Immunohistological observations of gonadotrophs in the anterior pituitary gland of the rat

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PURVES and GRIEBACH ('52, '54, '55)8-10 described gonadotrophs in the rat anterior pituitary glands as "peripheral" gonadotrophs and "central" gonadotrophs. They concluded that central gonadotrophs correspond to LH producers. Their results were supported by the experimental observations of BARNETT et al. ('56)1.

On the other hand, RENNELS ('56, '57)11,12) described the distribution patterns of two tinctorially different types of gonadotrophs by use of the PAS-methyl blue procedure, and he designated these cells as PAS-red and PAS-purple gonadotrophs. According to his description and the experimental studies of Hildbrand, RENNELS and FINERTY ('57)5) the PAS-red cells were distributed in the peripheral area and corresponded to LH producers, while PAS-purple cells were located in the central area and corresponded to FSH producers. Additionally, Rennels' observations were supported by the work of HELLBRANUM et al.('61)3). However, at present, this controversy concerning the identification of FSH and LH gonadotrophs in the pituitary gland of the rat has not been completely resolved.

It was, therefore, of interest to investigate the localizations of endogenous FSH and LH in the anterior pituitary gland of the rat by means of the fluorescent antibody technique.

Materials and Methods

Antiseras were prepared by immunizing rabbits with an emulsion of 5 mg of purified ovine FSH (NIH-FSH-S4) and LH (NIH-LH-S11) in Freund's complete adjuvant (Difco Lab). These injections were given once each week for 4 weeks. A final booster injection given one week later consisted of 1 mg of hormone preparation suspended in 1 ml of physiological saline solution. The rabbits were bled by cardiac puncture 3 days after the booster injection. The titer of antibody was estimated by interfacial ring tests (SHINO and RENNELS, '66)16). The contaminating antibodies were estimated by means of the Ouchterlony agar diffusion test (1% agar in phosphate buffered saline solution, at pH 7.1).

Contaminating antibodies in each antiserum were removed by precipitation with measured amounts of normal ovine serum powder and by addition of 0.5-1.0 mg of each hormone preparation which represented contaminating hormones. Incubation was carried out with 10 ml of antiserum in a cold room over night. The incubated antisera were centrifuged for 20 min. at 3,000 rpm, and the supernatant was retained for use. Gamma globulin fractions were prepared from the absorbed antisera by KEKWICK'S procedure ('40)6), and after dialysis these preparations were stored in a deep freezer until use.
The pituitary glands were removed in the laboratory from several female normal and pregnant albino rats (3-19 days). The animals were obtained from Cheek-Jones Company of Houston, Texas. The glands were placed in isopentane suspended in a dry ice and acetone mixture. These frozen glands were lyophilized for a week in a freeze-dry apparatus and embedded in paraffin. Sections were cut in the horizontal plane at a thickness of 5 μ and sections from the midregion were spread, by using 70% alcohol, on glass slides which were coated with a little egg albumin-glycerine mixture.

The indirect fluorescent antibody procedure was used. After incubating the sections with the purified globulin preparations, the sections were thoroughly rinsed in phosphate-buffered saline and incubated with rabbit globulin antiglobulin (goat) conjugated with fluorescein isothiocyanate (Bact FA rabbit globulin antiglobulin, Difco Lab). The details of this procedure, including a description of the several types of control preparations, have been reported (Shino and Rennels '66)16). Our fluorescent microscope was a Carl Zeiss photomicroscope equipped with an immersion type darkfield condenser. Evaluation of the fluorochrome was made by using primary filters UG-2, 2 BG-12, BG-38 and barrier filter # 47 or 50.

Results

In the Ouchterlony agar diffusion test, at least four precipitin lines formed between FSH and its antiserum, and the same number of lines formed between this antiserum and the LH preparation. On the other hand, only two lines were formed between LH antiserum and LH. The same two lines (lines of identity) were formed between the LH antiserum and the purified preparations of FSH and TSH. These facts indicate that NIH-FSH-S4 and NIH-LH-S11 contained measurable amounts of contaminating hormones and that these hormonal components caused the production of antibodies.

