Hyperplasia of the Parathyroids and the Pituitary in Rats with Experimental Leydigomas and Inhibitory Effect of Several Steroids

I. Petrea and N. Guéritée

Department of Internal Medicine, Griffin Hospital, Derby, Connecticut, 06418

SUMMARY

Bilateral orchidectomy performed on 10- to 15-day-old Wistar rats, immediately followed by the homograft of one testis into the splenopancreatic epiploon, resulted 6 months later in leydigiomas at the site of the graft in 100% of the animals. After this latency period, the tumors continued to grow proportionately to time elapsed and were associated with both pituitary microadenomas of the aldehyde-fuchsin-, aldehyde-thionin-periodic acid-Schiff-, and periodic acid-Schiff-positive cells and diffuse hyperplasia of the parathyroids, affecting in particular the chief cells of the latter.

After a 6- or 12-month leydigioma latency period, the rats were given a daily treatment by gavage for 45 days of one on two of the following steroids: Compound A, 17α-ethynyl, (5α)-androst-2-en, 17β-ol (17β-acetate) (5 mg/kg when alone; 2.5 mg/kg when combined); Compound B, 17α-ethynyl, (5α)-androst-2-en, 17β-ol (17β-nicotinate) (always given in combined form at 2 mg/kg); Compound C, 16-oximinoestronene, 3-allyl ether (2 mg/kg when combined). The treatments caused, in various degrees, (a) involution of the leydigiomas until complete disappearance in up to 50% of the cases; (b) recovery of the characteristic morphological pattern of the pituitaries; and (c) cytolysis and interstitial fibrosis in the previously hyperplastic parathyroids.

INTRODUCTION

Spontaneous testicular tumors can exist in certain strains of laboratory animals (10); however, experimentally induced tumors have obvious advantages in endocrine oncology (12, 18, 33). These tumors are most frequently induced by the administration of various products such as estrogens (8, 11, 15, 21), triphenylethylene (9), or methylcholanthrene (22). Other methods relate either to experimental cryptorchid (3) or to auto- (29) or heterografts (4). In particular, the leydigiomas were obtained practically exclusively by chronic endogenous stimulation of the gonadotrophic pituitary (2). As reported in the papers of Milcu et al. (20) and Petrea and Zimel (29), bilateral orchidectomy performed on rats less than 15 days old, immediately followed by the autograft of only 1 testis into the splenopancreatic epiploon, causes the development of a testicular tumor with interstitial cells within 6 months. Twelve months after the graft, compression of the abdominal organs with ascites is observed. Death can also be caused by the concomitant development of gonadotrophic extensive and hemorrhagic pituitary adenomas.

In order to determine the activity of some specific steroids (Table 1) on the evolution of leydigiomas, we observed not only the existence of gonadotrophic pituitary hyperplasias but also the development of extensive parathyroid hyperplasias that regressed under the effect of the treatment, at the same time as the involution of the leydigiomas and the pituitary hyperplasias. Intriguing but possible, this reaction could be related to the proliferative polyglandular syndromes, the biological and clinical peculiarities of which are recently starting to be better understood (17).

MATERIALS AND METHODS

Male Wistar rats were castrated between the ages of 10 and 15 days, and 1 of the 2 testes was immediately grafted into the splenopancreatic epiploon.

The development of the graft was controlled by exploratory laparotomy and biopsies before the onset of the treatment, either 6 months later (rats of Groups 3 and 4), or 12 months later (rats of Groups 5 to 8; see Table 1).

The animals thus prepared, as well as the uncastrated controls of the same age, were grouped and treated daily by gavage for 45 days (Table 1). The specific androgens and estrogen compounds (Lasdon Foundation, Colorado Springs, Colo., and Theramex S.A., Paris, France), as previously studied, were utilized because of their gonad-inhibiting activities (13, 30, 34, 35).

