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# Heterogeneity of Gonadotropins and Levels of Uncombined Luteinizing Hormone Subunits in Pituitaries of Cryptorchid Rams

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**ABSTRACT:** Pituitaries were collected from intact rams and rams that had been rendered bilaterally cryptorchid by surgery to examine the effects of cryptorchidism on gonadotropin heterogeneity, levels of uncombined luteinizing hormone (LH) subunits, and the apparent molecular sizes of LH and follicle-stimulating hormone (FSH). Cryptorchid rams had higher pituitary contents of LH and FSH as well as reduced testicular weights. The levels of uncombined LH subunits, their apparent molecular weights, and the apparent molecular weights of intrapituitary LH were similar in control and cryptorchid rams. However, the apparent molecular weight of intrapituitary FSH was slightly larger in cryptorchid rams. Cryptorchidism altered the pattern of gonadotropin heterogeneity by shifting the distribution of LH isoforms towards basic components and shifting the distribution of FSH isoforms towards acidic components. Thus, it appears that the altered gonadal feedback mechanisms resulting from cryptorchidism modify the pattern of both LH and FSH heterogeneity by shifting the distribution of isoforms in opposite directions.

Key words: Cryptorchidism, heterogeneity, gonadotropins, luteinizing hormone, follicle-stimulating hormone.

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Cryptorchidism is an abnormal condition in which one or both testes fail to descend into the scrotum. The undescended testes exhibit reduced spermatogenesis and are associated with a higher incidence of testicular tumors (reviewed by Abney and Keel, 1989). As might be anticipated, cryptorchidism alters the normal feedback relationships between the testes and the hypothalamic-anterior pituitary axis. Serum concentrations of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) are typically elevated as a result of bilateral cryptorchidism, but testosterone concentrations change minimally (Swerdloff et al, 1971; Rager et al, 1975; Gomes and Jain, 1976; Keel and Abney, 1980; Bhasin et al, 1987). The marked increases in serum FSH in conjuction with less dramatic changes in LH and minimal changes in testosterone have generally been interpreted to indicate that cryptorchidism primarily alters the feedback loop that regulates FSH secretion via nonsteroidal substances secreted by the seminiferous epithelium (Blanc et al, 1978; de Kretser and Robertson, 1989). In contrast to bilateral cryptorchidism, unilateral cryptorchidism is usually associated with minimal changes in serum concentrations of FSH, LH, and testosterone, suggesting compensation by the eutopic scrotal testis (Gomes and Jain, 1976; Keel and Abney, 1981).

Rather than existing as single molecular entities, the pituitary gonadotropins exist as families of similar molecular forms frequently termed "isohormones" (reviewed by Keel and Grotjan, 1989; Robertson, 1989). The distribution of LH or FSH among its isohormones appears to be regulated by endocrine feedback because castration yields a higher percentage of basic forms whereas estrogen administration shifts the distribution towards acidic forms (Keel and Grotjan, 1985a; Keel et al, 1987a; Keel and Schanbacher, 1987). In view of the more profound effects of cryptorchism on FSH secretion, we originally hypothesized that cryptorchidism would preferentially alter FSH heterogeneity. However, experiments with rats suggested cryptorchidism markedly altered the charge heterogeneity of LH as well as FSH (Keel and Grotjan, 1985b). Hence, the primary objective of the present study was to determine if cryptorchidism induces changes in the heterogeneity of both FSH and LH in the sheep. In addition, the apparent molecular sizes of the gonadotropins and levels of uncombined LH subunits were assessed. A preliminary report containing some of the data presented herein has appeared previously (Schanbacher et al, 1989).

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## Materials and Methods

### Animals

Ten Suffolk-sired crossbred ram lambs were assigned to this study at 3 months of age. Five lambs served as intact controls, and the remaining five were rendered cryptorchid by bilateral inguinal surgery. After the animals had reached sexual maturity (approximately 4 months later), the animals were sacrificed at the USDA Meat Animal Research Center abattoir. Anterior pituitaries were collected on ice, trimmed of extraneous blood vessels, and weighed.

