Stimulation of Growth of Carcinogen-induced Mammary Cancers in Rats by Thyrotropin-releasing Hormone

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SUMMARY

Twice-daily injections of three different doses of synthetic thyrotropin-releasing hormone (TRH), a hormone normally produced by the hypothalamus and released by the posterior pituitary, have significant effects on the growth of mammary tumors in rats. When thyroidectomized rats bearing 7,12-dimethylbenz(a)anthracene-induced mammary tumors were given twice-daily injections of TRH, mammary tumor growth was increased to the same extent as in intact rats given TRH, showing that the effects of TRH were not exerted via stimulation of thyroid function. The TRH-induced increments in mammary tumor growth were accompanied by significant increases in serum prolactin levels over 0.87% NaCl solution-injected controls. A single daily injection of 16-bromo-α-ergocryptine (CB-154), a prolactin-release inhibitor, completely blocked TRH-induced mammary tumor growth and reduced serum prolactin values. These results indicate that a twice-daily pulse of TRH can stimulate mammary tumor growth by releasing prolactin from the anterior pituitary.

INTRODUCTION

Prolactin and estrogen are believed to be the 2 primary hormones involved in carcinogen-induced mammary cancer development and growth in rats (2, 4, 8). Placement of lesions in the median eminence of rats can promote growth of carcinogen-induced mammary cancers (9) and hasten development of spontaneous mammary tumors in rats (10) by increasing release of prolactin from the anterior pituitary (4, 11). Drugs that act on the hypothalamus to alter prolactin release also can influence mammary tumor growth. Thus l-dopa, pargyline, and iproniazid decrease prolactin release and produce regression in size and number of carcinogen-induced mammary cancers in rats, whereas methyldopa, reserpine, and haloperidol increase prolactin release and promote mammary cancer growth (5).

Hypothalamic hormones have not previously been tested for their effects on mammary tumor growth. However, TRH administration is known to induce rapid release of prolactin as well as thyrotropin from the anterior pituitary, reaching peak values in the serum in about 10 to 15 min, followed by a decline (1, 3, 6). The half-life of TRH in the circulation has been estimated to be only 3 to 4 min (3). It was of interest, therefore, to determine whether a twice-daily pulse of synthetic TRH could increase growth of DMBA-induced mammary cancer in rats.

MATERIALS AND METHODS

Female Sprague-Dawley rats were housed in a temperature (75 ± 10°F) and light (14 hr/day) controlled room. Each rat was given a single injection of 5 mg DMBA through the tail vein at 50 to 55 days of age. About 2 months later, rats bearing 2 to 5 palpable mammary tumors each were used for experiments. In the 1st experiment, 4 groups of 7 to 8 rats each were treated as follows: Group 1 (controls) received 2 daily injections of 0.2 ml of 0.87% NaCl solution; Groups 2, 3, and 4 (experimental rats) received injections twice daily for 3 weeks of 0.5, 1, or 10 µg of synthetic TRH in 0.2 ml of 0.87% NaCl solution. In a 2nd experiment, 5 groups, each comprised of 6 to 13 rats, were treated as follows: Group 1 (intact controls) received injections of 0.2 ml of 0.87% NaCl solution; Group 2 (thyroidectomized controls) received s.c. injections of 0.2 ml of 0.87% NaCl solution; Group 3 (intact) received 10 µg synthetic TRH twice daily; Group 4 (thyroidectomized) received 10 µg synthetic TRH twice daily; Group 5 (intact) received 10 µg synthetic TRH twice daily and 0.4 mg CB-154 once daily in 0.2 ml of 3% alcohol dilut-o with distilled water. All TRH injections were given s.c. Between 1000 and 1100 hr and again between 1600 and 1700 hr. Bilateral thyroidectomized rats, maintained on 1% calcium lactate in drinking water, were used 10 days after removal of the thyroid gland.

At the beginning and at weekly intervals for 3 weeks, the largest 2 perpendicular diameters of each tumor were measured with calipers after the animals had been placed under light ether anesthesia. The total number of tumors per rat also were counted at these times. Ten min after the last injection of TRH or CB-154, at the end of the 3rd week, blood samples were collected by ocular sinus puncture under light ether anesthesia and serum prolactin levels were measured by radioimmunoassay (7). Results were analyzed by 1-way analysis of variance and by Student’s t test.