On the 46th day after treatment, all the animals were sacrificed, including the controls of the same age, were grouped and treated daily by gavage for 45 days (Table 1). The specific androgens and estrogen compounds (Lasdon Foundation, Colorado Springs, Colo., and Theramex S.A., Paris, France), as previously studied, were utilized because of their gonad-inhibiting activities (13, 30, 34, 35).

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On the 46th day after treatment, all the animals were sacrificed, including the controls of the same age (8 and 14 months, respectively). The leydigiomas were removed and weighed. Fragments of leydigiomas, pituitaries, and thyro-parathyroid complex were removed and prepared for histological and cytological study with light and electron microscopy (fixation in 3.5% glutaraldehyde buffered with Veronal phosphate at pH 7.4, postfixed in 1% OsO₄ and then embedded in Epon. The blocks were sliced with a lead citrate-uranyl acetate sequence and examined with a B₁₁ Hitachi electron microscope at an accelerating voltage of 50 to 80 kV).

1 Present address: Clinique Endocrinologique, Faculté de Médecine Pitié-Salpêtrière, Hôpital de la Pitié, 75013 Paris, France.

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Parathyroid and Pituitary Hyperplasia in Leydigomas

Some pituitary sections were stained with fuchsin-aldehyde (23) and thionin-PAS aldehyde (5).

Fragments of Leydigomas were also prepared for fluorescent microscopy according to the method of Bertalanffi and Nagy, as modified in Ref. 28. Sections were stained with 0.10% acridine orange, diluted to 1/10,000 in a phosphate solution buffered at pH 6, and then incubated in a 0.1 M cent lamp (400-watt HBO and UG 1/35 filter).

Because the parathyroids are embedded in the thyroid parenchyma, we have done serial histological sections of the whole thyroid-parathyroid complex, and we selected only the slides on which the parathyroids were the largest in diameter. The slides were projected with a Leitz projector with an identical objective lens and focal distance. Thus it was possible to trace the contours of the thyroid and parathyroids and to compare their differences planimetrically (25).

RESULTS

Leydigomas. All the controls sacrificed 8 months after the graft of the testis (Table 1, Group 3) developed tumors with a mean weight of 0.887 ± 0.248 g. There was no spermatogenesis; the seminiferous tubules were atrophic and the interstitial cells showed active proliferation (Fig. 1). After 45 days of treatment with Compounds A + C (Table 1, Group 4), the testicular tumors were inhibited up to total disappearance of the grafts in 8 of 15 cases. Hence, the mean weight of the tumors was reduced (0.034 ± 0.0049 g) in the treated group in a highly significant manner (p < 0.001) in comparison with nontreated controls. The mean weight of the Leydigomas of the controls sacrificed 14 months after the graft (Table 1, Group 5) was 2.533 ± 0.260 g (Chart 1). The histological examination of the tumor showed rare seminiferous tubules with no epithelial content and advanced tubular fibrosis. The tumor consisted practically entirely of large cells with either dense or vacuolar cytoplasm and ovoid or round nuclei resembling luteal-like cells (Fig. 2). In electron microscopy (Figs. 3, 4), 2 categories of Leydig cells are apparent. The first are large with dense cytoplasm, a dense ergastoplasmic system, numerous mitochondria, but a few secretory granules. The others, on the contrary, are clear cells and smaller, probably corresponding to aging or due to functional depletion factors. Here the ergastoplasm is scanty, the vacuolization is sometimes observed, and the mitochondrias are far less numerous and often seem degenerated. Inhibition of the Leydigomas after 45 days of treatment with Compounds A, A + C, and B + C (Table 1, Groups 6 to 8) often caused disappearance of the grafts, as can be seen in Table 2.