### Preparation of Pituitary Extracts

Pituitaries were homogenized vigorously using a Polytron at a setting of 6 for approximately 15 seconds in 150 mM NaCl buffered with 50 mM Tris (pH 7.4) containing 1% (v/v) Triton X-100, 5 mM disodium ethylenediaminetetraacetic acid, 1 mM phenylmethylsulfonyl fluoride, and 200 U/ml aprotinin (1.0 ml per 100 mg wet weight). Tissue extracts were clarified by centrifugation at 100,000 × *g* for 1 hour, aliquoted, and stored at −70°C until assayed or subjected to chromatography.

### Analytical Gel Permeation Chromatography

In order to quantitate uncombined LH subunits with minimal interference from native LH, pituitary extracts were subjected to gel permeation chromatography using methods previously described (Keel et al, 1987b) except that Bio-Gel P-100 was substituted for Sephadex G-100 Superfine. On both gels, the glycoprotein hormones elute with apparent molecular weights larger than expected. This difference is minimal on Bio-Gel P-100 but pronounced on Sephadex G-100, where they elute at apparent molecular weights of approximately 150% of true molecular weight (Grotjan, unpublished data).

Briefly, an aliquot (0.2 ml representing 20 mg tissue equivalents) was supplemented with 1.4 mg thyroglobulin (which eluted in the void volume, Vo), 4.0 mg ovalbumin (OV, mol. wt. 45,000), 1.8 mg carbonic anhydrase (CA, mol. wt. 29,000), 2.4 mg cytochrome c (CC, mol. wt. 12,400) and approximately 100,000 cpm $^{125}I$ (which eluted in the included volume, Vi) to monitor the efficacy of separation. Samples were loaded onto 1.5 × 185 cm columns of Bio-Gel P-100 (100-200 mesh; Bio-Rad, Richmond, California) and eluted at 3 ml/hour with 0.15 M ammonium bicarbonate, pH 7.8, at 4°C. Three-ml fractions were collected and analyzed for LH, LHα, LHβ, and FSH by radioimmunoassays. Recovery of immunoreactive LH and FSH averaged 76% and 90%, respectively; neither was significantly different ($P > 0.05$) between experimental groups. Distribution coefficients (Kds) were calculated from the elution volume (Ve) as defined by the fraction with the highest concentration of each hormone using the formula Kd = (Ve − Vo)/(Vi − Vo). Apparent molecular weights were interpolated from a graph of Kd versus log molecular weight.

Native LH (α-β dimers) and uncombined subunits were identified by their elution profiles during gel filtration. Two peaks of immunoreactivity, corresponding to the elution position of native LH and uncombined subunits, respectively, were observed in the radioimmunoassays for LH and uncombined subunits (see Figs. 1 and 2 for examples). The nadir of the two peaks was used to define the point separating native LH from uncombined subunits. Approximate molar ratios of uncombined subunits to native LH were calculated under the following assumptions: 1) the standards employed in the radioimmunoassays were relatively pure, 2) there were no differential losses of subunits and native LH during gel filtration, and 3) the molecular weights of the uncombined subunits were approximately one-half those of native LH.

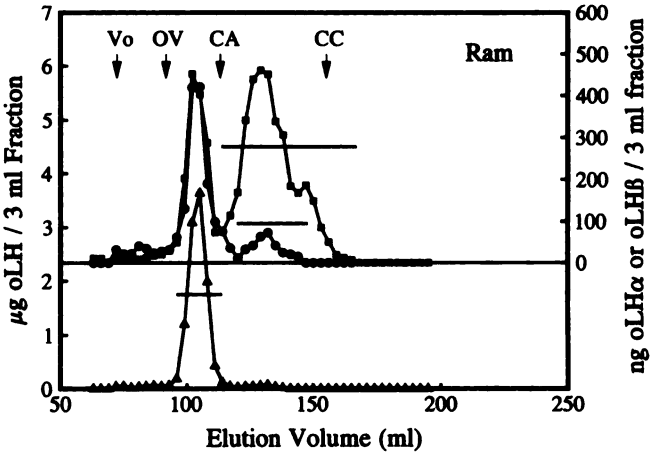


**FIG. 1.** Representative elution profile of the immunoreactive LH (▲), LHα (■), and LHβ (●) in the extract (20 mg tissue equivalents) of a pituitary from an intact ram subjected to analytical gel permeation chromatography. The 1.5 × 185 cm column of Bio-Gel P-100 was eluted at 3 ml/hour with 0.15 M ammonium bicarbonate, pH 7.8, and 3-ml fractions were collected. The Vi for this profile was 327 ml. The values summed to estimate LH and LH subunit concentrations (Table 3) are denoted by the bars. Extracts of four other pituitaries obtained from intact rams yielded similar results (data not illustrated).

