Plasma and pituitary gonadotrophins before and after LRH in normal, cryptorchid and castrated rats

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Abstract. Rats were either castrated or made cryptorchid at 1, 8, 21 or 49 days of age. Sham-operated animals were used as controls. At 70 days, all the animals were anaesthetized and then injected with saline or 100 ng of LRH (time 0). Blood samples were collected before and after the injection (time -15, 0, 8, 15, 45 min), and the animals were killed by decapitation. Plasma LH and FSH were measured by radioimmunoassays (RIA) and pituitary gonadotrophins were measured by bioassays and RIA.

Irrespective of the age of surgery, plasma gonadotrophin levels of cryptorchid rats were elevated: they were nearly tripled for LH and doubled for FSH but they always remained distinctly below those of castrated rats. The LH response to LRH injection was higher in cryptorchid rats than in controls, but this response was lower than in castrated rats. Plasma FSH response to LRH injection depended on the age of surgery and FSH levels were not always significantly increased.

In rats operated on at 49 days, following cryptorchidism, or castration, pituitary gonadotrophin content was increased. The highest value was found in castrated rats. After LRH injection pituitary LH content was unchanged in controls but decreased in other animals, pituitary FSH content was decreased in castrated rats and unchanged in cryptorchid rats.

These results showed, in the adult rat that at any time of operative cryptorchidism between 1 and 49 days of age, a negative feedback control persisted, exerted by the testicular system on the hypothalamo-hypophyseal system as regards the secretion of both LH and FSH.

Experimentally induced cryptorchidism resulted generally in an increase in plasma gonadotrophin levels, the magnitude of which depended on the age of surgery and postoperative delays (Amatayakul et al. 1971; Gomes & Jain 1976; Grizard et al. 1979; Grizard et al. 1980). The increase of plasma gonadotrophin levels was related to the decrease of negative feedback control produced by testicular factors on the pituitary (Blanc et al. 1978; Denef & Hautekeete 1978; Gupta et al. 1975) since cryptorchidism was accompanied by a progressive loss of germinal epithelium, and in some cases there was also a steroidogenic alteration.

To determine the capacity of the cryptorchid rat pituitary to produce LH and FSH, both plasma and pituitary gonadotrophins were measured before and after LRH in adult rats made cryptorchid at different stages of sexual development. The results have been compared with those obtained in rats which were castrated at identical ages.

Materials and Methods

Animals

Male Sprague Dawley rats were used. The animals were kept under controlled light (12 h light from 6.00 a.m. to 6.00 p.m.) and temperature (22 ± 2°C) conditions – laboratory chow and tap water were provided ad libitum.

The animals were divided into four groups, which had been operated on at 1, 8, 21 and 49 days, respectively. Each group was composed of 3 sub-groups: sham-operated, cryptorchid and castrated animals. Bilateral cryptorchidism and bilateral castration were produced in ether-anæsthetized rats. Artificial cryptorchidism was made by translocation of the testes into the abdominal cavity by means of an abdominal incision. Both testes were pushed into the body cavity and anchored by suturing the tunica albuginea to the peritoneum at the level of the apical zone of the kidney (Vera Cruz et al. 1970). In the control group, the rats were subjected to a sham-operation.
involving anaesthesia, mid-ventral incision and retracting the testes into the body cavity before returning them to the scrotum.

LRH stimulation test
At 70 days, the rats were anaesthetized with urethane (160 mg/100 g body weight, ip). Two hours before the beginning of LRH stimulation, heparinized cannulae were inserted towards the heart; one cannula inserted into the carotid artery was used for blood withdrawal, the other cannula implanted into the opposite jugular vein was used for intrajugular injection. All the animals operated at 1.8, 21 days and one part of animals operated at 49 days were injected with 100 ng of synthetic LRH (provided by the Roussel Laboratories, France) in 0.2 ml of saline. The other animals operated at 49 days were injected with saline.

