

Corticotropin-releasing hormone deficiency increases allergen-induced airway inflammation in a mouse model of asthma

Eric S. Silverman, MD,^{a,b} David T. Breault, MD, PhD,^c Joseph Vallone, BS,^a Srikanth Subramanian, BS,^c Ayca D. Yilmaz, BS, MA,^a Sheeba Mathew, BS,^a Venkat Subramaniam, BS,^a Kelan Tantisira, MD,^d Karel Pacák, MD, PhD,^e Scott T. Weiss, MD,^d and Joseph A. Majzoub, MD^c

Boston, Mass, and Bethesda, Md

Background: Corticotropin-releasing hormone (CRH) is a major regulator of adrenocorticotropic hormone and the production of glucocorticoids by the adrenal gland. Abnormal regulation of CRH and endogenous glucocorticoids has been implicated in the pathogenesis of asthma.

Objective: We postulated that CRH deficiency could increase asthma severity by disrupting hypothalamus-pituitary-adrenal axis function and the induction of glucocorticoids through inflammatory and physiologic stress. However, CRH is expressed by several types of immune cells and might be induced at sites of inflammation, where it has local immunostimulatory actions. Thus CRH deficiency could decrease asthma severity.

Methods: To test these possibilities, we subjected CRH-knockout mice to an ovalbumin-induced airway inflammation protocol that mimics many features of asthma.

Results: CRH-knockout mice had an increase in airway inflammation of approximately 80% to 300% and an increase in goblet cell hyperplasia of approximately 70% compared with wild-type mice. In contrast, IgE induction was unaffected by CRH deficiency. The increased inflammation in knockout mice was associated with increased tissue resistance, elastance, and hysteresivity. Levels of IL-4, IL-5, IL-13, RANTES, IFN- γ , and eotaxin were all increased in knockout mice. Serum corticosterone levels were decreased in knockout mice and might account for some of the differences between knockout and wild-type mice.

Conclusion: We conclude that CRH deficiency disrupts endogenous glucocorticoid production and enhances allergen-induced airway inflammation and lung mechanical dysfunction

in mice. Thus inherited or acquired CRH deficiency could increase asthma severity in human subjects. (*J Allergy Clin Immunol* 2004;114:747-54.)

Key words: Corticotropin-releasing hormone, asthma, airway responsiveness, glucocorticoids, rodent, eosinophils, knockout, lung, ovalbumin, catecholamines

Corticotropin-releasing hormone (CRH) is a major integrator of endocrine, autonomic, and immune responses to stress.¹⁻³ It is best known as the hypothalamic regulator of pituitary adrenocorticotropin secretion that stimulates adrenal glucocorticoid synthesis.⁴ CRH has important central anti-inflammatory actions throughout the body by stimulating the hypothalamic-pituitary-adrenal (HPA) axis and endogenous glucocorticoid production. However, CRH can also be expressed peripherally at sites of inflammation, where it might potentiate the local immune response.⁵ CRH has been implicated in the pathogenesis of asthma because it might stimulate mast cell degranulation, histamine release, and edema formation in the airways; however, this has never been proved.^{2,6}

We hypothesized that this balance between central anti-inflammatory and peripheral proinflammatory actions might be important in asthma. CRH deficiency could increase asthma severity by decreasing glucocorticoid production in response to inflammatory and physiologic stress. In contrast, it is possible that CRH deficiency would decrease local inflammation and asthma severity through peripheral mechanisms. To test these possibilities, we subjected CRH-knockout (KO) and wild-type (WT) control mice to an ovalbumin (OVA)-induced airway inflammation protocol and compared the effects of CRH deficiency on airway inflammation and lung function. We found that KO mice had increased airway inflammation and mechanical dysfunction of the lungs and decreased glucocorticoid levels compared with those seen in WT mice. CRH was not expressed in the lungs of asthmatic animals or mice with or without inflammation. These data provide evidence supporting the central anti-inflammatory effect of CRH in asthma.

From ^aPhysiology Program, Department of Environmental Health, Harvard School of Public Health, Boston; ^bPulmonary and Critical Care Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston; ^cthe Division of Endocrinology, Children's Hospital, Harvard Medical School, Boston; ^dChanning Laboratory, Department of Medicine, Brigham and Women's Hospital, Boston; and ^ethe Pediatric and Reproductive Endocrinology Branch, National Institute of Child Health and Development, National Institutes of Health, Bethesda.

Supported by National Institutes of Health grants HL70573, HL03827, and HL65899.

Received for publication March 2, 2004; revised June 9, 2004; accepted for publication June 24, 2004.

Reprint requests: Eric S. Silverman, MD, Physiology Program, Department of Environmental Health, Harvard School of Public Health, 665 Huntington Ave, Boston, MA 02115-6021. E-mail: esilverm@HSPH.harvard.edu.

