Immunoreactive Corticotropin-releasing Hormone and Its Binding Sites in the Rat Ovary

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Abstract

Corticotropin-releasing hormone (CRH), the principal neuropeptide regulator of pituitary ACTH secretion, is also produced at peripheral inflammatory sites, where it acts as a proinflammatory cytokine, and by the Leydig cell of the testis, where it exerts autocrine inhibition of testosterone biosynthesis. Because key ovarian functions, such as ovulation and luteolysis, represent aseptic inflammatory responses, and because the theca cell is the functional equivalent of the Leydig cell, we explored the CRH presence in the ovary, first, by specific CRH immunohistochemistry of adult cycling female Sprague-Dawley rat ovaries. We detected cytoplasmic immunoreactive CRH (IrCRH) in theca and stromal cells and in cells within the corpora lutea, at all phases of the estrous cycle. Using a specific radioimmunoassay, we measured IrCRH in extracts of rat ovaries (0.042–0.126 pmol/g wet tissue). The mobility of the ovarian IrCRH molecule was similar to that of rat/human CRH by reverse phase HPLC. To investigate the CRH action in the ovary, we identified, characterized, and localized CRH receptors in the rat ovary. Binding was linear with increasing tissue concentration, saturable, and of high affinity. Scatchard analysis of ^{125}I-Tyr-ovine CRH competitive displacement curves indicated a high affinity binding site with a K_d of $\approx$ 6 nM and a B_{max} value of $\approx$ 61 fM/mg protein. Autoradiographic studies revealed CRH receptors primarily in ovarian theca and stroma. We conclude that IrCRH and CRH receptors are present in rat ovaries, suggesting that this neuropeptide may play a regulatory role in this gonad, perhaps through its proinflammatory properties and/or by participating in the auto/paracrine regulation of steroid biosynthesis. Functional studies are necessary to define the role(s) of CRH in the ovary. (*J. Clin. Invest.* 1993. 92:961–968.) Key words: ovary • inflammation • corticotropin-releasing hormone • follicle • corpus luteum

Introduction

Corticotropin-releasing hormone (CRH),[1] a 41-amino acid neuropeptide, was originally isolated in the hypothalamus and named for its property to stimulate anterior pituitary secretion of ACTH (1–3). CRH and its receptors were subsequently identified in many extrahypothalamic sites of the central nervous system (4–7), as well as in several peripheral tissues, including active inflammatory sites (8–10) and the testes (11–14). In the central nervous system, CRH plays a major coordinative role for the stress response, including activation of the arousal and sympathetic systems and elicitation of stress-related behaviors (15–17). In inflammatory sites, CRH participates as an autocrine/paracrine proinflammatory regulator (8). Although the actual immune/inflammatory cell type that secretes CRH is not known, it is the immune accessory cells (macrophages, tissue fibroblasts, and endothelial cells) that contain immunoreactive CRH (IrCRH) (8–10). In the rat testis, CRH is produced by Leydig cells and appears to play an autocrine inhibitory role on testosterone secretion (13, 14).

Ovulation, luteolysis, and, perhaps, follicular atresia, three key ovarian functions, have characteristics of an aseptic/immune inflammatory reaction (18). In addition, resident macrophages constitute a major cellular component of the interstitial ovarian compartment (19–21) and macrophage-generated cytokines, such as interleukin 1, interleukin 6, and TNFα, have been demonstrated or suggested to participate in several ovarian functions, such as ovulation, luteinization, and steroidogenesis (22, 23). The theca cell in the ovary, on the other hand, has been proposed as the functional equivalent of the Leydig cell in the testis (24). The roles of CRH in inflammation and Leydig cell function suggested that CRH and its receptors might also be present in the rat ovary and might participate in inflammatory-like phenomena and/or steroidogenesis taking place in the female gonad. In this study we identified, quantitated, and localized CRH and its receptors in the ovaries of mature female Sprague-Dawley rats.

Methods

Animals

Adult (2–3 mo old), cycling, female Sprague-Dawley rats were obtained from Harlan-Sprague-Dawley (Indianapolis, IN). Animals were maintained on a 12-h light (lights on at 0600 h), 12-h dark cycle, with food and water available ad lib. Normal cycling was verified by daily vaginal smears. Animals were killed by CO_2 inhalation, after at least three normal cycles.

