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PUBLICATIONS

Distribution of β-Endorphin Immunoreactivity in Normal Human Pituitary

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ABSTRACT Recent immunohistochemical demonstration of calcitonin in rat pituitary has suggested that calcitonin, in addition to ACTH, endorphins, lipotropins, and melanocyte-stimulating hormones might be derived from a 31,000-dalton glycoprotein percursor molecule. This immunoperoxidase study demonstrates a similar distribution for β-endorphin and ACTH immunoreactivity in human pituitary; however, the two peptides are not necessarily present in the same cells at all times. Calcitonin could not be demonstrated in human pituitary under conditions suitable for demonstration of the peptide in thyroid C cells. Weakly positive immunostaining could be obtained only with much increase in antiserum concentration and length of incubation, and higher concentrations of calcitonin were needed to abolish staining in preabsorption studies. It thus appears that the immunoreactive calcitonin in human pituitary differs from that in thyroid C cells. Likewise, we could not demonstrate immunoreactive endorphin in any developmental stage of medullary thyroid carcinoma. Our study suggests that caution should be applied in considering a physiologic role for calcitonin in the pituitary and in postulating a common peptide origin for endorphin and calcitonin in humans.

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INTRODUCTION

Recent evidence indicates that the lipotrophic hormones (LPH),[1] α-LPH and β-LPH; the melanocyte-stimulating hormones (MSH), α-MSH and β-MSH; ACTH; and the peptides with opiate-like activity, enkephalins and endorphins, are derived from a large 31,000-dalton glycoprotein molecule (31 K precursor molecule) (1–6). Immunocytochemical studies have shown that in normal human pituitary glands, ACTH, α-MSH, and β-MSH are present within the same cells in the anterior and posterior lobes (7); in the rat pituitary, ACTH, β-LPH, α-endorphin, and β-endorphin are present within the same cells in the anterior and intermediate lobes (3). Guillemin et al. (2) have recently shown that in the rat, ACTH and β-endorphin are secreted concomitantly by the pituitary, and it has been suggested that in the human too, the enkephalin-endorphin peptides and ACTH might be secreted in parallel under both physiological and pathological conditions (6).

More recently, Deftos et al. (8) demonstrated immunoreactive calcitonin within the intermediate and anterior lobes of the rat pituitary gland. This finding suggests that calcitonin might also be part of the 31K precursor molecule. To investigate this possibility further and to define the distribution of β-endorphin immunoreactivity in the normal human pituitary, we undertook to localize ACTH, β-endorphin, and calcitonin with immunoper-

[1] *Abbreviations used in this paper*: 31K precursor molecule, 31,000-dalton glycoprotein molecule; LPH, lipotrophic hormones; MSH, melanocyte-stimulating hormones.

oxidase techniques; we also studied the relationship of β-endorphin to the calcitonin-producing tumor, medullary thyroid carcinoma. The data define the distribution of β-endorphin immunoreactivity in the human pituitary and reveal that in this gland, calcitonin is not present in the same form in which it occurs in normal and abnormal thyroid C cells.

METHODS

Normal human pituitary glands were obtained from 11 patients at autopsy; two were children (3 mo and 3 yr of age) and nine were adults (22–78 yr of age). No patient had evidence of endocrine disease either clinically or at autopsy, and no patient received hormone therapy. All glands were obtained within 20 h of death, and in seven cases, within 12 h of death; the causes of death included acute myocardial infarction (1 patient), complex congenital cardiac malformations (1 patient), death during cardiac surgery (2 patients), liver failure (1 patient), septicemia (1 patient), and carcinoma (5 patients).

Immunohistochemical techniques. All pituitary glands were sectioned in the horizontal plane, fixed in 10% formalin, and routinely processed for histologic examination. Localization of ACTH, β-endorphin, and calcitonin was carried out on serial 5-micron sections with the immunoperoxidase technique as previously modified for calcitonin localization (9), with rabbit antisera to synthetic 1-24 ACTH (a gift from Dr. Paul Wolfe, Rochester University School of Medicine and Dentistry, Rochester, N. Y.), β-endorphin (Immunonuclear Corp., Stillwater, Minn.), and two rabbit antisera to human calcitonin (one prepared to synthetic human calcitonin by the technique of Vaitukaitis et al. (10) and previously used in our assay and immunoperoxidase studies (9), and the other a gift from Dr. Bernard A. Roos, Case Western Reserve University, Cleveland, Ohio). The β-endorphin antibody (RB 145A) used in this study has been shown to cross-react with β-endorphin.[2] Sections were incubated with the antisera for 1 h and for 24 h, and control studies included substitution of normal rabbit serum for the specific antisera and preabsorption of each antiserum with ACTH, β-endorphin, and synthetic human calcitonin. Absorption was carried out by taking each antiserum at the dilution used in the immunoperoxidase studies and adding each antigen at a final concentration of 1 μg/ml. The antisera were then incubated with the different peptides at 37°C for 1 h, after which they were kept at 4°C for 48 h. Control antisera were carried through the same procedure, but with antigen excluded.

