The Effect of Hypophysectomy on the Experimental Production of Rat Thyroid Neoplasms

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SUMMARY

Hypophysectomized, sham-hypophysectomized, and nonoperated rats were injected with 3 μCi $^{131}$I or not injected and then placed on a low-iodine diet with or without iodine supplement. In 6 months, when follicular cell neoplasms (not necessarily light cell neoplasms) were expected to appear, the thyroid glands were examined under the light microscope and neoplasms were counted and measured by a histometric technique. The incidence of animals with follicular cell neoplasms in the sham-hypophysectomized group given 3 μCi $^{131}$I and the low-iodine diet was 96%, with a mean neoplasm number of 5.5/animal thyroid gland and a mean aggregate neoplasm volume of $0.57 \times 10^8$ cu μm/animal thyroid gland. Sham-hypophysectomized animals given $^{131}$I alone did not develop neoplasms; hypophysectomized animals given a low-iodine diet alone, $^{131}$I alone, or a combination of both, also did not develop thyroid neoplasms. It is concluded that, in the rat, in the absence of thyroid-stimulating hormone stimulation, thyroid follicular cell neoplasms do not appear following an injection of 3 μCi $^{131}$I and/or a low-iodine diet.

INTRODUCTION

Neoplasms of the follicular cells and light cells develop in the thyroid glands of rats given a low-iodine diet over a period of months (1). Confirming the observation that the level of circulating TSH was elevated in animals fed such a diet (2), it was postulated that, at least for the follicular cell neoplasms, it is the high level of circulating TSH which exerts the neoplastic effect on the thyroid gland (3). Later, it was demonstrated that the administration of a small dose of radioactive iodine (or other radiation) prior to the low-iodine diet greatly enhanced the formation of follicular cell neoplasms (4). It was suggested that these findings supported the “two-stage” hypothesis of thyroid carcinogenesis (5) which implicates radiation as the “mutagenic” agent and TSH as required to promote the development of neoplasms. With this hypothesis in mind, the following experiments were designed to determine the effect of the removal of circulating TSH by hypophysectomy on thyroid follicular cell carcinogenesis in animals fed a low-iodine diet alone or after 3 μCi $^{131}$I. The thyroid glands were examined at 6 months when follicular cell (and not necessarily light cell) neoplasms were expected to develop. In this manner, we hoped to decide whether TSH is prerequisite to follicular cell carcinogenesis in the rat thyroid gland.

MATERIALS AND METHODS

Of 10-week-old female albino CDF (inbred) Fischer rats, obtained from Charles River Breeding Laboratories, Boston, Mass., 100 rats had been hypophysectomized, 100 had had a sham hypophysectomy, and 100 had no operation. The animals were subdivided into 10 groups as shown in Table 1. Some rats were given injections i.p. of 3 μCi $^{131}$I; all rats were placed on a low-iodine diet; all rats were placed on a low-iodine diet alone; all operated animals were given 10% glucose in distilled water to drink (less than 0.5 μg iodide/100 ml); nonoperated animals drank distilled water. Control groups received an iodide supplement as 2 μg/ml NaI added to drinking water and these animals were housed separately from the low-iodine groups. At 6 months, the surviving animals were anesthetized with ether, and the thyroid glands, attached to trachea and esophagus, were removed and fixed in Bouin’s solution. Paraffin blocks were cut serially at 6 μm, and every 50th section was retained and stained with Masson’s trichrome and light green. Pituitary fossae were examined and body weights, as well as adrenal gland weights, were used to confirm hypophysectomy (Table 1).

The histological slides were examined under the microscope to locate neoplasms of the thyroid gland and to assess their number and size according to a technique described by Nadler et al. (10). Three parameters were obtained: (a) number of neoplasm-bearing thyroid glands per group (incidence); (b) mean number of neoplasms per thyroid gland in the group; (c) mean aggregate neoplasm volume per thyroid gland.

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2The abbreviation used is: TSH, thyroid-stimulating hormone.

