Ucn, the injection sites were pretreated with the H₁ receptor antagonists diphenhydramine or cyproheptadine. Dye extravasation in response to Ucn (10^{-6} M) was reduced ($p < .05$) by $85.9 \pm 4.1\%$ ($n = 6$), $67.7 \pm 14.4\%$ ($n = 5$), and $30.9 \pm 15.4\%$ ($n = 6$) with 10^{-4} , 10^{-5} , and 10^{-6} M diphenhydramine, respectively ($p < .05$). Cyproheptadine also inhibited dye extravasation induced by Ucn (10^{-6} M), with the inhibition being $70.9 \pm 6.6\%$ ($n = 5$), $59.7 \pm 15.6\%$ ($n = 5$), and $51.8 \pm 11.4\%$ ($n = 5$) with 10^{-4} , 10^{-5} , and 10^{-6} M cyproheptadine ($p < .05$). These results suggest that histamine is the major molecule that mediates Ucn-induced fluid extravasation.

Discussion

The present results indicate that Ucn can induce skin mast cell degranulation and increase vascular permeability; both actions appear to be more potent than those previously shown for CRH (Theoharides et al., 1998). The present results showed that mast cells were necessary for the effect of Ucn. Other studies using W/W^v mice also concluded that SP-induced vascular permeability was mast cell dependent (Matsuda et al., 1989), findings supported by the well known