Our usual schedule of blood withdrawals and injection was as follows: a baseline sample was followed 15 min later by a second sample immediately preceding the injection. Successive samples were then taken 8 min after injection and another 7 min later. Then 30 min later the animals were killed by decapitation and trunk blood was collected. To prevent bleeding caused by several blood withdrawals, animals were injected with 1 ml of heparinized succedaneous plasma substance (Haemaccel, Laboratories Hoeschst) 15 min before and 8 min after the injection of the test substance, and 0.2 ml of heparinized succedaneous plasma substance, just before, and 15 min after the test injection.

When the animals were decapitated, the testes, ventral prostate, seminal vesicles and anterior pituitary were dissected and weighed. The pituitary and plasma were stored at -20°C until the estimation of gonadotrophins.

Assays of LH and FSH
Pituitary: Just before the assay, the anterior pituitary of each animal was homogenized in ice-cold saline (5 ml/pituitary). LH and FSH radioimmunoassays (RIA) were performed in each pituitary homogenate. Gonadotrophin bioassays were performed with the pool of pituitary homogenates of each sub-group of animals.

Plasma: In each sub-group and every time, the plasma of 3 animals were pooled to determine LH and FSH by RIA.

For the RIA a double antibody method (André et al., 1976) and RIA kits for the determination of rat LH and FSH (supplied by the Rat Pituitary Program, NIAMD) were used. LH and FSH were expressed in terms of nanograms of NIAMDD rat LHRP-1 and nanograms of NIAMDD rat FSHRP-1. Our assay system could detect as low as 5 ng/ml of LHRP-1 and 70 ng/ml of FSHRP-1. The intra-assay coefficients of variation were respectively 5 ± 1% for LH and 3.0 ± 1.5% for FSH.

Details of the method of bioassays for pituitary LH and FSH as adapted in our laboratory have been published previously (Grizzard et al. 1975, 1978). The results were expressed in terms of oL.H-M4 [1 µg oL.H-M4 = 1.83 (1.54—2.48) µg NIH-LH-S1] and in terms of oFSH-M4 [1 µg oFSH-M4 = 14.0 (9.9—18.2) µg NIH-FSH-S1].

Statistical methods
Comparison of means between different groups or sub-groups was performed by means of an analysis of variance and the significance level was set at 0.05.

Results

Tests and sex reproductive organs weights (relative to body weight)

Data are shown in Table 1 and Fig. 1. In rats injected with LRH, the cryptorchid testes weights less than those of sham-operated animals (P < 0.001); the lowest and highest weights were observed in rats made cryptorchid at 1 and 49 days, respectively.

In animals made cryptorchid at 1, 8, 21 and 49 days and injected with LRH, the ventral prostate weight and seminal vesicle weight were lower than control values.

In animals made cryptorchid at 1 and 8 days, ventral prostate weight and seminal vesicle weight were lower (P < 0.05) than those of animals operated at 49 days.

Ventral prostate weight of rats made cryptorchid at 21 days was heavier than that of animals operated at 1 and 8 days but it was lower than that of rat operated at 49 days (P < 0.05). In animals castrated at 1, 8 and 21 days these sex reproductive organs weights were not significantly different, but they were lower than those of animals castrated at 49 days (P < 0.001).

Basal plasma levels of LH and FSH

In every sub-group, the two baseline values of LH or FSH (determined 15 min and just before the injection of LRH) were not significantly different, also in Tables 2 and 3, only the basal values determined just before the injection were reported. Irrespective of the age of surgery, in cryptorchid rats the levels of plasma LH and FSH were higher than those of control rats (P < 0.001): nearly tripped for LH and nearly doubled for FSH. In castrated animals, concentrations of plasma LH and FSH were higher than those of control rats (they were respectively increased about 20-fold
Table 1.

Body weight and relative testicular weight to body weight (mean ± SD) in 70-days old rats made sham-operated cryptorchid and castrated at different ages.