0091-6749

doi:10.1016/j.jaci.2004.06.055

Abbreviations used

BAL: Bronchoalveolar lavage
 CRH: Corticotropin-releasing hormone
 CRHR1: Corticotropin-releasing hormone type 1 receptor
 HPA axis: Hypothalamic-pituitary-adrenal axis
 KO: Knockout
 OVA: Ovalbumin
 WT: Wild-type

METHODS**Animal husbandry and inflammation protocol**

All animal protocols were approved by the Harvard Standing Committee on Animals. Male and female CRH-KO and WT mice of a 129SVJ/C57BL6 background were housed in isolation cages in a specific pathogen-free, 12-hour/12-hour light-dark cycle environment and were provided with sterilized rodent chow and water ad libitum.⁷ Corticosterone (10 µg/mL) was added to the drinking water of KO mice throughout pregnancy until the time of weaning at 21 days to prevent pulmonary dysplasia.⁸ At the age of 10 to 12 weeks, mice were subjected to an OVA-induced airway inflammation protocol, as described in detail in Appendix E1 (in the Journal's Online Repository at www.mosby.com/jaci). All mice were studied on day 21 of the protocol, approximately 24 hours after the last exposure to OVA.

Antalarmin was prepared as previously described.⁹ Mice were treated twice daily with antalarmin, 20 mg/kg, or ethanol/Cremophor (Sigma, St Louis, Mo) EL vehicle subcutaneously from day 13 to day 19.

Lung mechanics

On day 21, all mice were anesthetized and paralyzed with pentobarbital (70 mg/kg administered intraperitoneally) and doxacurium (0.5 mg/kg administered intraperitoneally), tracheostomized with a 20-gauge cannula, and ventilated with a small-animal ventilator (flexiVent; SCIREQ, Montreal, Quebec, Canada). Lung mechanics and responsiveness to methacholine were assessed by means of a forced oscillation technique described in detail in Appendix E2 (in the Journal's Online Repository at www.mosby.com/jaci).

Bronchoalveolar lavage, cell counts, differentials, and histologic evaluation

Immediately after methacholine challenges, mice were killed by means of exsanguination after cardiac puncture. Whole blood was collected for leukocyte counts, and smears were stained with Wright-Giemsa for differentials. Serum was stored at -80°C for IgE, corticosterone, and catecholamine quantification. Bronchoalveolar lavage (BAL) fluid and lung histologic analysis were performed in a masked fashion (Appendix E3 in the Journal's Online Repository at www.mosby.com/jaci).

RIA, ELISA, HPLC, RT-PCR, and immunohistochemistry

Serum corticosterone levels were measured by means of RIA (Inctar, Stillwater, Minn). Total IgE, IL-4, IL-5, IL-13, RANTES, IFN-γ, and eotaxin levels were measured by using ELISA (R&D Systems, Minneapolis, Minn). Catecholamine analysis was performed with HPLC.¹⁰ Quantitative PCR and immunostaining are described in detail in Appendix E4 (in the Journal's Online Repository at www.mosby.com/jaci).

Statistical analysis

Computations were performed with Statistica 6.0 software (StatSoft, Tulsa, Okla). Differences among genotypes and treatment groups were assessed by means of 1-way ANOVA with the Newman-Keuls post hoc test, factorial ANOVA, or repeated-measures ANOVA as indicated. IgE levels were log transformed to approximate a normal distribution. Tests were conducted at the 5% significance level, and results are expressed as means ± SEM.

RESULTS**Airway inflammation and goblet cell hyperplasia in CRH deficiency**

Allergen-induced airway inflammation in the absence of CRH was examined by means of sensitization of WT and CRH-KO mice to OVA through intraperitoneal injection and challenge with either nebulized PBS or OVA. WT and KO mice challenged with PBS had normal lung architecture and no lung inflammation or goblet cell hyperplasia (Fig 1, A and B, and Table E1 in the Journal's Online Repository at www.mosby.com/jaci). There were no differences in lung weight. In contrast, OVA-challenged mice had a patchy inflammatory process characterized by the accumulation of eosinophils and lymphocytes around airways (Fig 1, C and D) and extensive goblet cell hyperplasia (Fig 1, E and F). OVA-challenged KO mice had significantly increased (approximately 80%-110%) large-, medium-, and small-airway inflammation compared with WT mice. OVA-challenged KO mice also had increased (approximately 70%) goblet cell hypertrophy compared with WT mice.

Analysis of BAL confirmed that OVA-challenged KO mice had increased inflammation compared with that seen in WT mice. WT and KO mice challenged with PBS had low and equivalent numbers of leukocytes in BAL fluid (Fig 2, A). In contrast, OVA-challenged mice had a significant increase in total leukocytes in BAL fluid and a shift in the predominant cell type from macrophage to eosinophil (Fig 2, B). OVA-challenged KO mice had a greater increase in total BAL leukocytes compared with WT mice because of an increase in the percentage and total number of eosinophils (Fig 2, B). These data indicate that CRH deficiency increases eosinophilic inflammation of OVA-challenged mice.

Similar changes were evident in the blood leukocyte count and differential. PBS-challenged WT and KO mice had equivalent leukocyte counts. However, OVA-challenged KO mice had a significant increase in total blood leukocytes compared with that seen in OVA-challenged WT mice (Fig 2, C). Blood differential counts showed that this increase was caused in part by an increase in the percentage and total number of eosinophils (Fig 2, D). These data indicate that CRH deficiency increases eosinophilia in OVA-challenged mice.

