Endocrine investigations in two cases of feminizing Leydig cell tumour

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Abstract. Two patients, aged 32 and 35 years, presented with gynaecomastia and a unilateral testicular tumour which proved to be a Leydig cell tumour. Pre-operative samples taken at 08.00 h on different days showed marked elevation of plasma oestradiol in the first patient, and very slight irregular oestradiol elevation in the second, plasma oestrone within the normal range in both patients, reduced plasma testosterone in the first patient and reduced or normal testosterone in the second, and low or low-normal serum LH and FSH in both patients. One of the patients received an oral dose of 100 mg of clomiphene citrate for 3 consecutive days which induced a rise in LH and FSH and a decrease in the 17-hydroxyprogesterone/androstenedione ratio. These data suggest the inhibiting effect of endogenous hyperoestrogenism on testicular steroidogenesis owing to both the reduction of gonadotropin secretion and a direct local negative effect on C17,20-lyase. After human chorionic gonadotropin stimulation, oestradiol response was increased and abnormally prolonged, a finding which may be helpful when diagnosing a feminizing Leydig cell tumour; testosterone reached normal values. After removal of the tumoural testis, gynaecomastia regressed within a few days, gonadotropins increased, oestrogens dropped, testosterone and 5α-dihydrotestosterone normalized in one patient but remained low in the other at day 30. The Leydig cells outside the tumour appeared morphologically normal, but the count gave evidence of juxtatumoural Leydig cell hyperplasia in areas where the tumour was well encapsulated while showing a significant reduction at a distance from the tumour and in the contralateral testis by comparison with control testes. An incubation procedure with [³H]testosterone was performed in one patient and showed an aromatase activity 21 times greater in the tumour than in normal peritumoural tissue, whereas the Leydig cell number per field and the percentage of the volume occupied by Leydig cells was 11 and 35 times higher, respectively. This shows that the aromatase activity of a single tumour cell is very similar to that of a normal Leydig cell.

About one third of the 290 cases of Leydig cell tumours reported in adults are associated with a feminizing syndrome predominantly with gynaecomastia, in which tumour-induced production of oestradiol is involved (Gabrilove et al. 1975).

Two patients with feminizing Leydig cell tumour were investigated in order to study the variability of tumoural oestrogen secretion and the consequences of hyperoestrogenism on gonadotropic secretion and testicular function. In one of these patients, aromatizing activity was analysed in tumoural and peritumoural tissue and compared with Leydig cell count. Juxtatumoural Leydig cell hyperplasia was quantified in both patients.

Patients and Methods

Subjects
Patient 1*, aged 32, was hospitalized for painful right gynaecomastia which had developed within one year.

He had undergone surgery for left gynaecomastia 4 years previously. There was no abnormal testicular history for this patient, the father of two children, the younger of whom was 8. Libido was slightly diminished, but sexual potency was normal. There was no recurrence of left gynaecomastia. A well-defined tumour, about 15 mm in diameter, was palpated at the upper pole of the left testis.

Patient 2, aged 35, was hospitalized for painful bilateral gynaecomastia, which had appeared 8 months previously, and for impotence over a 3-month period. A well-defined tumour, about 15 mm in diameter, was palpated at the upper pole of the right testis.

In both patients, the contralateral testis was normal in size and consistency. The prostate, muscle development, body hair, and voice were also normal. The rest of the physical examination revealed no abnormalities.

The testis echogram confirmed the data of the clinical examination. The tumour was in both cases essentially hypoechoic. The epididymis, scrotum, and the contralateral testes were normal and there was no hydrocele.

The usual laboratory investigations and liver function tests were normal. Prolactin and the silla turca films were normal. T3, T4, and the free T4 index were normal.

hCG β, α-foetoprotein and the carcinoembryonic antigen were not detected.

In both cases, a tan-yellow tumour was found at surgery, which consisted of a homolateral orchietomy. A biopsy of the contralateral testis was also performed in patient 2.

Breast pain disappeared within a few hours after the operation and there was marked decrease of gynaecomastia by day 6 and 8. In addition to the chest films, the post-operative examinations consisted of an abdominal echogram, an iv pyelogram, a lymphogram, an abdominal CT-scan, and a scintigram of the bones, which were normal. The absence of detectable extension 7 years and 32 months, respectively, after the first symptoms argue in favour of a benign Leydig cell tumour in both patients.

**Endocrine studies**

In order to study the spontaneous variability of plasma steroid and serum gonadotropin concentrations, 17β-oestradiol (E2), oestrone (E1), testosterone (T), 5α-dihydrotestosterone (DHT), androstenedione (Δ4), LH and FSH were assayed at 08.00 h on different days, and in addition in patient 2 every 30 min on the same day from 08.00 to 14.00 h.

