American Journal of Pathology, Vol. 145, No. 5, November 1994

Presence of Immunoreactive Corticotropin Releasing Hormone in Thyroid Lesions

Chrisoula D. Scopa,*§ George Mastorakos,† Theodore C. Friedman,†‡ Maria Melachrinou,§ Maria J. Merino,* and George P. Chrousos†

From the Laboratory of Pathology, National Cancer Institute, the Developmental Endocrinology Branch† and the Laboratory of Developmental Neurobiology,‡ National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland, and the Department of Pathology,§ University of Patras Medical School, Patras, Greece*

Corticotropin-releasing hormone (CRH) functions as a regulator of the hypothalamic-pituitary-adrenal axis and coordinator of the stress response. Immunoreactive CRH (IrCRH) is also produced in a variety of inflammatory sites, where this peptide acts as a proinflammatory cytokine. To detect CRH in autoimmune thyroid disease as well as in disorders that may be associated with an inflammatory reaction within this gland, we examined immunohistochemically 45 thyroid lesions, including 12 nodular goiters, 9 cases of Hashimoto thyroiditis, 6 follicular adenomas, 4 follicular and 8 papillary carcinomas, 4 Hürthle cell tumors, 1 medullary cancer, and 1 insular thyroid carcinoma. We also examined the presence of IrCRH in the adjacent normal thyroid parenchyma. The avidin-biotin complex method was employed on formalin-fixed, paraffin-embedded tissue, using a highly specific, affinity-purified polyclonal rabbit anti-CRH antibody. Granular cytoplasmic immunostaining of follicular cells was observed in 100% of the cases of Hashimoto thyroiditis, 77% of the neoplasms and 42% of goiters. The intensity of the staining was more pronounced in Hashimoto thyroiditis and Hürthle cell tumors, whereas the remaining lesions exhibited a heterogeneous staining pattern. No IrCRH was observed in the normal thyroid parenchyma. Using a specific radioimmunoassay, the IrCRH in extracts of simple thyroid goiters, papillary carcinomas, and Hürthle cell tumors ranged between 0.031 and 0.224 pmol/g of wet tissue but was undetectable in normal thyroid parenchyma. The IrCRH molecule in the thyroid gland eluted at the same fraction as synthetic rat/human CRH 1-41 in reverse phase high pressure liquid chromatography. We conclude that IrCRH is present in thyroid lesions, predominantly in those related to autoimmune phenomena, suggesting that this neuropeptide may be directly and/or indirectly involved with inflammatory processes taking place in this gland. (Am J Pathol 1994, 145:1159–1167)

Corticotropin-releasing hormone (CRH), a 41-amino acid neuropeptide first isolated from the hypothalamus, is the principal regulator of adrenocorticotropin hormone (ACTH) secretion and coordinator of the stress response in the central nervous system.[1–4] CRH and its receptors are present in many extrahypothalamic sites of the central nervous system[5–8] as well as in several peripheral tissues, including active inflammatory sites in rats and humans,[9–12] human placenta and fetal membranes,[13–16] rat and ovine testes,[17–20] and rat and human ovaries.[21,22] In experimentally induced inflammatory sites, CRH participates as an autocrine/paracrine proinflammatory cytokine.[9,10] In these sites immune accessory cells (macrophages, tissue fibroblasts, and endothelial cells) contain immunoreactive CRH (IrCRH) and/or express CRH mRNA.[19–12] Furthermore, circulating monocytes and lymphocytes express CRH mRNA and secrete IrCRH when activated by exogenous stimuli *in vitro*.[23,24] CRH and/or its mRNA are also present in autoimmune lesions, such as those in adjuvant- and streptococcal cell wall-induced arthritis,[10] in interphotoreceptor retinoid binding protein-induced uveitis in rats,[12] and in rheumatoid arthritis in humans.[11] In addition, CRH and its mRNA are present in the dorsal root and sympathetic ganglia, possibly participating along with other substances such as substance P and somatostatin in the process of neu-

Accepted for publication August 10, 1994.

Address reprint requests to Dr. George P. Chrousos, National Institutes of Health, NICHD/DEB, Building 10, Room 10N244, 9000 Rockville Pike, Bethesda, MD 20892.

rogenic inflammation via secretion, respectively, by the sensory afferent fibers and the postganglionic sympathetic neurons.[25–27]

A variety of pathologic conditions afflict the thyroid gland, ranging from hyperplasia and/or hypertrophy to autoimmune disease and from benign to malignant neoplasias. Any of these conditions may be accompanied or characterized by a local inflammatory reaction. The purpose of this study was to examine thyroid samples from patients with various pathologic states of the thyroid for the presence of IrCRH.

