Action of Thyroid Hormones and Diethylstilbestrol on the Gonadotropic Activity of a Mouse Thyrotropic Tumor

BERNARD MESSIER

Institut du Cancer de Montréal, Laboratoires de Recherche, Hôpital Notre-Dame et Université de Montréal, Montréal, Canada

Summary

Mice bearing a grafted thyrotropic tumor manifested secondary gonadotropic effects. Ovaries and uterus increased in weight, whereas seminal vesicles were significantly atrophied. These changes were corrected when the growth of the tumor was inhibited with l-thyroxine (T₄) or l-triiodothyronine (T₃). Attempts to inhibit only the gonadotropic activity with diethylstilbestrol (DES) proved unsuccessful. On the contrary, DES enhanced tumor growth in females with concomitant increase in mean ovarian weight, whereas in males, tumor growth was decreased by DES.

Introduction

In a broad survey of the biologic characteristics of thyrotropic pituitary tumors in mice, Furth (3) reported gonadotropic side effects of the thyrotropin-secreting tumors. These effects are more evident in females than in males and invariably require an athyroid state for their manifestation (4, 7). A proper explanation of this phenomenon is still lacking. The present work was undertaken to study the action of T₄ and T₃, as well as that of DES, on the gonadotropic properties of such a thyrotropic tumor.

Materials and Methods

Male and female LAF₁ mice, obtained from Cumberland View Farms, were used when 3–4 months of age. The various experimental groups are listed in Table 1. The thyrotropic tumor employed had been induced by radiothyroidectomy a few years previously and was in its 3rd passage at the beginning of the present experiment. Previous generations of this tumor (LA 6113) have manifested marked gonadotropic side effects in athyroid females. The tumor was transplanted s.c. into the interscapular region.

Radiothyroidectomy was performed by injecting i.p. 200 μc of 131I into each mouse. T₄ and T₃ were administered in the drinking water at respective doses of 1.0 μg and 0.2 μg/ml. Pellets containing either 0.01, 0.1, or 1.0 mg of DES were implanted s.c. in the lower back.

In hormone-treated groups about 1 month elapsed between radiothyroidectomy and initiation of hormonal treatment. All animals were sacrificed when hormonal treatment had lasted 5 months.

For histologic examination, tissues were fixed in Bouin's fluid for 24—48 hr, after which the fixative was changed for 70% alcohol. The testes, seminal vesicles, ovaries, and uteri were dissected free of neighboring tissues and weighed. When growth of the grafted thyrotropic tumor occurred, it was also excised and weighed.

Paraffin sections of the above tissues were prepared and stained routinely with hematoxylin-eosin. In addition, pituitary sections were stained with an alcian blue-PAS-orange G technic to show thyrotropes distinctly (Messier, accepted for publication).

Results

Effects of T₄ and T₃

The thyrotropic tumor used was fully dependent in the sense that it invariably required an athyroid state to grow. Indeed, the tumor failed to grow in euthyroid hosts or in radiothyroidectomized hosts receiving T₄ (Group V). In the case of T₃, administered at a dose 0.20 that of T₄, only a small tumor growth occurred in males whereas none was observed in females.

In males, the presence of a growing thyrotropic tumor was associated with an important reduction in the weight of the seminal vesicles (Group IV). Histologically, the weight reduction was attributable to a decrease in secretory material (Fig. 1). This condition was directly related to the presence of the tumor, since radiothyroidectomy alone had no significant effect (Groups I and II, Fig. 2). Furthermore, when radiothyroidectomized animals were grafted with the tumor and the growth of the latter was prevented with T₄ or T₃ (Groups V and VII), their seminal vesicles did not differ significantly from those of normal mice.

Thus, it is quite evident that the function of seminal vesicles was hampered by the thyrotropic tumor under study. Similar observations have been reported (1, 7) for other thyrotropic tumors.

In females, a marked increase in ovarian size was almost invariably observed in each tumor-bearing animal. The mean ovarian weight for such animals (Group IV) significantly exceeded that of euthyroid or radiothyroidectomized mice. When
intermediate doses of DES led to a significant increase in tumor size. Data for the highest dose of DES in females are uncertain, since only 2 animals survived in this group.

