

31377

IgE-Mediated Inhalant Allergy against Human Corticotropin-Releasing Hormone

J. Kleine-Tebbe^a, R. Wahl^b, K. Dierks^c, G. Kunkel^a

^a Department of Clinical Immunology and Asthma OPD, Rudolf Virchow University Hospital, Free University of Berlin;

^b Allergopharma Joachim Ganzer KG, Research and Development of Allergen Extract Preparations, Reinbek;

^c Henning Pharma, Research Unit, Berlin, FRG

Abstract. A 25-year-old female developed IgE-mediated sensitization against human recombinant corticotropin-releasing hormone (CRH) with symptoms of allergic rhinoconjunctivitis and bronchial asthma. The occupational allergy was proved by positive skin prick test, bronchial provocation, dose-dependent histamine release, RAST measurements with CRH allergen (RAST class 3) and RAST inhibition. Using the immunoblot technique, a single allergen band with a molecular weight of <14.4 kD in the range between the isoelectric point 5.2 and 5.7 was detected for the CRH extract. Since no endocrinological and behavioral disorders were found, increased CRH-specific IgE was not able to influence the regulatory control of this neuropeptide. After 18 months of avoiding the occupational CRH exposure allergen-specific histamine release and RAST were negative.

Introduction

Corticotropin-releasing hormone (CRH), a peptide composed of 41 amino acids (molecular weight 4,670 D), is established as the principal physiological regulator of the pituitary-adrenal axis [1] by releasing adrenal corticotrophic hormone (ACTH), β -endorphin and other proopiomelanocortin peptides from the anterior pituitary gland. Furthermore, CRH demonstrates potent neuropharmacological actions, that are independent of its pituitary effects [2]. Combined anatomical, pharmacological, and physiological evidence supports its role in mediating the integrated endocrine, autonomic, and cardiovascular responses to stress [3, 4]. Since highly sensitive CRH radioim-

munoassays are available, CRH had not only been detected in hypophyseal portal blood and cerebrospinal fluids of several species, but also in peripheral plasma and other sites outside the central nervous system (lungs, liver, stomach, pancreas, adrenal glands, and placenta). Plasma levels of CRH in normal subjects were found to be 2–28 pg/ml [5]. In recent years, bovine and human CRH had been administered in investigational clinical studies [6, 7], since the intravenous application of 100 μ g/ml CRH and repeated measurement of plasma ACTH and cortisol allows the differentiation between the primary and secondary Cushing syndrome and the differentiation between suprarrenal, pituitary, and hypothalamic insufficiency [4].

Case Report

After 5 months of daily exposure to human recombinant CRH, a 25-year-old atopic female medical technician developed signs of an allergic rhinoconjunctivitis and asthma strongly associated to her work. Additionally, her case history is characterized by a well-known seasonal rhinoconjunctivitis without asthma symptoms in summer. A positive skin prick test to grass pollen, horse and cat dander reflects the atopic status of the patient. The handling at work with the de novo synthesized neuropeptide included analytical procedures and purifications steps (i.e. dissolving, weighing, HPLC analysis). After the appearance of an increasing cough during the night and exercise-induced dyspnea with white-colored sputum, the patient was examined in our department. The clinical examination did not show any pathological findings except a weak wheezing during forced expiration. Serum cortisol (988 nmol/l) and 24-hour urine cortisol (67 nmol/vol) were found to be in the normal range.

Materials and Methods

Purification of CRH by HPLC

After automatic synthesis of the neuropeptide, CRH was purified by HPLC on a column of Vydac C18 (10 μ m, 300 Å pore size), using a gradient of 10–90% CH₃CN in 0.1% TFA over 30 min. A major peak was detected photometrically at 215 nm, which represents the pure neuropeptide. After purification, CRH was lyophilized, weighed, and stored at room temperature before dissolving it on the day of its use.

Skin Prick Test

After dissolving the purified CRH (98% pure) in PAG buffer, a skin prick test was performed on the volar side of the patient's forearm with several concentrations of the solution (2, 6, 20, 60, 200, 1,000 μ g/ml CRH). Histamine (1 mg/ml) and the pure PAG buffer were tested as positive and negative control. After 15 min the wheals were compared to the histamine-induced reactions. The allergen-induced wheals were marked by a pencil and transferred to the tape. Using the formula $(\text{diameter}_{\text{MAX}} + \text{diameter}_{\text{MIN}})/2$ the mean diameter of each wheal was calculated to find the allergen concentration corresponding to the histamine-induced wheal.