Materials

Synthetic rat/human (r/h) CRH 1-41 was obtained from Peninsula Laboratories Inc. (Belmont, CA) (25, 26); HPLC purified ^{125}I-r/hCRH and ^{125}I-Tyr-ovine(o) CRH from New England Nuclear Co. (Boston, MA); cyanogen bromide–activated Sepharose 4B and 3,3-diaminobenzidine tetra-hydrochloride 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); light green SF from Roboz Surgical Instrument Co. Inc. (Washington, DC); octadecylsilyl-silica (ODS-sil-

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1. *Abbreviations used in this paper:* CRH, corticotropin-releasing hormone; IrCRH, immunoreactive CRH; r/hCRH, rat/human CRH; oCRH, ovine CRH; TFA, trifluoroacetic acid.

ica) cartridges (C-18 Sep-Pak) from Waters Associates (Marlboro, MA); BCA protein assay kits from Pierce Chemical Co. (Rockford, IL); tritium-sensitive Ultrofilms from LKB (Rockville, MD). The HPLC LKB 2150 system used was purchased from Pharmacia LKB (Piscataway, NJ) and was coupled to a HiPore RP-318 column (C-18) (5 × 250 mm) obtained from Bio-Rad Co. (Richmond, CA).

Tissue preparation

The ovaries were dissected immediately after killing. Ovaries prepared for immunohistochemistry were fixed in 10% formalin and embedded in paraffin. Ovaries prepared for CRH extraction and radioimmunoassay, CRH binding assay, or autoradiographic mapping of ^{125}I-Tyr-oCRH-binding sites, were frozen on dry ice immediately and stored at −70°C until assay.

Immunohistochemistry

Peptide antisera production and purification. Polyclonal antiserum (TS-2) against r/hCRH was produced in rabbits as previously described (27). 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 h. The suspension was packed in a 4 × 0.7-cm column and washed with phosphate buffer (20 mM sodium phosphate, 0.5 M sodium chloride, pH 7.3) until the OD_{280} 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 M potassium thiocyanate, 0.5 M ammonium hydroxide) and dialyzed against several changes of cold PBS.

Immunohistochemistry. Tissue specimens were preserved in 10% formalin. They were embedded in paraffin and sectioned onto gelatin-coated microscope slides at a thickness of 6 μm. Immunoperoxidase staining was performed with the Vectastain ABC kit (Vector Laboratories), using the manufacturer's suggested protocol and reagents (28, 29). All subsequent procedures took place at room temperature. The sections were deparaffinized with two 5-min 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% peroxide in methanol for 45 min. The sections were then incubated sequentially with 0.1% BSA in PBS for 20 min and with diluted goat serum (1/66.7) for 20 min, and in a humid chamber with the affinity-purified rabbit antibody to r/hCRH (50 μg/ml), the control, affinity-negative IgG fraction (50 μg/ml) or nonimmune normal rabbit IgG (50 μg/ml). After 40 min, the sections were washed with PBS and incubated with biotinylated goat anti-rabbit IgG for 30 min. The sections were further washed with PBS and incubated with avidin and a biotinylated horseradish peroxidase complex for 45 min. Finally, the sections were washed and color was developed by immersing sections in a solution of 0.05% wt/vol 3,3-diaminobenzidine tetra-hydrochloride, 0.04% wt/vol nickel chloride, and 0.01% hydrogen peroxide in 0.05 M Tris, pH 7.4, for 2 min. The sections were counterstained with 0.5% light green SF. Positive staining was revealed as black-dark green spots, whereas light green color was seen in r/hCRH-free areas, affinity-negative control sections, and nonimmune normal rabbit IgG control sections. A different staining was also obtained by using the DAB solution without nickel chloride, and Meyer's hematoxyline as nonspecific counterstaining of cell nuclei. In this case, positive staining was revealed as brown color, and nonspecific staining of all cell nuclei as light blue color.

Radioimmunoassay

Ovarian concentrations of CRH were measured by radioimmunoassay after acid extraction. Briefly, 10 vol of boiling 2 M acetic acid was added to whole ovaries and the mixture was incubated at 95°C for 10 min. Samples were then sonicated on ice for 1 min and centrifuged at 15,000 *g* for 30 min. 3 vol of acetone was added to the supernatants, and they were again centrifuged at 15,000 *g* for 30 min. The supernatants were collected, lyophilized, and reconstituted in radioimmunoassay buffer for measurement, as previously described (30). The CRH antiserum (TS-2) used has been characterized in detail previously (27). ^{125}I-r/h CRH 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/liter, respectively.

Reverse phase HPLC

The samples obtained from acid-extraction of the rat ovaries were reconstituted in 200 μl of 0.1% trifluoroacetic acid (TFA). The sample was then passed through an ODS-silica cartridge (C-18 Sep-Pak; Waters Associates), and the cartridge was washed with 0.1% TFA, and subsequently by 20% acetonitrile containing 0.1% TFA. The peptides were then 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 column 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/min, was applied over 45 min. 1-ml samples of the eluate were collected in an automatic collector, lyophilized to dryness, reconstituted with radioimmunoassay buffer, and assayed for CRH content, as described above. A standard of 1 μg of synthetic r/hCRH was injected after each experiment to determine its chromatography profile.

