FOLLICLE-STIMULATING HORMONE IN THE PITUITARY OF CAstrate AND CRYPTorchid Parabiotic Male Rats

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Summary. The follicle-stimulating hormone (FSH) content of the pituitaries of normal, castrated and cryptorchid parabiotic male rats was determined by the augmented ovarian weight bio-assay. These values were compared with those obtained from the same types of animals joined to hypophysectomized—intact, hypophysectomized—castrated, or hypophysectomized—cryptorchid males.

The total amount of FSH in the pituitary was reduced by 38% 1 week after castration of 45-day-old males. Neither increased spermatogenesis plus androgen in scrotal testes, nor increased androgen alone in cryptorchid testes of hypophysectomized parabiotic partners, had any effect upon this reduction. Loss of the germ cells by artificial cryptorchidism did not alter the total amount of pituitary FSH, but the concentration was increased by 19%. Active spermatogenesis in the testes of a hypophysectomized partner had no effect upon pituitary FSH in the cryptorchid male. In no case did a castrated hypophysectomized male influence pituitary FSH in his partner. The results indicate that nothing produced by the testes passed through the blood to alter the FSH content of the pituitary in a parabiotic partner.

INTRODUCTION

Attempts to explain control of follicle-stimulating hormone (FSH) in the pituitary of males on the basis of a negative feedback by testicular androgens have not been completely satisfactory. This has led to the formation of theories involving the germinal epithelium of the testes in FSH physiology; either directly by production of an inhibitor, or indirectly by utilization of gonadotrophin, and hence its removal from the circulation. Johnsen (1964) reviewed the evidence for a pituitary inhibitor and offered the cytoplasmic remnants of spermiogenesis as a possible source for this ambiguous material, generally referred to as 'inhibin'. According to his hypothesis, active spermatogenesis and sperm formation produce increased amounts of 'inhibin' which reduce the output of FSH. Recently, Steinberger & Duckett (1966) have proposed another hypothesis for control of FSH which involves the germinal epithelium. They assign to androgen the function of inhibiting FSH release, while an
inhibitor produced by the germinal epithelium is responsible for checking FSH production.

The present experiments were undertaken to study the effects of the testicular germinal epithelium of a hypophysectomized male on the FSH content in the pituitary of a castrated, or cryptorchid, parabiotic partner. It was hoped that if an 'inhibin' was produced by the testes, it might pass through the blood, as do the gonadotrophins, into a contralateral castrated or cryptorchid male partner and influence pituitary FSH. In addition, we sought information regarding gonadotrophin release in cryptorchid males.

MATERIALS AND METHODS

Holtzman-strain rats were used for experimental as well as assay animals. They were kept in air-conditioned rooms and had free access to food and water. When 40 days old, males were joined in parabiosis and, 5 days later, one member was hypophysectomized by the parapharyngeal approach under ether anaesthesia. On the day of hypophysectomy, and the following day, the operated animal received an aqueous suspension of 2.5 mg cortisone acetate subcutaneously. Pairs received 2.5% glucose in the drinking water following hypophysectomy.

Males were made cryptorchid or castrated a few minutes before the hypophysectomy operation. Testes were removed trans-scrotally, but were rendered cryptorchid by a mid-ventral abdominal incision. In the latter operation, the testes were pushed into the abdominal cavity, the gubernacula were cut and the inguinal canals were closed with sutures. With this method the testes themselves were not injured.

At the time of autopsy, on the 8th day following hypophysectomy (animals 53 days old), the success of the operations was evaluated. Attempts were made to force the testes into the scrotum to verify that they had remained intra-abdominal during the experimental period. In cases where there was doubt, the animals were discarded. Hypophysectomy was established by visual examination of the sella turcica and all incomplete operations were excluded from the study. Gonads and sex accessory organs were weighed on a torsion balance and then preserved in Bouin's fixative, sectioned in paraffin, and stained with haematoxylin and congo red. Pituitaries were weighed, homogenized in cold saline and stored frozen until they were assayed.