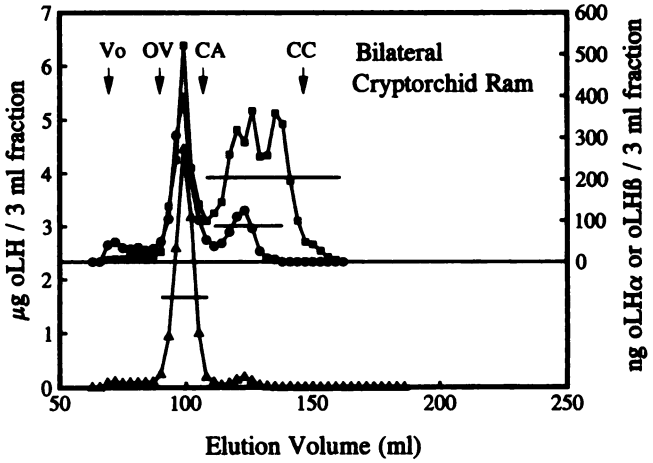


**FIG. 2.** Representative elution profile of the immunoreactive LH (▲), LHα (■), and LHβ (●) in the extract (20 mg tissue equivalents) of a pituitary from a bilateral cryptorchid ram subjected to analytical gel permeation chromatography. Experimental details were similar to Figure 1 except that the Vi was 306 ml. The values summed to estimate LH and LH subunit concentrations (Table 3) are denoted by the bars. Extracts of four other pituitaries from bilateral cryptorchid rams yielded similar results (data not illustrated).

Table 1. *Organ weights and gonadotropins in intact and cryptorchid rams**

| Variable (units) | Intact rams | Bilateral cryptorchid rams |
|---|---|---|
| Anterior pituitary weight (g) | 0.50 ± 0.05 | 0.65 ± 0.11 |
| Testes weight (g) | 272 ± 11 | 53* ± 7 |
| Pituitary LH concentration (μg/mg) | 0.96 ± 0.13 | 1.32 ± 0.21 |
| Pituitary LH content (μg/pituitary) | 470 ± 60 | 796* ± 98 |
| Pituitary FSH concentration (μg/mg) | 39 ± 8 | 105* ± 12 |
| Pituitary FSH content (μg/pituitary) | 18 ± 2 | 61* ± 7 |
| Serum LH (ng/ml)† | 0.23 ± 0.06 | 3.34 ± 1.56 |

* Mean ± SE for five animals in each category. Means for cryptorchid rams identified by an asterisk are significantly different by one-way analysis of variance ($P < 0.05$).

† Serum LH concentrations were significantly higher in bilateral cryptorchid rams when the data were log transformed prior to analysis ($F_{1,8} = 19.9$, $P = 0.0022$).

Table 2. *Molecular characteristics of gonadotropins and LH subunits in pituitary extracts of intact and bilateral cryptorchid rams subjected to analytical gel permeation chromatography**

| Variable | Intact rams | Bilateral cryptorchid rams |
|---|---|---|
| Kd of LH | 0.127 ± 0.002 | 0.128 ± 0.001 |
| Apparent mol. wt., LH | 33,100 ± 300 | 34,000* ± 100 |
| Kd of LHα | 0.233 ± 0.004 | 0.242 ± 0.008 |
| Apparent mol. wt., LHα | 19,100 ± 400 | 19,200 ± 950 |
| Kd of LHβ | 0.230 ± 0.002 | 0.235 ± 0.002 |
| Apparent mol. wt., LHβ | 19,300 ± 150 | 19,900 ± 400 |
| Kd of FSH | 0.115 ± 0.004 | 0.104 ± 0.004 |
| Apparent mol. wt., FSH | 35,200 ± 590 | 38,400* ± 890 |

* Mean ± SE for five animals in each category. Means for cryptorchid rams identified by an asterisk are significantly different by one-way analysis of variance ($P < 0.05$).