CRH binding assay

The affinity and concentration of CRH binding sites were determined from competitive displacement binding experiments, in which rat ovarian tissue homogenates and ^{125}I-Tyr-oCRH were incubated with increasing concentrations of unlabeled synthetic r/hCRH. Both ovaries of the same animal were used to produce each displacement curve. At the time of the assay ovaries were weighed and subsequently homogenized in 20 vol of ice-cold homogenization buffer (50 mM Tris-HCl, 10 mM $MgCl_2$, and 2 mM EGTA, pH 7.2) using a Polytron (Brinkmann Instruments, Westbury, NY). The homogenate was then centrifuged at 38,000 *g* for 10 min, the supernatants were discarded, and the pellets were resuspended to a final concentration of 40 mg tissue wet wt/ml in homogenization buffer. 100 μl of the membrane preparation were then added to a 1.5-ml polypropylene microtube containing 100 μl of an r/hCRH solution (12 concentrations ranging from 0 to 1 μM) and 100 μl of ^{125}I-Tyr-oCRH (0.2 nM final concentration) in incubation buffer (50 mM Tris-HCl, 10 mM $MgCl_2$, 2 mM EGTA, 0.15% BSA, 100 kallikrein inhibitor units/ml aprotinin, 0.15 mM bacitracin, pH 7.2). The reaction was allowed to proceed, at room temperature, for 2 h. The membranes were then separated from the incubation medium by centrifugation in a microfuge at 12,000 *g* for 8 min. The supernatant was aspirated, the pellets washed gently with 1 ml of PBS containing 0.01% Triton X-100, and the radioactivity of the pellet was measured in a γ counter. The final protein concentration was determined using the BCA protein assay.

Autoradiographic mapping of ^{125}I-Tyr-oCRH-binding sites

Slide-mounted tissue sections from ovaries were incubated at room temperature with 0.1 nM ^{125}I-Tyr-oCRH in incubation buffer (50 mM Tris-HCl, 10 mM $MgCl_2$, 2 mM EGTA, 0.15% BSA, 100 kallikrein inhibitor units/ml aprotinin, 0.15 mM bacitracin, pH 7.2) for 2 h. To assess nonspecific binding parallel sections were also incubated in the same solution of ^{125}I-Tyr-oCRH in the presence of 1 μM r/hCRH. After incubation, tissue sections were washed in PBS containing 0.01% Triton X-100 at 4°C for two consecutive 5-min periods, dipped in a deionized water rinse, and dried rapidly, under a stream of cold dry air. Tritium-sensitive Ultrofilm was apposed to the tissue sections. After 4–5 d of exposure, the autoradiograms were developed, and the tissue was stained with thionin.