FSH assay. The FSH content of pooled pituitaries from each group was determined by a modified Steelman–Pohley (1953) augmentation assay. The modifications involved: (1) the use of 25-day-old animals, (2) augmentation with 50 i.u. chorionic gonadotrophin and (3) administration of the test material in two daily injections. Two assays were done, always using three doses of standard (NIH-FSH-S3) and two doses of unknown with six animals to a group. The regression curves for the two assays were similar as were the lambda values (0.134 and 0.138). The amount of FSH, with 95% confidence limits, in the pituitaries was determined, using routine statistical methods (Gaddum, 1953; Snedecor, 1956). When material was tested in both assays, the results were combined by the method of Sheps & Moore (1960).
RESULTS

The data from the thirteen groups in the study are summarized in Table 1. Groups 1, 2, 3 and 5 served as controls. Groups 1 and 5 were normal intact controls, their only difference being that one partner of Group 5 had no pituitary. While the testes, and particularly the sex accessory organs of the hypophysectomized partner (Group 5), regressed, these organs in the intact partner were not significantly different from those in the intact + intact pairs (Group 1). Also, the FSH content of the pituitary of the intact partner of a hypophysectomized male was not different from the control.

Groups 2 and 3 were castrated and cryptorchid controls respectively. With the loss of the testes the amount of stored FSH dropped 38% but it was not changed following cryptorchidism. The testes in the cryptorchid males were reduced 60% in weight and the tubules contained only Sertoli cells, spermatogonia and occasionally a spermatocyte, but interstitial cells were normal in appearance. Androgen production was somewhat reduced since ventral prostates were smaller (P<0.05) in cryptorchid males than in normal intact animals.

Pairs in Group 4 consisted of a castrated and cryptorchid male. The testes of the latter were slightly (P>0.05) and the prostates and seminal vesicles very significantly (P<0.01) heavier when compared with other cryptorchids (Group 3). The increased androgen production did not influence pituitary FSH in either partner; FSH was reduced in the castrate, and normal in the cryptorchid male.

The testes in hypophysectomized male partners of castrates (Group 6) were normal in size and distinctly larger (P>0.01) than in hypophysectomized partners of normal intact males (Group 5). Histologically, the tubules contained all stages of spermatogenesis and considerable numbers of spermatozoa were present. Sex accessory organs were much larger than those of normal intact males and equal to those of cryptorchid partners of castrates (Group 4). Pituitary FSH in the castrated partners was 27% below control, but not significantly (P>0.05) different from that found in castrate partners of hypophysectomized castrates (Group 7).

When the castrated male had a cryptorchid–hypophysectomized partner (Group 8) pituitary FSH was only 53% of control, lower than in the castrate partner of a castrate–hypophysectomized male (Group 7). However, the level was not significantly (P>0.05) below that found in castrate intact pairs (Group 2) or castrate partners of intact cryptorchid males (Group 4).