Bronchial Provocation Test

Using a body plethysmograph (Jäger, FRG), a bronchial provocation test was performed with several dilutions (0.03, 0.1, 1, 3 μ g/ml) of the purified CRH in physiological saline. After evaluation of the lung function before and after inhalation of saline, 15 min after inhalation of the CRH dilutions, the lung function measurements were repeated. An increase in total airway resistance (R_{aw}) of 100% and a decrease of the FEV₁ more than 20% was defined as a positive result.

Histamine Release from Peripheral Leukocytes

According to the method described by Siraganian and Hook [8], 20 ml whole blood was gently mixed with a solution containing 2 ml 0.1 M EDTA, 5 ml 6% dextran (Braun, FRG), 0.8 ml 50% glucose. After 90 min, the basophil-containing supernatant was drawn and washed twice by centrifugations (400 g, 4°C) with 50 ml

of the following buffers: (1) PAG/EDTA: 25 mM Pipes (Serva, FRG), 0.003% HSA (Mérieux, France), 0.1% glucose, supplemented with 0.01 M EDTA; (2) PAG: the same buffer without EDTA. For the experiments cells were resuspended in 10 ml prewarmed PAG CM buffer (PAG supplemented with 1 mM CaCl₂, 1 mM MgCl₂), equal samples of washed cells (0.25 ml) in duplicates were incubated for 40 min in a water bath (37°C) with different concentrations of CRH (0.25 ml) or with buffer, to evaluate the spontaneous histamine release. Perchloric acid was added to four samples for the determination of the total histamine content. The mediator release was stopped in an ice bath. After centrifugation (800 g, 4°C), to remove the cells, the supernatants were investigated with an automated fluorometric histamine assay [8]. The histamine release was expressed in percent of the total histamine after subtraction of the spontaneous released histamine. The same experiment was done with blood of 3 healthy volunteers, to avoid non-IgE-mediated, unspecific histamine release by the neuropeptide.

RAST

The sera of the patient and a nonallergic control pool serum ($n = 112$) were measured by RAST (Pharmacia, Freiburg, FRG) using allergen disks prepared with the unpurified and purified CRH extracts according to Ceska et al. [9] and Maasch et al. [10]. For the preparation of the allergen disks, 2 μ g protein [11] were used for the coupling to each BrCN-activated paper disk. After coupling, the amount of protein coupled to each disk was measured. For nonspecific binding, the allergen disks were measured with the nonallergic pooled control serum.

RAST Inhibition

The allergenic activity of the purified CRH extract was determined by RAST inhibition [12, 13] using allergen disks prepared with the purified CRH extract and the serum of the patient (1:5 diluted in sample diluent, Pharmacia, Sweden). As tracer for bound specific IgE ¹²⁵I-antihuman IgE (Pharmacia) was used.

SDS-PAGE

SDS-PAGE (gradient 3–22%) of the purified CRH extract was performed according to the instructions of the manufacturer (LKB, München, FRG). For SDS-PAGE, 25 μ g protein was applied in duplicate onto the gel. Marker proteins were obtained from Pharmacia. The proteins were stained with Coomassie brilliant blue R 250 (E. Merck, Darmstadt, FRG).

Immunoprint

Immunoprint was performed according to the method of Peltre et al. [14]. The proteins (50 μ g protein applied in duplicate) were transferred from the gel onto the nitrocellulose membrane (Schleicher and Schüll, Dassel, FRG) by capillary transfer. The membrane was incubated overnight with the serum of the patient (1:5 diluted in the sample diluent, Pharmacia), washed and incubated with ¹²⁵I-antihuman IgE (Pharmacia, 3 kBq/ml) overnight and washed. Then autoradiography was performed at –20°C over 1 and 2 weeks using Kodak X Omat AR films (Eastmann Kodak Co., Rochester, Minn., USA).

Western Blot

Utilizing Western blot analysis [15], the molecular weight of the allergen bands of the CRH extract and the IgE specificities of

the patient's serum were determined. In order to perform the Western blot, 25 μg of the protein of the CRH extract was separated via SDS-PAGE according to molecular weight. The protein was transferred from the gel to the nitrocellulose membrane (Schleicher and Schüll) by electrotransfer. The following steps were also done as described for the immunoprint.