<table>
<thead>
<tr>
<th>Age (days) of surgery</th>
<th>Body weight (g)</th>
<th>Relative testicular weight (mg/100 g body weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>Injection</td>
<td>LRH</td>
<td>LRH</td>
</tr>
<tr>
<td>Sham-operated (≥ 10)</td>
<td>303 ± 14</td>
<td>329 ± 14</td>
</tr>
<tr>
<td>Cryptorchid (≥ 10)</td>
<td>293 ± 31</td>
<td>343 ± 23a</td>
</tr>
<tr>
<td>Castrated (≥ 12)</td>
<td>251 ± 13a</td>
<td>285 ± 14a</td>
</tr>
</tbody>
</table>

The number of animals in each sub-group is indicated in brackets.

a: P < 0.01 as compared with the appropriate sham-operated group.
b: P < 0.001 as compared with the groups made cryptorchid at 8, 21, 49 days of age.
c: P < 0.001 as compared with the groups made cryptorchid at 8, 21 days of age.
and 4-fold) and those cryptorchid rats (respectively 6.5-fold and 2-fold).

In the 3 sub-groups of animals operated at 1 day, basal level of LH was more elevated than in other corresponding sub-groups of animals operated at 8, 21 and 49 days. On the other hand FSH was generally greater in rats operated at 21 and 49 days than in two other groups.
Table 2.
Basal plasma LH levels and response of LH to saline and LRH injections in 70-days old rats made sham-operated, cryptorchid and castrated at different ages. Mean data ± SD from several determinations are shown. Each determination is made on a pool of three plasmas.

<table>
<thead>
<tr>
<th>Age at surgery (days)</th>
<th>Sham-operated</th>
<th>Cryptorchids</th>
<th>Castrated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Basal</td>
<td>Δ</td>
</tr>
<tr>
<td>8</td>
<td>15</td>
<td>33</td>
<td>45</td>
</tr>
<tr>
<td>8</td>
<td>15</td>
<td>38a</td>
<td>28</td>
</tr>
<tr>
<td>LRH</td>
<td>± 10</td>
<td>± 11</td>
<td>± 100</td>
</tr>
<tr>
<td>21</td>
<td>12</td>
<td>19a</td>
<td>51</td>
</tr>
<tr>
<td>LRH</td>
<td>± 9</td>
<td>± 17</td>
<td>± 134</td>
</tr>
<tr>
<td>49</td>
<td>12</td>
<td>19a</td>
<td>76</td>
</tr>
<tr>
<td>LRH</td>
<td>± 3</td>
<td>± 8</td>
<td>± 78</td>
</tr>
<tr>
<td>49</td>
<td>9</td>
<td>18</td>
<td>-7</td>
</tr>
<tr>
<td>Saline</td>
<td>± 4</td>
<td>± 3</td>
<td>± 13</td>
</tr>
</tbody>
</table>

n: number of animals in each sub-group.
Δ: difference between plasma levels obtained 45 min after the injection and basal plasma levels.
Δ%: % change from the basal level determined 8 and 15 min after the injection.
*: significantly different from the value (Δ%) determined at the same time following LRH injection in appropriate sham-operated group.
a, b, c: significantly different from the values determined in animals respectively operated on at 1, 8, 21 days of age.
Table 3.

Basal plasma FSH levels and response of FSH to saline and LRH injections in 70-days old rats made sham-operated, cryptorchid and castrated at different ages. Mean data ± SD from several determinations are shown. Each determination is made on a pool of three plasmas.