Materials and Methods

Thyroid Tissue Specimens

Forty-five thyroid tissue specimens were obtained from the surgical pathology repository of the Pathology Branch of the National Cancer Institute (Bethesda, MD) and from fresh surgical material submitted to the Laboratory of Surgical Pathology of the University of Patras Medical School (Patras, Greece). All thyroid tissue specimens were retrieved from storage banks *post hoc* and studied anonymously and in a blind fashion. Specimens were from 12 nodular goiters, 9 cases of Hashimoto thyroiditis, 6 follicular adenomas, 4 follicular and 8 papillary carcinomas, 4 Hürthle cell tumors, 1 medullary carcinoma, and 1 insular carcinoma.

Materials

Synthetic rat/human CRH (r/hCRH) 1-41 was obtained from Peninsula Laboratories Inc. (Belmont, CA);[28,29] HPLC-purified ^{125}I-r/hCRH from New England Nuclear Co. (Boston, Ma); cyanogen bromide-activated Sepharose 4B and 3,3-diaminobenzidine tetrahydrochloride from Sigma Chemical Co. (St. Louis, MO); 10% formalin (Formalde-Fresh) from Fischer Scientific Co. (Pittsburgh, PA); Vectastain ABC kits from Vector Laboratories Inc. (Burlingame, CA); rabbit IgG from Jackson Immunoresearch Laboratories Inc. (West Grove, PA); octadecylsilyl-silica cartridges (C-18 Sep-Pak) from Waters Associates (Marlboro, MA). The high pressure liquid chromatography (HPLC) LKB 2150 system used was purchased from Pharmacia LKB (Piscataway, NJ) and was coupled to a HiPore RP-318 column (C-180 (5 × 250 mm) obtained from Bio-Rad Co. (Richmond, CA). Met(O)^{21,38}-CRH was generated by incubating synthetic r/hCRH 1-41 with 0.05% H_2O_2 for 10 minutes at room temperature at pH 9.0 before acidification with 10 μl of 1% trifluoroacetic acid (TFA).

Tissue Preparation

Thyroid tissue specimens prepared for immunohistochemistry were fixed in 10% formalin and embedded in paraffin. Thyroid tissue specimens prepared for CRH extraction, radioimmunoassay (RIA), and reverse phase HPLC were frozen on dry ice immediately after surgical removal and stored at −70 C until assay.

Production and Characteristics of CRH Antiserum Used for Immunohistochemistry and RIA

Antiserum (TS-2) against r/hCRH was produced in rabbits as previously described.[30] Briefly, rabbits were immunized with synthetic r/hCRH conjugated to bovine serum albumin by the carbodiimide reaction. Specific binding of [^{125}I]-labeled r/hCRH by the antiserum was 22.2 ± 1.2% (mean ± SE), and nonspecific binding was 2.2 ± 0.2%. No significant cross-reactivities (<0.0001%) were found for growth hormone-releasing hormone, luteinizing hormone-releasing hormone, arginine vasopressin, adrenocorticotrophic hormone, oxytocin, β-endorphin, human luteinizing hormone, human follicle-stimulating hormone, β-lipotropin, human chorionic gonadotropin, human growth hormone, thyroid-stimulating hormone or human protection. The specificity of the anti-r/hCRH in tissues was also examined by coincubation of the antibody with increasing concentrations of pure synthetic r/hCRH, which eliminated specific immunostaining. No crossreactivity of the TS-2 antibody with thyroglobulin was found.

Immunohistochemistry

Purification of CRH Antiserum

Before use it was affinity-purified by adsorption to synthetic r/hCRH 1-41 coupled to cyanogen bromide-activated Sepharose 4B. Briefly, the antiserum was added to the r/hCRH-Sepharose 4B conjugate (1 mg of r/hCRH 1-41 on 200 μg gel) and incubated at room temperature for 2 hours. The suspension was packed in a 4 × 0.7 cm column and washed with phosphate buffer (20 mmol/L sodium phosphate, 0.5 mmol/L sodium chloride, pH 7.3) until the OD280 returned to baseline. The elutions obtained from these washings were depleted of anti-r/hCRH IgG (affinity-negative IgG fraction) and were used in immunohistochemistry

staining as controls of the specificity of the anti-r/hCRH IgG. The antibody bound to the affinity column (affinity-positive IgG fraction) was eluted with thiocyanate buffer (3 mol/L potassium thiocyanate, 0.5 mol/L ammonium hydroxide) and dialyzed against several changes of cold phosphate buffer saline (PBS).