### *Chromatofocusing*

Ovine LH and FSH were fractionated into various isohormones by chromatofocusing on pH 10.5 to 7 and pH 7 to 4 chromatofocusing gradients, respectively (Keel et al, 1987a; Keel and Schanbacher, 1987). Briefly, a 0.5-ml aliquot of each pituitary extract was prepared for pH 10.5 to 7 chromatofocusing by flow dialysis against water (recovery of immunoreactive LH = 83%) and was applied to a 20-ml column of PBE118 resin in 2% Pharmalyte 8-10.5, pH 7.0 (Grotjan et al, 1991). The pH gradient was generated with Pharmalyte as previously described (Keel et al, 1987a). A 1.0-ml aliquot of each pituitary extract was prepared for pH 7 to 4 chromatofocusing by gel permeation chromatography on Sephadex G25 Superfine, applied to a 20-ml column of PBE94, and the pH gradient was generated with 1:10 Polybuffer 74, pH 4.0. Reagents used for chromatofocusing and Sephadex were purchased from Pharmacia/LKB (Piscataway, New Jersey). Recoveries of immunoreactive LH and FSH from the chromatofocusing columns averaged 78% and 76%, respectively.

### *Radioimmunassays*

The ovine (o) LH, LHα, and LHβ radioimmunassays have been described in detail previously (Keel et al, 1987a,b). In this experiment, oLH-DNW-HSN-10-124 (generously provided by Dr. D. N. Ward, Houston, Texas) was substituted for oLH-DNW-MES-1-171D (relative potency 1 ng oLH-DNW-HSN-10-124 = 1.11 ng oLH-DNW-MES-1-171D). A radioimmunoassay employing reagents obtained from the NIH Pituitary Hormone Distribution Program (Keel and Schanbacher, 1987) was used to quantitate ovine FSH in pituitary extracts and in chromatofocusing fractions. Concentrations of FSH in gel filtration fractions were quantitated using oFSH-LER-1976A2 as the standard and hormone for iodination (generously supplied by Dr. L. E. Reichert, Jr., Albany, New York) and an anti-oFSH (JAD-17-679) obtained from Dr. James A. Dias (Albany, New York) (Prewitt and Grotjan, 1992).

### *Statistics*

Statistical significance was established by one-way analysis of variance and Duncan's New Multiple Range Tests. A probability of less than 0.05 was considered to be statistically significant. Percentage values were subjected to arc sine transformations (square root of the arc sine of the percentage) prior to statistical analysis.

## Results

### *Physiological Characteristics of Cryptorchid Rams*

Testicular weights were markedly reduced in bilateral cryptorchid rams, and serum concentrations of LH were slightly elevated (Table 1). Anterior pituitary weights and pituitary LH concentrations were similar ($P > 0.05$) among the two treatment groups whereas pituitary content of LH was slightly elevated in cryptorchid rams ($P < 0.05$). More importantly, both the pituitary content and concentration of FSH were markedly elevated ($P < 0.01$) in cryptorchid rams. Thus, the cryptorchid animals used in this study responded as anticipated.

### *Apparent Molecular Sizes of Gonadotropins and Concentrations of Uncombined Subunits*

Bilateral cryptorchidism did not markedly alter ($P > 0.05$) the elution position of LH, LHα, or LHβ during gel permeation chromatography (i.e., their Kds) or the apparent molecular sizes of these gonadotropins interpolated from the elution of standard proteins (Figs. 1, 2; Table 2). Furthermore, neither the concentrations of uncombined LH subunits nor the molar ratios of uncombined LH subunits