| Age at surgery (days) | Sham-operated | | Cryptorchids | | Castrated |
|-----------------------|---------------|----------------|-------------|----------------|
| Injection             | n  | Basal | Δ | Δ% | n  | Basal | Δ | Δ% | n  | Basal | Δ | Δ% |
| 1                     | 15 | 238   | 44 | 11 | 33 | 12 | 601 | 106 | -1 | 1* | 15 | 1129 | 84 | -10 | 0* |
| LRH                   | ± 14 | ± 54 | ± 29 | ± 29 | ± 29 | ± 42 | ± 15 | ± 10 | ± 191 | ± 109 | ± 10 | ± 10 |
| 8                     | 18 | 315a  | 33 | 21 | 15 | 9  | 458 | 87  | 30a | 16 | 18 | 1126 | 173 | -13 | 1* |
| LRH                   | ± 79 | ± 85 | ± 39 | ± 45 | ± 32 | ± 48 | ± 22 | ± 4 | ± 287 | ± 195 | ± 12 | ± 19 |
| 21                    | 12 | 345a  | 244a,b | 11 | 13 | 18 | 637b | 254a,b | 7 | 11 | 12 | 1548a,b | 341 | -1 | -5* |
| LRH                   | ± 54 | ± 254 | ± 13 | ± 11 | ± 116 | ± 122 | ± 17 | ± 13 | ± 177 | ± 99 | ± 11 | ± 8 |
| 49                    | 12 | 369a  | 149a,b | -7 | 12 | 15 | 632b | 284a,b | 2b | 6 | 15 | 1488a,b | 705a,b,c | 5- | 4 |
| LRH                   | ± 41 | ± 38 | ± 7 | ± 19 | ± 93 | ± 130 | ± 19 | ± 24 | ± 180 | ± 247 | ± 7 | ± 16 |
| Saline                | ± 18 | ± 37 | ± 7 | ± 4 | ± 77 | ± 46 | ± 7 | ± 12 | ± 230 | ± 164 | ± 13 | ± 17 |

For legend: see Table 2.
Table 4.
Pituitary content of LH and FSH after injection of saline or LRH in 70-days old rats made sham-operated, cryptorchid or castrated at 49 days of age.

<table>
<thead>
<tr>
<th>Pituitary Content</th>
<th>LH</th>
<th>FSH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sham-operated</td>
<td>Cryptorchid</td>
</tr>
<tr>
<td></td>
<td>Saline (8)</td>
<td>LRH (14)</td>
</tr>
<tr>
<td></td>
<td>145 ± 35</td>
<td>185 ± 53</td>
</tr>
<tr>
<td></td>
<td>126 ± 37</td>
<td>144 ± 23</td>
</tr>
<tr>
<td>RIA 10</td>
<td>10</td>
<td>71a</td>
</tr>
<tr>
<td></td>
<td>24–45</td>
<td>25–49</td>
</tr>
</tbody>
</table>

n: number of animals used is in brackets.

*: P < 0.05;  **P < 0.001 as compared with the appropriate group injected with saline.
a: P < 0.01 as compared with the sham-operated group injected with saline.
b: P < 0.001 as compared with the cryptorchid group injected with saline.

RIA: radioimmunological assay (mean ± sd) in μg LHRP/pituitary and μg FSH RP/pituitary.

BIO: biological assay (mean with limits) in μg oLH-M4/pituitary and μg oFSH-M4/pituitary.
Plasma levels of LH and FSH after saline or LRH injections

Results are shown in Tables 2 and 3. In sham-operated, cryptorchid and castrated rats, plasma LH and plasma FSH levels were not significantly altered after saline injection, except plasma FSH level in sham-operated rats which was decreased 8 and 15 min after the injection.

In all the sub-groups, plasma LH levels were increased ($P < 0.05$) 8 min after the injection of LRH, and the greatest levels were reached 45 min after the administration of the releasing-factor ($P < 0.001$). Plasma FSH levels were increased ($P < 0.05$) 45 min after LRH injection only in the groups of animals operated on at 21 and 49 days (sham-operation, cryptorchidism and castration).

In the four groups studied, the response of plasma LH to LRH, expressed as the difference between plasma level obtained 45 min after the LRH injection and basal plasma level ($\Delta$LH), was greater in castrated and cryptorchid rats than in controls. However, for cryptorchid rats this value was lower than for castrated rats. In general, at 8, 15 and 45 min after LRH injection no significant difference existed in response, expressed in percentage change from basal level ($\Delta$% LH), when control, cryptorchid and castrated animals were compared. There was, however a significant difference for animals made cryptorchid at 49 days for which ($\Delta$% LH) was always lower than for controls ($P < 0.05$).