Results

The purification of the CRH resulted in 98% pure material.

Skin Prick Titration

Skin prick titration induced dose-dependent wheal and flare reactions (fig. 1). While the crude CRH provoked a histamine-equivalent wheal at about 10–20 $\mu\text{g}/\text{ml}$, the histamine-corresponding reaction induced by the pure CRH was found at about 5 $\mu\text{g}/\text{ml}$. Three nonatopic individuals without any symptoms did not show any positive reactions to the CRH dilutions.

Bronchial Provocation Test

The initial pulmonary function was normal except for a mild airflow obstruction indicated by a forced expiratory volume in 1 s (FEV_1) of 2.7 l (89% of normal) and a total airway resistance (R_{aw}) of 0.31 $\text{kPa} \times \text{s}/\text{l}$ (110% of normal). The other spirometric parameters were normal and did not change after inhalation of saline. Repeated inhalation of increasing doses of CRH were characterized by an elevation of the R_{aw} to 0.6 $\text{kPa} \times \text{s}/\text{l}$ (94% increase) at 1 $\mu\text{g}/\text{ml}$ CRH. After application of 3 $\mu\text{g}/\text{ml}$ CRH, FEV_1 decreased to 2.1 liters (22% decrease) and R_{aw} increased to 1.1 $\text{kPa} \times \text{s}/\text{l}$ (255% increase) (fig. 2). The sudden elevation of the bronchial obstruction – in parallel with clinical signs of wheezing and increasing dyspnea – was reversible after inhalation of a β_2 -mimetic bronchodilator (salbutamol 400 μg).

Histamine Release

The basophils of the nonatopic controls released histamine after incubation with 30 and 100 $\mu\text{g}/\text{ml}$ CRH. Therefore, this amount of probably unspecific release ($5 \pm 1.5\%$ of the total histamine at 30 $\mu\text{g}/\text{ml}$ CRH, $25 \pm 3.2\%$ at 100 $\mu\text{g}/\text{ml}$ CRH) was deducted from the dose-dependent histamine release, measured after CRH challenge of the patients' blood cells. The resulting curves (fig. 3) are characterized by a slow increase of the mediator release (unpurified material: histamine elevation at a range of 10^{-1} – 10 $\mu\text{g}/$

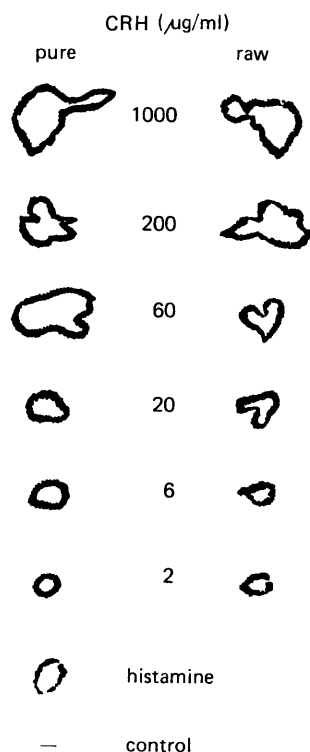


Fig. 1. Skin prick test performed with several concentrations of unpurified and purified CRH. 6 $\mu\text{g}/\text{ml}$ of the highly purified CRH induced a reaction comparable to the induced wheal.

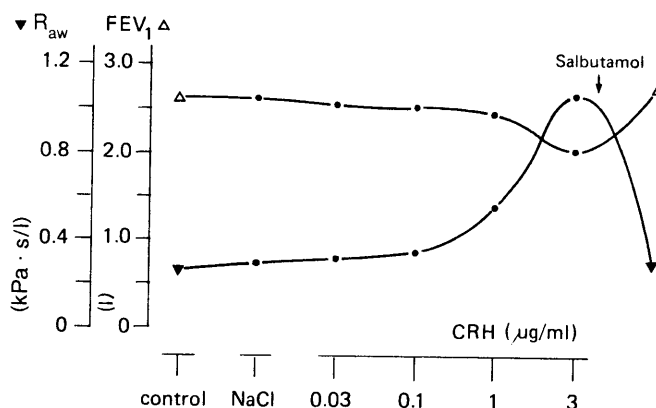


Fig. 2. Bronchial provocation test with increasing doses of pure CRH. A 100% increase of the R_{aw} was detectable after inhalation of 1 $\mu\text{g}/\text{ml}$ of CRH, while 3 $\mu\text{g}/\text{ml}$ CRH induced a 20% decrease of FEV_1 .