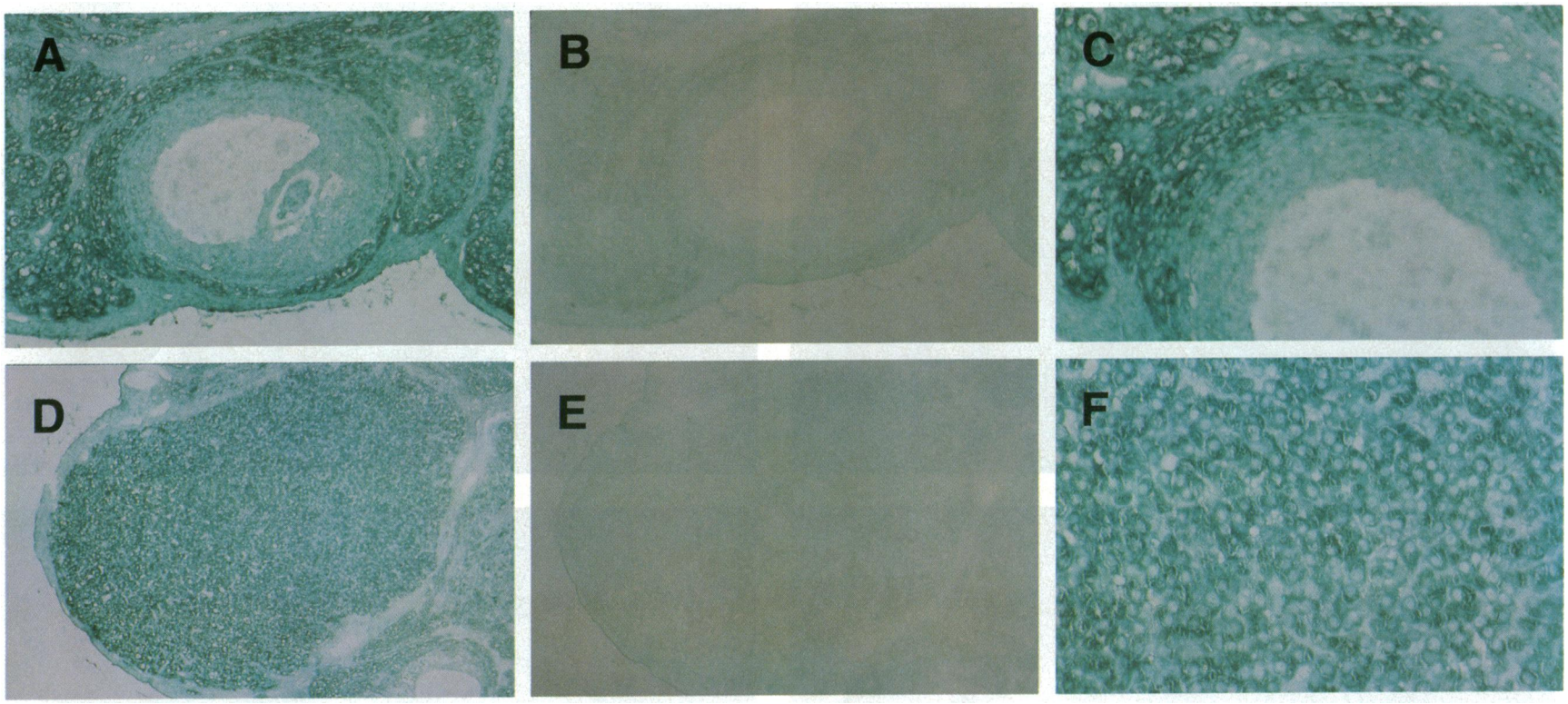

Figure 1. Immunohistochemical localization of IrCRH in the rat ovary. Positive staining is revealed as black-dark green spots, whereas light green color is used to reveal tissue architecture. (*A*) Positively stained theca and stromal cells around a follicle (×50). (*B*) No specific staining can be seen on a control adjacent tissue section incubated with the affinity-negative IgG fraction. (*C*) Positively stained theca cells on a higher magnification (×100). A small amount of CRH immunostaining is also seen in some granulosa and surface epithelial cells. (*D*) Positively stained cells of a corpus luteum (×25). (*E*) No specific staining can be seen on the same corpus luteum on a control adjacent tissue section incubated with the affinity-negative IgG fraction. (*F*) Positively stained cells of a corpus luteum on a higher magnification (×100).