Table 3. *Concentrations of LH and its subunits in pituitary extracts subjected to analytical gel permeation chromatography**

| Variable (units) | Intact rams | Bilateral cryptorchid rams |
|---|---|---|
| LHα (μg/mg) | 0.192 ± 0.028 | 0.154 ± 0.012 |
| LHα/LH molar ratio | 0.546 ± 0.047 | 0.380* ± 0.044 |
| LH (μg/mg) | 0.707 ± 0.084 | 0.907 ± 0.090 |
| LHβ (μg/mg) | 0.027 ± 0.005 | 0.032 ± 0.002 |
| LHβ/LH molar ratio | 0.076 ± 0.008 | 0.072 ± 0.006 |

* Mean ± SE for five animals in each category. Means for cryptorchid rams identified by an asterisk are significantly different by one-way analysis of variance ($P < 0.05$).

to native LH were altered ($P > 0.05$) as a result of cryptorchidism (Table 3). However, the apparent molecular size of intrapituitary FSH was slightly greater in cryptorchid rams as judged by Kds ($P = 0.075$) or after transformation of Kd values to apparent molecular weights ($P = 0.015$) (Fig. 3; Table 2).

### *Charge Heterogeneity of Intrapituitary LH*

Intrapituitary LH was resolved into nine peaks by chromatofocusing on pH 10.5 to 7 gradients (Fig. 4). A majority of the immunoreactive LH was associated with peaks

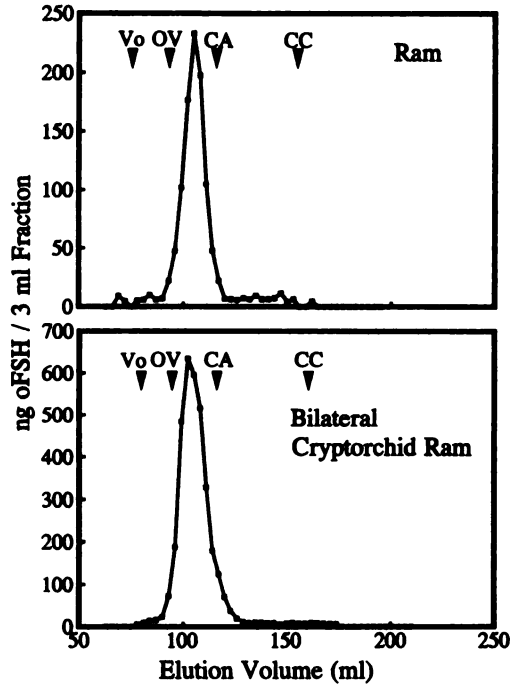


FIG. 3. Representative elution profile of the immunoreactive FSH in extracts (20 mg tissue equivalents) of pituitaries from intact (upper panel) and bilateral cryptorchid (lower panel) rams subjected to analytical gel permeation chromatography. Experimental details were similar to Figure 1 except that the respective Vi's were 333 and 306 ml. Extracts from four other pituitaries in each category yielded similar results (data not illustrated).

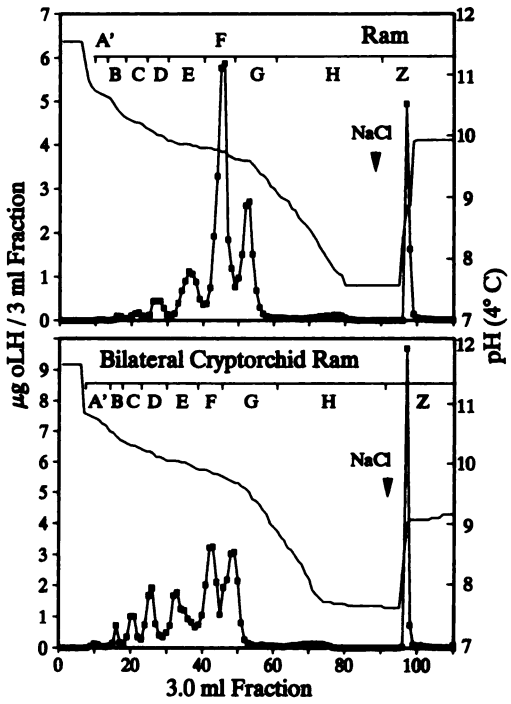


FIG. 4. Representative chromatofocusing elution profiles of immunoreactive LH in extracts of the anterior pituitaries of intact (upper panel) and bilateral cryptorchid (lower panel) rams on pH 10.5 to 7 gradients. The pH profiles are illustrated as solid lines. After the pH gradient reached its lower limiting plateau, bound components were eluted with 1 M NaCl (denoted as isohormone Z). Brackets correspond to fractions pooled to divide the profile into respective isohormones (designated with letters; see Table 4).