By absorption procedure (utilizing the Dean-Webb's optimal proportion=equivalence) with equivalent LH (0.5–1.0 mg/10 ml antiserum) and TSH (0.5 mg/10 ml antiserum) preparations the precipitin lines between FSH antiserum and FSH were changed to one distinct line when agar at pH 6.8 was used (Fig. 3). However, on some occasions when agar at pH 7.4–8.0 was used four lines (may be components) still appeared.

After absorption of the LH antiserum globulin with FSH and TSH (0.5–1.0 mg/10 ml antiserum) one strong and one weak line were formed between LH antiserum and the LH preparation; whereas only one line appeared between LH antiserum globulin and the FSH preparation (Fig. 3). The titer of each antiserum was considerably reduced by addition of each hormone to the antiserum to remove the contaminating antibodies.

Gamma globulins were separated from the antisera without reduction of the titer by the Kekwick's procedure. These globulin preparations were then ready for use in the indirect fluorescent antibody method. By this procedure, using the absorbed globulin from the FSH antiserum, brilliant yellow-green colored cells were concentrated in the central area of the anterior pituitary gland, although some fluorescent cells were also found in the peripheral area (Fig. 1). On the other hand, LH fluorescent cells were concentrated mainly in the peripheral area with lesser numbers of fluorescent cells located in the central areas of the gland (Fig. 2). Restaining of these preparations verified that the fluorescent cells corresponded to basophils.

While there was some variation from animal to animal in the number and distribution of the two types of fluorescent cells there was no marked alteration in the pattern seen for either type during the course of pregnancy.
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![Fig. 1. Normal rat anterior pituitary gland stained with fluorescent antiserum globulin to LH. Arrows show fluorescent LH cells in the peripheral area.](image)

![Fig. 2. Normal rat anterior pituitary gland stained with fluorescent antiserum globulin to FSH. Arrows show scattered fluorescent FSH cells to the central area.](image)

![Fig. 3. Precipitin reaction in Ouchterlony agar diffusion plate. Top cup, antiserum globulin to FSH; bottom, antiserum globulin to LH: right, 15 µg of FSH; left, 15 µg of LH.](image)

Discussion

PREER ('56) revealed three distinct precipitin lines between FSH and its antiserum, but one of these lines was shown to be due to serum albumin present in the FSH preparation. Four precipitin lines were demonstrated between FSH and its antibodies. One of them was found to be formed to ovine ICSH (LH). Recently, TAMADA et al. ('67) reported that antiserum to urinary FSH formed a number of precipitin lines when tested by agar diffusion and electrophoresis against FSH, or pediatric urine extract (Pednex) or HCG, but after absorption with 1.5 mg of HCG and 4 mg of Pednex per ml of antiserum, precipitin lines between the antiserum and Pednex or HCG were removed and only two lines formed between the FSH preparation and the absorbed antiserum.

On the other hand, HENRY and VAN DYKE ('58) showed several precipitin lines between ovine ICSH (LH) and its antibody on the Ouchterlony agar diffusion test, and after absorption
to remove nonspecific antibodies, only one precipitin line was observed. In another study in which ovine LH was used as the antigen (NIH-LH-S₁) Segal et al. (’62) demonstrated five lines between the antiserum and the homologous LH preparation. The Ouchterlony agar diffusion test by Ely and Chen (’66) showed seven precipitin lines between LH(NIH-LH-S₃) and antiserum to this hormone. However, consecutive absorption with FSH, PMS, HCG, and TSH resulted in removal of specific lines with each contaminating hormone, and they obtained a final serum which was no longer serologically reactive with any hormone except LH.