In the second patient, a clomiphene citrate (Clomid®) test was performed, consisting of a daily oral dose of 100 mg for 3 consecutive days. LH, FSH, T, E2, E1, Δ4 and 17-hydroxyprogesterone (17-OHP) were measured one and 4 days after the last dose.

In both patients, an hCG test was made, consisting of an im injection of human chorionic gonadotropin (5000 IU) on 3 consecutive days. T and E2 were assayed before each injection and 24 h after the third one.

All the assays were made in duplicate. FSH and LH were measured by RIA using a double-antibody separation technique. Inter-assay variations and detection limits were, respectively, 3% and 0.2 U/l for FSH, 3.5% and 0.3 U/l for LH. Results were expressed as international units per litre (FSH: second International Reference Preparation (IRP) MRC 78/549 and LH: second IRP MRC 68/40). T, DHT, Δ4, E2 and E1 were measured by RIA after column chromatography on celite (Abraham et al. 1970). Binding capacity of testosterone-oestradiol-binding globulin (TeBG) was measured as previously described (Corvol et al. 1971). Inter-assay variations and detection limits were, respectively, 10% and 0.35 nmol/l for T, DHT and Δ4, 10% and 35 pmol/l for E2, 13% and 0.05 nmol/l for E1, and 5% and 1.4 nmol/l for TeBG.

**Histologic examination**

The tumour-bearing testes were fixed with 15% formaldehyde; the sections were stained with haematoxylin-eosin-saffron and examined by light microscopy. The Leydig cells were counted (using magnification ×1000) by two methods in the tumour and in the homolateral testis, both adjacent to and at a distance from the tumourous tissue, and also in the biopsy of the contralateral testis for patient 2: 1) the Leydig cell number per field was considered to be the mean (± sd) for 36 fields; 2) the volume occupied by Leydig cells was also evaluated with a Zeiss ocular integrator (25 points) and expressed as a percentage and the mean (± sd) value was calculated for 100 fields (i.e. 2500 points). The findings were compared with those for two controls aged 20 and 31 years, who had died of a non-testicular disease.

**Incubation procedure**

In the first patient, we tested the tumourous tissue and a fragment of the homolateral testis removed at a distance from the tumour in an area which appeared normal by light microscopy. They were maintained at a temperature of −70°C until incubation. They were homogenized in 2 volumes of 0.25 mol/l sucrose. Homogenates were centrifuged at 900 × g for 20 min. Under air, 360 µl of supernatant (corresponding to 230 mg of tumoural or normal tissue) was incubated at 37°C for 1 h, in 2.5 ml of 0.07 mol/l phosphate buffer (pH 7.4) containing 4.5 nmol of [7-3H(N)]testosterone (NEN, 10–25 Ci/mmol/l) and an energy generating system consisting of 0.5 mmol/l NADPH, H+, 5 mmol/l of glucose-6-phosphate and 2 × 10³ U/l of glucose-6-phosphate dehydrogenase.

At the end of the incubation period, 4 vol of ethanol were added to each vial and kept overnight at −18°C. Steroids were extracted twice by 7 ml of ethyl acetate,
Steroid and gonadotropin concentrations in samples taken at 08.00 h on different days before surgery and on days 16 and 30 after surgery. Figures in parentheses give normal ranges for men.

<table>
<thead>
<tr>
<th></th>
<th>Patient 1</th>
<th>Patient 2</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Before surgery</td>
<td>Day 16</td>
</tr>
<tr>
<td>E₁ (0.13–0.31 nmol/l)</td>
<td>0.24–0.37</td>
<td>0.18</td>
</tr>
<tr>
<td>T (21–52 nmol/l)</td>
<td>7.6–17.3</td>
<td>31.2</td>
</tr>
<tr>
<td>DHT (1.7–8.5 nmol/l)</td>
<td>4.8</td>
<td>2.7</td>
</tr>
<tr>
<td>Δ₄ (1.7–7 nmol/l)</td>
<td>3.1–4.5–3.1</td>
<td>4.5</td>
</tr>
<tr>
<td>LH (3–10 U/l)</td>
<td>3.5</td>
<td>5.9</td>
</tr>
<tr>
<td>FSH (3–10 U/l)</td>
<td>&lt; 2</td>
<td>6.1</td>
</tr>
</tbody>
</table>

and the combined extracts taken to dryness under nitrogen.

Phenolic and neutral steroids were separated by ion-exchange chromatography on an AG 1 × 2 column (Goutte-Coussieu et al. 1979).

The 17β-oestradiol was isolated and identified by determination of its specific radioactivity using Adessi’s radio-gas-chromatographic method (Adessi & Jayle 1974) applied to high-pressure liquid chromatography (HPLC). After adding 25 μg of cold 17β-E₂ to the oestrogen fraction from the ion-exchange column, the specific radioactivity of each of the E₂ peaks following passage through two HPLC columns of different polarity, α, was measured. The columns used were R-SIL-PHENYL with methanol/water (46/54; v/v) as the mobile phase and LICHROSORB-DIOL with methanol/chloroform/cyclohexan (2/29/21; v/v/v) as the mobile phase. The measurement of identical specific radioactivity with the two columns demonstrated the identity of the 17β-E₂.