Staining Protocol

Tissue specimens embedded in paraffin, as described before, were sectioned onto gelatin-coated microscope slides at a thickness of 6 μ. Immunoperoxidase staining was performed with the Vectastain ABC kit, using the manufacturer's suggested protocol and reagents.[31,32] All subsequent procedures took place at room temperature. The sections were deparaffinized with two 5-minute washes in xylene and rehydrated by sequential rinses in absolute, 90%, 80%, and 70% ethanol. Endogenous peroxidase activity was exhausted by incubation with 0.3% hydrogen peroxidase in absolute methanol for 45 minutes. The slides were then incubated sequentially with 0.1% bovine serum albumin in PBS for 20 minutes, with diluted goat serum (1/66.7) for 20 minutes, and in a humid chamber with the affinity-purified rabbit antibody to r/hCRH (30 μg/ml), the control, affinity-negative IgG fraction (30 μg/ml), or nonimmune normal rabbit IgG (30 μg/ml). After 60 minutes, the sections were washed with PBS and incubated with biotinylated goat anti-rabbit IgG for 30 minutes. The sections were further washed with PBS and incubated with avidin and horseradish peroxidase complex for 45 minutes. Finally, sections were washed and color was developed by immersing sections in a solution of 0.05% weight/volume diaminobenzidine tetrahydrochloride and 0.01% hydrogen peroxide in 0.05 mol/L Tris, pH 7.4, for 3 minutes. The sections were counterstained with Meyer's hematoxylin, which stains all cell nuclei. Positive staining was revealed as brown color spots, and nonspecific staining of all cell nuclei as light blue color. Human ovary and placental sections as well as rat hypothalamus and rat inflammatory granuloma sections were used as positive controls. Because positive staining often varied, the slides were blindly examined by two independent observers and their results recorded on a scale of 0 to 3 for the IrCRH distribution (0 = none, 1 = <10%, 2 = 10 to 50%, and 3 = >50% of cells) and on a scale 1+ to 3+ for the intensity of the staining (1+ = mild, 2+ = moderate, and 3+ = marked).

Radioimmunoassay

Thyroid extract concentrations of CRH were measured by RIA after acid extraction. Briefly, 10 volumes of boiling 2 mol/L acetic acid was added to thyroid tissue specimens and the mixture was incubated at 95 C for 10 minutes. Samples were then sonicated on ice for 1 minute and centrifuged at 15,000 × *g* for 30 minutes. Three volumes of acetone were added to the supernatants, and they were again centrifuged at 15,000 × *g* for 30 minutes. The supernatants were collected, lyophilized, and reconstituted in RIA buffer, as previously described.[33] The CRH antiserum (TS-2) used has been characterized in detail previously.[30] ^{125}I-r/hCRH was used as the tracer, and synthetic r/hCRH as the standard. The within-assay coefficient of variation and sensitivity were 4% and 1.05 pmol/L, respectively. Human ovaries and placenta tissues as well as rat hypothalami and inflammatory granulomas, known to contain large amounts of CRH, were used as positive controls.

Reverse Phase HPLC

Samples obtained from acid extraction of thyroid tissues were reconstituted in 200 μl of 0.1% TFA and passed through an octadecylsilyl-silica cartridge (C-18 Sep-Pak). The cartridges were then washed with 0.1% TFA and subsequently with 20% acetonitrile containing 0.1% TFA. The peptides were finally eluted off the cartridge with 5 ml of 60% acetonitrile containing 0.1% TFA. The eluate was lyophilized, reconstituted in 200 μl of 0.1% TFA and analyzed by HPLC. An aliquot of 180 μl of the sample was injected into the HPLC system. Solvent A was 0.1% TFA and solvent B was 80% acetonitrile containing 0.1% TFA. A linear gradient from 40 to 65% of solvent B at a flow rate of 1 ml/minute was applied over 45 minutes. One-ml samples of the eluate were collected in an automatic fraction collector, lyophilized to dryness, reconstituted with RIA buffer, and assayed for CRH content, as described above. A standard of 1 μg synthetic r/hCRH and 1 μg Met(O)^{21,38}-CRH were injected after each experiment to determine their chromatographic profile.

