Neuroendocrinology 39: 93-95 (1984)

# Decreased Corticotropin-Releasing Factor-Like Immunoreactivity in Rat Intermediate and Posterior Pituitary after Stalk Section

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Key Words. Corticotropin-releasing factor · Pituitary gland · Intermediate pituitary · Posterior pituitary · Pituitary stalk section

Abstract. We determined the corticotropin-releasing factor(CRF)-like immunoreactivity by radioimmunoassay in intermediate and posterior pituitary lobes of stalk-sectioned and sham-operated control rats. The antigenic determinant read by the CRF antibody used was contained within the region of amino acids 26 (Gln) to 37 (Leu) of the molecule. Intermediate and posterior lobes of control rats contained similar amounts of CRF (591  $\pm$  78 and 487  $\pm$  34 pg/mg protein, respectively). The section of the pituitary stalk produced a marked decrease in CRF-like immunoreactivity in both structures. The CRF content on the intermediate lobe after stalk section was 62  $\pm$  17 pg/mg protein, a decrease of 90%, and that of the posterior lobe was 90  $\pm$  13 pg/mg protein, an 83% decrease. Our results suggest that most of the CRF-like immunoreactivity in the rat intermediate and posterior pituitary lobes is contained in nerve fibers of brain origin and support the hypothesis of a role of CRF in the release of intermediate and posterior lobe peptides.

Hypothalamic corticotropin-releasing factor (CRF) potently stimulates adrenocorticotropic hormone (ACTH) secretion from the rat anterior pituitary in vivo [8]. CRF fibers have been recently detected by immunocytochemical techniques in the pituitary stalk and the posterior pituitary [2, 3, 5]. CRF has also been shown to affect the release of intermediate lobe peptides [6]. These observations suggested that CRF could also play a role in the regulation of posterior and intermediate pituitary function. We studied the CRF-like content of the rat intermediate and posterior pituitary gland by radioimmunoassay, after a complete transection of the pituitary stalk which results in the section of all pituitary nerve fibers of brain origin. We attempted to determine what portion, if any, of the pituitary CRF was contained in nerve terminals of brain origin.

Received: February 1, 1984 Accepted after revision: April 11, 1984

#### Materials and Methods

Pituitary stalk section was performed via the parapharyngeal route under ether anesthesia in male Sprague Dawley rats, weighing 280–300 g. Sham-operated rats were similarly anesthetized, parapharyngeal incisions were made, but the pituitary stalk was not sectioned. Completeness of the stalk section was verified by microscopic visualization and by determination of the protein concentration in the posterior pituitary (see below).

Rats were killed by decapitation at 09.00 a.m., I week after surgery, and the posterior, intermediate, and anterior lobes were dissected immediately at 4 °C under stereomicroscopic control [7]. Tissues from single rats were homogenized by sonication at 4 °C in 250  $\mu$ I of 0.2 N hydrochloric acid, and aliquots of the homogenate were removed for protein determination [4].

CRF-like immunoreactivity was measured in duplicate determinations of two tissue dilutions from single rats, using a CRF antiscrum against ovine CRF (231-0607). The antigenic determinant read by the CRF antibody was contained within the region of amino acids 26 (Gln) to 37 (Leu) of the molecule [3]. Homogenates were centrifuged at 25,000 g for 30 min in a refrigerated centrifuge, and the supernatants were lyophilized. The residue was resuspended in 50 mM sodium phosphate buffer, pH 7.35, containing 100 mM NaCl, 25 mM EDTA, 0.1% Triton X-100, and 0.1% bovine

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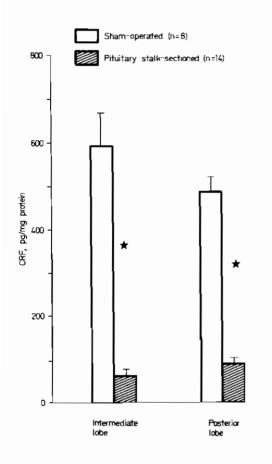


Fig. 1. Concentration of corticotropin-releasing factor-like immunoreactivity in rat intermediate and posterior pituitary lobes after stalk section. Values represent mean  $\pm$  SEM of duplicate determinations from two tissue dilutions taken from individual animals. \* p < 0.01, Student's t test.

serum albumin. Aliquots of the supernatant (100  $\mu$ l) were incubated at 4 °C in the presence of 100  $\mu$ l of CRF antibody (final dilution 1:6,000) for 24 h. Subsequently, 100  $\mu$ l of <sup>123</sup>I-Tyr-CRF (ovine, 8,000 cpm) was added and the samples were incubated for 24 h at 4 °C. The antigen-bound antibody was separated from the free antigen by addition of 100  $\mu$ l of normal rabbit serum (1/100), 100  $\mu$ l of anti-rabbit  $\lambda$ -globulin from goat (1/10), and 1 ml of 6% polyethylene glycol 8000, at 0 °C. In a typical assay, ID<sub>50</sub> was 25 pg. Average of nonspecific binding was 1%. 4–6 pg could be reliably detected. Serial dilutions of pituitary tissue extracts produced parallel curves to ovine antigen standard [*Dray* et al., in preparation].

### Results

The intermediate and posterior lobes of sham-operated rats contained similar total amounts of CRF-like immunoreactivity (65.7  $\pm$  6.9 and 52  $\pm$  6.0 pg per lobe, respectively). Total transection of the pituitary stalk resulted in very marked decreases in CRF-like immunoreactivity, 7 days after the lesion. These changes were evident either when expressed as total tissue content (7.3  $\pm$  0.8 pg, an 89% decrease, in the intermediate lobe, and  $6.9 \pm 0.5$  pg, an 87%decrease, in the posterior lobe) or when expressed as peptide concentration per milligram of protein (fig. 1). Protein content did not change after stalk section in the intermediate lobe (from 111.2  $\pm$  8.1 to 117.2  $\pm$  6.1 µg), but decreased by 29% in the posterior lobe (from 107.6  $\pm$  4.4 to 76.9  $\pm$  7.0  $\mu$ g, p < 0.01, Student's t test). No CRF-like immunoreactivity could be detected in the anterior lobe of sham-operated or pituitary stalk-sectioned rats.

## Discussion

Our findings indicate that most of the intermediate and posterior pituitary CRF-like immunoreactivity was localized in nerve fibers of brain origin. Earlier studies [1] indicated the presence of peptidergic nerves in the posterior pituitary. CRF-positive immunoreactive fibers have been recently detected in the pituitary stalk and in the posterior pituitary [2, 3, 5]. These observations, together with ours, indicate that CRF could have a role in the regulation of posterior pituitary function.

On the contrary, no CRF-positive nerve terminals could be detected in the intermediate lobe by immunohistochemical techniques [3]. However, CRF stimulates the release of peptides from the intermediate lobe of the rat pituitary [6]. Our findings indicate that CRF-like material is indeed present in the rat intermediate pituitary lobe, and that most of the peptide is probably located in nerve fibers of brain origin. These observations support the hypothesis of a role of CRF-containing nerves in the regulation of intermediate lobe function.

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