Results

Pre-operative steroid and gonadotropin concentrations

Samples taken at 08.00 h on different days showed (Table 1) that in patient 1 E₂ was always high, E₁ was high in one sample and normal in the other one, T was low, DHT and Δ₄ were normal LH was low-normal, and FSH was low; in patient 2, E₂ was elevated only once and high-normal in the other three samples, E₁ was always normal, T was low in two samples and normal in two others, DHT and Δ₄ were normal, LH and FSH were low or low-normal. The binding capacity of TeBG, assayed at 08.00 h on the first day of the investigation, was slightly elevated in both cases: 34.7 nmol/l and 45.1 nmol/l (normal men: 7–28 nmol/l).

In patient 2, samples taken every 30 min on the same day between 08.00 and 14.00 h showed that E₂ was constantly elevated (between 158 and 209 pmol/l) except for the last two samples, whereas E₁ was constantly within the normal range except for the last sample; FSH and LH were reduced, with small variations in amplitude for LH (Fig. 1).

Clomiphene test

In patient 2, after clomiphene (Table 2), LH, FSH and T levels increased on day 7, respectively, by 132, 64 and 73% over the mean basal pre-opera-

**Pathological findings**

Histologically, the tumour displayed a very similar endocrine structure in both cases. The tumour cells had regular round nuclei with delicate chromatin, and large finely granular cytoplasm in the first case, presence of Reinke cristalloids in the second one. In both cases, the tumour had the aspect of a Leydig cell tumour, and much of it was encapsulated (Fig. 5). As in the contralateral testis in the second patient, no atrophy or structural alteration was observed in the testicular tissue adjacent to the tumour and at a distance from it. The seminiferous tubules appeared normal as did spermatogenesis. There was no tumoural extension in the epididymis and the spermatic cord.

Both methods showed that in both patients, the Leydig cell count inside the tumour was markedly higher than in the normal testis (Table 3). It was also markedly higher than at a distance from the tumour: 11 and 30 times higher in patient 1 and
from the tumour in both patients. They were significantly more numerous in patient 1 than in normal testes, and in patient 2 than in his contralateral testis (Table 3). These findings suggest juxtamural Leydig cell hyperplasia (Fig. 3) in both cases.

**Incubation**

In patient 1, aromatase activity, expressed as ng of oestradiol per mg of tissue at a 1-h incubation, was 2.14 (mean of 3 assays, range 1.96–2.25) for tumoural tissue, and 0.1 (mean of 5 assays, range 0.09–0.11) for homolateral normal tissue. This finding shows that incubations of tumoural tissue result in markedly (× 21) increased aromatase activity compared with incubations of homolateral normal tissue.

**Discussion**

Serum hormone data for patients with a benign feminizing Leydig cell tumour are rare in the literature. Recently in a series of 16 such patients, $E_2$ was high in 10 and T was reduced in 5 (Kuhn et al. 1986). It is interesting to note in our patients the spontaneous variability of the $E_2$ level on different days, and that $E_2$ on the same day can be normal in some samples, abnormal in others, which suggests intermittent tumoural $E_2$ secretion. This would justify making several assays of $E_2$ when the values are borderline. In the literature, the gonadotropin levels are more variable. The decrease in FSH is not constant (Kuhn et al. 1986), and LH is generally reduced or at least not elevated in spite of the lower T level.

The $E_2$ response to hCG is characterized by a delayed, but exaggerated peak which is still at a level above 280 pmol/l on day 4 (Gabrilove et al. 1975; Kuhn et al. 1986). The abnormalities found in our patients have been observed with the hCG protocol described above, as well as with a single 5000 IU hCG injection. This type of $E_2$ response may thus be helpful in diagnosing a feminizing Leydig cell tumour, particularly when the tumour is not detectable by testis echogram, and suggests that the $E_2$ response is mainly due to the tumour, showing that it is not completely autonomous. The T response to hCG appears normal or low and probably results from non-tumoural Leydig cell stimulation (Bercovici et al. 1984).
Fig. 3.
Juxtatumoral Leydig cell hyperplasia (arrows) (magnification × 90) in patient 1 (upper part) and in patient 2 (lower part). The Leydig cell tumor is seen on the right.
Table 3.

Leydig cell count in tumour, in homolateral and contralateral testis tissue.