IgE in CRH deficiency

Because CRH deficiency enhances airway inflammation, we postulated that levels of serum IgE would be increased in KO mice. To test this hypothesis, we measured total IgE levels in WT and KO mice. Serum

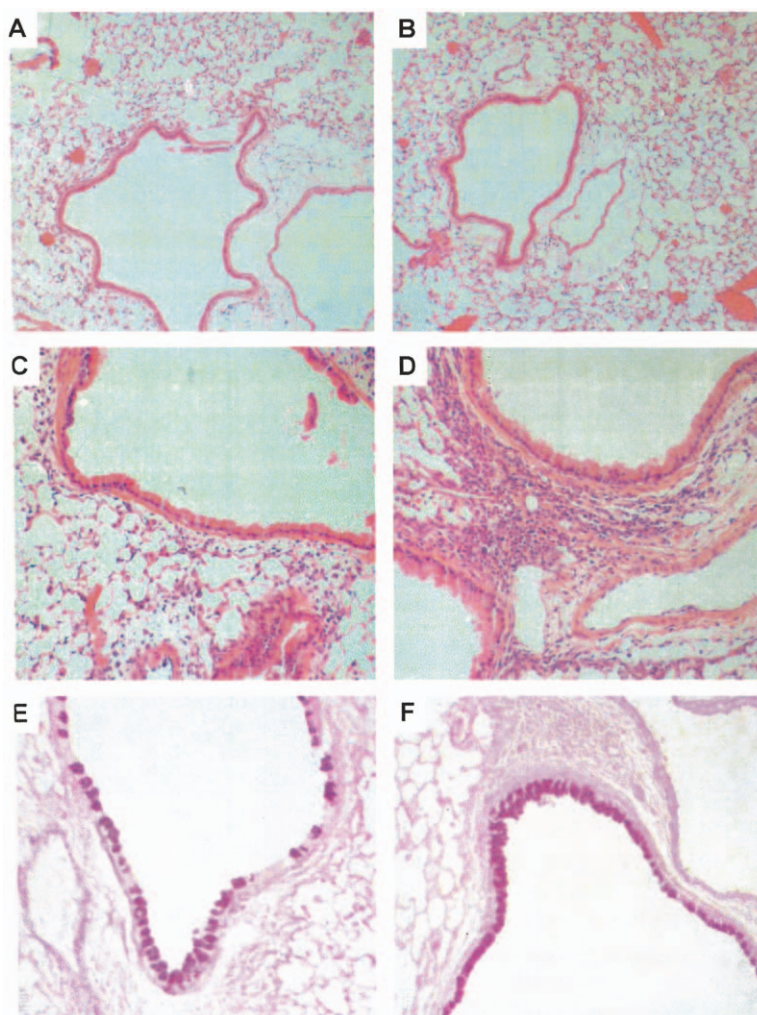


FIG 1. Lung sections: **A**, PBS-challenged WT mouse, hematoxylin and eosin stain (original magnification 100 $\times$); **B**, PBS-challenged KO mouse (original magnification 100 $\times$); **C**, OVA-challenged WT mice (original magnification 200 $\times$); **D**, OVA-challenged KO mice with increased inflammation (original magnification 200 $\times$); **E**, periodic acid-Schiff staining showing goblet cell hyperplasia after OVA challenge (original magnification 200 $\times$); and **F**, periodic acid-Schiff staining after OVA challenge showing increased goblet cell hyperplasia in KO mice (original magnification 200 $\times$).

IgE levels increased approximately 7-fold after OVA challenge in both WT and KO mice; however, there was no difference between the IgE levels of WT and KO mice (Fig E1 in the Journal's Online Repository at www.mosby.com/jaci). Although CRH deficiency increases airway inflammation, goblet cell hyperplasia, and peripheral blood eosinophilia after allergen challenge, serum IgE levels were not similarly increased.

Lung mechanics and airway hyperresponsiveness in CRH deficiency

We postulated that OVA-challenged KO mice would have increased pulmonary dysfunction compared with that seen in WT mice because KO mice have increased airway inflammation and goblet cell hyperplasia. To determine the effects of CRH deficiency on lung mechanics and airway hyperresponsiveness, we compared WT

and KO mice by using a forced oscillation technique and nebulized methacholine.^{11,12} The measured real and imaginary impedance data are shown in Fig E2 (in the Journal's Online Repository at www.mosby.com/jaci). The derived results describing lung mechanics are shown in Table I for each study group.

There were no differences in lung mechanics between PBS-challenged WT and KO mice. In contrast, KO mice had severe pulmonary dysfunction after OVA challenge compared with identically challenged WT mice. After OVA challenge, all derived values of lung mechanics except airway resistance were significantly increased in KO mice. These included total resistance, elastance, coefficient of tissue dampening, coefficient of tissue elastance, and hysteresivity. These data indicate that CRH deficiency has significant effects on the dissipative and elastic properties of the mouse lung after allergen challenge.