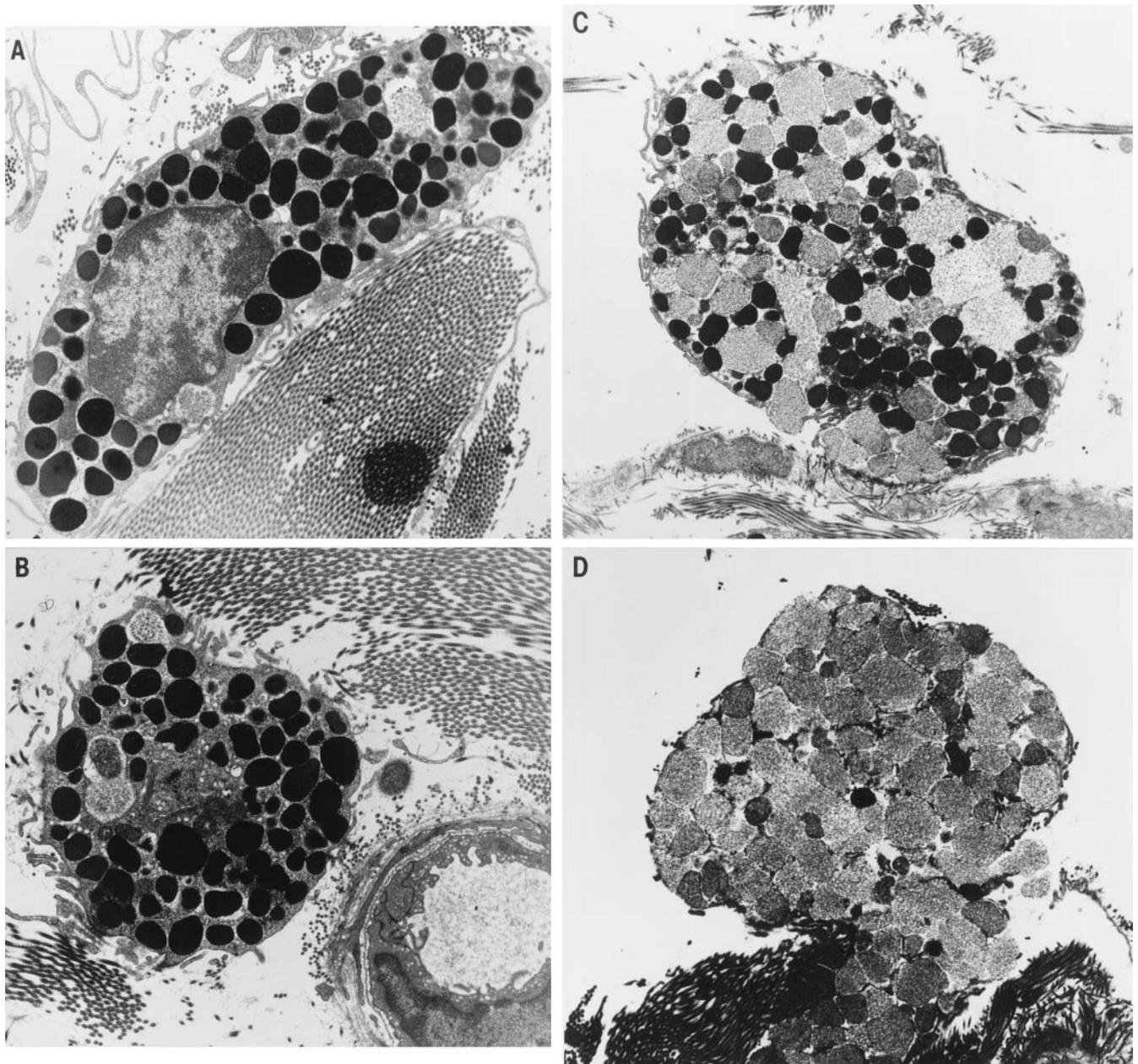


Fig. 3. Electron photomicrographs of rat mast cells from skin sites injected with saline (A and B) or with 10^{-6} M Ucn (C and D) showing extensive ultrastructural changes of the granule content with loss of electron density indicating secretory activity. Magnification: A and B, 15,600 \times ; C and D, 11,700 \times .

ability of cromolyn to inhibit connective tissue mast cell secretion (Theoharides et al., 1980). The in situ effect of Ucn did not depend on the peripheral nervous system because it was unaffected by treatment neonatally with capsaicin, the ganglionic blocker hexamethonium, or the local anesthetic lidocaine.

The results with CRH receptor antagonists indicate that the effect of Ucn is mediated through specific receptors but could not confirm the involvement of the known CRH receptors. For instance, antalarmin only partially inhibited the action of Ucn, consistent with the finding that cultured human leukemic mast cells express CRHR₁ (Theoharides et al., 1998). Antalarmin is a nonpeptide CRHR₁-selective analog of CP-154,526 (Pfizer), previously shown to reduce both carrageenin-induced s.c. inflammation (Webster et al., 1996) and the direct effect of CRH on skin mast cell degranulation and

vascular permeability (Theoharides et al., 1998). CRH (Roloff et al., 1998) and CRH receptor gene expression was recently shown in rodent (Slominski et al., 1996; Roloff et al., 1998) as well as human skin (Slominski et al., 1995, 1998). The nonselective peptide CRHR antagonist astressin (Martinez et al., 1997) also did not inhibit the Ucn-induced effects entirely. On the other hand, the peptide receptor antagonist α -helical CRH-(9–41) inhibited the action of both Ucn and CRH as well as that of C48/80, SP, and histamine, implying that there may be a systemic agonist effect as previously shown for another peptide CRHR antagonist (D-Phe¹², Nie^{21,38}, Ala³²) rCRH (12–41) (Theoharides et al., 1998). Taken altogether, the present results imply that skin mast cell degranulation and subsequent vascular permeability in rodents may involve a CRH receptor other than the known CRH₁ or CRH_{2 α} and CRH_{2 β} subtypes. This putative