Incubation studies with labeled peptides. To further assess the specificity of the antisera, each was incubated overnight, in the concentrations used in the immunohistochemical studies, with ^{125}I-labeled human calcitonin and similarly labeled human endorphin. The percentage of binding of peptide to antiserum was compared with normal rabbit serum, and 10,000 cpm of each labeled peptide was used for the studies. Only the calcitonin antiserum gave significant binding of the ^{125}I-calcitonin (78% binding compared with 2% for antiendorphin and 1% for anti-ACTH), and only the endorphin antibody gave significant binding for ^{125}I-endorphin (22% compared with 5% for anticalcitonin and 0% for anti-ACTH).

RESULTS

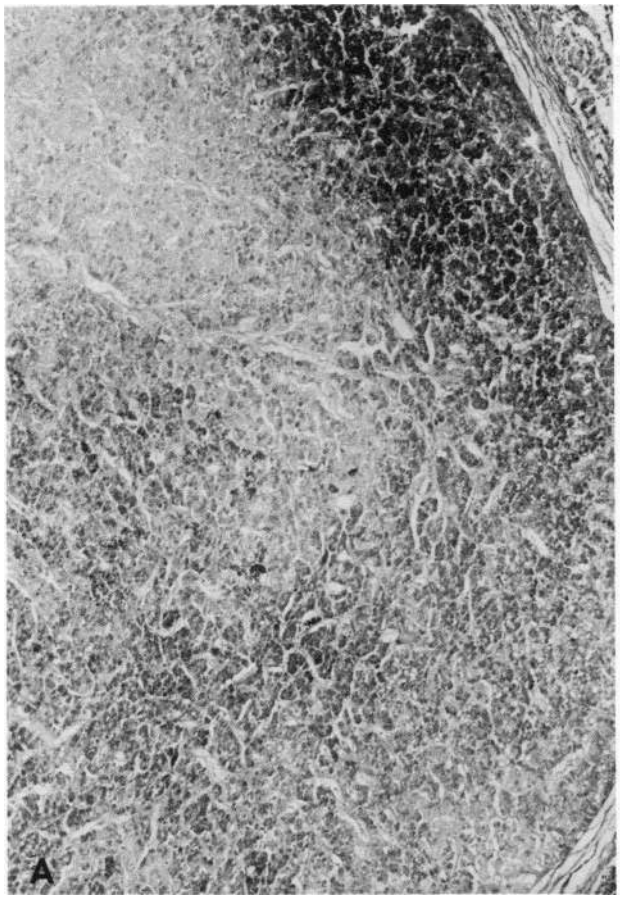
Our studies confirm the previously described distribution of ACTH within the anterior lobe of the normal human pituitary (7, 11). The majority of ACTH-containing cells are located within the anterior and medial portions of the anterior lobe (Fig. 1A) with scattered ACTH-positive cells in the lateral portions of the anterior lobe (acidophil wings). β-endorphin (or β-lipotropin) -containing cells are distributed primarily within the anterior lobe. They are most concentrated in the anterior rim of the anterior lobe (Figs. 1B and 2) and frequently around the follicles between the anterior and posterior lobes (Fig. 2). In one of the glands studied, ACTH and β-endorphin immunoreactivity were also present in cells of anterior pituitary incorporated within the posterior lobe (Fig. 2). Although, overall, the distribution of β-endorphin and ACTH was the same, in some instances there were more β-endorphin- than ACTH-reacting cells in the lateral acidophil wings (Fig. 1). β-endorphin and ACTH were not necessarily present in the same cells simultaneously; some ACTH-positive cells did not stain positively for β-endorphin in serial sections and, conversely, some β-endorphin-containing cells did not contain ACTH (Fig. 1). Neither the distribution nor the intensity of immunostaining was altered when sections were incubated with the ACTH and β-endorphin antisera for 24 h rather than for 1 h.

