Tamoxifen does not block the inhibitory effect of testosterone on FSH release in rats

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Abstract. The aim of the present experiments was to analyze whether the inhibitory effect exerted by testosterone on FSH release might be mediated by the intracerebral transformation of the hormone into oestrogenic metabolites. Advantage has been taken of the availability of the potent antiestrogen tamoxifen. Two series of experiments have been performed. In the first one, adult male rats have been castrated and submitted, beginning immediately after surgery, to a 6-day treatment with testosterone propionate (2 mg/rat/day), tamoxifen (50 or 200 μg/rat/day) or testosterone propionate (2 mg/rat/day) plus tamoxifen (either 50 or 200 μg/rat/day). In the second experiment, adult male rats have been castrated and submitted to the same 6-day treatments, beginning 4 weeks following orchidectomy. In both experiments, the animals were killed 24 h after the last injection, and serum levels of FSH and LH have been measured by radioimmunoassays. The results have clearly shown that, in both experiments, the administration of testosterone results in a significant decrease of serum FSH and in a total suppression of LH release. The administration of tamoxifen, in either dose, does not modify the elevated serum FSH and LH levels present in the orchidectomized animals, and does not antagonize the inhibitory effect on FSH and LH secretion exerted by the concomitant treatment with testosterone propionate. It is concluded that testosterone inhibits FSH secretion in orchidectomized rats acting as such, and not following aromatization to oestrogens.

It is now firmly established that in the brain (especially in the hypothalamus) and in the anterior pituitary, like in the peripheral androgen-sensitive structures, testosterone is metabolized to yield 5α-androstane-17β-ol-3-one (DHT), 5α-androstane-3α,17β-diol (3α-diol) and 5α-androstane-3β,17β-diol (3β-diol) (Martini 1982). These transformations occur under the influence of an enzymatic complex, which consists of a 5α-reductase and two (3α- and 3β-)hydroxysteroid dehydrogenases. In the brain (particularly in the hypothalamus and in the limbic system), but not in the anterior pituitary, testosterone may also be metabolized via the so-called aromatization pathway, which brings to the formation of oestradiol and oestrone (Martini 1982). Receptors binding oestrogens and androgens have been found both in the brain and in the anterior pituitary (Martini 1978).

In previous experiments of this laboratory, it has been clearly demonstrated that, when administered to castrated male rats, DHT, 3α- and 3β-diol are more efficient than testosterone in decreasing serum LH levels (Zanisi et al. 1973a,b). This observation, which has been subsequently confirmed by many other authors (Martini 1982), has brought to suggest that the negative feedback effect testosterone exerts on LH secretion might be mediated by the local conversion (occurring in the brain and/or in the anterior pituitary) of the hormone into DHT and the diols. The hypothesis is also supported by the fact that men exhibiting the 5α-reductase deficiency syndrome, and who are consequently unable to transform testosterone into DHT, have constantly elevated serum levels of LH (Imperato-McGinley et al. 1974; Martini et al. 1979).

Previous work of this and other laboratories has also shown that DHT, 3α- and 3β-diol are less
effective than testosterone in inhibiting FSH release in castrated animals (Zanisi et al. 1973a,b; Verjans et al. 1974; Celotti et al. 1977). These results have brought to assign to the 5α-reduced metabolites of testosterone only a marginal role in the inhibitory control of FSH secretion. Consequently, it appears possible to suggest that the negative feedback effect testosterone exerts on FSH secretion might be due either to the hormone as such, or to the oestrogenic molecules formed in the brain via the aromatization pathway. A large group of data support the view that oestrogens are good suppressors of FSH secretion (Martini et al. 1968; Celotti et al. 1979).