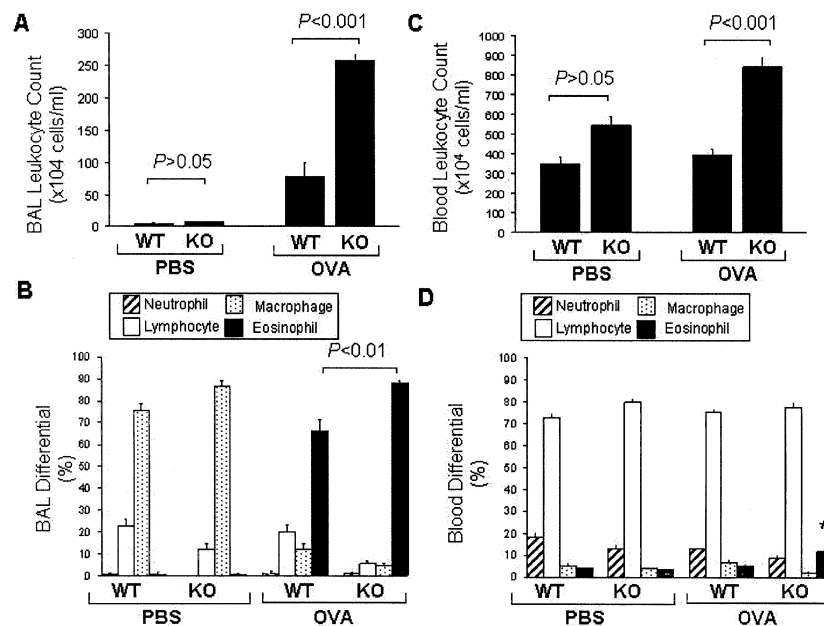


FIG 2. Cell counts and differentials: **A**, BAL leukocyte counts; **B**, BAL differentials; **C**, blood leukocyte counts; and **D**, blood differentials. Data are plotted as means $\pm$ SEM ($n \geq 13$ in each group). *P* values are shown (1-way ANOVA with post hoc comparisons). *Eosinophil percentage is increased compared with that seen in the other groups ($P < .01$).

TABLE I. Baseline lung mechanics ($n = 13$ for each group)

	WT/PBS	KO/PBS	WT/OVA	KO/OVA	<i>P</i> value*
Total resistance at 1 Hz (cm H ₂ O/mL/s)	1.12 $\pm$ 0.02	1.27 $\pm$ 0.06	1.20 $\pm$ 0.05	1.96 $\pm$ 0.33	<.05
Elastance at 1 Hz (cm H ₂ O/mL)	27.2 $\pm$ 0.39	32.2 $\pm$ 2.14	28.3 $\pm$ 0.76	39.5 $\pm$ 3.91	<.01
Airway resistance (cm H ₂ O/mL/s)	0.38 $\pm$ 0.03	0.38 $\pm$ 0.03	0.34 $\pm$ 0.03	0.41 $\pm$ 0.04	NS
Coefficient of tissue resistance (cm H ₂ O/mL)	4.54 $\pm$ 0.09	5.39 $\pm$ 0.33	4.99 $\pm$ 0.20	12.68 $\pm$ 3.78	<.05
Coefficient of Elastance (cm H ₂ O/mL)	29.5 $\pm$ 0.68	36.6 $\pm$ 2.36	30.8 $\pm$ 1.44	40.9 $\pm$ 3.32	<.01
Hysteresivity	0.15 $\pm$ 0.01	0.15 $\pm$ 0.01	0.16 $\pm$ 0.01	0.27 $\pm$ 0.06	<.05

WT, Wild-type mice; KO, CRH-knockout mice.

**P* values refer to post hoc comparisons of the WT/OVA versus KO/OVA groups. WT/PBS versus KO/PBS is not significantly different for any measurement.

We hypothesized that CRH deficiency would also enhance airway responsiveness to bronchoconstricting agonists. To test this hypothesis, we measured peak resistance after increasing concentrations of nebulized methacholine. KO mice had dose-response curves that were shifted upward, with higher resistance values at baseline (PBS) and at each methacholine dose (Fig 3). However, WT and KO mice were relatively unresponsive to methacholine and showed no increase in slope or maximal resistance value after OVA challenge compared with that after PBS challenge (Fig 3). In contrast, BALB/cJ control mice showed airway hyperresponsiveness after OVA challenge. Although CRH-deficient mice demonstrated severe pulmonary dysfunction after allergen challenge, the effects of CRH deficiency on airway hyperresponsiveness could not be assessed on the basis of the genetic background of these mice.

Inflammatory cytokines in CRH deficiency

We postulated that CRH-deficient mice have increased airway inflammation, goblet cell hyperplasia, and lung

dysfunction because of differences in the expression of inflammatory cytokines known to be important in asthma. To test this hypothesis, we measured levels of IL-4, IL-5, IL-13, RANTES, IFN- γ , and eotaxin in BAL fluid of WT and KO mice with ELISA. OVA challenge resulted in a significant increase in the BAL fluid levels of all of these mediators, regardless of genotype (Fig 4; $P < .001$, 2-way ANOVA; $n \geq 13$). We found no difference in levels of any mediator between WT and KO mice after PBS challenge. However, after OVA challenge, the levels of all mediators were significantly higher in KO mice. We found no differences in the levels of IL-1, IL-2, or TNF- α mRNA by means of real-time RT-PCR (data not shown). These data indicate that CRH deficiency increases BAL levels of some cytokines in OVA-challenged mice.

Glucocorticoids and catecholamines in CRH deficiency

KO mice have impaired adrenal responses to stress and lack the normal diurnal rhythm of glucocorticoid

production.¹³ In light of the well-established ability of glucocorticoids to suppress airway inflammation, we postulated that KO mice have increased levels of inflammation and inflammatory mediators because of deficiencies in glucocorticoid production. To test this hypothesis, we measured serum levels of corticosterone in WT and KO mice. As expected, corticosterone levels were significantly decreased in KO mice, irrespective of whether mice were challenged with PBS or OVA (Fig 5, A). There were no significant differences in corticosterone levels between the PBS- and OVA-challenged groups for either WT or KO mice. These data suggest that CRH deficiency decreases endogenous glucocorticoid levels in stressed mice and might account for some of the differences between WT and KO mice after allergen challenge.