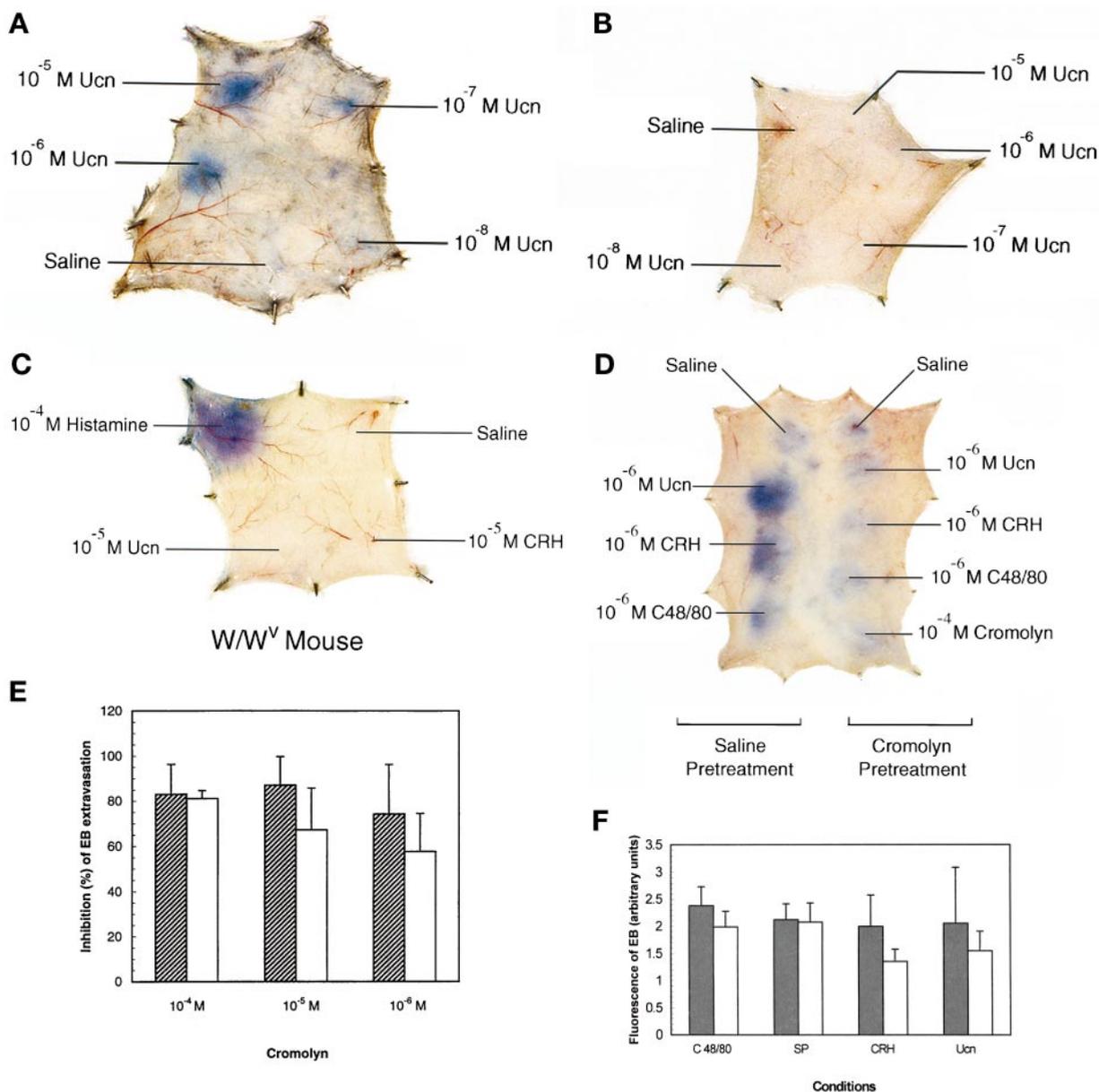


Fig. 4. Ability of Ucn to induce vascular permeability depends on mast cells ($n = 5$). Photographs of mouse skin from $+/+$ wild-type control (A); W/W^v mast cell-deficient mouse (B); W/W^v mouse showing that histamine (used as positive control) is capable of inducing vascular permeability indicating that the vasculature is intact (C); sites pretreated in situ with 10^{-4} M cromolyn for 5 min before intradermal administration of saline, C48/80, Ucn, or CRH (10^{-6} M) (D); and the inhibitory effect of in situ cromolyn (10^{-4} to 10^{-6} M) administration quantified by extracting Evans' blue with formamide (E). CRH (10^{-6} M; shaded bars) and Ucn (10^{-6} M; open bars) were injected 5 min after cromolyn. F, pretreatment i.p. with (15 mg/kg b.wt.) of the ganglionic blocker hexamethonium (shaded bars) or saline (open bars) 30 min before intradermal administration of Ucn, CRH, SP, or C48/80 (10^{-6} M) quantified by Evans' blue extracted in formamide ($n = 3$).

receptor may be of lower affinity than the ones known so far. A possible candidate may be the CRHR2 γ , identified in human brain and shown to exhibit low affinity (5 nM) for the CRHR (Sperle et al., 1997), which is in the range of the effect (10 nM) reported here.