ml CRH, pure material: 10^{-2} – 10 $\mu\text{g}/\text{ml}$ CRH). The released histamine values peaked at 100 $\mu\text{g}/\text{ml}$ unpurified CRH and at 30 $\mu\text{g}/\text{ml}$ (pure CRH), respectively. For the release of 20% of the total histamine, 10 $\mu\text{g}/\text{ml}$

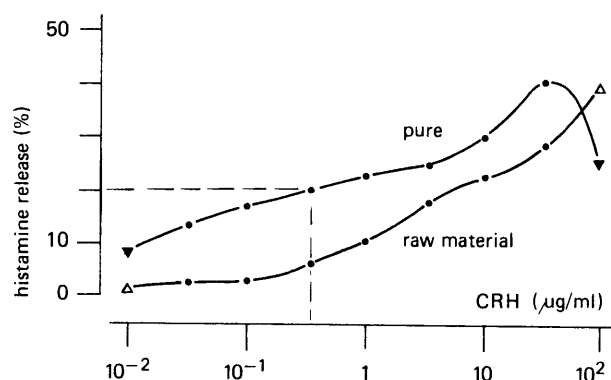


Fig. 3. CRH-induced histamine release from peripheral leukocytes of a CRH-sensitized individual. Highly purified CRH at a concentration of 0.3 µg/ml induced 20% release of the total histamine content, 40% histamine release was obtained as the maximum of mediator response to CRH.

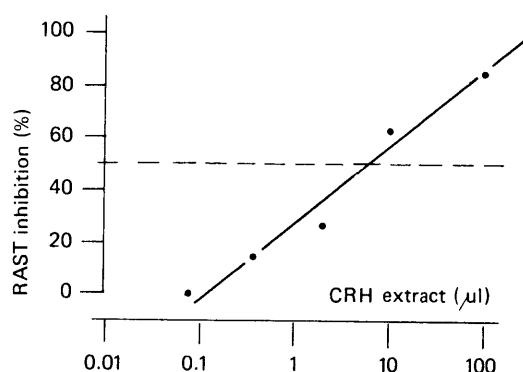


Fig. 4. Regression line of the RAST inhibition curve of the CRH extract.

Table 1. RAST classes of the patient's serum (10/5/88 and 24/1/90) and a nonallergic control pool serum using allergen disks prepared with the unpurified and purified CRH

Sera	Allergen disks	RAST class	Bindings, %
Reference system			
Reference A	reference	4	46.7
Reference B	reference	3	21.6
Reference C	reference	2	6.9
Reference D	reference	1	3.8
Test			
Serum (10/5/88)	unpurified	3	23.1
Serum	purified	3	25.0
Serum (24/1/90)	unpurified	0	3.0
Serum	purified	0	3.4
Nonallergic			
Pool serum	unpurified	0	0.6
Pool serum	purified	0	0.5

unpurified CRH and 0.3 µg/ml pure CRH were needed, indicating the increased basophil sensitivity to CRH as a specific allergen. The same experiment – repeated after 18 months of nonexposure to the synthesized neuropeptide – was negative: no histamine was detectable after CRH challenge, but a positive response to anti-IgE was obtained, indicating the capability of the basophils to react after IgE-mediated stimulation.

RAST

Preparing the allergen disks, 0.94 µg protein of unpurified CRH and 0.88 µg protein of purified CRH on each BrCN-activated paper disk were coupled. The serum of 10/5/88 showed a RAST class 3 for allergen disks prepared with the unpurified and purified CRH extracts (23.8 and 25% binding) (table 1). RAST class 0 was measured with the sera of 24/1/90 with both allergen disks. The nonallergic control pool serum did not contain specific CRH IgE on both occasions (RAST class 0). This shows that the positive RAST measured with the serum of the patient from 10/5/88 could not be related to unspecific binding.

RAST Inhibition

Allergenic activity could be measured with the purified CRH extract by RAST inhibition. In total 90% inhibition was obtained.