The response of plasma LH to LRH ($\Delta$LH or $\Delta$% LH) was weaker in animals castrated at 1 day than in the three other sub-groups.

For animals castrated at 49 days, the response of plasma FSH to LRH ($\Delta$FSH) was greater ($P < 0.05$) than for control and cryptorchid rats operated on at the same ages, but in other groups $\Delta$FSH in control, cryptorchid and castrated rats were not different. In the four groups, relative responses of FSH ($\Delta$% FSH) determined at 8, 15 and 45 min after LRH injection were not different in control, cryptorchid and castrated animals except 15 min after LRH injection in rats made cryptorchid at 1 day and in castrated rats operated on at 1, 8 and 21 days.

In sham-operated and cryptorchid rats operated at 21 and 49 days, $\Delta$FSH was greater than in rats operated at 1 and 8 days and in rats castrated at 49 days, $\Delta$FSH and $\Delta$% FSH were more elevated than in other groups.

Pituitary levels of LH and FSH

Pituitary content of gonadotrophin evaluated, after saline injection, with bioassay and radioimmunoassay was higher ($P < 0.01$) in cryptorchid or castrated animals than in controls. Moreover, immunological pituitary content of LH and FSH in cryptorchid rats was lower ($P < 0.001$) than in castrated rats.

LRH injection resulted in a decrease of pituitary content of LH in cryptorchid and castrated rats. Following LRH injection, pituitary content of FSH was increased in controls, unchanged in cryptorchid rats and decreased in castrated rats.

Discussion

The increase in plasma gonadotrophin levels observed in adult rats after castration, has been reported by other authors. This has been shown for rats castrated either at birth (Goomer et al. 1977) at 21 days of age (Swerdlow et al. 1971) or after puberty (Blackwell & Amoss 1971; Badger et al. 1978). The relative increase in LH was greater than FSH, irrespective of age at castration, since baseline values were increased 20-fold and 4-fold, respectively.

Cryptorchid rats operated on when 1 day old, or before and after puberty and subsequently killed when 70 days old, were found to have distinctly lower plasma gonadotrophin levels than was seen in castrated rats operated on at identical ages, since plasma LH and FSH levels were about 15% and 25%, respectively, of values obtained after castration. Additional studies with rats subjected to cryptorchidism when 21 days old, where the post-operative period varied between 14 and 70 days; or with rats subjected to cryptorchidism after puberty, where the post-operative period varied between 2 and 36 days, have shown that plasma LH and FSH levels were consistently lower than in castrated rats (Swerdlow et al. 1971; Amatayakul et al. 1971; Gomes & Jain 1976). These results show the persistence of negative feedback control exerted by testicular factors on the hypothalamo-hypophyseal system in adult cryptorchid rats. In contrast, Pelletier et al. 1977 have shown that plasma LH levels were the same for adult rams having undergone cryptorchidism or castration when 2 weeks old and suggest that before 19 weeks of age, there is a factor originating from the tubules which prompts
the ultimate maturation of the hypothalamo-hypophyseal system so far as LH release in concerned.

In cryptorchid rats, increased plasma FSH probably results in degeneration of the testicular germinal epithelium. Similar effects have been reported after testicular damage induced either by irradiation, heat or busulphan treatment (Aafjes et al. 1978; Debeljuk et al. 1973; Hopkinson et al. 1978; Main et al. 1976). According to these authors, plasma FSH levels are probably increased because of diminished secretion of a substance called 'inhibin' which controls the release of FSH in normal animals.