F and G. Cryptorchidism altered the distribution of intrapituitary LH among its isohormones (Fig. 4; Table 4), with bilateral cryptorchid rams exhibiting lower percentages of isoform F and higher percentages of LH isoforms E and D. Thus, cryptorchidism induced a subtle shift in the distribution of LH isohormones towards basic forms.

Table 4. *Distribution of immunoreactive LH in pituitary extracts among isohormones separated by chromatofocusing on pH 10.5 to 7 gradients**

| Isohormone (elution pH) | Intact rams | Bilateral cryptorchid rams |
|---|---|---|
| A' (10.74 ± 0.10)† | 0.3 ± 0.1 | 0.5 ± 0.1 |
| B (10.29 ± 0.06) | 0.7 ± 0.1 | 1.1 ± 0.2 |
| C (10.10 ± 0.05) | 1.1 ± 0.2 | 2.4 ± 0.8 |
| D (9.93 ± 0.05) | 3.6 ± 0.7 | 6.6* ± 0.9 |
| E (9.78 ± 0.04) | 8.5 ± 1.6 | 14.8* ± 0.7 |
| F (9.66 ± 0.04) | 42.9 ± 2.2 | 30.9* ± 2.7 |
| G (9.50 ± 0.04) | 22.6 ± 1.3 | 24.2 ± 0.7 |
| H (7.71 ± 0.03) | 3.0 ± 3.5 | 2.4 ± 0.3 |
| Z (<7.00) | 17.4 ± 2.2 | 17.1 ± 1.6 |

* Mean percentage of LH in each isohormone ± SE for five animals in each category. Means for cryptorchid rams identified by an asterisk are significantly different by one-way analysis of variance ($P < 0.05$).

† Elution pH values equal the mean ± SE of 10 observations.

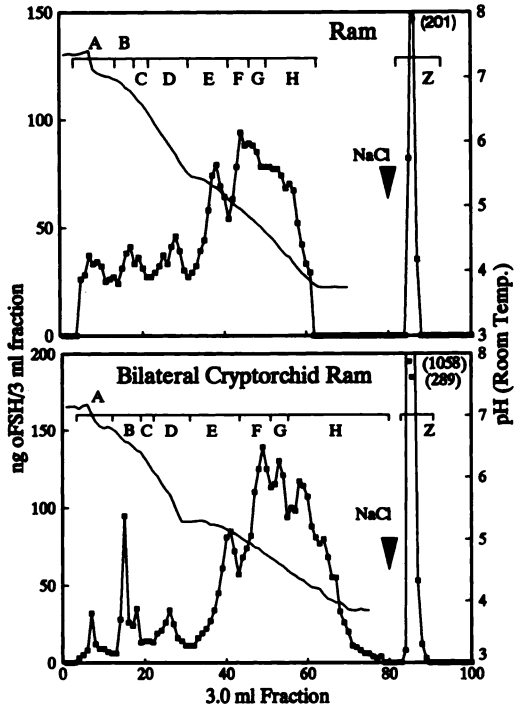


**FIG. 5.** Representative chromatofocusing elution profiles of immunoreactive FSH in extracts of the anterior pituitaries of intact (upper panel) and bilateral cryptorchid (lower panel) on pH 7 to 4 gradients. The pH profiles are illustrated as solid lines. After the pH gradient reached its lower limiting plateau, bound components were eluted with 1 M NaCl (denoted as isohormone Z). Brackets correspond to fractions pooled to divide the profile into respective isohormones (designated with letters; see Table 5).