SDS-PAGE

By SDS-PAGE one single protein band could be detected with a molecular weight <14.4 kD (fig. 1).

Immunoprint, Western Blot

Figures 5 and 6 show besides the SDS-PAGE, the immunoprint and Western blot performed with the purified CRH extract using the patient's serum. By immunoprint 'allergen bands' (IgE specificities) were detected in an isoelectric range of 5.2–5.7. By Western blot, one allergen band was detected with a molecular weight <14.4 kD (identical to the protein band measured by SDS-PAGE).

Discussion

Allergic reactions after repeated intravenous administration of bovine CRH have been described but never published. Therefore, the use of the CRH test for diagnostic purposes was limited in the past [7].

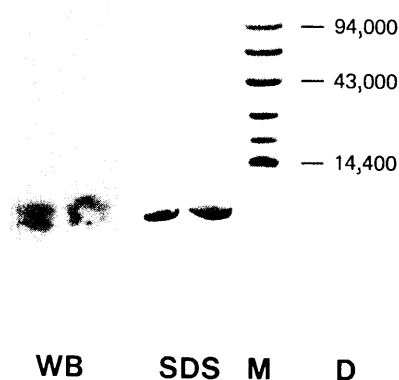


Fig. 5. Western blot (on the left) and SDS-PAGE (on the right) of CRH-purified extract. The extract was applied in duplicate; M = marker protein; time of autoradiography for the Western blot: 14 days.

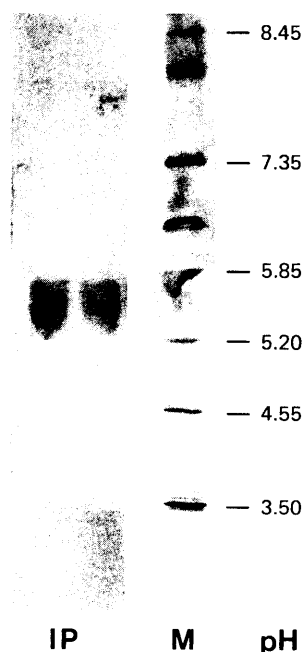


Fig. 6. Immunoprint of CRH purified extract. The extract was applied in duplicate; M = marker protein; time of autoradiography: 14 days.

The development and production of recombinant human CRH created better diagnostic conditions for the differentiation of pituitary-adrenal disorders [4]. The described case strongly suggests that the immediate type allergy to CRH was a result of a sensitization after continuous inhalation of the recombinant peptide. High levels of CRH-specific serum IgE – measured by the RAST – elucidate the extraordinary sensitization against the neuropeptide. In healthy volunteers, IgE against this peptide was not detectable, also the inhibition capacity of the patients' serum – investigated by RAST inhibition – underlines the specific character of this immediate-type sensitization. The separation by SDS-PAGE demonstrates the single-allergen character of CRH, indicating the high purity of the peptides used. Therefore, it seems rather unlikely that other testing products with lower molecular weights would have caused the immediate-type sensitization. The single band of CRH, found below the marker protein of 14.4 kD molecular weight corresponds to the real CRH molecular weight of 4,670 D [1]. The positive skin prick test results obtained with different CRH concentrations demonstrate a dose-dependent allergic response of mediator-containing cells. The hista-

mine-equivalent dose of 6 µg/ml CRH indicates a sensitization as well, which was proved by a titrated bronchial provocation test. In this in vivo experiment we found an increase of R_{aw} in a dose-dependent manner, paralleled by a decrease of FEV_1 . The threshold level of the neuropeptide causing a 100% increase of R_{aw} (R_{aw} PC 100 = 1 µg/ml CRH) and a 20% decrease of FEV_1 (FEV_1 PC₂₀ = 3 µg/ml CRH) demonstrates the definitive immediate-type inhalant allergy. This case leads to the question of how immune tolerance against molecules of human origin (as CRH) can change into a remarkable sensitization characterized by increased specific IgE levels. The phenomena which are responsible for a shift of the immunological activity are not well understood. In some cases, autoantibodies themselves – other than IgE – directly can cause pathological changes. In other autoimmune diseases, however, autoantibodies are associated with disease and indicate pathological damage, but alone may fail to initiate the disease process. Thus, the appearance of serum autoantibodies does not necessarily imply the existence of an autoimmune disease [16]. Increased IgE antibody levels to peptide hormones and endogenous proteins have also been observed in