Our present results show that i.v. administration of CRH can inhibit increased vascular permeability induced by in situ Ucn. Surprisingly, Ucn was recently shown to inhibit heat-induced paw edema but was associated with pronounced hypotension, which could explain those results (Turnbull et al., 1996). In this latter study, pretreatment with the α -helical CRH-(9–41) completely reversed Ucn and CRH-mediated inhibition of rat paw edema, whereas it had

no effect on ACTH levels (Turnbull et al., 1996). Most perplexing is the reported ability of local s.c. CRH (0.5–5 ng) to inhibit rat paw edema induced by phospholipase A₂ or carrageenin (Correa et al., 1997); in this study, the authors concluded that histamine was not involved because it could not be released by CRH from peritoneal mast cells (Correa et al., 1997). However, we have shown that even though rat peritoneal mast cells do not respond, rat pleural mast cells secrete about 30% histamine in response to 10^{-5} M CRH (Boucher et al., 1996), suggesting that mast cells from different sites vary in their responsiveness. Still, there is no obvious explanation of why our results in the rat flank are opposite from those obtained in the rat paw. It may be that the

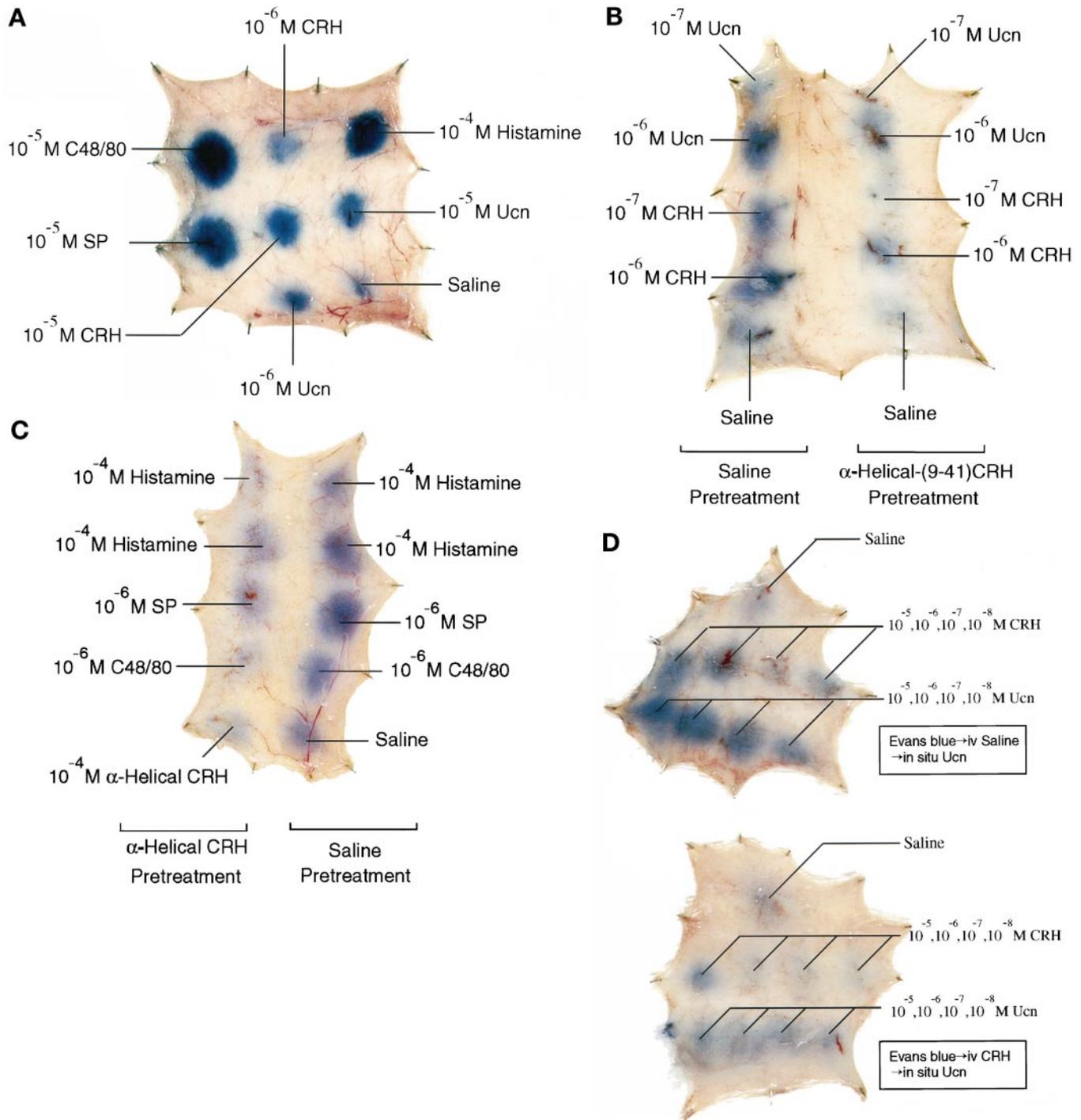


Fig. 5. Pretreatment with CRHR antagonists ($n = 4$) inhibits vascular permeability. Photographs of rat skin showing effects of 10 mg/kg b.wt. antalarmin from NIH dissolved as 20 mg/0.15 ml in absolute ethanol, from which the appropriate amount was then dissolved in saline and the final amount (0.6 ml) was injected via a tail vein 6 h before stimulation as shown (A). B, 10⁻⁴ M α-helical CRH-(9-41) injected intradermally for 5 min before stimulation by Ucn or CRH as shown. C, 10⁻⁴ M α-helical CRH-(9-41) injected intradermally 5 min before histamine, C48/80, or SP as shown. D, i.v. administration of saline or 7.6 nmol/kg b.wt. CRH for 5 min followed by intradermal Ucn for 15 min as shown.