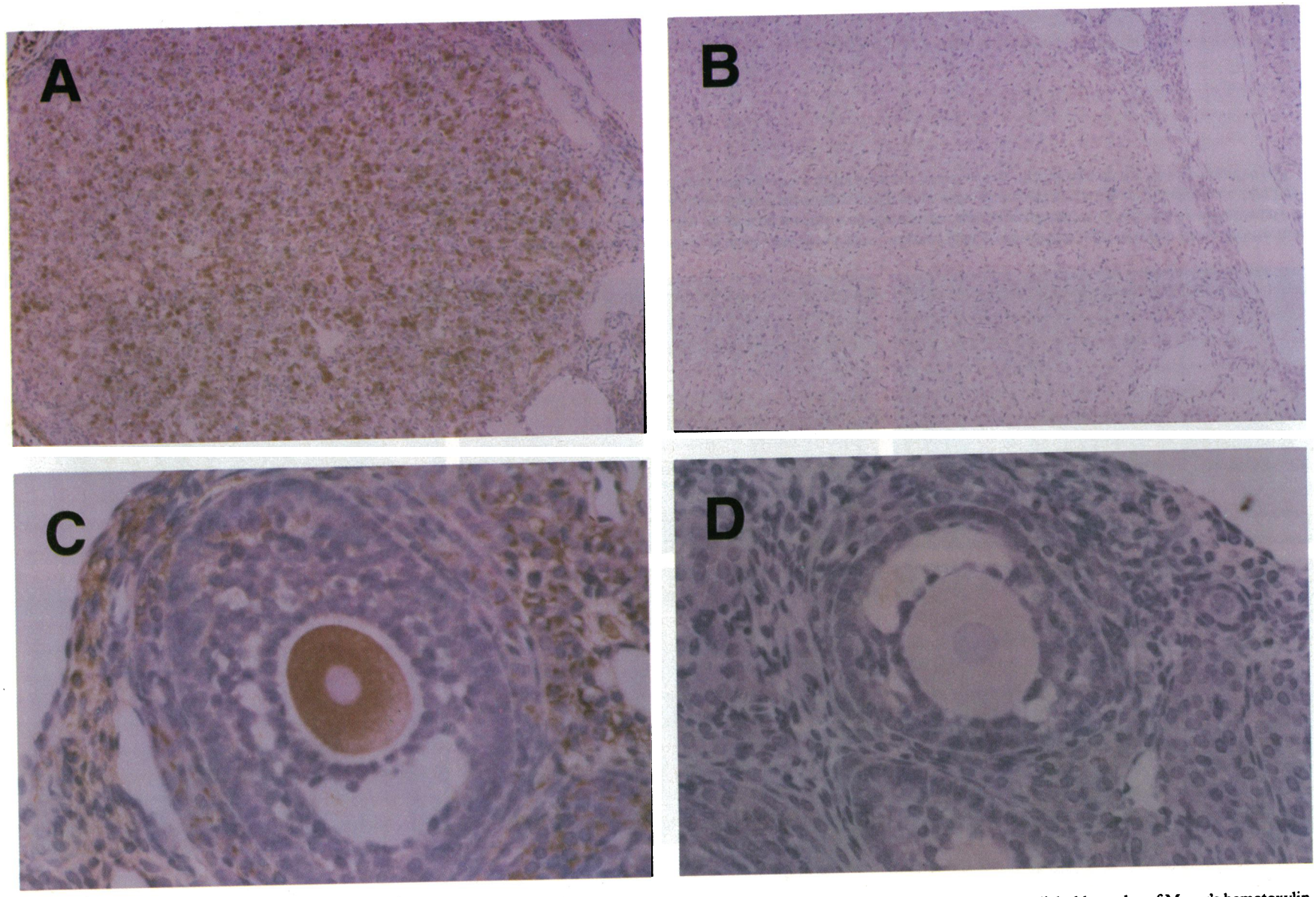

Figure 2. Immunohistochemical localization of IrCRH in rat ovary using alternative staining. Positive staining is revealed as brown color whereas the light blue color of Meyer's hematoxylin stains nonspecifically all cell nuclei. (*A*) Positively stained cells of a corpus luteum (×33). (*B*) No specific staining can be seen on a control adjacent tissue section of the same corpus luteum incubated with the affinity-negative IgG fraction. (*C*) Positively stained cytoplasm of an intrafollicular oocyte (×66). In this panel a small amount of CRH immunostaining is also seen in some granulosa and surface epithelial cells. (*D*) No specific staining can be seen in a control intrafollicular oocyte incubated with the affinity-negative IgG fraction.

Data analyses

^{125}I-Tyr-oCRH binding data were analyzed by the Macintosh-based nonlinear least squares curve fitting computer program LIGAND (31).

Results

Immunohistochemical detection of IrCRH in the rat ovary. The presence of IrCRH in the ovaries of adult, cycling Sprague-Dawley rats was examined by immunohistochemistry of sections obtained at proestrous, estrous, metestrous, and diestrous. IrCRH was detected in the cytoplasm of theca and stromal cells, regardless of size or maturity of ovarian follicles (Fig. 1, *A* and *C*), as well as in the oocytes of antral follicles (Fig. 2 *C*) but not the primary oocytes of primordial follicles (data not shown). A small amount of CRH immunostaining was also seen in some granulosa and surface epithelial cells (Figs. 1 *C* and 2 *C*). IrCRH was also detected in the cytoplasm of a subpopulation of cells within the corpora lutea (Fig. 1, *D* and *F*, and 2 *A*). These cells were also found through all stages of corpora lutea maturation and at all phases of the estrous cycle. Since the ≈ 14-d half-life of a rat corpus luteum is longer than the duration of the 4–5-d rat estrous cycle, corpora lutea of different maturation stages were found in the rat ovaries at all phases of the estrous cycle. The IrCRH-containing cells of the corpora lutea, however, were localized progressively from the periphery to the center with advancing maturation and there was less staining for IrCRH in older corpora lutea compared to younger. The maturation/aging of the corpora lutea was based on morphological criteria, including the progressively increasing presence of fibrotic tissue with advancing age of the corpus luteum.

IrCRH measurements and HPLC mobility in rat ovarian extracts. Total ovarian IrCRH content was measured in extracts of rat ovaries at all phases of the estrous cycle. The amount varied between 0.042 and 0.126 pmol/g of wet tissue. No significant differences in the CRH content were found among ovaries obtained at the different phases of the cycle. Ovarian IrCRH had similar chromatographic mobility to r/hCRH 1-41, the form produced by the rat and human hypothalamus and the human placenta (Fig. 3). There were also immunoreactive bands with higher mobility than r/hCRH 1-41, indicating the presence of CRH-degradation products and/or aggregates/precursors with different hydrophobicity. To examine whether the ovarian CRH levels reflected or were reflected by circulating CRH levels, we measured plasma concentrations of IrCRH in the same animals. Plasma levels of IrCRH remained < 2.101 pmol/liter (10^{-12} M) throughout the estrous cycle.