The increase in plasma LH levels may well be a consequence of degenerated testicular germinal epithelium, but could also be provoked by Leydig cell dysfunction. The suggestion that the quantity and type of testicular androgen secretion are modified in cryptorchid testis is supported by the observed decreases in prostate and seminal vesicle weights, in circulating testosterone and dihydrotestosterone levels (Amatayakul et al. 1971; Gupta et al. 1975) and in enzyme activity associated with androgen biosynthesis (Inano & Tamaoki 1968). Furthermore, stimulation of the cryptorchid testis with hCG results in an abnormal increase in serum testosterone levels (Kerr et al. 1979).

In our experimental conditions, the plasma LH level was unchanged following following saline injection in sham-operated, cryptorchid or castrated rats, consequently the increase of plasma LH to the LRH injection was probably specific to this one. Moreover, Masken et al. (1976) have previously reported in female rats that urethane does not affect pituitary responsiveness to LRH. Plasma LH profile following LRH injection agree with previous studies in anaesthetized male rats (Lotz 1975; Viguier-Martinez 1976). However, any differences related to the LH pattern were found with rats injected with LRH according to other experimental methods (André et al. 1979); these discrepancies are probably due to differences in methodology (anaesthesia, mode of injection).

The increase in plasma LH levels shows the ability of the pituitary in adult cryptorchid rats to secrete LH in response to an iv injection of LRH. Verjans & Eik-Nes 1976 have also noted that after X-irradiation of the testes in rats, the increment in circulating LH following LRH injection was higher than in controls but lower than in castrated rats and the relative response measured 15 and 60 min after LRH injection appears normal in the X-ray treated rats. Regardless of age of cryptorchidism, the early releasable pituitary LH storage and/or the susceptibility of the pituitary to release LH following LRH injection is intermediate between those observed for control and castrated rats. Also, there is a good relationship between basal plasma LH levels and the increase of LH to LRH as shows the relative response which is identical, generally, in all the animals, except in rats made cryptorchid at 49 days of age in which the release of LH to LRH is lower than in other groups.

In our study plasma FSH levels following LRH administration were either unchanged or became slightly increased only 45 min after the injection in control, cryptorchid and castrated rats. The finding that patterns of FSH and LH release are different has been previously described for normal rats (Gupta & Eichner 1977), castrated rats (Debeljuk et al. 1974) and testicular X-irradiated rats (Verjans & Eik-Nes 1976). The similar response of FSH to LRH in sham-operated, cryptorchid or castrated rats can be partly explained by the experimental conditions i.e. the amount of LRH injected, the delay between injection and sacrifice of animals being perhaps too short to allow significant modifications to appear.

The increase in gonadotrophins, both for pituitary content and plasma levels, following cryptorchidism and castration is probably related to increased synthesis of these hormones since the negative feedback control due to testicular factors has been decreased or suppressed.

The release of LH and FSH into the blood stream after LRH injection might explain the decrease in pituitary LH and FSH content in castrated rats, and the fall in pituitary LH content in cryptorchid rat. However after LRH injection pituitary content of both LH and FSH in controls and pituitary FSH content in cryptorchid rats are unchanged or increased. Similar results have been previously reported in intact rats (Debeljuk et al. 1972). The release of gonadotrophins is perhaps not sufficient to provoke a significant modification in pituitary content, and/or the synthesis of gonadotrophins is stimulated either under the action of LRH or in consequence to the release of these hormones.

It can be concluded from this study that (1) Irrespective of the time of surgery plasma gonadotrophin levels were increased in cryptorchid rats, but these levels always remained below those observed in castrated rats. Similar results were ob-
tained with the LH response to LRH injection expressed as the difference between the level measured 45 min after LRH injection and basal level. However, the relative response determined 8, 15 and 45 min after LRH injection was generally the same in cryptorchid rats and controls, except in animals made cryptorchid at 49 days where it was smaller than in controls. The FSH response to LRH was unchanged or slightly increased.

(2) The pituitary FSH and LH content in 70-days old animals made cryptorchid at 49 days was higher than in controls, but lower than in castrated rats. In these animals LRH injection caused a decrease in pituitary LH content but it did not alter pituitary FSH content.

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