The aim of the present experiments was to analyze whether the effect exerted by testosterone on FSH release might be mediated by the intracerebral transformation of the hormone into oestrogenic metabolites. Advantage has been taken of the availability of tamoxifen (TMX), a potent antioestrogen, which has been shown to antagonize the effects of oestrogens both in the peripheral and in the central neuroendocrine structures (Roy & McEwen 1979; Dix & Jordan 1980; Gogan et al. 1980). Two series of experiments have been performed. In the first one, adult male rats have been castrated and submitted, beginning immediately after surgery, to a 6-day treatment with testosterone, TMX, or testosterone plus TMX. In the second experiment, adult male rats have been castrated and submitted to the same treatments (testosterone, TMX, testosterone plus TMX) beginning 4 weeks following orchidectomy. In both experiments, the animals were sacrificed 24 h after the last injection, and serum levels of FSH and LH have been measured by radioimmunoassays. It was reasoned that the inhibitory effect of testosterone on FSH release would have been abolished or diminished by the concomitant administration of TMX, only if its effects were due to the conversion into oestrogens. In experiments similar to the present ones, TMX and other antioestrogens proved effective in abolishing the effects testosterone exerts in directing the neonatal organization of the brain toward male patterns and in facilitating male sex behaviour in adult male rats, two processes which are believed to be due to the intracerebral conversion of testosterone into oestrogens (McEwen et al. 1977; Södersten & Eneroth 1980).

**Materials and Methods**

Adult male Sprague-Dawley rats (Charles River, Italy) weighing 125–150 g were used throughout the present study. They were housed in an animal quarter with

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>FSH NIADDK-rat FSH (ng/ml)</th>
<th>LH NIH-LH-S17 (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal controls</td>
<td>281.7 ± 20.0b</td>
<td>&lt; 0.1</td>
</tr>
<tr>
<td>Castrated</td>
<td>786.7 ± 38.5c</td>
<td>4.0 ± 0.3c</td>
</tr>
<tr>
<td>Castrated controls</td>
<td>986.4 ± 46.6c</td>
<td>7.6 ± 1.5c</td>
</tr>
<tr>
<td>TP 2 mg (10)</td>
<td>260.3 ± 22.1d</td>
<td>&lt; 0.1d</td>
</tr>
<tr>
<td>TMX 50 µg (10)</td>
<td>959.9 ± 53.9</td>
<td>4.6 ± 1.3</td>
</tr>
<tr>
<td>TMX 200 µg (9)</td>
<td>950.1 ± 39.9</td>
<td>4.9 ± 0.7</td>
</tr>
<tr>
<td>TP + TMX 50 µg (10)</td>
<td>280.1 ± 15.4d</td>
<td>&lt; 0.1d</td>
</tr>
<tr>
<td>TP + TMX 200 µg (9)</td>
<td>296.9 ± 22.3d</td>
<td>&lt; 0.1d</td>
</tr>
</tbody>
</table>

a: Number of animals in parentheses.
b: Values are means ± SEM.
c: Significant $P < 0.05$ vs normal controls.
d: Significant $P < 0.05$ vs castrated controls.
controlled temperature and humidity (light schedule: 14 h light, 10 h dark; lights on at 06.30 h). The animals were fed with a standard pellet diet; water was provided ad libitum. All animals were castrated under a light ether anaesthesia. In the first experiment, the various treatments were initiated on the same day in which castration was performed. In the second experiment, the treatments were initiated 4 weeks after orchidectomy. The treatment consisted of a daily sc injection, for 6 consecutive days, of: group 1) testosterone propionate (TP) (Fluka, Switzerland) (2 mg/rat dissolved in 0.2 ml of peanut oil); groups 2) and 3) TMX base (IC-Pharma, Milano, Italy) respectively in the dose of 50 and 200 µg/rat (dissolved in 0.2 ml of a 3% mixture of absolute ethanol and distilled water); groups 4) and 5) a combined treatment with 2 mg/rat of TP associated with either 50 or 200 µg/rat of TMX. Normal rats and castrated animals treated with 0.2 ml of oil or with 0.2 ml of the vehicle used to dissolve TMX served as controls. The animals were killed with a guillotine 24 h after the last injection, and the trunk blood was collected in centrifuge tubes. Serum was kept at -20°C until FSH and LH assays. Serum FSH concentrations were assayed by the method of Daane & Parlow (1971), using the kit obtained from the Rat Pituitary Hormone Distribution Program of the NIADDK. Serum LH concentrations were assayed by the method of Niswender et al. (1968) using the anti-ovine LH (GDN 15) serum provided by Dr Niswender. Assays were carried out in 100 µl plasma samples, in duplicate. The limits of sensitivity were 10 ng FSH/ml and 0.1 ng LH/ml, respectively. Intra-assay coefficients of variation were 2.42 and 2.45% for FSH and LH, respectively. The data were statistically analyzed utilizing the Scheffé's test (Scheffé 1955) for multiple comparison after one way analysis of variance, performed utilizing an appropriate programme and a Digital MINC II computer.