Catecholamine production by the adrenal medulla is also impaired in KO mice.¹⁴ Epinephrine and other catecholamines might have anti-inflammatory or proinflammatory effects on airway inflammation.¹⁵ Thus to explore differences between WT and KO mice that might account for the proinflammatory effects of CRH deficiency, we also measured the levels of serum catecholamines and catecholamine metabolites. We found a significant effect of CRH deficiency on levels ($P = .02$, factorial ANOVA) and an interactive effect between genotype and challenge regimen ($P = .015$; Fig 5, B). OVA-challenged KO mice had increased levels of dihydroxyphenylglycol, norepinephrine, and 3,4-dihydroxyphenylacetic compared with those seen in WT mice. Epinephrine levels were also increased in OVA-challenged KO mice but did not reach statistical significance ($P = .35$). Dopamine levels were increased in KO mice ($P = .004$), but OVA challenge had no effect.

Absence of peripheral actions of CRH in airway inflammation

Antalarmin, a specific antagonist of corticotropin-releasing hormone type 1 receptor (CRHR1), can be used to block the peripheral effects of CRH and unmask its proinflammatory actions.^{16,17} To test the hypothesis that CRH has proinflammatory actions in the lungs of mice with asthma-like inflammation, we subjected BALB/cJ mice to OVA challenge and compared the effects of antalarmin treatment with those of vehicle control. The antalarmin protocol used for these experiments did not result in a decrease in corticosterone levels (data not shown). We found that antalarmin treatment had no effect on inflammation and goblet cell hyperplasia (Table EII in the Journal's Online Repository at www.mosby.com/jaci), BAL and blood total leukocyte count and differential count, or airway hyperresponsiveness to methacholine (data not shown).

Consistent with the absence of a peripheral effect of CRH, CRH mRNA was expressed at very low levels in the lungs of BALB/c mice ($1.49 \times 10^{-5} \pm 2.48 \times 10^{-6}$, relative copy number to reduced glyceraldehyde-phosphate dehydrogenase) and did not increase after OVA challenge ($1.28 \times 10^{-5} \pm 1.92 \times 10^{-6}$, $P = .52$; Fig E3 in the Journal's Online Repository at www.mosby.com/jaci).

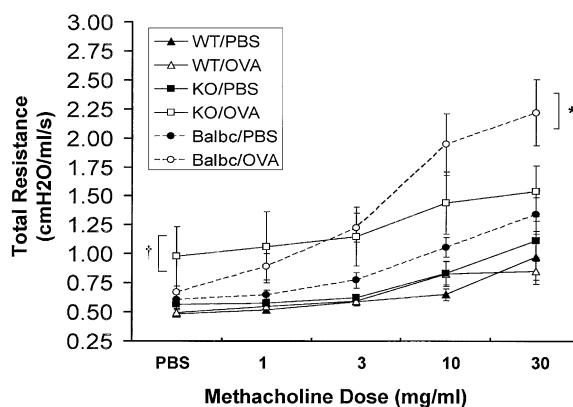


FIG 3. Methacholine dose-response curves plotted as means $\pm$ SEM ($n \geq 13$ in each group, $n = 8$ for Balb/cJ mice). *Balb/cJ mice show airway hyperresponsiveness with increased slope and maximal resistance value ($P = .01$). †KO mice demonstrated pulmonary dysfunction after OVA challenge consistent with the baseline lung mechanics.

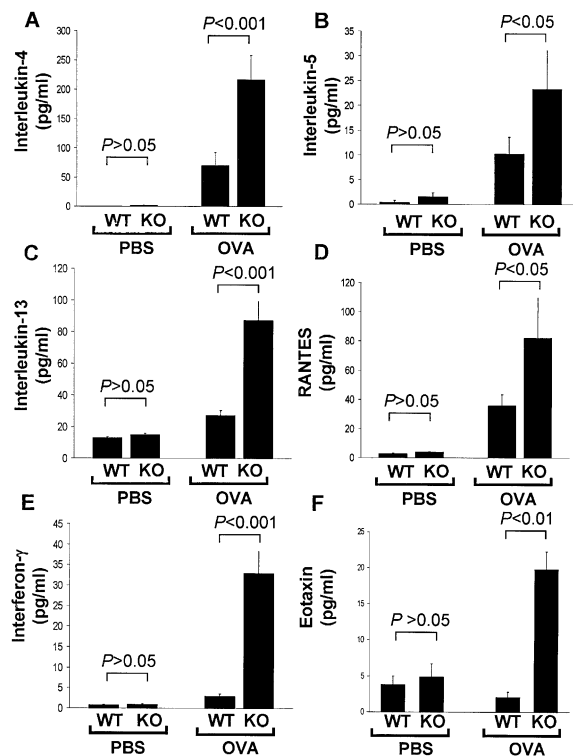


FIG 4. BAL fluid cytokine levels for WT and KO mice after challenge with PBS and OVA, as measured by means of ELISA. Data are plotted as means $\pm$ SEM ($n \geq 13$ in each group). P values are shown (1-way ANOVA with post hoc comparisons).