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Table 1

<table>
<thead>
<tr>
<th>No. of rats surviving/ No. of rats at start</th>
<th>Mean body weight gain (mg ± S.D.)</th>
<th>Mean adrenal gland weight (mg ± S.D.)</th>
<th>No. of rats with neoplasm</th>
<th>Mean No. of neoplasms/gland</th>
<th>Mean aggregate volume of neoplasm/gland (X 10^8 cu μ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Hypox + low I</td>
<td>17/25</td>
<td>28 ± 10</td>
<td>10.9 ± 2.9</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2. Hypox + I + 131I + low I</td>
<td>19/25</td>
<td>32 ± 12</td>
<td>11.1 ± 3.7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3. Sham-hypox + low I</td>
<td>25/25</td>
<td>68 ± 16</td>
<td>39.3 ± 2.8</td>
<td>13</td>
<td>0.75</td>
</tr>
<tr>
<td>4. Sham-hypox + 131I + low I</td>
<td>24/25</td>
<td>70 ± 13</td>
<td>39.3 ± 2.6</td>
<td>23</td>
<td>5.5</td>
</tr>
<tr>
<td>5. Hypox + low I + I</td>
<td>19/25</td>
<td>30 ± 10</td>
<td>11.6 ± 1.5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6. Hypox + 131I + I + low I</td>
<td>21/25</td>
<td>32 ± 15</td>
<td>13.0 ± 2.1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7. Sham-hypox + low I + I</td>
<td>24/25</td>
<td>74 ± 11</td>
<td>45.6 ± 6.1</td>
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<td>0</td>
</tr>
<tr>
<td>8. Sham-hypox + 131I + low I + I</td>
<td>24/25</td>
<td>68 ± 14</td>
<td>51.7 ± 3.1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>9. Nonop + 131I + low I</td>
<td>48/50</td>
<td>47</td>
<td>5.5</td>
<td>9.6</td>
<td></td>
</tr>
<tr>
<td>10. Nonop + 131I + low I + I</td>
<td>47/50</td>
<td>47</td>
<td>5.5</td>
<td>9.6</td>
<td></td>
</tr>
</tbody>
</table>

aHypox, hypophysectomized; I, iodine; Sham-hypox, sham-hypophysectomized; Nonop, nonoperated.

and (c) mean aggregate volume of neoplasm per thyroid gland in the group. The 1st parameter was analyzed by the chi-square method for differences between groups; the 2nd and 3rd parameters were analyzed by t test.

RESULTS

Observation of the thyroid glands in the surviving animals disclosed relatively small glands in those hypophysectomized and larger glands in those not hypophysectomized and placed on a low-iodine diet. Microscopic examination of thyroid tissue in hypophysectomized animals revealed the typical appearance of follicles with flattened epithelium and relatively abundant dense colloid in the lumens. Where neoplasms of the follicular cells were found, they were as described previously (1). Neoplasms of the light cells were found only in Groups 4 (sham-hypophysectomized + 131I + low-iodine, 4 glands) and 9 (nonoperated + 131I + low-iodine, 1 gland). The quantitative parameters for follicular cell neoplasms are reported in Table I.

DISCUSSION

Those animals with an intact pituitary gland given a low-iodine diet for 6 months (Group 3, sham-hypophysectomized + low iodine) developed follicular cell neoplasms in 13 of 24 rats (54%), with a mean neoplasm number of 0.75/thyroid gland and a mean aggregate neoplasm volume of 8.8 x 10^8 cu μ/thyroid gland, whereas those animals which had been hypophysectomized and given the same diet (Group 1, hypophysectomized + low iodine) developed no neoplasms at all (Table 1). In addition, intact animals given 3 μCi 131I and then placed on a low-iodine diet for 6 months (Group 4, sham-hypophysectomized + 131I + low iodine; Group 9, nonoperated + 131I + low iodine) developed follicular cell neoplasms in 23 of 24 animals (96%) and 47 of 48 animals (98%), respectively, with a mean neoplasm number of 5.5/gland for both cases, and a mean aggregate neoplasm volume of 8.8 x 10^8 and 9.6 x 10^8 cu μ/thyroid gland, respectively. Hypophysectomized animals given the same treatment (hypophysectomy + 131I + low iodine) failed to show any neoplasm formation (Table 1). Clearly (and confirmed statistically), the pituitary gland is essential for the development of follicular cell neoplasms by a low-iodine diet for 6 months, with or without preirradiation by an injection of 3 μCi 131I.

The dose of 131I was injected 6 days after the animals had been hypophysectomized, a sufficiently long interval to allow for an alteration in 131I uptake by the thyroid glands in the hypophysectomized animals. Therefore, it was decided to conduct a supplementary experiment in order to determine the difference in the total radiation dose delivered to the thyroid glands of hypophysectomized and sham-hypophysectomized rats. It was demonstrated that the hypophysectomized animals received an average of 54% of the radiation delivered to the thyroid glands, compared to the sham-hypophysectomized animals. To have overcome this discrepancy it would have been necessary either to administer the 131I prior to hypophysectomy, or to inject a sufficiently larger dose of 131I into the hypophysectomized animals. Nonetheless, the overall conclusion remains valid concerning the role of the pituitary gland, since the same conclusion can be based on the observations made in the hypophysectomized versus nonhypophysectomized animals which did not receive any preirradiation.