RESULTS

The effects of the 3 doses of TRH on mammary cancer growth are shown in Table 1. It can be seen that, in controls,
### Table 1

**Dose-response effect of TRH on growth of DMBA-induced mammary tumors**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of rats</th>
<th>Av. tumor diameter/rat (cm) Wk 0</th>
<th>Av. tumor diameter/rat (cm) Wk 1</th>
<th>Av. tumor diameter/rat (cm) Wk 2</th>
<th>Av. tumor diameter/rat (cm) Wk 3</th>
<th>Av. no of Tumors/rat Wk 0</th>
<th>Av. no of Tumors/rat Wk 3</th>
<th>Prolactin concentration min after TRH (ng/ml) Wk 0</th>
<th>Prolactin concentration min after TRH (ng/ml) Wk 3</th>
<th>Body wt (g) Wk 0</th>
<th>Body wt (g) Wk 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.87% NaCl solution</td>
<td>8</td>
<td>8.1 ± 1.3</td>
<td>9.5 ± 1.3</td>
<td>11.1 ± 1.3</td>
<td>12.0 ± 1.6</td>
<td>4.8 ± 0.4</td>
<td>6.6 ± 0.8</td>
<td>35 ± 8</td>
<td>280 ± 4</td>
<td>286 ± 4</td>
<td></td>
</tr>
<tr>
<td>TRH, 0.5 µg</td>
<td>8</td>
<td>8.3 ± 1.0</td>
<td>11.0 ± 1.2</td>
<td>14.0 ± 1.5</td>
<td>16.0 ± 2.2</td>
<td>5.0 ± 0.5</td>
<td>7.8 ± 0.7</td>
<td>113 ± 20</td>
<td>282 ± 5</td>
<td>291 ± 7</td>
<td></td>
</tr>
<tr>
<td>TRH, 1.0 µg</td>
<td>8</td>
<td>8.1 ± 1.1</td>
<td>11.0 ± 2.0</td>
<td>13.6 ± 3.0</td>
<td>16.3 ± 3.0</td>
<td>5.0 ± 0.5</td>
<td>8.4 ± 0.4</td>
<td>142 ± 24</td>
<td>282 ± 6</td>
<td>281 ± 10</td>
<td></td>
</tr>
<tr>
<td>TRH, 10 µg</td>
<td>7</td>
<td>8.3 ± 1.0</td>
<td>11.0 ± 1.7</td>
<td>16.2 ± 2.0</td>
<td>19.0 ± 2.5</td>
<td>5.1 ± 0.4</td>
<td>10.0 ± 1.1</td>
<td>133 ± 23</td>
<td>292 ± 5</td>
<td>301 ± 6</td>
<td></td>
</tr>
</tbody>
</table>

* Mean ± S.E.

**p < 0.05.**

**p < 0.01.

### Table 2

**Effects of TRH and TRH plus CB-154 on growth of DMBA-induced mammary tumors in intact or thyroidectomized rats**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of rats</th>
<th>Av. tumor diameter/rat (cm) Wk 0</th>
<th>Av. tumor diameter/rat (cm) Wk 1</th>
<th>Av. tumor diameter/rat (cm) Wk 2</th>
<th>Av. tumor diameter/rat (cm) Wk 3</th>
<th>Av. no of tumors/rat Wk 0</th>
<th>Av. no of tumors/rat Wk 3</th>
<th>Prolactin concentration 10 min after TRH or CB-154 (ng/ml) Wk 0</th>
<th>Prolactin concentration 10 min after TRH or CB-154 (ng/ml) Wk 3</th>
<th>Body wt (g) Wk 1</th>
<th>Body wt (g) Wk 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact controls</td>
<td>10</td>
<td>5.7 ± 0.6</td>
<td>6.5 ± 0.8</td>
<td>8.1 ± 1.0</td>
<td>7.8 ± 0.6</td>
<td>3.6 ± 0.3</td>
<td>5.1 ± 0.6</td>
<td>36 ± 3</td>
<td>280 ± 4</td>
<td>305 ± 10</td>
<td>310 ± 14</td>
</tr>
<tr>
<td>Thyroidectomized controls</td>
<td>12</td>
<td>5.5 ± 0.7</td>
<td>7.3 ± 1.1</td>
<td>7.5 ± 1.1</td>
<td>9.8 ± 1.5</td>
<td>3.7 ± 0.4</td>
<td>5.7 ± 0.6</td>
<td>39 ± 10</td>
<td>290 ± 7</td>
<td>305 ± 7</td>
<td>310 ± 12</td>
</tr>
<tr>
<td>Thyroidectomized controls and 10 µg TRH</td>
<td>13</td>
<td>5.7 ± 0.6</td>
<td>8.9 ± 1.3</td>
<td>11.2 ± 1.5</td>
<td>14.9 ± 1.3</td>
<td>3.1 ± 0.3</td>
<td>7.9 ± 0.6</td>
<td>88 ± 6</td>
<td>307 ± 12</td>
<td>320 ± 10</td>
<td></td>
</tr>
<tr>
<td>Intact and 10 µg TRH</td>
<td>13</td>
<td>5.7 ± 0.9</td>
<td>8.6 ± 1.2</td>
<td>11.0 ± 1.6</td>
<td>14.1 ± 1.0</td>
<td>3.3 ± 0.9</td>
<td>7.1 ± 0.7</td>
<td>108 ± 12</td>
<td>280 ± 4</td>
<td>291 ± 9</td>
<td></td>
</tr>
<tr>
<td>Intact, 10 µg TRH and 0.4 mg CB-154</td>
<td>6</td>
<td>4.4 ± 1.1</td>
<td>3.9 ± 1.3</td>
<td>4.1 ± 1.5</td>
<td>4.6 ± 1.1</td>
<td>3.2 ± 0.6</td>
<td>3.0 ± 0.7</td>
<td>54 ± 7</td>
<td>295 ± 13</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* p < 0.05, compared with intact controls.

