A COMPARISON OF ALCIAN BLUE-ALDEHYDE FUCHSIN AND PEROXIDASE-LABELED ANTIBODY STAINING TECHNIQUES IN THE RAT ADENOHYPOPHYSIS

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The peroxidase-labeled antibody (P-Ab) technique was compared on adjacent sections with a permanganate-Alcian Blue (AB)-aldehyde fuchsin (AF) procedure on the anterior pituitary gland of young adult, female rats. The cells that stained with both AB and AF (AB, AF+) were large and polygonal and frequently possessed long processes; these cells correspond to those which reacted with the TSH antibody. The cells that stained only with AF reacted with the FSH antibody in the P-Ab technique and the cells which reacted with the LH antibody were not stained with either AB or AF. AB, AF+ cells ("TSH" cells) were distributed throughout the anterior lobe except along the lateral and dorsal peripheries of the gland and adjacent to the intermediate lobe, while both the AF+ ("FSH" cells) and the "LH" cells were distributed throughout the anterior lobe.

Pituitary cytologists have tried to correlate specific adenohypophyseal cell types with particular hormonal secretions. This has been difficult until now because classical staining techniques may not be specific for a particular hormone, may stain only the storage form of the hormone or may not be sensitive enough. Although the problem of hormone specificity has been aided by the use of fluorescent-antibody techniques, such methods do not always provide a direct means of correlating fluorescent cells with cell types long identified by classical procedures. The advent of the peroxidase-labeled antibody method for identifying pituitary hormones in situ (7,8), however, incorporates not only the specificity of an antibody technique but also provides a means of comparison with classical procedures. Unlike the fluorescent-antibody technique, this method is used with a light rather than a fluorescent microscope and provides permanent microscopic slides. Thus, correlation of function with the structure previously established by classical stains is greatly facilitated.

MATERIALS AND METHODS

All pituitaries were taken from young adult, female Sprague-Dawley rats on the day of proestrus or estrus. The rats were decapitated and the pituitaries were immediately removed and placed in Bouin's solution at room temperature for either 5 or 24 hr. The glands were embedded in paraffin, cut at 4 μ and mounted.

Although Bouin's fixation is necessary for the peroxidase-labeled antibody (P-Ab) technique, it is not suitable for the more common classical procedures used to identify mucoid cells in the rat anterior pituitary, e.g., those using periodic Acid-Schiff staining. Consequently, a permanganate-Alcian Blue-aldehyde fuchsin (AB-AF) technique, which can be used on Bouin's-fixed tissues and which identifies both the thyrotrophic cells and at least one of the gonadotrophic cell types, was used here. Alternate sections were hydrated, oxidized for 1 min in a 1:1 solution of 0.2% KMnO₄ and 0.5% H₂SO₄ and reduced in 2% NaHSO₃. After a distilled water rinse, the slides were placed in 0.5% solution of Alcian Blue (AB) in 3% acetic acid (pH 2.7) for 30 min and rinsed in distilled water and 70% alcohol. The same sections were then stained for 2-3 min in aldehyde fuchsin (AF) (2) and rinsed at least three times in 70%

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2 Alcianblau SGS, Chroma-Gesellschaft, Schmid and Company, was obtained from Roboz Surgical Instrument Company, Inc., Washington, D.C. AF was made from basic fuchsin, Allied Chemicals, C.I. 42500.
alcohol. Routine dehydration and mounting followed.

The P-Ab technique (7-9), in which 3,3′-diaminobenzidine is used to demonstrate peroxidase activity, was applied to sections adjacent to those stained with the AB-AF technique. This method is based on the fact that an antibody in rabbit serum to a particular anterior pituitary hormone becomes attached to that hormone when applied to microscopic slides containing anterior pituitary sections. An anti-rabbit γ-globulin, to which a peroxidase is coupled, is then applied to these same sections and becomes attached to the antigen-antibody complex. The site of this reaction is then made visible by allowing the peroxidase to oxidize a substrate (in this case, 3,3′-diaminobenzidine) which leaves an insoluble, brown precipitate at the site of the reaction.

The specificity of such a procedure, however, depends on the purity of the antibodies. The antibodies used in this study (rabbit, anti-ovine luteinizing hormone (LH) and follicle-stimulating hormone (FSH) and rabbit, anti-human thyroid-stimulating hormone (TSH)) were absorbed with normal, male rat serum (1:1) and with rat liver powder (1) to reduce nonspecific, background staining. However, these antibodies were not absorbed with normal ovine or normal human serum or with other anterior pituitary hormones.

RESULTS

Two mucoid cell types were stained using the Alcian Blue-aldehyde fuchsin procedure (AB-AF). The first was stained with both AB and AF (AB,AF+) and was a deep blue in color. These large cells were usually ovoid or polygonal and frequently possessed long processes. AB,AF+ cells were uniformly scattered throughout the sections examined except along the dorsal and lateral peripheries and immediately adjacent to the intermediate lobe. The second cell type was stained only with AF (AF+) and appeared a very pale purple. These AF+ cells were smaller than AB,AF+ cells and were randomly distributed throughout each section examined.