Thus, the mean weight of the tumors of the treated animals was reduced in a highly significant manner (Chart 1; Table 2). The inhibition of the Leydigomas, which had not completely disappeared under the influence of the treatment when examined histologically, consisted of the presence of degenerative phenomena, karyopyknosis, and hyalinofibrosis (Figs. 5, 6, and 9). The fluorescence of the Leydigoma cells stained with acridine orange is slight in the

Table 1
Animal groups, experimental conditions, and treatment applied

<table>
<thead>
<tr>
<th>Group</th>
<th>Experimental conditions and treatment applied</th>
<th>No. of rats</th>
<th>Age of animals at end of experiment (mos.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Intact controls</td>
<td>15</td>
<td>8</td>
</tr>
<tr>
<td>2</td>
<td>Intact controls</td>
<td>15</td>
<td>14</td>
</tr>
<tr>
<td>3</td>
<td>Leydigomas after 6 months; nontreated controls</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>4</td>
<td>As in Group 3; treatment with Compounds A + C</td>
<td>15</td>
<td>8</td>
</tr>
<tr>
<td>5</td>
<td>Leydigomas after 12 months; nontreated controls</td>
<td>3</td>
<td>14</td>
</tr>
<tr>
<td>6</td>
<td>As in Group 5; treatment with Compound</td>
<td>8</td>
<td>14</td>
</tr>
<tr>
<td>7</td>
<td>As in Group 5; treatment with Compounds A + C</td>
<td>8</td>
<td>14</td>
</tr>
<tr>
<td>8</td>
<td>As in Group 5; treatment with Compounds B + C</td>
<td>8</td>
<td>14</td>
</tr>
</tbody>
</table>

* Compound A: 17α-ethynyl, (5α)-androst-2-en, 17β-ol (17β-acetate) (5 mg/kg when alone, or 2.5 mg/kg when combined); Compound B: 17α-ethynyl, (5α)-androst-2-en, 17β-ol (17β-nicotinate) (given only in combined form at 2 mg/kg); Compound C: 16-oximinoestrone, 3-allyl ether (given only in combined form at 2 mg/kg).

Table 2
Ratio of the number of testicular graft lyses and the number of animals/group

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of animals</th>
<th>Treatment</th>
<th>No. of testicular graft lyes</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>3</td>
<td>Control</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>8</td>
<td>Compound A</td>
<td>4</td>
</tr>
<tr>
<td>7</td>
<td>8</td>
<td>Compounds A + C</td>
<td>3</td>
</tr>
<tr>
<td>8</td>
<td>8</td>
<td>Compounds B + C</td>
<td>4</td>
</tr>
</tbody>
</table>

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graffs with a latency period of 8 months (Table 1, Group 3; Fig. 7). However, it was intensive in the leydigioma cells after a latency period of 14 months (Table 1, Group 5; Fig. 8). Under the influence of the treatments, when fibrosis was extensive, the fluorescence was practically nonexistent (Fig. 10), marking possibly an inhibition of the RNA synthesis (28).

**Pituitary.** The histological study of the animals of Groups 3 and 5 (Fig. 11; Table 1) revealed the presence of certain large cells that are sometimes completely vacuolar, sometimes containing PAS-, aldehyde-fuchsin-, and thionin-aldehyde-positive granules. The cellular distribution is diffuse or sometimes nodular.

In electron microscopy (Figs. 12 to 15), the secretory granules sometimes exceed 200 nm and are usually situated near the cellular membrane. The number of granules is inversely proportional to the volume and active proliferation of the leydigioma. The ergastoplasmic system of the pituitary cells is progressively inhibited. The reticular membranes can break, thus transforming the cytoplasm into a multivacuolar structure which gives a “clear cell” appearance.

The pituitaries of the treated animals (Figs. 16 to 18) show various degrees of remission, up to the complete disappearance of the “signet ring-like cells” which, in the nontreated controls, showed different degrees of cellular hypertrophy and secretory activity. Electron microscopy reveals the abundance of secretory granules which seem relatively uniform and remain within taxonomic limits characteristic of granules of the gonadotrophic type, with their 2-dimensional variables, $SG_1 < 200$ nm; $SG_2 > 200$ nm (Figs. 17 and 18).