### *Charge Heterogeneity of Intrapituitary FSH*

Intrapituitary FSH was also resolved into nine peaks by chromatofocusing on pH 7 to 4 gradients (Fig. 5). As observed previously (Keel and Schanbacher, 1987), FSH primarily elutes in the low acidic range, with a significant amount remaining bound to the column at a lower limiting pH of 4. In contrast to LH, there were no major differences in the percentage of intrapituitary FSH in each peak between intact and cryptorchid rams except that a higher percentage of the FSH was bound to the column at the lower limiting pH of 4.0 (Table 5). This suggests that cryptorchidism induces a subtle shift in the distribution of FSH isoforms yielding a higher percentage of acidic forms.

## *Discussion*

Gonadotropin heterogeneity is thought to be regulated by feedback mechanisms involving gonadal substances (reviewed in Keel and Grotjan, 1989; Robertson, 1989). The pattern of isoforms changes after castration (Robertson et al, 1982; Ulloa-Aguirre and Chappel, 1982; Keel and Grotjan, 1985a; Keel et al, 1987a; Keel and Schanbacher, 1987), during pubertal development (Chappel et al, 1983; Chappel and Ramaley, 1985), and throughout the normal reproductive cycle of females (Robertson et al, 1982; Cameron and Chappel, 1985; Ulloa-Agurrie et al, 1988). Presumably, an intricate, fine-tuned control mechanism exists between the gonads and the pituitary to regulate both the amounts (quantities) of the gonadotropins synthesized as well as to induce subtle changes in their heterogeneities (qualities). For example, castration leads to increased rates of LH synthesis and secretion in the sheep. The net result is an increased percentage of basic isoforms in the pituitary (Keel et al, 1987a). This pattern can be attributed to increased production of the extremely basic isoforms (coded with the letters A′ through E), some of which are secreted in minimal amounts, as well as to the selective secretion of the mid-alkaline isoforms we have coded with the letters F and G (Zalesky and Grotjan, 1991). Analogous changes in LH heterogeneity appear to result from cryptorchidism (present study).

Table 5. *Distribution of immunoreactive FSH in pituitary extracts among isohormones separated by chromatofocusing on pH 7 to 4 gradients**

| Isohormone (elution pH) | Intact rams | Bilateral cryptorchid rams |
|---|---|---|
| A (>7.4) | 7.6 ± 1.8 | 4.1 ± 1.0 |
| B (6.71 ± 0.03) | 5.2 ± 1.4 | 5.6 ± 1.0 |
| C (6.51 ± 0.02) | 4.6 ± 0.7 | 3.2 ± 0.7 |
| D (5.76 ± 0.02) | 10.1 ± 2.2 | 7.9 ± 1.3 |
| E (5.15 ± 0.02) | 13.7 ± 1.1 | 12.8 ± 0.9 |
| F (4.82 ± 0.03) | 10.6 ± 1.0 | 14.6 ± 2.2 |
| G (4.62 ± 0.04) | 12.1 ± 1.3 | 11.1 ± 0.9 |
| H (4.34 ± 0.04) | 17.1 ± 4.8 | 14.0 ± 2.9 |
| Z (<4.00) | 18.3 ± 3.4 | 27.2* ± 1.1 |

* Mean percentage of FSH in each isohormone ± SE for five animals in each category. Means for cryptorchid rams identified by an asterisk are significantly different by one-way analysis of variance ($P < 0.05$).
† Elution pH values equal the mean ± SE of 10 observations.

In the rat, cryptorchidism causes a marked increase in circulating LH concentrations (Keel and Abney, 1980) and shifts the isohormone pattern of pituitary LH towards the more alkaline forms that exhibit the highest B/I ratios (ratio of activities in an *in vitro* bioassay versus radioimmunoassay) (Keel and Grotjan, 1985b). This change can be interpreted as shift in the distribution towards more biologically active forms as a mechanism to maintain normal circulating levels of androgens. In the sheep, the shift towards more basic isoforms is much less pronounced than in the rat. Furthermore, the basic isoforms of ovine LH appear to be less potent that the mid-alkaline isohormones F and G (Keel et al, 1987a). Thus, the reason for the observed changes in LH heterogeneity in cryptorchid rams is not as obvious and may simply be a consequence of increased LH secretion.