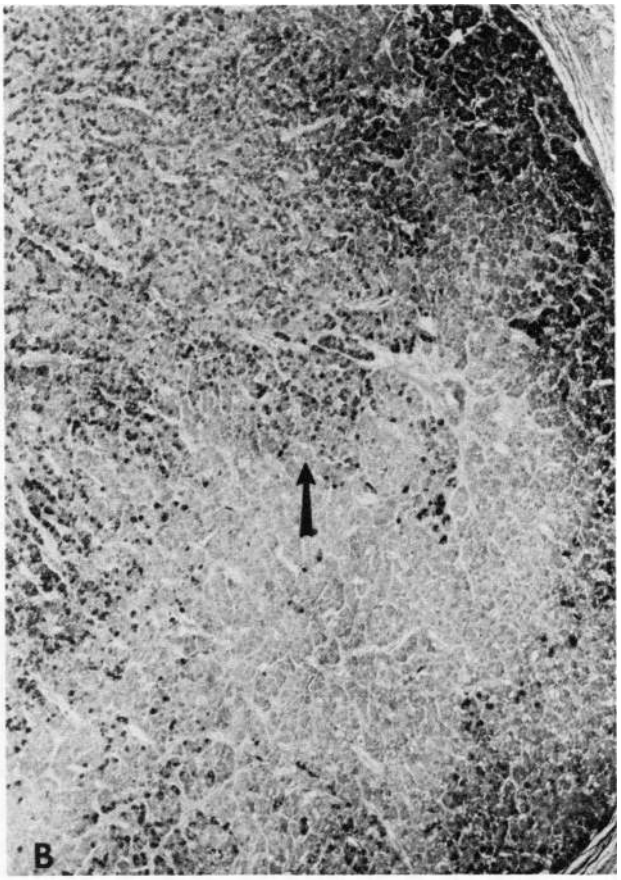
All developmental stages of medullary thyroid carcinoma used in our previous work (9) were studied for β-endorphin. We were not able to obtain positive immunostaining in thyroid glands with C-cell hyperplasia, microscopic or gross medullary carcinoma with either 1- or 24-h incubations.

With two different antisera to human calcitonin, we were unable to show positive staining when pituitary sections were incubated for 1 h. Both antisera gave strongly positive staining for calcitonin in thyroid glands with C-cell hyperplasia and medullary carcinoma under these conditions. When the pituitary sections were incubated for 24 h with higher concentrations of the antisera (1:20 rather than 1:100), weakly positive immunostaining was obtained. The distribution of this staining was similar to that of ACTH (Fig. 1C), it being most concentrated in the medial and anterior portions of the anterior lobe. Positive staining was present in many of the cells that contained ACTH.

Substitution of normal rabbit serum for the ACTH, β-endorphin, and calcitonin antisera resulted in a loss of positive immunostaining, which was similarly abolished when the ACTH and β-endorphin antisera were preabsorbed with their respective antigens. Preabsorption of the calcitonin antisera with synthetic human calcitonin at 0.001 and 0.01 mg/ml resulted in a loss of staining in control thyroid glands with C-cell hyperplasia and medullary carcinoma. However, only a higher concentration of calcitonin (0.5 mg/ml) in preabsorption studies abolished staining in the pituitary glands. Immunostaining was not altered when the antisera used were preabsorbed with the other two peptides, and no

[2] Personal communication on studies performed by Dr. B. A. Roos.