**Parathyroids.** In leydigioma-bearing animals, the parathyroids are obviously enlarged and expand in the thyroid tissue. The treatment with the applied steroids, whatever the type, causes a decrease in volume of the parathyroids and of their proliferation into the thyroid gland (Fig. 19).

The histological examination (Table 1, Group 5) of the leydigioma-bearing controls shows diffuse hyperplasia of the parathyroids (Figs. 20 and 21) with numerous chief-like cells having dense, basophilic cytoplasm, alternating with large, light, slightly eosinophilic cells (Fig. 21). In the treated animals (Figs. 22 to 24, 28, and 29), the various degrees of inhibition of the parathyroid hyperplasia are observed. The inhibition is characterized by nuclear and cytoplasmic degenerative phenomena leading to interstitial fibrosis.

In electron microscopy (Figs. 25 to 27), the presence of variable cells, which are probably forms of a functional transition, was observed in the parathyroids of the leydigioma-bearing controls. Some of the chief cells contain secretory granules (Fig. 25) that measure about 200 nm. However, other “dark”-like cells with practically no secretory granules contain a dense endoplasmic reticulum, numerous ribosomes and polysomes, an important system of microvesicles with a sometimes osmiophilic content, and active, round, or oval mitochondrias (Figs. 26 and 27). The “light” cells, on the contrary, more frequently show degenerative and involutional signs such as reduction of the ergastoplasmic system and ribosomes and complete absence of the secretory granules (Fig. 27). The most characteristic aspects of the cellular involution of the parathyroids under the influence of the treatment are the bizarrely shaped nuclei and the presence of numerous lysosomes (Figs. 30 and 31).

**DISCUSSION**

We have just shown how the induction in rats of a Leydig cell tumor, after early castration and immediate autograft of 1 testis in the portal vein area, causes the development of hyperplasias in 2 other glands, the pituitary and parathyroids, and how these other hyperplasias regress at the same time as the leydigioma under the effect of the appropriate treatment.

The development of pituitary microadenomas with large, light cells containing dilated endoplasmic reticulum with progressive loss of secretory granules could be considered in the context of chronic pituitary stimulation triggered off by castration (2, 14, 18, 19, 26-29, 33). One of the histological expressions of this stimulation is the appearance of “signet ring cells” (6) which correspond to a pituitary cellular exhaustion due to their holocrine-like function.

The new phenomenon observed consists of a constant parathyroid hyperplasia which evolves at the same time as does the testicular tumor (and the pituitary hyperplasia) and which might even be dependent. Otherwise, it would be difficult to understand the active cytolysis and interstitial fibrosis observed in the parathyroids of animals in which leydigiomas regressed or disappeared under the effect of the various applied treatments.

One of the questions to be raised could regard the biology of the hyperplastic parathyroid. A parathyroid-like hormone secreted by a spontaneous transplanted Leydig cell tumor has recently been reported in rats. This hormone causes lethal hypercalcemia and hypercalciuria and, in thyroparathyroidectomized animals, the increase of the phosphorus clearance. This substance, which does not pass the parabiotic barrier, is neither analogous to the parathormone, nor to the D vitamins, nor to an osteolytic phytosterol. Furthermore, it does not seem that the disturbances in the calcium metabolism of these rats are associated with anatomic modifications of the parathyroids (31). We, therefore, find no analogy between this later observation and ours, which, however, does not exclude the intervention of the parathormone on calcemia (16), nor that, possibly, of the leydigioma (37). A paraneoplasia-like syndrome (17, 24) could be linked with the development of the leydigioma and revealed by both diffuse hyperplasia of the parathyroids and hypercalcemia. However, another unknown mechanism cannot be ruled out.

A gonadotrophic stimulation as intense as the one caused by very early castration, and continually progressive, could end in a modified hormone-secreting phenotype (36). Such cells would be likely to secrete intermediate hormone substances which, deviated from the normal sequence of physiological end hormones, could stimulate others than the specific ones, the parathyroids for instance (32).