In contrast, to what was observed for LH, the pattern

of FSH heterogeneity was shifted towards acidic components in cryptorchid rams. Thus, cryptorchidism shifted the pattern of LH and FSH isohormones in opposite directions. This would appear to signify precise and hormone-specific control of gonadotropin heterogeneity via gonadal feedback mechanisms. Experimentally induced cryptorchidism causes degeneration of the seminiferous epithelium, yielding increased circulating concentrations of FSH and LH in conjunction with normal or only slightly reduced testosterone levels. The increases in circulating (Keel and Abney, 1980) and pituitary (present study) levels of FSH after cryptorchidism are particularly pronounced. In view of the convincing argument that inhibin participates in the regulation of FSH secretion by the pituitary (Schanbacher, 1988; de Kretser and Robertson, 1989), the observed reduction in the testicular inhibin levels associated with cryptorchidism (Au et al, 1983) and the divergent changes in the pattern of FSH heterogeneity in response to cryptorchidism and castration, putative regulators of FSH heterogeneity can be suggested. The major change in the heterogeneity of FSH in the pituitaries of cryptorchid rams was an increase in the extremely acidic forms (isohormone Z, Table 5). In the sheep, castration also increases the percentage of FSH present as isohormone Z (Keel and Schanbacher, 1987). However, castration induces other changes in the pattern of FSH isohormones (Keel and Schanbacher, 1987). Assuming that the two major regulators of FSH secretion are testosterone and inhibin, it would appear that the removal of both substances (i.e., castration) yeilds an increase in acidic components primarily in response to reduced inhibin secretion. Hypothetically, testosterone would appear to change the distribution of FSH isoforms with elution pHs greater than 5. In any case, further experimentation will be required to delineate the precise role of inhibin in controlling FSH microheterogeneity.

LH and FSH exist as heterodimers comprised of a hormone-specific beta subunit and a common alpha subunit. The subunits are synthesized individually and subsequently combine to form the dimers found in the native hormone. The synthesis of the subunits is influenced by the endocrine environment of the animal (reviewed by Nett, 1990). We have reported that the absolute levels of uncombined LH subunits in the sheep pituitary are altered as a result of castration and endocrine treatment (Keel et al, 1987b). However, in the present study, we observed that neither the concentrations of uncombined LH subunits nor the molar ratios of subunits to native dimer are changed as a result of bilateral cryptorchidism. Thus, the dynamic changes observed in the absolute levels of gonadotropins in response to cryptorchidism do not appear to reflect alterations in the relative synthesis of the LH subunits or the rate of LH subunit combination.

The apparent molecular size of monkey LH and rat gonadotropins is altered as a result of castration and admistration of estrogens (Bogdanove et al, 1974; Peckham and Knobil, 1976a,b), suggesting that gonadal feedback mechanisms can alter the molecular size of the gonadotropins in addition to charge heterogeneity. However, we did not observe any significant alterations in the apparent molecular size of pituitary LH (Keel et al, 1987b) or FSH (Prewitt and Grotjan, 1992; unpublished data) in sheep as a result of castration or administration of estrogen. Although bilateral cryptorchidism significantly alters the pituitary levels of pituitary LH and FSH, this condition does not appear to cause a major change in the apparent molecular size of LH. However, the FSH in the pituitaries of cryptorchid rams eluted earlier during gel permeation chromatography (slightly larger apparent molecular size) than that from intact rams. This observation is consistent with the observed shift towards acidic components presuming both changes reflect terminal sialylation of the hormone.

In summary, bilateral cryptorchidism, like castration, produces a marked increase in the circulating levels of gonadotropins, suggesting that translocation of the testes to the abdomen upsets the normal feedback relationship between the hypothalamus, pituitary, and the testes. The resulting change in feedback relationships in cryptorchid animals alter the distribution of both LH and FSH among their isoelectric variants by shifting the distribution of LH and FSH isohormones towards basic and acidic components, respectively. Although these changes are subtle, they may be required to maintain normal testosterone production by the abdominal testes and may be a physiological response aimed at maintaining normal spermatogenesis.

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