The yields for neoplasm in the sham-hypophysectomized and nonoperated animals injected with 3 μCi 131I and subsequently placed on a low-iodine diet (Group 4, sham-
hypophysectomy + $^{131}$I + low iodine; Group 9, nonoperated + $^{131}$I + low iodine) were statistically identical (Table 1). Therefore the sham operation had no effect on follicular cell carcinogenesis.

A low-iodine diet results in a high level of circulating TSH and therefore it was postulated that oversecretion of pituitary TSH induced by iodine deficiency is responsible for thyroid carcinogenesis (8). Indeed, even prior to our experiment, follicular cell neoplasms in the rat thyroid gland had been induced by feeding animals thiouracil for 1 year (12, 13), an observation compatible with the hypothesis that elevated TSH is responsible for the formation of these neoplasms. More recently, follicular cell neoplasms were induced in the isthmus of rats that had both lobes of the thyroid gland removed by operation (4). It was concluded that the extensive partial thyroidectomy caused a decrease in thyroid hormone production with a reciprocal rise in TSH secretion and stimulation of the remaining thyroid tissue. It was also demonstrated that destruction of the thyroid gland in mice with $^{131}$I resulted in the development of gross pituitary neoplasms (3). Then, when such neoplasms were transplanted into hosts pretreated with thyroid-destructive doses of $^{131}$I (100 to 400 μCi injected), after 2 or more transplants the neoplasms were found able to survive in untreated animals. The ability of these latter neoplasms to secrete TSH was demonstrated by stimulation of the thyroid parenchyma, which resulted in papillary tumor formation similar to the follicular cell neoplasms described in this paper (2).

Hence, the postulate that TSH is necessary for follicular cell carcinogenesis is entirely reasonable. The fact that hypophysectomy prevents the development of these neoplasms would confirm the theory. Of course, hypophysectomy results in the elimination of anterior pituitary hormones other than TSH; and, perhaps the absence of other hormones, particularly growth hormone and adrenocorticotropic hormone (and therefore adrenal steroids) may have also played some part in preventing neoplasia.

It has been suggested that TSH is also needed for maintenance of thyroid neoplasm. It was demonstrated that small follicular cell neoplasms disappeared following the addition of an iodide supplement to a low-iodine diet, and the large follicular cell neoplasms decreased after that continued iodide supplementation (6). Moreover, when fragments from an established line of transplanted thyroid neoplasms were implanted into rats which had been hypophysectomized, thyroidecotomized, or left intact, it was observed that there was no tumor growth in the hypophysectomized group (7). Thus, it was inferred that follicular cell neoplasms require a sustained presence of TSH for maintenance and growth (11).

However, this does not exclude the possibility that such neoplasms still retain a latent autonomous capacity. Follicular cell neoplasms induced by a low-iodine diet were transplanted into rats which had been thyroidecotomized and placed on a low-iodine diet (9). After 2 transplants, these neoplasms were able to survive in hosts with intact thyroids, on an adequate iodine diet. In other words, at a certain stage, the neoplasms no longer required an elevated level of TSH, and were able to survive in the presence of normal levels of circulating TSH. It was thought that similar neoplasms of the rat thyroid were unable to survive transplantation into hypophysectomized hosts, and were therefore still dependent on some TSH (7). However, after repeated transplants over 3 years, follicular cell neoplasms induced originally by a low-iodine diet have been found to take and grow in hypophysectomized hosts (9).

Similarly, thyroid neoplasms were induced in rats fed a "moderately low" iodine diet in combination with thiouracil. It was observed that after repeated transplants over a 3-year period, the implants began to grow in hosts fed a diet to which no thiouracil was added, and then, after further transplants over a 4-year interval, tumor implants were able to survive in hypophysectomized hosts (13). Thus, thyroid follicular cell neoplasms, at first dependent on TSH, can become autonomous.

In conclusion, the anterior pituitary gland, presumably through the secretion of TSH, is prerequisite for the development of neoplasm of the thyroid follicular cells and is probably essential to maintain them and make them grow, but would appear not to be necessary once the neoplasms attain autonomy.

REFERENCES

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