Statistical Analyses

Differences between the mean of IrCRH content of Hürthle cell tumors, papillary carcinomas and simple thyroid goiters were examined by ANOVA followed by Fisher's least significant difference *post hoc* test. Statistical significance was set at $P < 0.05$.

Results

Immunohistochemical Detection of IrCRH in Human Thyroid Tissues

The results are summarized in Table I. The distribution and intensity of immunohistochemical staining are depicted graphically in Figure 1.

Autoimmune Thyroiditis and Simple Goiters

Immunoreactive CRH was detected in the cytoplasm of the follicular cells in 100% of specimens with Hashimoto thyroiditis and in 42% of simple goiters. The intensity of the staining was more pronounced in the former than the latter (Figures 2, 3, and 4). Varying degrees of inflammatory reaction, ranging from heavy lymphocytic infiltration in Hashimoto thyroiditis to mild leukocytic infiltration in some simple goiters, were seen. The 7 of the 12 nodular goiters that failed to show any positive IrCRH had no inflammatory infiltrate.

Thyroid Neoplasms

Immunoreactive CRH was detected in the cytoplasm of neoplastic follicular cells in 100% of follicular adenomas and Hürthle cell tumors, in 25% of follicular carcinomas, and in 50% of papillary carcinomas (Figures 5 and 6). One medullary and one insular carcinoma were positive for IrCRH. The intensity of the staining was most pronounced in Hürthle cell tumors (Figures 7 and 8) and in metaplastic oxyphilic cells interspersed among tumor cells in papillary carcinomas. Prominent staining was also seen in malignant cells infiltrating the fibrous bands in tumors with desmoplastic reaction. Peritumoral thyroiditis and moderate to extensive fibrosis was observed in all but three neoplasms. A small number of CRH-positive mononuclear cells was also identified in most neoplasms (Figure 9). All three neoplastic lesions, which were negative for IrCRH staining, were follicular carcinomas with hardly any detectable inflammatory infiltration.

Table 1. *CRH Immunoreactivity in Thyroid Lesions*

Histology	No. positive/total cases	%
Hashimoto thyroiditis	9/9	100
Nodular goiter	5/12	42
Follicular adenoma	6/6	100
Follicular carcinoma	1/4	25
Papillary carcinoma	4/8	50
Hürthle cell tumor	4/4	100
Medullary carcinoma	1/1	100
Insular carcinoma	1/1	100

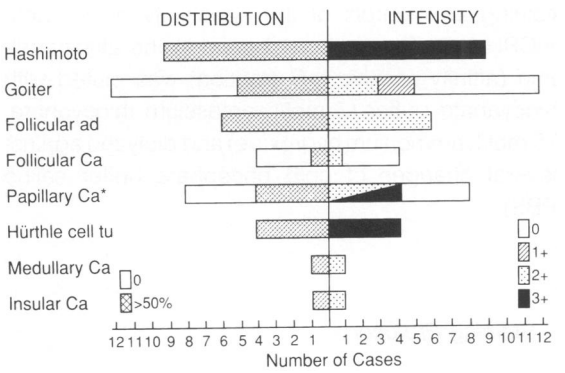

Figure 1. *Distribution and intensity of staining for IrCRH in all thyroid lesions examined. Each section of a bar represents the number of lesions from a given category that stained positively with a particular distribution or intensity. ad, adenoma; Ca, carcinoma; tu, tumor. *The 3+ intensity in papillary carcinomas refers to the metaplastic oxyphilic cells (Hürthle cells) interspersed among tumor cells.*

In all cases the immunostaining pattern was diffuse and/or granular. Follicular cells of normal thyroid parenchyma in specimens from goiters, autoimmune thyroiditis, or adjacent to the neoplastic lesions examined were completely negative for IrCRH. CRH immunostaining was also detected in the smooth muscles of the small size blood vessels as well as in endothelial cells.