CRH protein was not detected by means of immunohistochemical staining or BAL RIA with or without inflammation (data not shown). Moreover, we found that CRH is not detectable in lung tissue of asthmatic subjects by using immunohistochemical staining (data not shown).

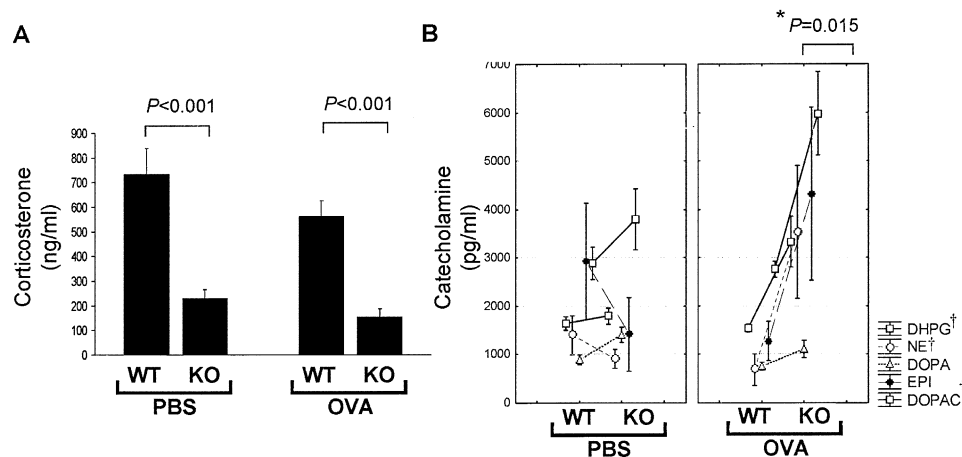


FIG 5. A, Corticosterone levels as means $\pm$ SEM ($n \geq 13$ in each group). **B,** Norepinephrine (NE), epinephrine (EPI), dopamine (DOPA), 3,4-dihydroxyphenylacetic acid (DOPAC), and dihydroxyphenylglycol (DHPG) levels as means $\pm$ SEM ($n \geq 12$). *Factorial ANOVA indicated an interaction between genotype and challenge groups, with OVA-treated KO mice having increased levels of DHPG ($\dagger P = .006$), NE ($\dagger P = .04$), and DOPAC ($\dagger P = .001$).

These data suggest that there is no peripheral proinflammatory action of CRH in asthma.

DISCUSSION

CRH is a 41-amino-acid peptide produced by the hypothalamus and secreted into the hypophyseal portal system in response to inflammatory and physiologic forms of stress.¹⁻⁴ CRH binds CRHR1 on corticotrophs of the anterior pituitary gland and stimulates the release of adrenocorticotropin, which is an essential regulator of glucocorticoid secretion by the adrenal glands. Mice deficient in CRH or CRHR1 show marked impairment of the HPA axis, adrenal gland atrophy, and an inability to increase glucocorticoid production in response to stress.^{7,18,19} Glucocorticoids have powerful anti-inflammatory effects, and their production, as modulated by CRH and the HPA axis, might represent one mechanism by which excessive activation of the immune system is restrained.³

CRH and CRHR1 are also distributed outside the brain and can be found in the lungs, blood leukocytes, lymph nodes, spleen, and thymus.²⁰ CRH can be expressed by leukocytes, epithelial cells, and nerve terminals and induced in local tissues at sites of inflammation. CRH has been shown to stimulate mast cell degranulation, T-lymphocyte proliferation, antibody production, natural killer cell activity, leukocyte chemotaxis, vascular permeability, angiogenesis, and expression of cytokines and reactive oxygen metabolites.³ CRH might inhibit IFN- γ expression and skew inflammation in a T_H2 direction *in vitro*.²¹ Inhibition of CRH by the administration of antiserum or CRHR1 antagonists generally decreases inflammation in animal models of disease, indicating that CRH has proinflammatory effects *in vivo*.^{3,5} It has been

suggested that CRH might have proinflammatory effects in asthma by stimulating T_H2 lymphocyte activity and mast cell degranulation; however, this has never been demonstrated.²

We hypothesized that CRH might affect asthma by means of 2 opposing mechanisms: (1) central anti-inflammatory actions involving glucocorticoid production by the adrenal cortex and (2) peripheral proinflammatory actions involving local immune cell stimulation in the lungs. We tested this hypothesis by comparing KO mice with WT mice in an airway inflammation model. We found that OVA-challenged KO mice had an increase in the severity of many asthma-like features. These data support the hypothesis that central anti-inflammatory actions of CRH exist. There was no evidence of decreased inflammation in the absence of CRH to support the peripheral hypothesis. Moreover, inhibition of CRHR1 with antalarmin did not decrease inflammation, and we could find no CRH in the lungs of OVA-challenged mice or asthmatic subjects. These data suggest that CRH does not have a peripheral proinflammatory action in asthma. However, these studies do not unequivocally exclude some peripheral effects.