**p < 0.05, compared with thyroidectomized controls.

**p < 0.02, compared with thyroidectomized controls.

* p < 0.01, compared with intact controls.
there was an increase in average tumor diameter and in average tumor number. TRH induced significantly greater increases in average tumor diameter over the controls at all 3 dose levels given, with the greatest increase seen in the rats given 10 μg TRH. The 10-μg dose of TRH also produced a significant enhancement in average tumor number over the control rats. TRH significantly raised serum prolactin concentration above control values at all 3 dose levels by 10 min after injection. There were no significant effects of TRH on body weight.

Table 2 shows the effects of injecting 0.87% NaCl solution, 10 μg TRH per rat, or 10 μg TRH with 0.4 mg CB-154 per rat on growth of mammary cancers in intact and thyroidectomized rats. By the end of 3 weeks of 0.87% NaCl solution treatment, average tumor diameter in the intact and thyroidectomized controls showed similar increases in average tumor diameter and tumor number. It can be seen that TRH injections produced similar increases in average tumor diameter and tumor number in both intact and thyroidectomized rats over 0.87% NaCl solution-injected controls. In the group given TRH and CB-154, average mammary tumor diameter and number were significantly reduced, compared with the 0.87% NaCl solution-injected controls. TRH produced similar increases in serum prolactin over control values both in the intact and thyroidectomized rats, but when TRH was given together with CB-154, only a small increase in serum prolactin was seen. None of these treatments significantly altered body weight.

DISCUSSION

These studies show that twice-daily injection of TRH, a hypothalamic hormone, produced a significant increase in size and number of DMBA-induced mammary cancers in rats over 0.87% NaCl solution-injected controls. These actions of TRH are believed to be mediated through an increase in prolactin release from the anterior pituitary, since simultaneous administration of CB-154, an ergot prolactin release inhibitor (4), blocked the stimulatory effects of TRH on mammary tumor growth. The possibility that TRH stimulation of the thyrotropin-thyroid system may have influenced these results was ruled out by the thyroidectomy experiment. TRH stimulated mammary cancer growth in the thyroidectomized animals to about the same extent as in intact animals.

Twice-daily injections of TRH were effective in stimulating mammary cancer growth in these rats despite the short half-life of TRH of about 3 to 4 min in the circulation (3). This suggests that a relatively brief, twice-daily pulse of prolactin in the circulation is sufficient to stimulate growth of these mammary cancers, and emphasizes the sensitivity of the DMBA-induced mammary cancers to prolactin stimulation. Whether each injection of TRH produced the same increase in prolactin release in these rats during the 21 days of the experiment is unknown, but there is no reason to believe that the daily injections of TRH failed to increase prolactin release. This is believed to be the first demonstration that administration of a synthetic hypothalamic hormone can increase mammary tumor growth. The possible implications of these observations to the problem of human breast cancer is unknown at present, but there is ample evidence that the hypothalamus regulates prolactin secretion in humans as well as in animals (1, 3, 4).

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REFERENCES

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