Whatever the case, careful verification of the intermediate
hormonal substances' nature or of the ectopic hormones is necessary. Confirming this hypothesis are the observations of the Leeds team (1, 7) which report the frequency of osteolytic hyperparathyroidism in postmenopausal women or after ovariectomy, comparable to those found in leydigiomabearing rats after chronic and progressive runaway activity of the gonadotrophic pituitary.

As for the action of the 3 steroid compounds (Compounds A, B, and C) (Table 1), used alone or in combined forms, the latter had the most potent inhibitory effect against leydigioisms, micronodular gonadotrophic pituitaries, and associated hyperplasia of the parathyroid glands. It would appear that the steroids are mainly acting at the pituitary level, on which leydigioisms are dependent. However, at this time we have no proof that the atrophy of leydigioisms alone could lead to the involution of the hyperplastic parathyroids or how extensive the role played by the steroids in these experimental conditions could be.

REFERENCES

Fig. 1. Leydigoma 8 months after bilateral orchidectomy and testicular graft, showing complete inhibition of spermatogenesis, seminiferous tubules (ST) with degenerate Sertoli cells (DS), and proliferation of the interstitial cells (IC). Trichromic stain, × 350.

Fig. 2. Leydigoma 14 months after bilateral orchidectomy and testicular graft. The microscopic field is completely occupied by clear interstitial cells (CIC), as well as large cells with dark cytoplasm. Some mitotic figures (MF) could be observed. Trichromic stain, × 350.

Figs. 3 and 4. Same as in Fig. 2. The ultrastructure of leydigoma cells with dense cytoplasm (DIC) and numerous mitochondria (M). Other cells are clear interstitial cells (CIC) with free ribosomes (FR) or lacunar endoplasmic reticulum (LER). N, nucleus; CM, cell membrane. ER, endoplasmic reticulum, × 24,000 and × 12,000.

Fig. 5. Leydigoma 8 months after bilateral orchidectomy and testicular graft. The animal was treated 45 days with the combination of Compounds A + C (Table 1, Group 4). An atrophic seminiferous tubule (ST) surrounded by numerous pyknotic interstitial cells (IC) with vacuolization and hyalinosis of the cytoplasm. Trichromic stain, × 350.

Fig. 6. Leydigoma 14 months after bilateral orchidectomy and testicular graft. The animal was treated for 45 days with Compounds A + C (Table 1, Group 7). There is obvious fibrosis (IF) progressing into the remnant of degenerated interstitial cells (IC). Trichromic stain, × 350.

Fig. 7. The same as in Fig. 1. Pale fluorescence of the interstitial cells (IC). ST, seminiferous tubule. UV, × 300.

Fig. 8. Same case as in Fig. 2. Intense fluorescence of interstitial cell cytoplasm (IC). UV, × 300.

Fig. 9. Leydigoma 14 months after bilateral orchidectomy and testicular graft. The animal was treated for 45 days with Compounds B + C (Table 1, Group 8). There is intensive fibrosis (IF) of the tumor with almost complete degeneration and replacement of the interstitial cells (IC) by connective tissue. ST, seminiferous tubule. Trichromic stain, × 350.

Fig. 10. The same as in Fig. 9. The fluorescence in the interstitial fibrosis (IF) and degenerated remnant interstitial cells is minimal. UV, × 300.

Fig. 11. Pituitary of the case presented in Fig. 2. There are numerous gonadotrophic cells (GC) with signet ring-like appearance. Trichromic stain, × 300.

Figs. 12 to 15. Pituitary gonadotrophic cells of different rats bearing leydigoma tumor 14 months after the testicular graft and bilateral orchidectomy. The cells have a clear cytoplasm with lacunar endoplasmic reticulum (LER) and scanty secretory granules (SG), generally located at the cellular periphery. Mitochondria (M) are round, some degenerated; ER, endoplasmic reticulum; L, lysosome; NM, nuclear membrane; NP, nuclear pore; N, nucleus. × 8,000 to 24,000.