HPLC Mobility and IrCRH Measurements in Thyroid Extracts

A large proportion of IrCRH from simple thyroid goiter and papillary thyroid carcinoma specimens had similar chromatographic mobility to that of synthetic r/hCRH 1-41 (Figure 10). In addition, a peak of IrCRH corresponding to the retention-time (18 min) of the sulfoxide form of CRH, Met(O)^{21,38}-CRH, was observed as well as immunoreactive bands with more mobility than r/hCRH 1-41. The latter may be CRH metabolites and/or aggregates/precursors with different hydrophobicity. The levels of IrCRH by RIA in simple thyroid goiters (0.045 ± 0.006 pmol per gram of wet tissue, mean ± SE, n = 6), papillary thyroid carcinomas (0.144 ± 0.013 pmol per gram of wet tissue, n = 5), and Hürthle cell tumors (0.204 ± 0.015 pmol per gram of wet tissue, n = 4) extracts were between 0.031 and 0.224 pmol per gram of wet tissue. No IrCRH was measurable by RIA in normal thyroid parenchyma.

Discussion

We demonstrated that IrCRH is localized in the follicular cells of various thyroid lesions, such as Hashimoto thyroiditis, simple goiters, and benign and malignant neoplastic lesions, but not in the follicular cells

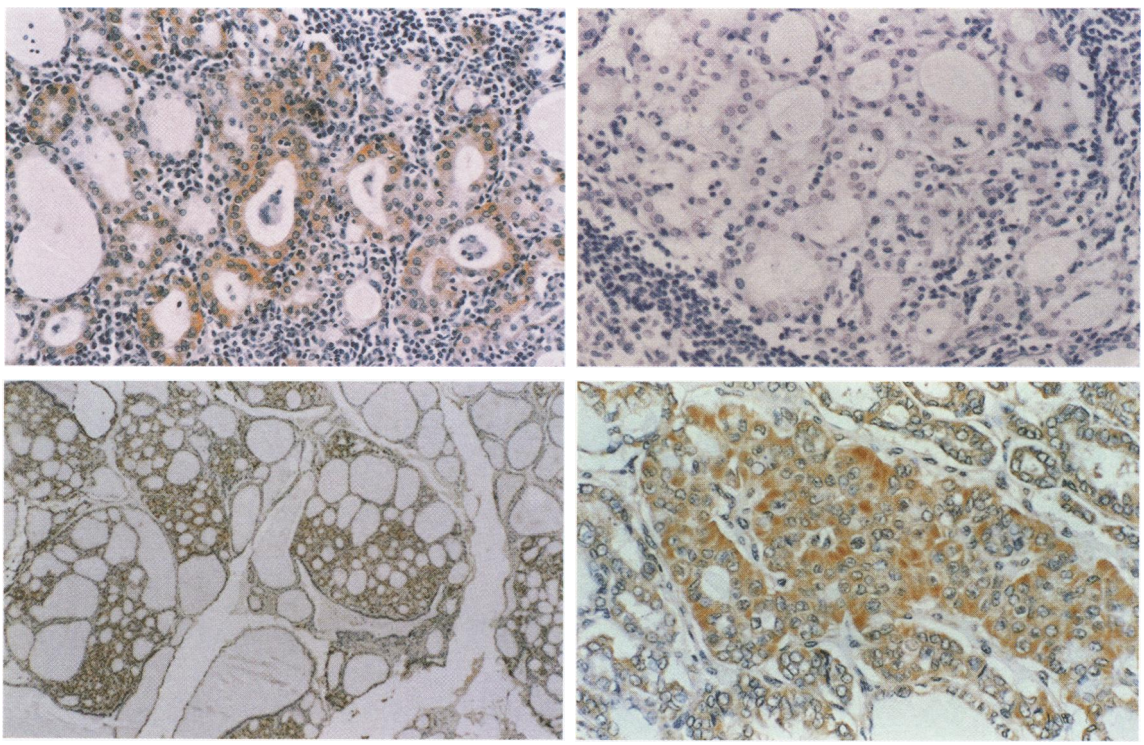

Figure 2. *Immunohistochemical localization of IrCRH in follicular cells of a thyroid section with Hashimoto thyroiditis (magnification, ×1225). Positive staining is revealed as brown color, whereas the light blue color of Meyer's hematoxylin stains nonspecifically all cell nuclei.*
Figure 3. *No specific staining can be seen in follicular cells of a control thyroid section from the thyroid with Hashimoto thyroiditis of Figure 2 incubated with the affinity-negative IgG fraction (magnification, ×1225).*
Figure 4. *Immunohistochemical localization of IrCRH in follicular cells of a thyroid section with simple thyroid goiter (magnification, ×241). Positive staining is revealed as brown color, whereas the light blue color of Meyer's hematoxylin stains nonspecifically all cell nuclei.*
Figure 5. *Immunohistochemical localization of IrCRH in follicular cells of a thyroid section with papillary carcinoma (magnification, ×1851). Positive staining is revealed as brown color, whereas the light blue color of Meyer's hematoxylin stains nonspecifically all cell nuclei.*