The castrated males of Group 9 received 50 µg testosterone propionate (TP) daily and the testes and sex accessory organ weights of their hypophysectomized partners were reduced considerably (compare with Group 6). The large variation in prostate and seminal vesicle weights was due to the fact that in one of the five pairs the pituitary of the castrate apparently was not influenced by the androgen, and the sex accessory organs of his hypophysectomized partner were as large as those in untreated pairs. The prostates and seminal vesicles of castrates in Group 9 were similar in size to those of normal intact controls (Group 1), suggesting that the dose of androgen used was within the physiologi-
<table>
<thead>
<tr>
<th>Group No.</th>
<th>No. of pairs</th>
<th>Type of animals</th>
<th>Body weight (g)</th>
<th>Testes (mg)</th>
<th>Ventral prostate (mg)</th>
<th>Seminal vesicle (mg)</th>
<th>Pituitary (mg)</th>
<th>FSH (µg of NIH-FSH-S3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>Normal intact</td>
<td>183 ± 9</td>
<td>2585 ± 59</td>
<td>154 ± 13</td>
<td>63±4 ± 5</td>
<td>9±8 ± 0·3</td>
<td>414 (377–451)*†</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>Castrate intact</td>
<td>150 ± 4</td>
<td>–</td>
<td>22 ± 3</td>
<td>12±0 ± 1</td>
<td>7±4 ± 0·2</td>
<td>256 (192–319)</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>Cryptorchid intact</td>
<td>167 ± 12</td>
<td>1032 ± 113</td>
<td>94 ± 16</td>
<td>41±1 ± 7</td>
<td>7±8 ± 0·2</td>
<td>417 (342–493)</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>Castrate intact</td>
<td>183 ± 8</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>Normal intact</td>
<td>171 ± 7</td>
<td>2474 ± 110</td>
<td>136 ± 15</td>
<td>45±5 ± 8</td>
<td>7±8 ± 0·7</td>
<td>402 (333–451)†</td>
</tr>
<tr>
<td>6</td>
<td>11</td>
<td>Castrate intact</td>
<td>160 ± 7</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>7</td>
<td>6</td>
<td>Normal hypophysecomized</td>
<td>140 ± 3</td>
<td>253 ± 42</td>
<td>216 ± 13</td>
<td>96±9 ± 7</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>8</td>
<td>8</td>
<td>Castrate intact hypophysecomized</td>
<td>164 ± 2</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>9</td>
<td>5</td>
<td>Castrate intact + TP†</td>
<td>172 ± 4</td>
<td>–</td>
<td>138 ± 4</td>
<td>52±1 ± 6</td>
<td>7±7 ± 0·4</td>
<td>343 (263–423)</td>
</tr>
<tr>
<td>10</td>
<td>6</td>
<td>Normal hypophysecomized</td>
<td>146 ± 4</td>
<td>2187 ± 63</td>
<td>88±27</td>
<td>37±5 ± 12</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>11</td>
<td>5</td>
<td>Cryptorchid intact</td>
<td>182 ± 4</td>
<td>997 ± 60</td>
<td>101±9</td>
<td>47±4 ± 5</td>
<td>8±3 ± 0·4</td>
<td>448 (409–487)</td>
</tr>
<tr>
<td>12</td>
<td>5</td>
<td>Cryptorchid hypophysecomized</td>
<td>165 ± 2</td>
<td>1093 ± 31</td>
<td>123±10</td>
<td>54±5 ± 4</td>
<td>7±4 ± 0·2</td>
<td>455 (402–508)</td>
</tr>
</tbody>
</table>

*95% confidence limits. † TP = 50 µg testosterone propionate daily given to castrate.
‡ indicates that material was assayed twice.
FSH in male rats

Pituitary FSH in the treated males did not fall as it did in untreated castrates.

Testes in hypophysectomized partners of cryptorchid males were smaller than those in intact controls, but significantly \((P<0.01)\) larger than in hypophysectomized partners of intact normal males. All stages of spermatogenesis, including mature spermatozoa, were present in the tubules, and the interstitial cells appeared normal. Androgen production was not normal, however, and prostates and seminal vesicles were not maintained at intact control levels. Seminal vesicles \((P<0.01)\) but not prostates \((P>0.05)\) in hypophysectomized males joined to cryptorchid males were significantly heavier than in partners of normal intact males (compare Groups 10 and 5). The cryptorchid male partners in Group 10 had atrophic testes and the ventral prostates were below normal intact male control values \((P<0.01)\). Pituitary FSH was not significantly above the control level.

When the testes of both the hypophysectomized and intact male were cryptorchid, androgen production decreased further, as seen in reduced seminal vesicle weights (Group 11). The testes in these hypophysectomized males were the smallest of any group and the tubules showed the greatest damage, containing only Sertoli cells and spermatogonia. The organ weights in the non-hypophysectomized partners were not different from those of males in Group 10 and total pituitary FSH was not significantly elevated above control values.

In Group 12 the hypophysectomized partner was castrated. The organ weights in the cryptorchid partner were the same as in other cryptorchid males and pituitary FSH was only slightly below normal \((P>0.05)\).