The marked shrinkage of seminal vesicles in Groups IX and X can be attributed to the estrogenic action of DES rather than to the presence of a thyrotrophic tumor, since DES alone (Groups XI and XII) caused a comparable atrophy. The histologic picture of these atrophic seminal vesicles testifies for the estrogenic action of DES: flattening of the epithelium, paucity of secretory material, relative abundance of stroma.

The testes also showed a weight reduction in DES-treated animals, whether a grafted thyrotropic tumor was present or not (Groups IX—XII). Histologic examination revealed that spermatogenesis was only rarely affected (1 animal showed azoospermia).

The significant stimulation of tumor growth in females by DES caused a proportional increase in the mean weight of the ovary, but values for Groups VIII and IX are not significantly different from those for Group IV. Furthermore, the histologic appearance of the ovaries was not modified by DES, i.e., many cystic and hemorrhagic follicles were present as in controls (Group IV). The endometrium, however, manifested signs of estrogenization, since the epithelium was often heightened, with occasional squamous metaplasia.

In contrast to T4 and T3, DES was unable to correct the pituitary changes brought about by radiothyroidectomy. Thus, the loss of alcianophilic material from pituitary thyrotropes, as observed following thyroid-destructive doses of 131I, was not restored by the DES implants.

**Discussion**

The observation by Furth (3) that mouse thyrotropic tumors also elicit a gonadal response constitutes a challenge to the concept of a separate homeostatic regulation for each anterior pituitary hormone. Indeed, radiothyroidectomy should theoretically augment TSH (thyroid-stimulating hormone) production only, without the frequently associated gonadotropic effects. So far, dissociation of this dual action spontaneously occurred in only 1 case (7). Attempts to suppress the thyrotropic character of the tumor without affecting the gonadotropic property have

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**TABLE 1**

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>MALES</th>
<th>FEMALES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of mice</td>
<td>Average weights (mg ± S.D.)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TtT</td>
</tr>
<tr>
<td>1 Euthyroid</td>
<td>8</td>
<td>210 ± 9</td>
</tr>
<tr>
<td>II Rte</td>
<td>8</td>
<td>202 ± 12</td>
</tr>
<tr>
<td>III Euthyroid + TtT</td>
<td>8</td>
<td>Not found</td>
</tr>
<tr>
<td>IV Rte + TtT</td>
<td>24</td>
<td>3286 ± 4022</td>
</tr>
<tr>
<td>V Rte + TtT + T4</td>
<td>10</td>
<td>Not found</td>
</tr>
<tr>
<td>VI Rte + T4</td>
<td>7</td>
<td>221 ± 19</td>
</tr>
<tr>
<td>VII Rte + TtT + T4</td>
<td>8</td>
<td>48 ± 53</td>
</tr>
<tr>
<td>VIII Rte + TtT + 0.01 mg DES</td>
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<td>2506 ± 3243</td>
</tr>
<tr>
<td>X Rte + TtT + 1.0 mg DES</td>
<td>8</td>
<td>549 ± 837</td>
</tr>
<tr>
<td>XI Rte + 1.0 mg DES</td>
<td>8</td>
<td>155 ± 34</td>
</tr>
<tr>
<td>XII Euthyroid + 1.0 mg DES</td>
<td>7</td>
<td>169 ± 21</td>
</tr>
</tbody>
</table>

* The abbreviations used are: TtT, I-thyroxine; TtT, I-triiodothyronine; DES, diethylstilbestrol; TtT, thyrotropic tumor; and Rte, radiothyroidectomized.

T4 or T3 was administered to tumor-implanted hosts (Groups V and VII), the exogenous thyroid hormones effectively prevented tumor growth, but a moderate increase in ovarian weight was nevertheless observed. The latter effect cannot be entirely attributed to T4, since T3 alone did not promote ovarian growth in radiothyroidectomized animals (compare Groups II and VI).

The histologic aspect of the stimulated ovaries presented a diversified picture. Numerous cystic or hemorrhagic follicles made up a large part of the hypertrophied ovaries of tumor-bearing animals (Fig. 3). In contrast, the moderate ovarian hypertrophy observed in animals that received T4 or T3 was largely due to the presence of lutein cells (Fig. 4). Ovaries from euthyroid (Fig. 5) or radiothyroidectomized mice were composed of more equally distributed granulosa and lutein cells.