different CRH/Ucn actions observed were due to different cytokines released from leukocytes, such as interleukin-1 (Karalis et al., 1997), which can then stimulate mast cell secretion (Kaplan et al., 1991). However, pretreatment of rats for 5 h with sufficient dexamethasone (0.1 mg/kg b.wt.) to inhibit immune cells, but not mast cells (Marquardt et al., 1983), did not affect Ucn-induced vasodilation, indicating that cytokines are most likely not involved (results not

shown). Alternatively, different CRH receptors may be involved at different sites, as discussed. Nevertheless, histamine appears to be the main mediator involved in situ because two H₁ receptor antagonists inhibited most of the vasodilatory response.

The involvement of the peripheral nervous system in the in situ effect of Ucn was excluded because neither neonatal capsaicin treatment nor pretreatment with the local anes-

thetic lidocaine or the ganglionic blocker hexamethonium reduced the effects of intradermal administration of Ucn or CRH. These results are in contrast to the *in vivo* effect of acute psychological stress, which was partially dependent on the peripheral nervous system in the dura (Theoharides et al., 1995) and in the intestinal tract (Castagliuolo et al., 1996). The fact that CRH is found in sympathetic chain ganglia (Merchenthaler et al., 1983; Suda et al., 1984) and in primary sensory afferent fibers (Merchenthaler et al., 1983), whereas Ucn is present in leukocytes (Bamberger et al., 1998), suggests that Ucn could be released *in vivo*, along with other neuropeptides, such as CGRP, VIP, or NT. This possibility is supported by the facts that 1) skin mast cells degranulated in response to electrical stimulation (ES) of sensory nerves (Kowalski et al., 1988), 2) dura mast cells degranulated after ES of the trigeminal (Dimitriadou et al., 1991) or cervical (Keller et al., 1991) ganglion, and 3) dura mast cells degranulated after acute psychological stress (Theoharides et al., 1995).

Our results could have direct relevance to clinical syndromes exacerbated by stress; these may include psoriasis (Al'Abadie et al., 1994), where CRHR may be overexpressed by an increased number of mast cells at the affected sites (Harvima et al., 1990). CRHR antagonists may prove to be potential therapeutic agents.

