Orchidectomy selectively increases follicle-stimulating hormone secretion in gonadotropin-releasing hormone antagonist-treated male rats

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The pituitary component of the feedback mechanisms exerted by testicular factors on gonadotropin secretion was analyzed in adult male rats treated with a potent gonadotropin-releasing hormone (GnRH) antagonist. In order to discriminate between androgens and testicular peptides, groups of males were orchidectomized (to eliminate androgens) and injected with ethylene dimethane sulfonate (EDS), a selective toxin for Leydig cells (to eliminate selectively androgens) and treated for 15 days with vehicle or the GnRH antagonist Ac-u-pClPhe-pClPhe-u-Trp-Ser-Tyr-u-Arg-Leu-Arg-Pro-u-Alk-NH2CH3COOH (Org.30276. 5 mg/kg/72 hours). Serum concentrations of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) were measured 7 and 14 days after the beginning of treatment. We found that in males treated with GnRH antagonist, orchidectomy or EDS treatment did not induce any increase in LH secretion; and orchidectomy, but not EDS treatment, increased FSH secretion in GnRH-treated males. The present results show that negative feedback of testicular factors on LH secretion is mediated completely through changes in GnRH actions. In contrast, a part of the inhibitory action of the testis on FSH secretion is exerted directly at the pituitary level. It can be hypothesized that non-Leydig cell testicular factor(s) inputs at different levels of the hypothalamic–pituitary axis in controlling LH and FSH secretion.

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The synthesis and secretion of gonadotropins is the final result of the interactions between central and peripheral signals. Hypothalamic GnRH stimulates the synthesis and secretion of FSH and LH (1). Testis inhibits gonadotropin secretion, as revealed by FSH and LH increase after orchidectomy (2–4). Testicular factors involved in the regulation of gonadotropin secretion include androgens and peptides (inhibins, activins and follistatin) (5, 6). These factors target their actions to hypothalamus and pituitary in controlling FSH and LH secretion (6, 7).

In order to discriminate between the site of action of testicular factors, two different experimental approaches have been used: analysis of in vitro gonadotropin secretion by pituitaries challenged by androgens and/or peptides (7–11); and in vivo models with impaired GnRH actions by administration of potent GnRH antagonists (12–18) or hypothalamic deafferentation (19). In addition, the existence of specific antiandrogens, such as flutamide (20) or selective toxins, against Leydig cells, such as ethylene dimethane sulphonate (EDS) (21, 22), has allowed the effects of androgens and peptides in the control of gonadotropin secretion to be differentiated (23).

Testicular androgens acting at the hypothalamic level decrease FSH and LH secretion (1). However, in vitro testosterone enhances FSH secretion and β-FSH gene expression by perfused pituitary cells (24–26); in vivo a similar stimulatory effect has been observed in males, where the action of GnRH was blocked with GnRH antagonist (12–18). These direct actions of androgens at the pituitary level were not observed for LH (12–14). Inhibins and activins control FSH secretion, acting mainly at the pituitary level (27, 28). Although the lack of action of testicular peptides on LH secretion has been reported, GnRH-induced LH secretion can be modulated by inhibin (7, 29).

We have proposed recently that non-Leydig cell non-androgenic testicular factor(s) modulates LH and FSH secretion (23). In order to elucidate the site(s) of action of this factor(s) on the hypothalamic–pituitary axis in controlling LH and FSH secretion, we analyzed the effects of orchidectomy (which eliminates androgens and testicular peptides) and EDS treatment (which eliminates exclusively androgens) on gonadotropin secretion in males with or without a GnRH clamp. The actions of non-androgenic testicular factor(s) would be blocked by a GnRH clamp if they...
were mediated exclusively through changes in GnRH actions.

Materials and methods

Animals and drugs

Wistar male rats (300–325 g) were maintained under controlled conditions of light (12 h of light, 12 h of darkness) and temperature (21–22°C), with free access to pelleted food (Pasca Sanders, Seville, Spain) and tap water.