CRH binding characteristics of rat ovaries. Specific binding of ^{125}I-Tyr-oCRH was measured in homogenates of rat ovaries using assay conditions identical to those described for membrane preparations from mouse pituitaries and spleens (32). Specific binding of radiolabeled oCRH was linear with increasing tissue concentrations. Competitive displacement curves of ^{125}I-Tyr-oCRH binding in ovarian homogenates incubated with increasing concentrations of r/hCRH demonstrated that the specific binding was saturable (Fig. 4). Scatchard analyses using the nonlinear curve fitting program LIGAND (31), revealed a single, high affinity binding site (Fig. 4, *inset*). The apparent K_d of ovarian CRH binding sites was 6.34±2.3 nM (mean±SEM, n = 13) and the receptor concentration (B_{max}) was 61.17±15.6 fmol/mg protein.

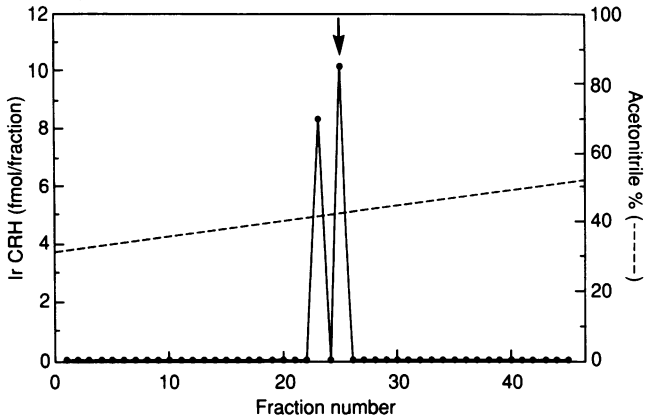

Figure 3. Reverse HPLC characterization of IrCRH extracted from rat ovaries. The amount of CRH in individual fractions was determined by RIA. Synthetic r/hCRH 1-41 eluted from this column in the fraction indicated by arrow.

Autoradiographic distribution of ^{125}I-Tyr-oCRH-binding sites in the rat ovary. The regional distribution of CRH receptors within the rat ovary was investigated using in vitro autoradiographic techniques. Autoradiograms on tritium-sensitive Ultrofilm (Fig. 5 *B*) were compared to photomicrographs of the same tissue section stained with thionin (Fig. 5 *A*). CRH receptors were diffusely distributed in ovarian theca and stroma areas surrounding follicles as well as on the cumulus oophorus (Fig. 5 *B*). There was a notable absence of specific binding in the granulosa areas while specific ^{125}I-Tyr-oCRH binding sites in the corpora lutea were sparse and not conclu-

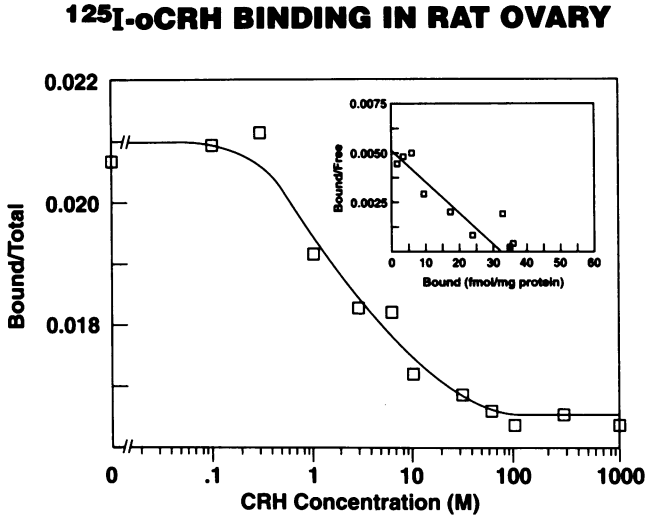

Figure 4. Competitive displacement binding of ^{125}I-Tyr-oCRH demonstrating specific binding sites in rat ovarian homogenates incubated in the presence of increasing concentrations of r/hCRH. Competitive displacement binding of ^{125}I-Tyr-oCRH demonstrates total binding as a function of increasing ligand concentration. The Scatchard plot (see inset) represents specific binding data (total nonspecific binding). Scatchard analysis of the displacement curves by the program LIGAND (31) revealed a single high affinity binding site. The data shown are from a representative experiment. The dissociation constant of 6.34±2.3 nM and the B_{max} of 61.17±15.6 fmol/mg protein (mean±SEM) were determined from the results of 13 independent experiments.