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References

- Al'Abadie MS, Kent GG and Gawkrödger DJ (1994) The relationship between stress and the onset and exacerbation of psoriasis and other skin conditions. *Br J Dermatol* **130**:199–203.
- Bamberger CM, Wald M, Bamberger A-M, Ergun S, Beil FU and Schulte HM (1998) Human lymphocytes produce urocortin, but not corticotropin-releasing hormone. *J Clin Endocrinol Metab* **83**:708–711.
- Boucher W, Singh L and Theoharides TC (1996) Differential effect of corticotropin-releasing hormone (CRH) on rat mast cell (MC) secretion (Abstract). *FASEB J* **10**:A1290.
- Castagliuolo I, LaMont JT, Qiu B, Fleming SM, Bhaskar KR, Nikulasson ST, Kornetsky C and Pothoulakis C (1996) Acute stress causes mucin release from rat colon: role of corticotropin releasing factor and mast cells. *Am J Physiol* **271**:884–892.
- Chrousos GP (1995) The hypothalamic-pituitary-adrenal axis and immune-mediated inflammation. *N Engl J Med* **332**:1351–1362.
- Correa SG, Riera CM, Spiess J and Bianco ID (1997) Modulation of the inflammatory response by corticotropin-releasing factor. *Eur J Pharmacol* **319**:85–90.
- Dimitriadou V, Buzzi MG, Moskowitz MA and Theoharides TC (1991) Trigeminal sensory fiber stimulation induces morphologic changes reflecting secretion in rat dura mast cells. *Neuroscience* **44**:97–112.
- Donaldson CJ, Sutton SW, Perrin MH, Corrigan AZ, Lewis KA, Rivier JE, Vaughan JM and Vale WW (1996) Cloning and characterization of human urocortin. *Endocrinology* **137**:2167–2170.
- Foreman JC (1987) Neuropeptides and the pathogenesis of allergy. *Allergy* **42**:1–11.
- Galli SJ (1993) New concepts about the mast cell. *N Engl J Med* **328**:257–265.
- Harvima IT, Naukkarinen A, Harvima RJ, Aalto M-L, Neittaanmaki H and Horsmanheimo M (1990) Quantitative enzyme-histochemical analysis of tryptase- and chymase-containing mast cells in psoriatic skin. *Arch Dermatol Res* **282**:428–433.
- Kaplan AP, Reddigari S, Baeza M and Kuna P (1991) Histamine releasing factors and cytokine-dependent activation of basophils and mast cells. *Adv Immunol* **50**:237–260.
- Karalis K, Louis JM, Bae D, Hilderbrand H and Majzoub JA (1997) CRH and the immune system. *J Neuroimmunol* **72**:131–136.
- Karalis K, Sano H, Redwine J, Listwak S, Wilder RL and Chrousos GP (1991) Autocrine or paracrine inflammatory actions of corticotropin-releasing hormone *in vivo*. *Science (Wash DC)* **254**:421–423.
- Keller JT, Dimlich RV, Zuccarello M, Lanker L, Strauss TA and Fritts MJ (1991) Influence of the sympathetic nervous system as well as trigeminal sensory fibres on rat dural mast cells. *Cephalalgia* **11**:215–221.
- Kowalski ML and Kaliner MA (1988) Neurogenic inflammation, vascular permeability and mast cells. *J Immunol* **140**:3905–3911.
- Lovenberg TW, Chalmers DT, Liu C and DeSouza EB (1995) CRF_{2α} and CRF_{2β} receptor mRNAs are differentially distributed between the rat central nervous system and peripheral tissues. *Endocrinology* **136**:4139–4142.
- Markowitz S, Saito K and Moskowitz MA (1987) Neurogenically mediated leakage of plasma protein occurs from blood vessels in dura mater but not brain. *J Neurosci* **7**:4129–4136.
- Marquardt DL and Wasserman SI (1983) Modulation of rat serosal mast cell biochemistry by *in vivo* dexamethasone administration. *J Immunol* **131**:934–939.
- Martinez V, Rivier J, Wang LX and Tache Y (1997) Central injection of a new corticotropin-releasing factor (CRF) antagonist, astressin, blocks CRF- and stress-related alterations of gastric and colonic motor function. *J Pharmacol Exp Ther* **280**:754–760.
- Matsuda H, Kawakita K, Kiso Y, Nakano T and Kitamura Y (1989) Substance P induces granulocyte infiltration through degranulation of mast cells. *J Immunol* **142**:927–931.