DISCUSSION

The present experiments clearly demonstrate that nothing produced by scrotal or cryptorchid testes in hypophysectomized males passed into intact, castrate or cryptorchid male parabiotic partners to influence pituitary stores of FSH. If the testicular germinal elements produced an inhibitor (inhibin) it must have been quickly inactivated, as was androgen, before reaching physiologically effective levels in a parabiotic partner.

In castrates (Groups 2, 4, 6, 7 and 8) total pituitary FSH was below that found in intact normal males (Groups 1 and 5). Relative to the weight of the pituitary, the concentration for the six groups was \(31.7 \pm 2.5 \mu g/mg\) compared with \(46.4 \pm 1.9 \mu g/mg\) for controls. Such an effect following castration has previously been reported. In 30-day-old males (Yasuda & Johnson, 1965), at an age when testicular development is very active, castration resulted in a 77% reduction in FSH, while in old mature males (Steinberger & Duckett 1966), where testicular development is completed, the fall was only 29%. In the puberal males of the present study (Table 1), in which testicular development is nearly complete, the decline in FSH was 38%. Several reports (van Rees, 1964; Bogdanove, 1964) indicate that plasma FSH increases quickly after gonadectomy, and thus at least some of the loss from the pituitary can be accounted for by the increase in blood. The important question is: are these changes in FSH due to a loss of androgen or germ cells or both? Testosterone propionate inhibited much of the
FSH reduction associated with castration (Yasuda & Johnson, 1965; van Rees, 1964, and group 10, Table 1) and would seem to support the belief that androgen plays a role in release of this gonadotrophin. However, this cannot be accepted unequivocally since recent studies in parabionts (Schutz, Sager & Meyer, 1964) failed to show an inhibitory effect upon release of FSH, even with large doses of TP.

The profound change in pituitary and blood FSH seen in the castrate are not apparent in the cryptorchid male, who lacks only germinal elements. Steinberger & Duckett (1966) found a 25% increase in total pituitary FSH 1 week after making adult males cryptorchid. In the present study, the average concentration of total hormone was elevated 19%: in the five groups with cryptorchid males pituitary FSH averaged 55.2±2.2 µg/mg compared with 46.4±1.9 µg/mg for controls (P<0.02). Plasma levels are unknown, but previous work with parabionts by Taira & Tarkham (1962) indicated that circulating FSH in cryptorchid males was higher than in normal males, but much lower than in castrates.

The question of normal androgen production by cryptorchid testes is of paramount importance in understanding testicular–pituitary relationships. Several facts contradict the often quoted view that interstitial tissue and androgen output are completely normal (see Llaurado & Dominquez, 1963; Clegg, 1965, for discussion). The average prostate weight for the twenty cryptorchid males in the present study was 105.4±6.1 mg and their seminal vesicles weighed 45.7±1.3 mg. In the sixteen normal males these organs weighed 147.3±9.6 and 56.4±4.2 mg respectively, which is significantly (P<0.02) heavier. Increased plasma LH (Johnson, 1966) in cryptorchid males suggests a decrease in androgen production. This can be seen in the present study also: hypophysectomized male partners of cryptorchid males (Group 10) had heavier sex accessory organs than partners of intact normal males (Group 5), but only the increased seminal vesicle weight had statistical significance. The effect of cryptorchidism on LH control is emphasized by the fact that this hormone increases 17-fold within 1 year after the operation, while the gland content of castrates goes up 6-fold in the same period (Steinberger & Duckett, 1966).

Altered androgen production by the testes and a subsequent change in pituitary LH control could be the result of either a decrease in testosterone synthesis, or a qualitative change in androgen synthesized, or both. Neither of the recent theories (Johnsen, 1964; Steinberger & Duckett, 1966) involving the germinal epithelium in inhibition of pituitary FSH takes into consideration possible alterations in production of steroids or conjugates, in abnormal testes (such as in Kleinfelter's syndrome) or in cryptorchid testes. With the data currently available, a study of the effects of different steroids upon both FSH and LH storage and release would make a significant contribution to our understanding of normal testicular pituitary physiology.

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REFERENCES