Results

Table 1 summarizes the results obtained in the first experiment, and shows that, as expected, 7 days following orchidectomy serum levels of FSH are significantly higher than those of normal animals. Since the administration of the vehicle used to dissolve respectively TP and TMX did not significantly modify the serum levels of the two hormones in the orchidectomized animals, the results obtained in the two groups of vehicle-treated animals have been combined, and the results obtained in the animals receiving TP, TMX, or TP + TMX have been compared to those obtained in the combined group of controls. The sc treatment with 2 mg/rat of TP for 6 days, initiated immediately after castration, induced a significant decrease of serum FSH levels in castrated animals (Table 1). On the contrary, the

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>FSH</th>
<th>LH</th>
</tr>
</thead>
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<tr>
<td></td>
<td>NIADDK-rat FSH</td>
<td>NIH-LH-S17</td>
</tr>
<tr>
<td></td>
<td>(ng/ml)</td>
<td>(ng/ml)</td>
</tr>
<tr>
<td>Normal controls</td>
<td>(7)³</td>
<td>272.9 ± 27.5b</td>
</tr>
<tr>
<td>Castrated</td>
<td>(7)</td>
<td>1428.8 ± 67.1c</td>
</tr>
<tr>
<td>Castrated controls</td>
<td>(11)</td>
<td>1705.6 ± 151.7c</td>
</tr>
<tr>
<td>TP</td>
<td>2 mg (10)</td>
<td>898.6 ± 42.7d</td>
</tr>
<tr>
<td>TMX</td>
<td>50 µg (10)</td>
<td>1361.6 ± 86.1</td>
</tr>
<tr>
<td>TMX</td>
<td>200 µg (6)</td>
<td>1417.5 ± 65.0</td>
</tr>
<tr>
<td>TP + TMX</td>
<td>50 µg (8)</td>
<td>1003.8 ± 146.9d</td>
</tr>
<tr>
<td>TP + TMX</td>
<td>200 µg (10)</td>
<td>907.2 ± 48.8d</td>
</tr>
</tbody>
</table>

a: Number of animals in parentheses.
b: Values are means ± SEM.
c: Significant P < 0.05 vs normal controls.
d: Significant P < 0.05 vs castrated controls.
6-day treatment with either 50 or 200 µg/rat of TMX did not exert any significant effect on serum FSH levels. When TMX (at the two dose levels considered) was given together with 2 mg/rat of TP, the antioestrogen proved unable to counteract the inhibiting effect exerted by TP on FSH release. The results obtained in the animals treated with TP plus either dose of TMX were not significantly different from those found in the animals treated only with TP.

Table 1 also shows that, in normal animals, serum LH levels were below the sensitivity of the radioimmunoassay used. Serum LH obviously increased following castration, and its levels were suppressed below the detection limit of the assay by the 6-day treatment with TP. Treatment with either dose of TMX tested did not induce any significant modification of serum LH levels. Finally, in the animals treated with TP + TMX the LH levels stayed below the detection limit of the assay.

Table 2 shows the results of the second experiment, in which the animals have been submitted to castration 4 weeks before the initiation of the various treatments. It is clear that also in this experiment orchidectomy induced a marked and significant increase of serum FSH and LH levels. The levels of the two gonadotropins were considerably higher than those recorded in animals castrated since 1 week (see Table 1). Also in this experiment, the sc administration of TP (2 mg/rat) for 6 days proved capable of significantly decreasing serum FSH levels, and of completely blocking LH secretion. As in the previous experiment, the two doses (50 and 200 µg/rat) of TMX tested did not significantly alter serum levels of either FSH or LH. The administration of TMX (either in the dose of 50 µg/rat or in the dose of 200 µg/rat), in association with TP, proved unable to counteract the inhibiting effect exerted by TP on FSH and LH release.