<table>
<thead>
<tr>
<th>Localization</th>
<th>Method 1*</th>
<th>Method 2** (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal testes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20-year-old control</td>
<td>3.1 ± 3.6</td>
<td>1.37 ± 3</td>
</tr>
<tr>
<td>31-year-old control</td>
<td>4.4 ± 3.7</td>
<td>1.24 ± 3</td>
</tr>
<tr>
<td>Patient 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumour</td>
<td>42.3 ± 8.2</td>
<td>24 ± 10.70</td>
</tr>
<tr>
<td>Testis tissue adjacent to tumour</td>
<td>9.2 ± 12.3</td>
<td>1.64 ± 2.84</td>
</tr>
<tr>
<td>Testis tissue at a distance from tumour</td>
<td>3.9 ± 6.9</td>
<td>0.68 ± 1.80</td>
</tr>
<tr>
<td>Patient 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumour</td>
<td>47.6 ± 12.0</td>
<td>31.20 ± 12.20</td>
</tr>
<tr>
<td>Testis tissue adjacent to tumour</td>
<td>4.3 ± 5.0</td>
<td>2.10 ± 4.16</td>
</tr>
<tr>
<td>Testis tissue at a distance from tumour</td>
<td>1.6 ± 1.9</td>
<td>1.44 ± 2.90</td>
</tr>
<tr>
<td>Contralateral testis</td>
<td>2.1 ± 2.8</td>
<td>0.80 ± 2.20</td>
</tr>
</tbody>
</table>

Mean ± sd values for *36 fields, **100 fields.

Comparisons (unpaired Student's t-test, P value) with the 20-year-old control testis:

1 < 0.05, 2 < 0.01, with the 31-year-old control testis:

3 < 0.05, 4 < 0.005, 5 < 0.001, and with testis adjacent to the tumour:

6 < 0.05, 7 < 0.025, 8 < 0.01, 9 < 0.005.

The pre-operative reduced T level reflects non-tumoural Leydig cell dysfunction, and may result from hyperoestrogenism through two mechanisms. The first one is indirect and corresponds to a decrease in LH secretion related to the high E2 level; it is suggested not only by the frequently low pre-operative LH levels, but also in patient 2 by LH spikes lower than in normal men (Boyar et al. 1978), by the clomiphene-induced LH and T rise, and in both patients by the post-operative LH rise. The other mechanism corresponds to the direct inhibiting effect of the E2 rise on Leydig cell steroidogenesis: E2 could act by reducing the enzymatic activities of 17β-hydroxylase, C17,20-lyase, and 17β-hydroxysteroid dehydrogenase (Kalla et al. 1980; Kremers et al. 1977). In a feminizing Leydig cell tumour, a rise in the P/17-OHP and 17-OHP/Δ4 ratios only in the tumoural spermatic vein (Bercovici et al. 1984) argues in favour of a reduction in the activity of 17α-hydroxylase and C17,20-lyase owing to a high local E2 concentration. In the present report, the reduction of the 17-OHP/Δ4 ratio induced by clomiphene shows the local inhibiting effect of hyperoestrogenism on C17,20-lyase.

Excessive E2 secretion in the tumour-bearing testis has been shown by E2 assay in the tumoural spermatic vein (Bercovici et al. 1984). Studies using labelled testosterone clearly demonstrated the presence of aromatase activity in the tumour (Weill et al. 1978; Bercovici et al. 1981) and confirmed the capacity of these tumours to produce oestrogens in abnormal quantities from androgenic precursors, in particular testosterone. However, increase in the aromatase activity of the tumour could be due to the large number of Leydig cells.

In the present report, the fact that most of the tumour was well encapsulated made it possible to compare the Leydig cell count with aromatase activity. In patient 1, the aromatase activity inside the tumour was 21 times higher than at a distance from it, whereas the Leydig cell count was 11 and 35 times higher depending on the method. This together with the fact that the tumour Leydig cells were apparently normal in size suggests that the aromatase activity of a single tumour cell is very similar to that of a normal Leydig cell.

The mechanism of tumoural development remains to be determined. Exogenous oestrogens can reduce the number of Leydig cells and change their structure (Oshima et al. 1974; Payer et al.)
Outside the tumour, no changes in Leydig cell size were observed, but at a distance from the tumour the Leydig cell count was lower than in controls. On the other hand, evidence of juxtatu-
moural Leydig hyperplasia, observed but not quantified by other authors (Bercovici et al. 1981; Weill et al. 1978), was confirmed and quantified in our two patients. This suggests the existence of a stimulating factor or the suppression of an inhibiting factor of Leydig cell proliferation. Peritumoural seminiferous tubule and Sertoli cell involution has often been reported and attributed to the decrease in FSH. However, the high incidence of cryptorchidia in patients with a Leydig cell tumour (Desmukh 1983; Gabrilove et al. 1975) suggests that tubular lesions precede the tumour development and that the resulting imbalance between Sertoli and Leydig cells could be involved in Leydig cell hyperplasia (Risbridger et al. 1981; Sharpe 1983).

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