of adjacent normal parenchyma. Thyroid IrCRH had similar chromatographic mobility as r/hCRH 1-41, the form produced by the rat and human hypothalamus, rat and human peripheral inflammatory sites, rat Leydig cells, human placenta, and rat and human ovarian stromal, thecal, and luteinized cells.[9–17,21,22,29,33–35] The early peak of IrCRH (Figure 10) corresponds to the sulfoxide form of CRH, Met(O)[21,38]-CRH, which has been reported to elute before CRH in many different tissues.[11,22,35,36] This finding indicates that sulfoxidation of CRH takes place in inflammatory thyroid tissues, contributing thus to the inactivation of this molecule and/or, less likely, that oxidized CRH participates somehow in the pathophysiology of inflammatory phenomena.

The concentrations of IrCRH (0.031 to 0.224 pmol per gram of wet tissue) in the thyroid lesions studied (simple thyroid goiter, papillary thyroid carcinoma, and Hürthle cell tumors) were in the range of those found in the extracts of rat inflammatory tissues (0.084 to 0.105 pmol per gram of wet tissue)[9,10] or human arthritic joints,[11] and rat (0.042 to 0.126 pmol per gram of wet tissue)[21] or human (0.055 to 0.095 pmol per gram of wet tissue)[22] ovaries, but lower than that in the rat testis (10.719 to 15.554 pmol per gram of wet tissue)[19] and human hypothalamus (10.931 to 18.919 pmol per gram of wet tissue).[35]

Thyroid CRH could be derived from several sources. First, it might be produced by the follicular cells of the thyroid lesions, which contained IrCRH, and/or by the Hürthle cells and Hürthle cell tumors, which showed an intense and prominent CRH immunostaining. Indeed, Hürthle cell tumors were shown by RIA to contain increased levels of IrCRH (0.204 ± 0.015 pmol per gram of wet tissue, mean ± SE) compared to that of papillary carcinomas (0.144 ± 0.013 pmol per gram of wet tissue $P < 0.05$) and simple thyroid goiters (0.045 ± 0.006 pmol per gram of wet tissue, $P < 0.01$). It should be noted that several nonneoplastic and neoplastic thyroid disorders, such as nodular goiter, thyroiditis, and follicular, papillary, and undifferentiated tumors may contain Hürthle cells.[37–39] Hürthle cells have electron-dense mitochondrial core matrical granules,[37,40] which are also present in human and animal tissues, such as adrenal cortex, Leydig cells, uterine epithelium, lu-