The cells which reacted either with anti-LH or with anti-FSH were round or ovoid in shape. Both cell groups were scattered throughout the sections examined; i.e., they were not localized to either the center or periphery of the sections. Cells which reacted with anti-TSH, however, were infrequently seen along the lateral and dorsal surfaces of the gland or immediately adjacent to the pars intermedia. These polygonal cells were larger than either of the presumptive gonadotrophs and frequently possessed long processes.

The comparison of adjacent sections showed that cells identified as “FSH cells” in the P-Ab sections corresponded to the AF+ cells in the next section (compare Fig. 1 with Fig. 2). The cells identified as “TSH cells” with the P-Ab technique appeared to be the same as the AB,AF+ cells (compare Fig. 4 with Fig. 5) while cells identified with the LH antibody were not stained with either dye (compare Fig. 2 with Fig. 3).

DISCUSSION

The results of this study both corroborate and contradict the work of previous investigators. Aldehyde fuchsin, as used here, i.e., following oxidation with acidified KMnO4, stains both the presumptive TSH and FSH cells but not the presumptive LH cells. Although Herlant obtained similar results with AF following KMnO4 oxidation, several investigators report different findings. For example, according to some reports, only the thyrotrophs are stained with AF following oxidation either with Lugol’s solution (10, 14, 15) or performic acid (6). However, Halmi and Davies (4) stated that the affinity of the thyrotrophs for AF diminishes following permanganate oxidation while FSH cells become AF++; Purves (12) reported that AF after permanganate oxidation stains both the Halmi (3) β cell (thyrotrhop) and the Halmi (3) δ cell (gonadotroph).

Conflict also exists as to the specificity of Alcian Blue as a means of identifying a particular cell type in the anterior pituitary. Purves (11) stated that Alcian Blue is specific for thyrotrrophs in the rat. However, other investigators claimed that both the thyrotrrophs and the gonadotrophs stained with AB after oxidation either with buffered performic acid (18) or permanganate (5). In our own study only the “TSH cells” displayed any affinity for AB.

The localization of the mucoid cells within the adenohypophysis is another point of controversy. After injecting rats with testosterone propionate, which supposedly depletes the LH content of the pituitary, Purves and Griesbach (13, 16) found that the central gonadotrophs were degranulated while the peripheral gonadotrophs were unaffected by the treatment; they con-

4 The horseradish peroxidase was type II from Sigma Chemical Company, St. Louis, Mo., while the 3,3′-diaminobenzidine (free base) was purchased from K and K Laboratories, Inc., Plainview, N.Y.

5 Cells thought to be secreting FSH, TSH or LH are referred to as “FSH cells,” “TSH cells” or “LH cells,” respectively.
Figs. 1-3 show the same area from adjacent sections. ×700.
Fig. 1. The peroxidase-labeled antibody technique shows seven FSH cells situated around the periphery of a blood vessel (BV). Two of these cells are labeled F. Note that the FSH antibody identifies the same cells as does aldehyde fuchsin (AF) in Figure 2.
cluded, therefore, that the central gonadotrophs secreted LH while the peripheral gonadotrophs secreted FSH. In contrast, Rennels (17) and Hildebrand et al. (6) reported that FSH is secreted by the central gonadotrophs and LH by the peripheral gonadotrophs; their findings were based on the distribution and pituitary hormone content of the two gonadotrophs at various intervals after castration. In our study, however, both the peroxidase and AB-AF techniques showed no localization for either of the two gonadotrophs. Conversely, the thyrotrophic cells do not appear in a band along the intermediate lobe or along the lateral and dorsal borders of the anterior lobe. This distribution, obvious with both procedures used here, was suggested by other investigators (13, 17).

The specificity of any immunologic technique depends on the purity of the antibodies used. Such antibodies should be absorbed with sera from the animal species involved in preparing the antibody, with serum and/or tissue extract of the animal from which the histologic tissue was obtained and also with other pituitary hormones. Although only a portion of these absorptions were performed here, the agreement seen after comparing sections indicated that little contamination was present and that the "TSH cells" were the only cells showing an affinity for both AB and AF while the "FSH cells" were only AF+.

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REFERENCES


FIG. 2. The KMnO4-AB-AF technique demonstrates six AF+ cells, two of which are labeled with an arrow.

FIG. 3. The peroxidase technique shows four LH (L) cells which are not identical with the AF+ cells in Figure 2. The LH cell indicated by the arrow morphologically resembles the cell above the AF+ cell in a similar location in Figure 2.

Figs. 4 and 5 show the same area from adjacent sections, ×700.

FIG. 4. The peroxidase-labeled antibody technique illustrates eight cells identified by the TSH antibody (two are labeled "T").

FIG. 5. KMnO4-AB-AF technique shows a cluster of nine AB, AF+ cells (two are indicated with an arrow) which correspond to those cells stained in Figure 4.