Fig. 16. Pituitary of the case presented in Fig. 9. There is a restitution of the pituitary gland parenchyma with absence of the signet ring-like cells, 45 days of treatment with Compounds B + C (Table 1, Group 8). Trichromic stain, × 300.

Figs. 17 and 18. Gonadotrophic cells in leydigoma-bearing animals after 45 days of treatment with Compounds A + C (Fig. 17) and B + C (Fig. 18). The granular content of the cytoplasm is increased and both SGa and SGb secretory granules are abundantly secreted as well as enlarged mitochondria (M). N, nucleus; NP, nuclear pore; ER, endoplasmic reticulum; FR, free ribosomes. × 24,000.

Fig. 19. Thyroparathyroidian complex. Representative serial sections involving the largest circumference of the parathyroid glands in: I, controls; II, leydigomas; III, leydigomas; treatment with 17α-ethinyl- (5α)-androst-2-en, 17b-3-ol (17α-acetate) + 16-oximinoestrone, 3-ethyl ether. Leitz microprojector, objective, × 15 to 26 from 15-cm distance.

Fig. 20. Parathyroid gland hyperplasia (P) infiltrating the thyroidian parenchyma (TF) in an animal bearing leydigoma 14 months after bilateral orchidectomy and graft of the testis (Table 1, Group 5). The parathyroidian hyperplastic cells are proliferating in solid sheets and consist of elements with dense cytoplasm. Trichromic stain, × 500.

Fig. 21. Parathyroidian gland hyperplasia in an animal (same experimental group as in Fig. 20) where a diffuse proliferation with clear (CPC) and dark chief-like cells (PCC) are seen. Semitine section after Epon embedding. Toluidine blue, × 500.

Fig. 22. Hyperplastic parathyroid gland in a rat with leydigoma treated with the Compound A (Table 1, Group 6). The parathyroid cells (P) are pyknotic and dislocated by connective trabeculae (IF). Trichromic stain, × 275.

Fig. 23. Hyperplastic parathyroid gland in a rat with leydigoma treated with combination of Compounds A + C (Table 1, Group 7). The remnant degenerated parathyroidian cells (P) are replaced or surrounded by extensive fibrosis (IF). TF, thyroidian follicle. Trichromic stain, × 275.

Fig. 24. Hyperplastic parathyroidian gland in a rat with leydigoma treated with a combination of Compounds B + C (Table 1, Group 8). The parathyroidian cells (P) are pyknotic and replaced by intensive fibrosis (IF). Trichromic stain, × 375.

Fig. 25. Chief cell-like parathyroidian tissue with secretory granules (SG) in a rat with leydigoma; N, nucleus. × 24,000.

Fig. 26. Hyperplastic parathyroidian cell in a rat with leydigoma. The cytoplasm has a well-developed ergastoplasm (ER), numerous mitochondria (M), and several microvesicles (MV). CM, cellular membrane. × 24,000.

Fig. 27. Rat with leydigoma. Clear (CPC) and dark cells (PCC) without secretory granules in a hyperplastic parathyroid. The dark cell contains a dense matrix with numerous ribosomes (FR), mitochondria (M), and polyribosomes (PR), as well as microvesicles (MV). Some filled with a dense osmiophilic material. FR, free ribosomes (FR); ER, endoplasmic reticulum; CM, cellular membrane. × 24,000.

Figs. 28 and 29. Involvement of hyperplastic parathyroid in rats with leydigomas after 45 days of treatment with Compounds A + C (Fig. 28) and B + C (Fig. 29). P, parathyroidian cells; IF, interstitial fibrosis. Trichromic stain, × 350.

Figs. 30 and 31. Same cases as in Figs. 28 and 29. Bizarre forms of nuclei (N) and numerous lysosomes (L) with degenerate mitochondria (M) characterize the cellular ultrastructure of the hyperplastic parathyroidian cells in leydigomas after treatment. DC, dense cytoplasm; V, microvesicles. × 18,000.
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[Images of cellular structures, labeled with GC, LER, SG, ER, N, M, and other markers.]

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