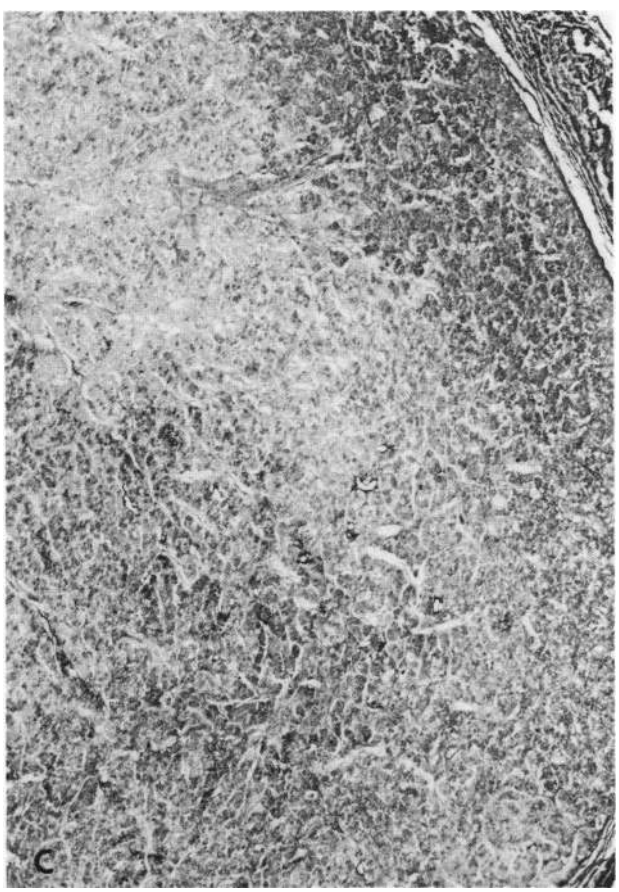


FIGURE 1 Comparative distribution of (A) ACTH, (B) β-endorphin, and (C) calcitonin immunoreactivity in the lateral portion of the anterior lobe of the normal human pituitary, with immunoperoxidase localization. Positive immunoreactivity is indicated by dark staining. Note similar distribution of ACTH (A) and calcitonin (C) and increased number of β-endorphin-containing cells in lateral acidophil wing (arrow, B). (Light green counterstain.) A, B, and C, ×60.