The GnRH antagonist Org.30276 (Ac-D-pClPhe-D-pClPhe-D-Trp-Ser-Tyr-D-Arg-Leu-Arg-Pro-D-Ala-NH₃ COOH), kindly supplied by Organon (Oss, The Netherlands), was dissolved in physiological saline. Ethylene dimethane sulfonate was synthesized as described previously (21, 22), dissolved in dimethylsulfoxide (DMSO) and water (1:3) and injected in a single dose of 75 mg/kg body wt (ip).

Experimental designs

Experiment 1. In order to stimulate the duration of
gonadotropin secretion blockade, intact male rats were injected ip with a single dose of 5 mg/kg body wt of GnRH antagonist. Blood samples were obtained by jugular venipuncture under light ether anesthesia 0, 8, 24, 48 and 72 h after injection.

Experiment 2. To check the effectiveness of GnRH blockade after a single GnRH antagonist injection in a 72-h period, six groups of male rats (10 animals/each, 5-7 days old) received the following treatments for 15 days: vehicle (controls), orchidectomy, EDS, GnRH antagonist (5 mg/kg body wt each 72 h), orchidectomy + GnRH antagonist, EDS + GnRH antagonist. The injected doses of GnRH antagonist are higher than described previously as being needed to abolish GnRH action (17). The dose of EDS used has been reported to destroy completely the Leydig cells (23).

Blood samples were taken by jugular venipuncture after light ether anesthesia on days 7 and 14 (2 h after the corresponding injection of GnRH antagonist or vehicle). At the end of the experiment, animals were decapitated, pituitaries collected for LH and FSH measurement and the weight of testis and sexual accessory gland recorded. Pituitaries were dissected, weighed and homogenized in 1 ml of physiological saline containing urea (2.5 mol/l) and subjected to ultrasonic treatment (30). Samples were centrifuged for 20 min at 2800 g and the supernatant was frozen at −25°C until analysis for hormone content.

Hormone measurements

After centrifugation, serum was collected, frozen and stored at −20°C until use. The concentrations of FSH and LH were determined by a double RIA method using kits supplied by the NIDDK (Bethesda, MD). Rat FSH-I-6

Fig. 1. The LH and FSH serum concentrations in adult male rats at different hours after the administration of GnRH antagonist (5 mg/kg). Values are given as means ± SEM (the SEM is not given when the bar is smaller than the symbols) of the 10 animals; **p ≤ 0.01 compared to preinjection levels (ANOVA followed by Tukey's test) (Data from Experiment 1.)

Fig. 2. The LH (open circles) and FSH (solid circles) serum concentrations in adult intact male rats at different days after the administration of GnRH antagonist (5 mg/kg every 72 h). Values are given as means ± SEM (the SEM is not given when the bar is smaller than the symbols) of 10 animals; **p ≤ 0.01 compared to preinjection levels (ANOVA followed by Tukey's test). Note the different scale for LH and FSH levels. (Data from Experiment 2.)
and rat LH-I-6 were labeled with $^{125}$I by the chloramine T method (31). The FSH and LH concentrations are expressed using FSH-RP-2 and LH-RP-3 as standards. All samples were measured in duplicate in the same assay; the intra-assay variations were 7% and 6%, respectively. The sensitivities were 20 and 3.5 pg/tube for FSH and LH.

**Statistics**

Data are expressed as the mean ± SEM. Statistically significant differences between groups were determined by the analysis of variance (ANOVA) followed by Tukey's test.

**Results**

In Experiment 1, serum concentrations of LH and FSH remained significantly lower (p < 0.01) than controls during the 72-h period following the GnRH antagonist injection (Fig. 1). In Experiment 2, serum LH and FSH concentrations decreased (p < 0.01) by 88% and 75%, respectively, 7 days after treatment with GnRH antagonist and by 93% for both gonadotropins 14 days after treatment (Fig. 2). At the end of the treatment the pituitary contents of FSH was decreased (p < 0.05) (1.12 ± 0.08 µg/gland vs. 1.99 ± 0.24 in the vehicle-injected group), while that of LH remained unchanged (0.50 ± 0.06 µg/gland vs. 0.62 ± 0.07 in the vehicle-injected group).