The ovarian stimulation observed in tumor-bearing animals was paralleled by an increase in the size of the uterus (Group IV). However, such increase was prevented when tumor growth was inhibited by T4 or T3 (Groups V and VII).

Radiothyroidectomy brought about characteristic changes in the pituitary cytology of both males and females (6). Histologically, the alcian-blue-positive material contained in thyrotropes disappeared completely in 131I-treated animals, and this was true whether a grafted tumor was present or not (Groups II and IV). The administration of T4 or T3 strikingly corrected the effects of radiothyroidectomy, with restoration of alcian-blue-positive material in thyrotropes.

**Effects of DES**

Implantation of DES pellets to tumor-grafted mice had opposite effects on the tumor according to the sex of the animal. In males, the highest dose of DES significantly depressed the growth of the tumor, whereas in females, both the lowest and the intermediate doses of DES led to a significant increase in tumor size. Data for the highest dose of DES in females are uncertain, since only 2 animals survived in this group.

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Action of Diethylstilbestrol on Thyrotropic Tumor

been unsuccessful. The work of Money et al. (8) and the results presented here demonstrate that T₄ and T₃ will inhibit tumor growth but will cause a simultaneous disappearance of the gonadal response. It is quite clear, therefore, that the gonadal stimulus goes hand in hand with the presence of tumor cells.

A different approach consisted in the specific obliteration of the gonadotropic effects with DES. Indeed, estrogens are known as effective inhibitors of gonadotropin secretion, particularly of FSH (follicle-stimulating hormone) (5). The choice of DES was justified here since previous observations (3, 7) had shown that the ovaries of tumor-bearing mice most frequently exhibited an FSH-type stimulation.

It should be pointed out (Group IV) that the gonadotropic effect is stimulatory in the female and inhibitory in the male, a picture also reported by Bates et al. (1). This is perplexing since gonadotropins are usually considered as being stimulatory in both sexes (5). It is difficult, therefore, to conceive of the gonadotropic effect of the tumor as due to ordinary pituitary gonadotropins. Yet, a "positive" result for gonadotropins was obtained when crude preparations of thyrotropic tumors were assayed in immature female mice (Messier, unpublished). Quantitative assays of TSH in these tumors have revealed high levels of the hormone (2), but no data on gonadotropin levels are available.

DES exerted a significant influence on the growth of the thyrotropic tumor but proved to be an inadequate inhibitor of the gonadotropic effect. This failure of DES to obliterate the gonadotropic effect would indicate that the tumor does not secrete FSH as such and that it is essentially thyrotropic. The latter possibility is confirmed in the present work by the inability of DES to correct the formation of thyroidectomy cells in the pituitaries of radiothyroidectomized mice.

In view of the above results, serious consideration should now be given to possible inherent gonadotropic properties of the TSH molecule elaborated by thyrotropic tumors.

Acknowledgments

We wish to thank Dr. Jacob Furth for originally supplying us with the thyrotropic tumor. The gift of the DES pellets from Dr. U. Kim, Roswell Park Memorial Institute, is gratefully acknowledged.

References

Fig. 1. Seminal vesicle from a radiothyroidectomized mouse bearing a grafted thyrotropic tumor. The lesser amount of secretory material brings about multiple folds of the epithelium. H & E, X 30.

Fig. 2. Seminal vesicle from a radiothyroidectomized mouse. The gland is distended with secretory material. H & E, X 30.

Fig. 3. Part of an ovary from a radiothyroidectomized mouse bearing a grafted thyrotropic tumor. The size of the whole ovary may be estimated from the short segment of the capsule seen in the upper right. Several cystic or hemorrhagic follicles are present in the ovary. H & E, X 30.

Fig. 4. Ovary from a radiothyroidectomized mouse in which the growth of the grafted thyrotropic tumor was inhibited with thyroxine. Groups of lutein cells make up most of the ovarian substance. H & E, X 30.

Fig. 5. Ovary from an euthyroid mouse. H & E, X 30.
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