OVA-challenged KO mice had an increase in airway inflammation of approximately 80% to 300% and an increase in goblet cell hyperplasia of approximately 70%. The increased inflammation occurred in the large and small airways and was due primarily to an increase in the number of eosinophils. Eosinophil numbers are increased in the sputum, BAL fluid, airway walls, and blood of asthmatic subjects and generally correlate with clinical symptoms, decreased lung function, and airway hyperresponsiveness, implying that eosinophils have a direct role in asthma pathogenesis.²² Increased levels of IL-3, IL-5, and GM-CSF in asthmatic subjects might prolong eosinophil survival. Glucocorticoids might decrease

eosinophil levels in asthmatic subjects by decreasing these cytokines and stimulating apoptosis.²³ Glucocorticoids might also decrease the release of eosinophils into the blood, decrease eosinophil chemotaxis to sites of inflammation, and increase phagocytosis of apoptotic eosinophils.²³⁻²⁵ The decrease in glucocorticoids observed in KO mice is probably responsible for the increased eosinophilia found in these mice. The generally increased levels of catecholamine observed in KO mice are unlikely to have had a proinflammatory effect in our asthma model and might represent a response to increased stress in these mice. These data strongly support the central anti-inflammatory hypothesis of CRH.

The increased inflammation we observed in KO mice was associated with increased levels of IL-4, IL-5, IL-13, RANTES, IFN- γ , and eotaxin in BAL fluid. All of these mediators are increased in the airways of asthmatic subjects and play a role in asthma.²⁶ It is likely that differences in the levels of these mediators have contributed to the increased inflammation in KO mice and that the decreased levels of glucocorticoids in the KO mice contributed to the increased expression of these mediators because glucocorticoids are known to decrease the transcription of IFN- γ , IL-4, IL-5, IL-13, RANTES, and eotaxin *in vitro*.²⁷ Moreover, asthmatic subjects and allergen-challenged mice treated with glucocorticoids have a marked decrease in inflammation and levels of these cytokines in BAL fluid.^{27,28} These data also strongly support the central anti-inflammatory hypothesis of CRH.

Not all indices of the asthmatic phenotype were increased in the KO mice. Serum levels of IgE were not significantly different, although IgE levels did increase markedly after OVA challenge in both groups. Findings similar to ours were observed in mice treated with metyrapone, an inhibitor of 11 β -hydroxylase and glucocorticoid production.²⁹ These and our findings are not unexpected given the complex and variable effects of glucocorticoids on IgE synthesis *in vivo*.^{30,31}

Differences in airway hyperresponsiveness could not be assessed because neither WT nor KO mice had airway hyperresponsiveness after OVA challenge. The 129SVJ/C57BL6 background of these mice is known to make them relatively hyporesponsive.³² We found no differences in lung mechanics (or histology) between PBS-challenged KO and WT mice, indicating that CRH deficiency has no significant effect on lung function in adult mice in the absence of inflammation. However, OVA-challenged KO mice had severely compromised lung function. The lung dysfunction was characterized by an increase in tissue resistance, elastance, and hysteresivity and is likely a reflection of early airway closure and airflow heterogeneity, such as that found in asthmatic subjects during flares.¹² These data suggest that CRH helps preserve lung function in asthma.

In conclusion, our data strongly suggest that CRH and its regulation of glucocorticoids are essential for the control of airway inflammation and the maintenance of lung homeostasis in a mouse model of asthma. There was no evidence to support the peripheral proinflammatory

hypothesis of CRH in asthma. If there is a balance between central anti-inflammatory actions and peripheral proinflammatory actions of CRH in asthma, the proinflammatory actions were masked by the anti-inflammatory actions in our mouse model. These data support the hypothesis that genetic, developmental, or acquired abnormalities of the CRH pathway might be factors in asthma susceptibility or severity.³³