man. Persons with elevated total serum IgE have a chance of about 10% to develop specific IgE antibodies to single hormones [17]. IgE antibodies to human growth hormone were found in 13% prior to as well as during treatment with the recombinant material not indicating any immunizing process but underlining the natural occurrence of these antibodies. Clinical manifestations of allergy to the biosynthetic hormone were not reported in 245 patients treated for 1 year [18]. Local or systemic IgE-mediated hypersensitivity reactions after administration of insulin had been described in rare cases, especially after therapy with bovine and porcine insulins [19]. In 2 cases of an autoimmune progesterone dermatitis, positive skin tests and indirect basophil degranulation tests were demonstrated, indicating the presence of circulating autoantibodies to the patient's own progesterone [20]. These findings suggest that the circulation of the hormones as well as systemic treatment with human peptides and proteins could be accompanied by an increase in specific serum IgE levels, provoking symptoms of hypersensitivity only in rare cases. In our case, the atopic status of the patient combined with the continuous exposure to the extraordinary inhalant allergen might be a reason for the development of the described IgE-mediated allergic reactions. However, decreasing levels of specific serum IgE antibodies against CRH and a negative histamine release test after 18 months of nonexposure to the inhaled CRH favors the possibility of a diminished allergic sensitization. Obviously, the inhalant allergy against CRH – related to the atopic status in combination with the extraordinary environmental conditions – occurred only in a transient manner. The nonappearance of other symptoms, which might indicate other immunological or neuroendocrinological disorders, can be explained by calculating the dose relationship for the physiological response of CRH in different sites and the dose-dependent conditions for the cross-linking of IgE-antibodies on the surface of mediator-containing cells. While CRH plasma levels were found to be 2–28 pg/ml in normal subjects [5], the threshold level to induce an IgE-mediated response was 10^4 - to 10^6 -fold higher (basophil sensitivity Ag 20:0.3 µg/ml). The extremely low plasma levels of the peptide hormone found in man could explain the lack of disruption of CRH function, as it may be speculated that circulating IgE might bind endogenous CRH and possibly modify its action. Regarding the several-fold higher CRH plasma levels measured in hypophysial portal

blood in rats (470 pg/ml) [21], the difference between these amounts and the dose necessary to cause allergic reactions will have to be multiplied by thousand. Furthermore, cells containing mediators or carrying IgE antibodies are only found in a small amount in the hypophysial area or the central nervous system under physiological conditions [22, 23]. Mast cells (so-called type 1) had been described along the pial and intracerebral vessels and had been observed in the leptomeningeal tissues, suggesting that the meninges contain more mast cells than the brain itself [24]. The close relationship between another cell type (type 2 mast cells) and the nerve fibres may indicate a neurogenic control of the cerebrovascular mast cell secretion [23]. Since in our case no other clinical symptoms or psychological changes were found, we conclude that the transient appearance of the immediate type allergy against CRH was not able to influence the regulatory control of the neuropeptide and its integrative and behavioral functions in the central nervous system. As the increasing use of recombinant human peptides and proteins involves the risk of autoimmune-like sensitizations, the conditions of peptide and protein production and their clinical use should be carefully controlled to prevent unpredictable allergic diseases.

Acknowledgement

For technical assistance we thank Mrs. M. Bormann and Mrs. A. Zieren.

References

- 1 Vale W, Rivier C, Brown MR, Spiess J, Koob G, Swanson L, Bilezikjian L, Bloom F, Rivier J: Chemical and biological characterization of corticotropin-releasing factor. *Recent Prog Horm Res* 1983;39:245–270.
- 2 Smith MA, Kling MA, Whitfield JH, Brandt HA, Demitrack MA, Geraciotti TD, Chrousos GP, Gold PW: Corticotropin-releasing hormone: From endocrinology to psychobiology. *Horm Res* 1989;31:66–71.
- 3 Fisher LA: Corticotropin-releasing factor: Endocrine and autonomic integration of responses to stress. *TIPS* 1989;10:189–193.
- 4 Taylor AL, Fishman LM: Corticotropin-releasing hormone. *N Engl J Med* 1988;319:213–222.
- 5 Suda T, Tomori N, Yajima F, Sumitomo F, Nakagami Y, Ushiyama T, Demura H, Shizume K: Immunoreactive corticotropin-releasing factor in human plasma. *J Clin Invest* 1985;76:2026–2029.
- 6 Chrousos GP, Schuermeyer TH, Doppmann J: Clinical appli-