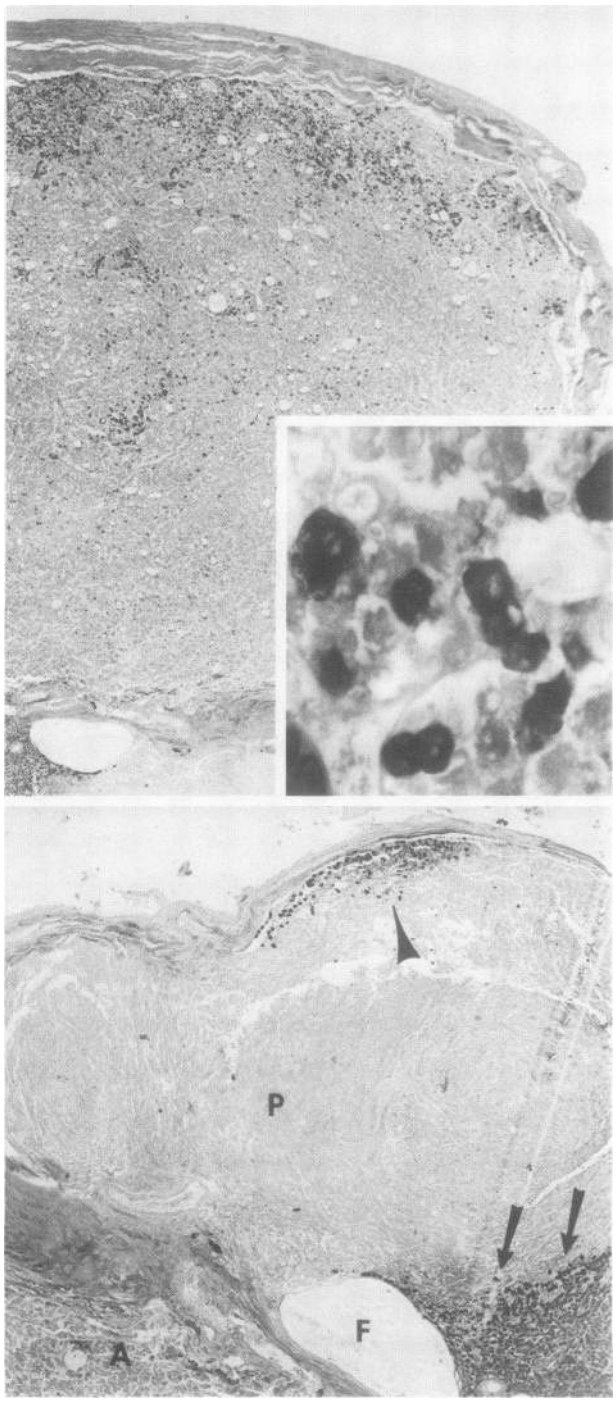


FIGURE 2 Distribution of β-endorphin immunoreactivity in normal human pituitary. (Top) Low-power photomicrograph demonstrating β-endorphin-reacting cells in anterolateral rim of anterior lobe with scattered cells in lateral acidophil wings. (Inset) High-power photomicrograph showing cytoplasmic staining in cells containing β-endorphin. (Bottom) Note concentration of β-endorphin-containing cells around follicles between anterior and posterior lobes (arrows), and at posterior rim of posterior lobe (arrowhead). These represent cells of the anterior lobe invading, or included within, the posterior lobe. P, posterior lobe; A, anterior lobe; F, follicles between anterior and posterior lobes. (Light green counterstain.) Top, ×27; Inset ×450; Bottom ×80.

differences in immunostaining could be related to either the age of the patients, the causes of death, or to the postmortem interval.

DISCUSSION

The comparative localization of ACTH and β-endorphin immunoreactivity within the normal human pituitary has implications for the relationship between these two peptides and their 31K precursor molecule (1–6). We have confirmed the previously described distribution of ACTH and have now localized β-endorphin immunoreactivity for the first stime in the normal human pituitary. Our data cannot settle the issue of whether native β-endorphin is actually stored in the pituitary (11); in a recent study, Liotta et al. (12) demonstrated that in the human and rat pituitary, immunoreactive β-endorphin was present predominantly as β-lipotropin, the presumed precursor for this molecule (13, 14). In common with other β-endorphin antisera (12, 15), the antibody used in our study recognizes both β-endorphin and β-lipotropin, and we therefore are looking at β-lipotropin and endorphin distribution simultaneously. Nevertheless, β-endorphin-reacting cells are present within the same region of the pituitary as those which contain ACTH, and most corticotropin cells contain both hormones. Our data suggest, however, that although both peptides may be derived from the same 31K precursor molecule, they are not necessarily stored or present in the same cells at any one time.

With regard to calcitonin immunoreactivity, we find somewhat different results in the human pituitary than did Deftos et al. (8) in rat pituitary. With two different antisera which stain calcitonin well in all stages of medullary carcinoma, we were unable to demonstrate positive immunostaining for calcitonin in normal human pituitary glands with the usual conditions we use for thyroid C cells. Only with much increase in the concentration of the calcitonin antisera and long incubation times was weakly positive immunoperoxidase staining obtained. Most importantly, preabsorption with low concentrations of synthetic calcitonin did not abolish staining.

The immunostaining we have obtained for calcitonin in human pituitary is thus significantly different from the staining for calcitonin which we routinely obtain in normal and abnormal thyroid C cells. In their recent immunohistochemical study in rat pituitary, Deftos et al. (8) indicated that positive staining for calcitonin was obtained with only 1 of 25 screened antisera against human calcitonin; the incubation conditions, including

time and antisera titers, under which positive immunostaining was obtained were not discussed. Thus it appears that a peptide having partial cross-reaction with calcitonin exists in the human pituitary; this peptide is either immunogenically different from the calcitonin normally present in thyroid C cells, or is stored such that its antigenic sites are not fully available for interaction with antibody. Whether calcitonin is present, but partially obscured within the 31K precursor molecule, or whether some other pituitary protein contains a peptide sequence which has immunogenic cross-reactivity with calcitonin is a matter for conjecture and further study at this stage. It is interesting, in this regard, that Xavier et al. (16) have recently presented evidence that in small cell carcinoma of the lung, calcitonin and endorphin have separate genetic determinants. Furthermore, our data showing that any calcitonin-like peptide present in the human pituitary differs either immunogenically or in its storage form from that in thyroid C cells stress that caution must be exercised in suggesting a biologic role for this peptide in the pituitary gland.

The relationship between β-endorphin in the normal human pituitary and any that may be present in thyroid C cells is also different. Our inability to stain this peptide in the thyroid suggests that either there is no cross-reactive sequence in C cells or again that the peptide is stored in such a form that antigenic sites on the molecule are not available for staining. Further studies of pituitary and medullary thyroid carcinoma with peptide cleavage analyses and immunoextraction techniques will be necessary before a direct relationship between β-endorphin molecule and calcitonin is established.

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