REFERENCES

- Chrousos GP. The hypothalamic-pituitary-adrenal axis and immune-mediated inflammation. *N Engl J Med* 1995;332:1351-62.
- Webster EL, Torpy DJ, Elenkov IJ, Chrousos GP. Corticotropin-releasing hormone and inflammation. *Ann N Y Acad Sci* 1998;840:21-32.
- Karalis K, Muglia LJ, Bae D, Hilderbrand H, Majzoub JA. CRH and the immune system. *J Neuroimmunol* 1997;72:131-6.
- Vale W, Spiess J, Rivier C, Rivier J. Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and beta-endorphin. *Science* 1981;213:1394-7.
- Karalis K, Sano H, Redwine J, Listwak S, Wilder RL, Chrousos GP. Autocrine or paracrine inflammatory actions of corticotropin-releasing hormone *in vivo*. *Science* 1991;254:421-3.
- Theoharides TC, Spanos C, Pang X, Alferes L, Ligris K, Letourneau R, et al. Stress-induced intracranial mast cell degranulation: a corticotropin-releasing hormone-mediated effect. *Endocrinology* 1995;136:5745-50.
- Muglia L, Jacobson L, Dikkes P, Majzoub JA. Corticotropin-releasing hormone deficiency reveals major fetal but not adult glucocorticoid need. *Nature* 1995;373:427-32.
- Venihaki M, Carrigan A, Dikkes P, Majzoub JA. Circadian rise in maternal glucocorticoid prevents pulmonary dysplasia in fetal mice with adrenal insufficiency. *Proc Natl Acad Sci U S A* 2000;97:7336-41.
- Bornstein SR, Webster EL, Torpy DJ, Richman SJ, Mitsiades N, Igel M, et al. Chronic effects of a nonpeptide corticotropin-releasing hormone type I receptor antagonist on pituitary-adrenal function, body weight, and metabolic regulation. *Endocrinology* 1998;139:1546-55.
- Pacak K, Palkovits M, Kvtnansky R, Matern P, Hart C, Kopin IJ, et al. Catecholaminergic inhibition by hypercortisolemia in the paraventricular nucleus of conscious rats. *Endocrinology* 1995;136:4814-9.
- Hantos Z, Daroczy B, Suki B, Nagy S, Fredberg JJ. Input impedance and peripheral inhomogeneity of dog lungs. *J Appl Physiol* 1992;72:168-78.
- Tomioka S, Bates JH, Irvin CG. Airway and tissue mechanics in a murine model of asthma: alveolar capsule vs. forced oscillations. *J Appl Physiol* 2002;93:263-70.
- Muglia LJ, Bethin KE, Jacobson L, Vogt SK, Majzoub JA. Pituitary-adrenal axis regulation in CRH-deficient mice. *Endocr Res* 2000;26:1057-66.
- Muglia LJ, Jacobson L, Weninger SC, Karalis KP, Jeong K, Majzoub JA. The physiology of corticotropin-releasing hormone deficiency in mice. *Peptides* 2001;22:725-31.
- Karalis KP, Kontopoulos E, Muglia LJ, Majzoub JA. Corticotropin-releasing hormone deficiency unmasks the proinflammatory effect of epinephrine. *Proc Natl Acad Sci U S A* 1999;96:7093-7.
- Webster EL, Lewis DB, Torpy DJ, Zachman EK, Rice KC, Chrousos GP. *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* 1996;137:5747-50.
- Webster EL, Barrientos RM, Contoreggi C, Isaac MG, Ligier S, Gabry KE, et al. Corticotropin releasing hormone (CRH) antagonist attenuates adjuvant induced arthritis: role of CRH in peripheral inflammation. *J Rheumatol* 2002;29:1252-61.
- Timpl P, Spanagel R, Sillaber I, Kresse A, Reul JM, Stalla GK, et al. Impaired stress response and reduced anxiety in mice lacking a functional corticotropin-releasing hormone receptor 1. *Nat Genet* 1998;19:162-6.
- Smith GW, Aubry JM, Dellu F, Contarino A, Bilezikjian LM, Gold LH, et al. Corticotropin releasing factor receptor 1-deficient mice display decreased anxiety, impaired stress response, and aberrant neuroendocrine development. *Neuron* 1998;20:1093-102.

20. Baigent SM. Peripheral corticotropin-releasing hormone and urocortin in the control of the immune response. *Peptides* 2001;22:809-20.
21. Angioni S, Petraglia F, Gallinelli A, Cossarizza A, Franceschi C, Muscettola M, et al. Corticotropin-releasing hormone modulates cytokines release in cultured human peripheral blood mononuclear cells. *Life Sci* 1993;53:1735-42.
22. Smith DL, Deshazo RD. Bronchoalveolar lavage in asthma. An update and perspective. *Am Rev Respir Dis* 1993;148:523-32.
23. Wallen N, Kita H, Weiler D, Gleich GJ. Glucocorticoids inhibit cytokine-mediated eosinophil survival. *J Immunol* 1991;147:3490-5.
24. Walsh GM, Sexton DW, Blaylock MG. Corticosteroids, eosinophils and bronchial epithelial cells: new insights into the resolution of inflammation in asthma. *J Endocrinol* 2003;178:37-43.
25. Beeson PB, Bass DA. The eosinophil. *Major Probl Intern Med* 1977;14:1-269.
26. Drazen JM, Arm JP, Austen KF. Sorting out the cytokines of asthma. *J Exp Med* 1996;183:1-5.
27. Barnes PJ. Corticosteroids. In: Barnes P, Drazen J, Rennard S, Thomson N, editors. *Asthma and COPD*. San Diego: Elsevier Science; 2002. p. 547-64.
28. Trifilieff A, El-Hashim A, Bertrand C. Time course of inflammatory and remodeling events in a murine model of asthma: effect of steroid treatment. *Am J Physiol Lung Cell Mol Physiol* 2000;279:L1120-8.
29. De Bie JJ, Hessel EM, Van Ark I, Van Esch B, Hofman G, Nijkamp FP, et al. Effect of dexamethasone and endogenous corticosterone on airway hyperresponsiveness and eosinophilia in the mouse. *Br J Pharmacol* 1996;119:1484-90.
30. Salvi SS, Babu KS, Holgate ST. Glucocorticoids enhance IgE synthesis. Are we heading towards new paradigms? *Clin Exp Allergy* 2000;30:1499-505.
31. Pullerits T, Lotvall J, Togias A. Glucocorticoids and immunoglobulin E: no new in vivo paradigms. *Clin Exp Allergy* 2002;32:345-6.
32. Silverman ES, De Sanctis GT, Boyce J, Maclean JA, Jiao A, Green FH, et al. The transcription factor early growth-response factor 1 modulates tumor necrosis factor-alpha, immunoglobulin E, and airway responsiveness in mice. *Am J Respir Crit Care Med* 2001;163:778-85.
33. Tantisira KG, Lake S, Silverman ES, Palmer LJ, Lazarus R, Silverman EK, et al. Corticosteroid Pharmacogenetics: Association of sequence variants in CRHR1 with improved lung function in asthmatics treated with inhaled corticosteroids. *Hum Mol Genet* 2004;13:1353-9.