- cations of corticotropin-releasing factor. *Ann Intern Med* 1985;102:344-358.
- 7 Marsiske C, Haack D, Kunkel G, Grabnitzki K, Vescei P, Liese B: Corticotropin-Releasing-Factor-(CRF)-Stimulationstest bei Asthmatikern mit unterschiedlicher Steroidmedikation. *Med Klin* 1986;21:663-667.
- 8 Siraganian RP, Hook WA: Histamine release and assay methods for the study of human allergy; in Rose NR, Friedman H (eds): *Manual of Clinical Immunology*. Washington, American Society for Microbiology, 1980, pp 808-821.
- 9 Ceska M, Eriksson R, Varga JM: Radioimmunosorbent assay of allergens. *J Allergy Clin Immunol* 1972;49:1-9.
- 10 Maasch HJ, Wihl JA, Schultze-Werninghaus G, Geissler W, Wahl R: A manufacturer's criteria for in house reference preparations for RAST inhibition. *Ann Allergy* 1987;59:29-33.
- 11 Wahl R, Geissler W, Maasch HJ: Comparison of classical Lowry, modified Lowry and a dye-binding assay for the estimation of protein in allergen extracts and influence of different parameters on the modified Lowry assay. *Anal Biochem* 1983;134:189-194.
- 13 Yman L, Ponterius B, Brandt R: RAST-based allergen assay methods. *Dev Biol Stand. Basel, Karger*, 1975, vol 20, pp 151-165.
- 14 Peltre G, Lapeyre J, David B: Heterogeneity of grass pollen allergens (*Dactylis glomerata*) by IgE antibodies in human patients sera by a new nitrocellulose technique. *Immunol Lett* 1982;5:127-131.
- 15 Burnete WN: 'Western blotting': Electrophoretic transfer of proteins from sodium dodecyl-polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radioiodinated protein A. *Anal Biochem* 1981;112:195-203.
- 16 Gleichmann E, Kimber I, Purchase IFH: Immunotoxicology: Suppressive and stimulatory effects of drugs and environmental chemicals on the immune system. *Arch Toxicol* 1989;63:257-267.
- 17 Khalaf AN, Kerp L, Schliebs B, Jägle M, Petersen KG: Natural occurrence of IgE antibodies to peptide hormones in man. *Eur J Clin Invest* 1989;19:464-465.
- 18 Petersen KG, Khalaf A, Zeisel HJ, Stryjek-Kaminska D, Kerp L: IgE antibodies to human growth hormone prior to and during treatment. *Acta Endocrinol* 1989;121:501-503.
- 19 Patterson R, Roberts M, Grammer LC: Insulin allergy. Reevaluation after two decades. *Ann Allergy* 1990;64:459-462.
- 20 Miura T, Matsuda M, Yanbe H, Sugiyama S: Two cases of autoimmune progesterone dermatitis: Immunohistochemical and serological studies. *Acta Derm Venereol (Stockh)* 1989;69:308-310.
- 21 Gibbs DM: Measurement of hypothalamic corticotropin-releasing factors in hypophysial portal blood. *Fed Proc* 1985;44:203-206.
- 22 Campbell DJ, Kiernan JA: Mast cells in the central nervous system. *Nature* 1986;210:756-757.
- 23 Dimitriadou V, Aubineau P, Taxi J, Seylaz J: Ultrastructural evidence for a functional unit between nerve fibers and type II cerebral mast cells in the cerebral vascular wall. *Neuroscience* 1987;22:621-630.
- 24 Edvinsson L, Cervos-Navarro J, Larsson LI, Owman C, Ronnberg AL: Regional distribution of mast cells containing histamine, dopamine, or 5-HT in the mammalian brain. *Neurology* 1977;27:878-883.

Received: February 25, 1991

Accepted after revision: March 18, 1991

Correspondence to: Dr. Jörg Kleine-Tebbe
Universitätsklinikum Rudolf Virchow
Abteilung für klinische Immunologie und Asthma-Poliklinik
Augustenburger Platz 1
D-W-1000 Berlin 65 (FRG)