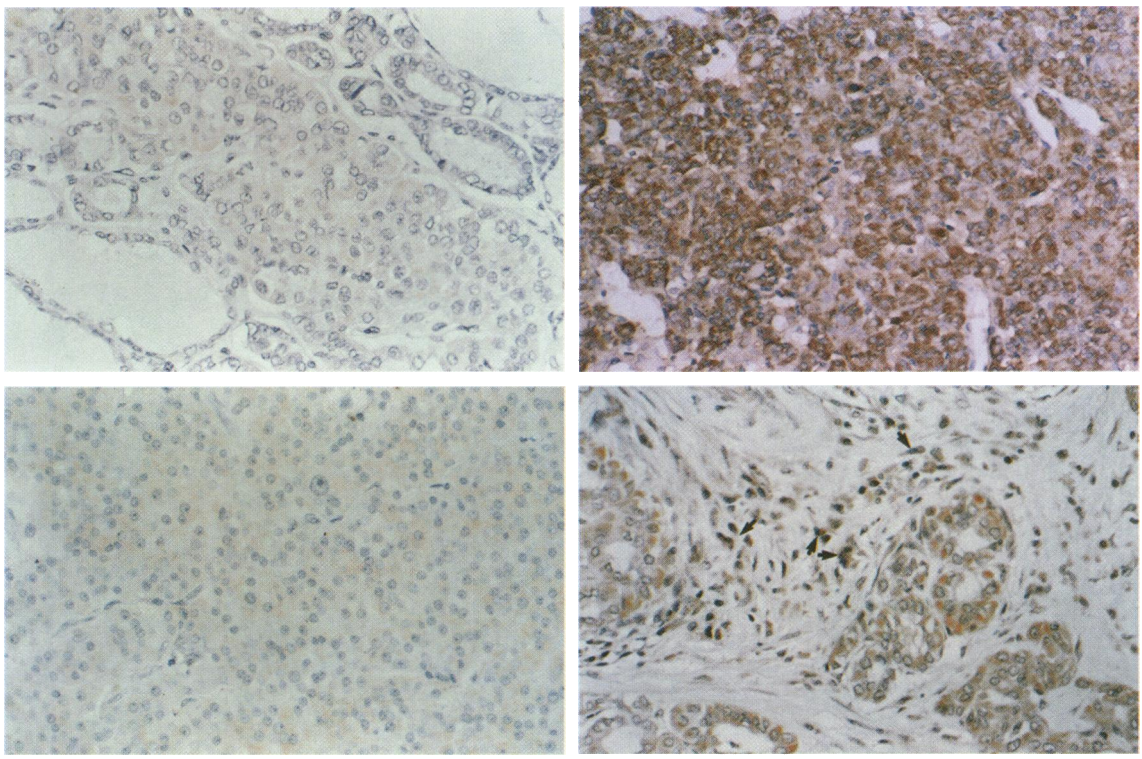

Figure 6. *No specific staining can be seen in follicular cells of a control thyroid section from the thyroid with papillary carcinoma of Figure 5 incubated with the affinity-negative IgG fraction (magnification, ×1851).*
Figure 7. *Immunobistochemical localization of IrCRH in metaplastic oxyphilic cells (Hürthle cells) of a section from a Hürthle cell tumor (magnification, ×1225). Positive staining is revealed as brown color, whereas the light blue color of Meyer's hematoxylin stains nonspecifically all cell nuclei.*
Figure 8. *No specific staining can be seen in metaplastic oxyphilic cells (Hürthle cells) of a control thyroid section from the thyroid with H[<]-lite)rthle cell tumor of Figure 7 incubated with the affinity-negative IgG fraction (magnification, ×225).*
Figure 9. *Mononuclear cells on a thyroid section with IrCRH-positive papillary carcinoma (magnification, ×1851). Positive staining is revealed as brown color, whereas the light blue color of Meyer's hematoxylin stains nonspecifically all cell nuclei. The arrows indicate positively stained mononuclear cells.*

teinized cells, peripheral ganglia, and neurons of the frontal lobe,[40–42] also shown to contain IrCRH.[17,21,22,26,35,36] This unexplained association is quite interesting.

Second, thyroid CRH could be derived from immune accessory cells, such as monocytes/macrophages, and from lymphocytes participating in the inflammatory reaction in the thyroid lesions. CRH mRNA and peptide have been shown in peripheral blood mononuclear cells and in mouse spleen[23,43] as well as in inflammatory synovia from patients with rheumatoid arthritis and arthritic Lewis rats.[10,11] Also, CRH mRNA has been shown in human T lymphocytes.[24] Third, another potential source of thyroid CRH might be the terminals of postganglionic sympathetic neurons and/or sensory afferent fibers, which form a network ending near the follicular basement membrane.[44] Immunoreactive CRH is present at large concentrations in the ganglia of the sympathetic chain and the dorsal root ganglia, where the cell bodies of these neurons respectively reside.[25,26]

The presence of CRH in human thyroid lesions, juxtaposed to its absence in adjacent normal thyroid tissue, raises the question of local involvement of this peptide in thyroid disorders. A likely potential role can be envisioned by extrapolation from existing information. Thus, thyroid CRH might act as a proinflammatory cytokine during the inflammatory phenomena accompanying thyroid disorders. This action of CRH was recently established in experimentally induced subcutaneous chemical inflammation.[9] Immunoreactive CRH was localized in monocytes, tissue fibroblasts, and endothelial cells and pretreatment of the animals with neutralizing r/hCRH antisera led to a decrease of inflammation by approximately 50%. Peritumoral thyroiditis, moderate to extensive fibrosis, and a small number of IrCRH-positive mononuclear cells were observed in all IrCRH-positive neoplastic lesions (Figure 9). On the other hand, inflammation was absent or minimal in the IrCRH-negative thyroid lesions. Endothelial cells also showed IrCRH-positive staining in human thyroid lesions and, in addition,