Testis weight was decreased significantly (p < 0.01) in males injected with EDS. Administration of GnRH antagonist reduced testicular weight (p < 0.05) in control males and induced a further decrease in testis weight in EDS-treated males (p < 0.05). The weights of prostate and seminal vesicles were reduced significantly (p < 0.01) after orchidectomy, treatment with GnRH antagonist or EDS injection (Table 1).

The effect of orchidectomy and GnRH antagonist administration is shown in Fig. 3. Male rats injected with vehicle showed significantly increased serum concentrations of LH and FSH 7 and 14 days after orchidectomy (p < 0.01). In contrast, serum concentration of FSH but not of LH was increased significantly by orchidectomy in males injected with GnRH antagonist (p < 0.01). The FSH and LH concentrations were lower (p < 0.01) in intact and orchidectomized rats injected with GnRH antagonist than in those injected with vehicle.

The effect of EDS treatment and GnRH administration is shown in Fig. 4. Males injected with vehicle showed significantly increased serum concentrations of LH and

![Table 1. Effect of GnRH blockade on the relative weights (mg%,g body weight) of testes and accessory sex organs.]

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Testes</th>
<th>Prostate</th>
<th>Seminal vesicle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control + vehicle</td>
<td>813 ± 16</td>
<td>89.3 ± 5.1</td>
<td>98.0 ± 5.5</td>
</tr>
<tr>
<td>Control + GnRH antagonist</td>
<td>719 ± 37*</td>
<td>23.2 ± 2.9**</td>
<td>44.1 ± 4.3**</td>
</tr>
<tr>
<td>Orchidectomized + vehicle</td>
<td>-</td>
<td>12.4 ± 0.9**</td>
<td>31.7 ± 1.5**</td>
</tr>
<tr>
<td>Orchidectomized + GnRH antagonist</td>
<td>-</td>
<td>14.1 ± 1.5**</td>
<td>31.8 ± 1.2**</td>
</tr>
<tr>
<td>EDS + vehicle</td>
<td>412 ± 22**</td>
<td>15.9 ± 1.0**</td>
<td>34.8 ± 2.8**</td>
</tr>
<tr>
<td>EDS + GnRH antagonist</td>
<td>265 ± 24***</td>
<td>16.5 ± 1.0**</td>
<td>35.0 ± 1.6**</td>
</tr>
</tbody>
</table>

*Values are the mean ± SEM (N = 8–10/group). *p < 0.05 and **p < 0.01 vs control males injected with vehicle.

*b: GnRH: ethyle dimethane sulfonate.

c: p < 0.05 vs EDS-treated males injected with vehicle.
FSH 7 and 14 days after EDS treatment \((p \leq 0.01)\). In contrast, serum concentrations of FSH and LH were not increased significantly by EDS treatment in males rats injected with GnRH antagonist. The FSH and LH concentrations were lower \((p \leq 0.01)\) in intact and EDS-treated males injected with GnRH antagonist than in those injected with vehicle.

Thus, results from Experiment 2 show that GnRH antagonist treatment completed blocked the increase in LH secretion caused by orchidectomy and the increase in LH and FSH secretion caused by EDS administration.

**Discussion**

The present study extends our previous data about the control of gonadotropin secretion in EDS-treated male rats (23). The main observations are: in male rats with GnRH action blockade, orchidectomy or EDS treatment was unable to increase LH secretion; and orchidectomy but not EDS treatment increased FSH secretion in males with a GnRH clamp. The dose of GnRH antagonist used to induce GnRH blockade is higher than reported previously (17) and was able to reduce gonadotropin secretion in terms of 80% from controls 1 week after beginning the treatment. This strong reduction ensures the effectiveness of the GnRH clamp. Although it has been reported that GnRH antagonist suppresses LH more than FSH secretion (32), our results showed a similar effectiveness on the blockade of both gonadotropins. The decreased content in pituitary FSH agrees with previous references that point out the reduction of \(\beta\)-FSH mRNA and immunoreactive FSH in GnRH antagonist-treated males (33). Also, a reduction of \(\beta\)-LH mRNA after administration of GnRH antagonist (17) or GnRH agonist-induced desensitization (34) has been reported.