- Merchenthaler I, Hynes MA, Vingh S, Schally AV and Petrusz P (1983) Immunocytochemical localization of corticotropin-releasing factor (CRF) in the rat spinal cord. *Brain Res* **275**:373–377.
- Reichlin S (1993) Neuroendocrine-immune interactions. *N Engl J Med* **329**:1246–1253.
- Rivest S, Laflamme N and Nappi RE (1995) Immune challenge and immobilization stress induce transcription of the gene encoding the CRF receptor in selective nuclei of the rat hypothalamus. *J Neurosci* **15**:2680–2695.
- Roloff B, Fechner K, Slominski A, Furkert J, Botchkarev VA, Bulfone-Paus S, Zipper J, Krause E and Paus R (1998) Hair cycle-dependent expression of corticotropin-releasing factor (CRF) and CRF receptors in murine skin. *FASEB J* **12**:287–297.
- Skofitsch G, Zamir N, Helke CJ, Savitt JM, Jacobowitz DM (1985) Corticotropin-releasing factor-like immunoreactivity in sensory ganglia and capsaicin sensitive neurons of the rat central nervous system: Colocalization with other neuropeptides. *Peptides* **6**:307–318.
- Slominski A, Ermak G, Hwang J, Chakraborty A, Mazurkiewicz JE and Mihm M (1995) Proopiomelanocortin, corticotropin releasing hormone and corticotropin releasing hormone receptor genes are expressed in human skin. *FEBS Lett* **374**:113–116.
- Slominski A, Ermak G, Hwang J, Mazurkiewicz J, Corliss D and Eastman A (1996) The expression of proopiomelanocortin (POMC) and of corticotropin releasing hormone receptor (CRH-R) genes in mouse skin. *Biochim Biophys Acta* **1289**:247–251.
- Slominski AJ, Ermak G, Mazurkiewicz JE, Baker J and Wortsman J (1998) Characterization of corticotropin-releasing hormone (CRH) in human skin. *J Clin Endocrinol Metab* **83**:1020–1024.
- Sperle K, Chen A, Kostich W, and Largent BL (1997) CRH2G: A novel CRH2 receptor isoform found in human brain (Abstract). *Soc Neurosci Abstr* **23**:1765.
- Suda T, Tomori N, Tozawa F, Mouri T, Demura H and Shizume K (1984) Distribution and characterization of immunoreactive corticotropin-releasing factor in human tissues. *J Clin Endocrinol Metab* **59**:861–866.
- Theoharides TC, Sieghart W, Greengard P and Douglas WW (1980) Antiallergic drug cromolyn may inhibit histamine secretion by regulating phosphorylation of a mast cell protein. *Science (Wash DC)* **207**:80–82.
- Theoharides TC (1990) Mast cells: The immune gate to the brain. *Life Sci* **46**:607–617.
- Theoharides TC, Singh L, Boucher W, Pang X, Letourneau R, Webster E and 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.
- Theoharides TC, Spanos CP, Pang X, Alferes L, Ligris K, Letourneau R, Rozniecki JJ, Webster E and Chrousos G (1995) Stress-induced intracranial mast cell degranulation: A corticotropin releasing hormone-mediated effect. *Endocrinology* **136**:5745–5750.
- Turnbull AV, Vale W and Rivier C (1996) Urocortin, a corticotropin-releasing factor-related mammalian peptide, inhibits edema due to thermal injury in rats. *Eur J Pharmacol* **303**:213–216.
- Vaughan J, Donaldson C, Bittencourt J, Perrin MH, Lewis K, Sutton S, Chan R, Turnbull AV, Lovejoy D and Rivier C (1995) Urocortin, a mammalian neuropeptide related to fish urotensin I and to corticotropin-releasing factor. *Nature (London)* **378**:287–292.
- Webster EL, Lewis DB, Torpy DJ, Zachman EK, Rice KC and Chrousos GP (1996) *In vivo* and *in vitro* characterization of antalarmin, a nonpeptide corticotropin-releasing hormone (CRH) receptor antagonist: Suppression of pituitary ACTH release and peripheral inflammation. *Endocrinology* **137**:5747–5750.
- Williams RM, Bienenstock J and Stead RH (1995) Mast cells: The neuroimmune connection. *Chem Immunol* **61**:208–235.

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