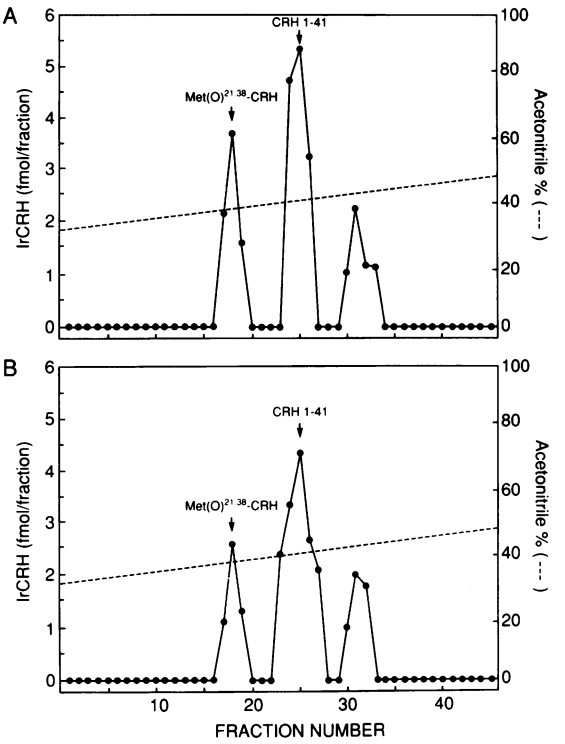

Figure 10. *Reverse phase HPLC characterization of IrCRH extracted from* (A) *a thyroid simple goiter, and* (B) *a thyroid papillary carcinoma. The amount of IrCRH in individual fractions was determined by RIA. Synthetic r/hCRH 1-41 and Met(O)^{21,38}-CRH were eluted from this column in fractions 25 and 18, respectively, as indicated.*

IrCRH was detected in smooth muscle cells of small blood vessels. It is possible that the type and intensity of the inflammatory reaction may affect the presence of CRH in these two types of vascular cells, since in previous reports and in this study, endothelial and smooth muscle vessel wall cells of noninflamed areas did not stain positively for CRH.

The immune cells involved in autoimmune disorders include not only the monocyte/macrophage system but also both members of the T and B lymphocyte series.[45,46] Both monocytes/macrophages and T cells produce CRH[23,24] and intense IrCRH staining was found in all cases of Hashimoto thyroiditis. In this condition, the histologic features include intrathyroidal infiltration with lymphocytes and plasma cells, whereas the follicular epithelium is frequently altered, with formation of reactive follicular centers, follicular atrophy, and varying degrees of fibrosis, and the normal epithelium is replaced by a metaplastic oxyphilic epithelium known as Hürthle cell change.

It is likely that all leukocytic infiltrations of the thyroid, whether seen in classic Hashimoto thyroiditis, nodular goiter, peritumorally, or in otherwise unremarkable thyroid glands, may represent mild forms of autoimmune disease, in which antithyroid antibody titers are very low or unmeasurable.[47] CRH and CRH mRNA have been localized in inflamed tissues of other autoimmune conditions, including the arthritic joints of patients with rheumatoid arthritis.[11] In addition, physiological levels of CRH have been shown to stimulate *in vitro* the migration of human monocytes.[48] Therefore, thyroid CRH may exert its inflammatory effects directly and/or indirectly via cytokines, because many of the CRH effects on human peripheral blood mononuclear cells are mediated by IL-1β and IL-6 secreted from monocytes/macrophages and/or other peripheral blood mononuclear cells.[49,50] Other cytokines secreted by immune/inflammatory and thyroid follicular cells could also mediate the effects of thyroid CRH.[51,52]

Recently the human CRH gene was shown to possess estrogen-responsive elements and to be activated by estradiol.[53] Interestingly, estrogen receptors are present immunohistochemically in both nonneoplastic and neoplastic thyroid follicular cells, regardless of a patient's hormonal status.[54–56] Because there is major female preponderance in the incidence of simple goiter, Hashimoto thyroiditis, and thyroid neoplasms, it is tempting to speculate that the estrogen environment might facilitate CRH expression and involvement of this neuropeptide in the autoimmune phenomena of the thyroid.

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