Serum LH levels in males treated with GnRH antagonist were similar in presence or absence of testis, which suggests that the inhibitory action of the testis on LH secretion is mediated completely through changes in GnRH action. These findings closely agree with the prevention of the postcastration rise in serum LH after GnRH desensitization (34). If it is assumed that testicular control of LH secretion involves testosterone and non-androgenic factor(s) (23), it can be postulated that non-androgenic factor(s) modulates GnRH action in controlling LH secretion. This hypothesis is supported by the inability of EDS or orchidectomy to enhance LH secretion in males with GnRH clamp.

On the contrary, the inhibitory action of the testis on FSH release included two components: a GnRH-dependent and a GnRH-independent mechanism. The FSH serum levels were lower in males orchidectomized and treated with GnRH antagonist than in intact males, which indicates that the inhibitory effect of the testis is dependent partially on GnRH action. However, the existence of a GnRH-independent inhibitory mechanism is supported by the presence of higher FSH levels in males orchidectomized and treated with GnRH antagonist than in males treated exclusively with GnRH antagonist. Thus, a part of the inhibitory input of the testis on FSH secretion is not mediated by changes in GnRH action. The GnRH-dependent component of the inhibitory action of the testis on FSH secretion is exerted probably by testicular androgens. The GnRH-independent mechanism is probably carried out by non-androgenic factors (inhibins). The ability of orchidectomy (which removes androgens and peptides) but not of EDS treatment (which removes exclusively androgenic factors) to increase FSH levels in males treated with GnRH antagonist confirmed this view.

The increase of FSH after orchidectomy in males treated with GnRH antagonist suggests that non-androgenic testicular factor(s) involved in the negative control of FSH (inhibin) is produced and secreted even when the secretion of gonadotropins is reduced by GnRH antagonist. A possible explanation for this phenomenon is that this factor (inhibin) is released.
with a certain degree of autonomy from pituitary secretion. In this sense, it has been shown that after EDS treatment inhibin increases even if FSH levels are lowered experimentally by exogenous administration of testosterone to EDS-treated animals (35). Another possibility is that this factor can be secreted under the control of low amounts of gonadotropins, because FSH and LH did not disappear completely after GnRH antagonist treatment.

The results presented herein are in agreement with previous references pointing out that inhibin acts mainly at the pituitary level in controlling FSH secretion (27, 28), although central actions of inhibins also have been reported (36) and cannot be ruled out by this experimental approach. Because the existence of a specific FSH-releasing factor has been suggested repeatedly (37, 38), the selective increase in FSH secretion after orchidectomy in male rats treated with a GnRH antagonist may be due to the release of a specific FSH-releasing factor. Alternatively, the pituitary secretion of FSH isoforms with higher half-lives in the absence of testis (39) also should be considered.

It has been reported previously that after blockade of GnRH action, testosterone increased FSH secretion through a direct pituitary action (12–16). Based on these findings, it would be expected that in males treated with GnRH antagonist the destruction of Leydig cells decreased FSH levels. This was not the case, probably because testosterone secretion was reduced similarly by GnRH antagonist, EDS or orchidectomy. In males treated with GnRH antagonist, EDS or orchidectomy did not result in further decrease in testosterone secretion, as revealed by the weights of sexual accessory glands, and so EDS treatment did not change FSH secretion in males with a GnRH clamp.

In conclusion, we have shown that testicular regulation of LH secretion is mediated completely through changes in hypothalamic GnRH actions, while that of FSH included hypothalamic and pituitary effects. Non-androgenic testicular factor(s) involved in the control of gonadotropin secretion likely inputs at different levels of the hypothalamic–pituitary axis in controlling LH and FSH secretion.

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