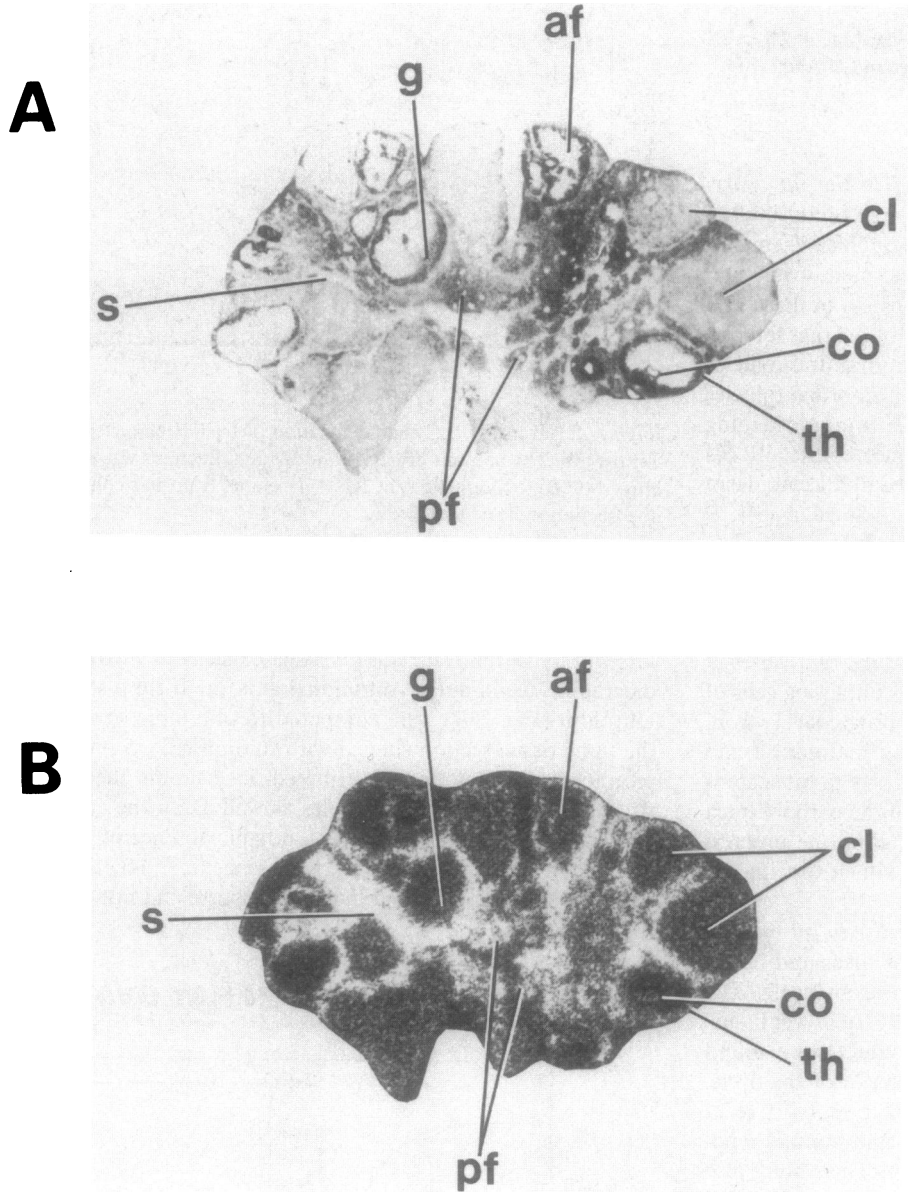

Figure 5. Autoradiographic localization of CRH receptors in the rat ovary. (*A*) Bright-field photomicrograph showing the histology of the section of rat ovary used to generate the autoradiogram shown in *B*. After exposure to Ultrofilm as described in Methods, the tissue was stained with thionin. (*B*) Direct print of the autoradiogram generated on tritium-sensitive Ultrofilm, which shows the distribution of autoradiographic grains (*white spots*) in the section of ovary shown in *A*. (*C*) Nonspecific binding determined in the presence of 1 mM r/hCRH was uniform. Proestrous ovary sections are shown here. Ovaries at all phases of the estrous cycle were examined, however. *s*, stroma; *g*, granulosa; *af*, atretic follicle; *cl*, corpus luteum; *co*, cumulus oophorus; *th*, theca; *pf*, primordial follicles.

sively seen. Binding in the presence of 1 μM r/hCRH was low and uniformly distributed (Fig. 5 *C*).