In the present study, the FSH (NIH-S₄) was found to contain measurable amounts of LH and TSH by means of serological methods. As a result, four precipitin lines formed between FSH and its antiserum, and four lines appeared between the FSH antiserum and LH (NIH-S₃). By the absorption procedure with equivalent amounts of LH and TSH some of the precipitin lines seen in Ouchterlony plates were removed and when agar at pH 6.8 was used only one line remained between FSH and its antibody. This antibody preparation no longer reacted with LH. From these facts we considered this antiserum to be adequately purified serologically for use in the fluorescent antibody procedure.

One precipitin line was formed between LH antiserum and FSH, although the reaction between antiserum to LH and the LH preparation showed two lines (one strong and one weak line) after absorption with FSH and TSH. The single precipitin line between the absorbed LH antiserum and FSH was identified with the strong line representing the reaction of LH and its antibody. Therefore, this precipitin reaction of an antigen and antibody seems to be due to contaminating LH hormone in the FSH preparation. Consequently, we assumed that the absorbed antiserum to LH was purified enough to use in visualizing endogenous LH by the fluorescent antibody procedure.

Different distributions in the rat anterior pituitary gland of cells thought to be FSH and LH gonadotrophs were demonstrated by Purves and Griesbach (’52), Siperstein et al. (’54), Rennels (’56, ’57), Hellbaum et al. (’16). Purves and Griesbach were supported by Barnett et al. (’55) who found that extraction with 2.5% trichloroacetic acid prior to fixation removed all FSH but left some LH in the gland, and under such condition the central zone of the gland kept PAS-positive materials. However, Rennels (’63) found that this procedure did not give consistent results. Additionally, he (’61) reported that injections of morphine sulfate resulted in a 3-fold increase in the pituitary content of FSH but caused no change in the level of pituitary LH of the rat. In this status, marked increase in the number and size of the PAS-purple gonadotrophs (central PAS cells) were observed.

The antibody preparations used in the present study were purified serologically, and their use in the indirect fluorescent antibody procedure resulted in the visualization of two distinct populations of fluorescent cells, one reacting with the FSH antibodies and the other with the LH antibodies. The FSH fluorescent cells were concentrated mainly in the central area of the anterior pituitary gland and LH fluorescent cells distributed mainly in the peripheral area in the gland. From these facts and the fact that the fluorescent cells corresponded to basophils, it seems clear that the peripheral fluorescent cells are LH producers and the central fluorescent cells correspond to FSH producers. Consequently, these observations concerning the distributions of FSH and LH producers support the Rennels’ concept as to the identification of the FSH and LH gonadotrophs in the pituitary gland of the rat.
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Summary

In the Ouchterlony agar diffusion test, at least four precipitin lines formed between FSH (NIH-FSH-S4) and its antiserum, and the same numbers of lines formed between this antiserum and the LH preparation (NIH-LH-S11). Regarding the LH preparation, two lines were formed between LH antiserum and the LH.

However, after absorption of both antisera with each contaminating hormone, the lines changed to one distinct line when agar at pH 6.8 was used.

By means of fluorescent antibody technique, using the globulin from such absorbed antisera, LH cells were mainly localized in the peripheral area, while FSH cells were mainly concentrated in the central area of the anterior pituitary gland of the rat.

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References

白ネズミ下垂体前葉の Gonadotrophs の免疫組織学的観察

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NIH-FSH (NIH-FSH-S4) を用いて作成した抗体血清はその抗原との間にオータロニーの二重拡散法によると少なくとも4本の沈降線が明らかである。またこの抗体血清は LH (NIH-LH-S11) を抗原とした場合でも同じく4本の沈降線を認めた。

NIH-LH (S11) を抗原として作成した抗体血清ではその抗原との間に2本の沈降線を観察した。しかし両抗体血清とも吸収法により pH 6.8 の寒天に明瞭な一本の染色線を認めた。

これら吸収血清から分離したグロブリンを用い観察抗体血清により LH 含有細胞は主に、腺体の周辺部に、FSH 含有細胞は主に腺体の中央部に集合するのを認めた。