Discussion

The present data confirm, first of all, the classical finding that orchidectomy exerts a strong stimulatory effect on LH and FSH release. The larger increase of LH and FSH secretion observed 5 weeks following castration (when compared to that obtained at one week) was also expected. The present results also confirm previous evidence indicating that the treatment of orchidectomized rats with testosterone is able to prevent (partially in the case of FSH, and totally in the case of LH) the increase of the serum levels of the two gonadotropins induced by castration (Martini 1982). The data have also clearly shown that the administration of TMX, in the two doses selected, does not significantly influence serum FSH and LH levels in orchidectomized animals. This result agrees with previous findings in the literature. Lamberts et al. (1981) have found that the administration of TMX (in the dose of 200 µg/kg) for 12 days to rats bearing an oestrogen-induced prolactin secreting pituitary tumour does not alter plasma FSH and LH levels and does not modify the pituitary content of the two gonadotropins. Bartke et al. (1978) have found that following a 5-day treatment of normal male rats with TMX (in the doses of 2 or 10 mg/rat) plasma concentrations of FSH were unmodified, while plasma LH levels were decreased. The small discrepancy with the data here presented may easily be explained by the fact that the doses of TMX used by these authors were much higher than the ones used in the present study; moreover, their experiments have been performed in normal and not in castrated animals.

It is clear from the present data that the administration of TMX is unable to modify the inhibitory effect exerted by TP on the castration-induced hypersecretion of FSH. This has been shown both in the experiment in which the treatment with TP and TMX was initiated immediately after castration, and in that in which treatment was performed in long-term castrated animals. The present data, which demonstrate that TMX does not alter the inhibitory effect of TP on the castration-induced hypersecretion of FSH, strongly suggest that testosterone inhibits the release of FSH acting as such, and not following aromatization to oestrogenic molecules. The present data obviously do not rule out the possibility that a portion of the inhibitory effect testosterone exerts on FSH secretion might be linked to its conversion into 5α-reduced androgens. However, this possibility appears a remote one since, as mentioned in the Introduction, DHT, 3α- and 3β-diol are very poor suppressors of FSH release (Martini 1982).

The fact that LH stayed below the detection limit of the assay, when TMX was injected with
TP was not unexpected since the experimental findings quoted in the Introduction strongly suggest that the inhibitory effect exerted by testosterone on LH secretion might be explained by its conversion into DHT and the diols. The present data, however, confirm that the intracerebral aromatization of testosterone does not play a significant effect in the control of LH release. The present results, which show that TMX does not alter the effects of testosterone on the gonadotropin response following castration, agree with a previous finding in the literature. Kalra & Kalra (1980) have found that nafoxidine, another potent antioestrogen, fails to block the inhibitory effect of testosterone on the release of LH and its ability to increase intrahypothalamic LRH stores.

The question may arise on whether the doses of TMX utilized in the present experiments were adequate to counteract the effects of the oestrogens formed in the neuroendocrine structures from the TP administered. This appears to be certainly the case. The doses of TMX used in the present study have been previously shown to be fully antioestrogenic. Jordan et al. (1975) have found that 50 and 200 µg of TMX (i.e., the two doses selected for this study) significantly decrease the effect of the administration of 5 µg of oestradiol on prolactin secretion and on uterine growth. Jordan et al. (1978) have found that doses of TMX between 2 and 16 µg/day are able to counteract the stimulatory effect exerted by 0.8 µg of oestradiol on the uterine weight of the immature rat. Nagy et al. (1980), de Quijada et al. (1980) and Lamberts et al. (1981) have found that the administration of TMX in doses ranging from 20 to 200 µg/kg/day significantly inhibits the growth of an oestrogen-induced prolactin-secreting rat pituitary tumour.

Acknowledgments

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