Discussion

We have demonstrated that IrCRH is localized in the normal rat ovary. Ovarian IrCRH has similar chromatographic mobility to that of r/hCRH 1-41, the form produced by the rat and human hypothalamus, rat and human peripheral inflammatory sites, rat Leydig cells, and human placenta (30, 33, 26, 34, 8, 9, 10, 13, 35). The concentrations of IrCRH in the rat ovary (0.042–0.126 pmol/g of wet tissue) are similar to those found in the extracts from rat inflammatory tissues (0.084–0.105 pmol/g of wet tissue) (8, 9) or human arthritic joints (10), but are lower than those in the rat testis (10.719–15.554 pmol/g of wet tissue) (11). Ovarian CRH could be derived from several sources. First, it might be produced by immune accessory cells normally residing in the ovary, such as macrophages, tissue fibroblasts, and endothelial cells (19–21). CRH mRNA and peptide have been shown in peripheral blood mononuclear cells (36) and in spleen and inflammatory synovia from arthritic Lewis rats (9), whereas the presence of a large number of extravascular macrophages has been well established in the ovarian stroma and corpora lutea (19–21, 37). These macrophages constitute a major cellular component of the interstitial ovarian compartment and are mostly concentrated near perifollicular capillaries (19–21). Second, another potential source of ovarian CRH might be the terminals of postganglionic sympathetic and/or unmyelinated sensory neurons type C and Aδ. IrCRH is present at large concentrations in both the sympathetic chain and the dorsal root ganglia, where the cell bodies of these neurons reside (38, 39) and is depleted in the rat spinal cord and dorsal root ganglia in response to capsaicin, which is toxic to sensory afferent fibers (40). Third, we must consider that theca and stroma cells, luteinized cells in the corpora lutea, and mature oocytes, all of which contained IrCRH, might also themselves produce this peptide. The plasma is an unlikely source of ovarian CRH, since the levels of this peptide in rat circulation are extremely low (8).

In the present study, we also identified specific, high affinity CRH receptors in homogenates of rat ovaries. ^{125}I-Tyr-oCRH binding was saturable and increased linearly with increasing protein concentration. Scatchard analysis of competitive displacement curves revealed a high affinity receptor for CRH in rat ovary (K_d = 6.34 nM), corresponding to the high affinity receptor previously identified in rat pituitary, hypothalamus, brain (6, 7), Leydig cells (14) and arthritic synovial tissues (9), and mouse spleen (32). The binding capacity of the rat ovary homogenates (B_{max} = 61.17 fmol/mg protein) was in the same order of magnitude as that of rat brain cortex homogenates (B_{max} = 62.19 fmol/mg protein) (32, 6), one-tenth of that shown in pituitary gland (41, 42), and 6 times and 20 times higher than that shown in mouse spleen (B_{max} = 8.74 fmol/mg protein) (32) and in rat Leydig cells (B_{max} = 2.6 fmol/mg protein) (14), respectively. We examined the distribution of ^{125}I-Tyr-oCRH binding sites by in vitro autoradiography to determine whether CRH receptors are expressed by specific cell types within the ovary. The receptors were primarily found in stroma and theca cells surrounding follicles, regions known to contain macrophages and other immune accessory cells (19–21), but also in the cumulus oophorus, which might indicate interactions between the IrCRH-containing mature oocyte and cells of the cumulus. There were sparse, dispersed CRH receptors in the corpora lutea, which might also be attributed to presence of resident immune accessory cells in these areas (20, 21, 37). Neither in the theca/stroma nor in the corpora lutea can we exclude the presence of receptors for CRH on the actual steroidogenic cells, however.

The presence of CRH and its receptors in the rat ovary raises the question of local involvement of this peptide in ovarian physiology. Two potential roles can be envisioned by extrapolation from existing information. First, ovarian CRH might act as a proinflammatory cytokine during the inflammatory phenomena of the ovarian physiology, which include ovulation, luteolysis, and, perhaps, follicular atresia. Theca CRH might participate in the actual process of ovulation and in the vascularization of the granulosa during formation of the corpus luteum, when eosinophils and T lymphocytes chemoattracted into the follicle produce lymphokines that attract and activate monocytes/macrophages (43). During luteolysis, phagocytically active macrophages appear to be involved in digestion of luteal cells (19, 44). The general presence of CRH in peripheral blood mononuclear leucocytes (36) and other inflammatory cells, and its proinflammatory properties (8–10) suggest that the peptide of the corpora lutea might be involved in the immune aspects of luteolysis. Ovarian CRH might exert its inflammatory actions directly and/or indirectly via cytokines, since many of the effects of this neuropeptide on peripheral blood mononuclear cells are mediated by IL-1β (45). The second role that might be ascribed to ovarian CRH is its participation in ovarian steroid hormone biosynthesis, in analogy to its effect on testosterone biosynthesis by Leydig cells (13, 14). Again, this effect might be direct or mediated by local secretion of cytokines and/or lipid mediators of inflammation, known to influence hormonogenesis. Indeed, IL-1 mediates inhibition of hCG-stimulated androsterone accumulation by cultured whole rat ovarian dispersates and might, thus, be involved in the actions of CRH (46). Further studies are needed to define the biological role(s) of ovarian CRH.

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