Influence of Prolactin on Carcinogen-induced Leukemogenesis in Long-Evans Rats

Clifford W. Welsch, Sally Horowitz, and Charles B. Huggins

Department of Anatomy, Michigan State University, East Lansing, Michigan 48824 [C. W. W., S. H.], and the Ben May Laboratory for Cancer Research, University of Chicago, Chicago, Illinois 60637 [C. B. H.]

SUMMARY

Hypophysectomy of rats bearing 7,12-dimethylbenzanthracene-induced leukemia has been reported to result in a prompt and persistent regression of the leukemia. The purpose of this study was to determine whether or not marked alterations in prolactin secretion would influence this neoplastic process. To determine this, immature male and female Long-Evans rats were divided into three groups: Group 1, controls; Group 2, pituitary grafted (hyperprolactinemia); and Group 3, 2-bromo-α-ergocryptine-treated (hypoprolactinemia). Two weeks after the initial treatment and at 2-week intervals thereafter (6 total), each rat was given a single intragastric intubation of 7,12-dimethylbenzanthracene (10 mg/rat). Two months after the initial carcinogen treatment and at 2- to 3-week intervals thereafter, all rats had liver biopsies for the identification of leukemia. Results cleanly show that despite nearly 10-fold difference in mean serum prolactin levels in the three groups of female rats and nearly a 20-fold difference in the level of this hormone in male rats, no significant differences in the magnitude of this leukemogenic process could be detected. Thus, striking changes in prolactin secretion do not appear to influence significantly this leukemogenic process.

INTRODUCTION

In 1972, Huggins and Oka (7) observed that hypophysectomy of Long-Evans rats bearing 7,8,12-trimethylbenzanthracene-induced leukemia resulted in a prompt and prolonged regression of this hematopoietic disease. More recently, Bentley et al. (1) reported that hypophysectomy of Sprague-Dawley rats treated with the Gross virus (passage A) totally prevented the induction of leukemia in this species, providing evidence that hydrocarbon-induced leukemia is not unique in its responsiveness to pituitary ablative procedures. Although it has been known for a number of years that many leukemias, both experimental and clinical, respond to certain adrenal steroids, little attention has been directed toward pituitary hormones in leukemogenesis. Thus, the pituitary hormone or hormonal combination prerequisite for either carcinogen- or viral-induced leukemogenesis is not known.

Pituitary prolactin has been shown to have an influential role in normal murine hemopoiesis (2, 10–12, 14). The secretion of this hormone can be easily and effectively inhibited by the use of a number of ergot alkaloids or ergoline derivatives (3). Furthermore, a number of rodent studies have provided evidence suggesting that the ergot alkaloids may be relatively specific for prolactin. For example, CB-154 does not appear to influence growth hormone secretion (21), and rodents chronically treated with the drug have normal estrous cycles (17) suggesting a lack of effect of the ergot on gonadotrophin secretion. Because of the reported role of prolactin in normal hemopoiesis and the availability of a means whereby the secretion of this hormone can be controlled, it was compelling to design and conduct a study to determine whether or not this hormone is one of the pituitary hormones important in murine leukemogenesis.

MATERIALS AND METHODS

Male and female Long-Evans rats were used in this study. They were housed in a temperature (24 ± 1°) and light (14 hr/day)-controlled room and provided a diet of Wayne Lab Blox (Allied Mills, Inc., Chicago, Ill.) and water ad libitum.

Induction of Leukemia

Thirty-six- to 55-day-old female and male rats were given an initial single intragastric intubation of DMBA (10 mg/rat, dissolved in sesame oil) and at 2-week intervals thereafter for a total of 6 gastric intubations. Two months after the initial carcinogen treatment and at 2-week (females) and 3-week (males) intervals thereafter, all rats had liver biopsies. For biopsy, 30- to 50-mg wedges of liver were excised, fixed in Bouin's fluid, embedded in paraffin, and stained with hematoxylin and eosin as previously...
Prolactin and Murine Leukemogenesis

Modification of Prolactin Secretion

Hyperprolactinemia. Twelve days prior to carcinogen treatment, 3 pituitary homografts were transplanted under the kidney capsule of male and female rats. The donor animals were of the same strain and age as the recipients, but of opposite sex. Pituitaries grafted to sites distant from the diencephalon persistently secrete increased amounts of prolactin and very small amounts, if any, of all other pituitary hormones (20).

Hypoprolactinemia. Twelve days prior to carcinogen treatment, male and female rats were injected once a day 4 times weekly (Monday, Wednesday, Friday, and Saturday or Sunday) for the duration of the study, with CB-154, at a dose of 0.4 mg/100 g body wt. CB-154 solution was prepared by dissolving the ergot in a minimal amount of 100% ethanol and diluting with 0.9% NaCl solution so that the final concentration was 2.0 mg CB-154 per ml.

Control animals and the pituitary grafted animals were given 4 injections weekly with the diluent only. All treatments were for 20 (females) and 35 weeks (males) at the conclusion of which all surviving rats were terminated. At that time, livers and spleens were excised, weighed, and prepared for histological evaluation. Blood was obtained from each rat and analyzed by radioimmunoassay for prolactin. Mean differences among organ weights, serum prolactin levels, and mean latency period of leukemia appearance were evaluated by Student's t test. Percentages of rats surviving in each group at the end of the study and leukemia incidence were evaluated by χ² analysis.

RESULTS

Female Rats (Tables 1 and 2). Leukemia incidence, mean latency period of leukemia appearance, mean liver, spleen, and body weights, and percentage of surviving rats at termination of the study were not shown to be significantly different among the 3 groups of animals (controls, pituitary grafted, and CB-154 treated). Mean serum prolactin levels, however, were found to be significantly (p < 0.001) elevated in the pituitary-grafted rats and significantly (p < 0.05) reduced in the CB-154-treated rats. All leukemias, regardless of groups or sex of animals were morphologically stem-cell leukemias as previously described (7-9, 13).

Male Rats (Tables 3 and 4). Leukemia incidence; mean liver, spleen, and body weights; and percentage of surviving rats at termination of the study were not shown to be significantly different among the 3 groups of animals. Mean latency period of leukemia appearance, however, was slightly lengthened (p < 0.05) in the pituitary-grafted rats.

Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>No. of rats (beginning of study)</th>
<th>Mean ± SE serum prolactin (ng/ml)</th>
<th>No. of rats with leukemia (%)</th>
<th>Mean ± SE latency period of leukemia appearance (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Controls</td>
<td>40</td>
<td>39.9 ± 10.5 (a)</td>
<td>13 (33.3) (d)</td>
<td>98.2 ± 5.3 (d)</td>
</tr>
<tr>
<td>2</td>
<td>Pituitary grafts (high prolactin)</td>
<td>38</td>
<td>196.4 ± 35.1 (b)</td>
<td>15 (39.5) (e)</td>
<td>91.7 ± 6.0 (e)</td>
</tr>
<tr>
<td>3</td>
<td>CB-154-treated (low prolactin)</td>
<td>41</td>
<td>20.9 ± 3.8 (c)</td>
<td>15 (36.6) (f)</td>
<td>85.8 ± 6.2 (f)</td>
</tr>
</tbody>
</table>

* Mean ± S.E. p < 0.001, a/b, b/c; p < 0.05, a/c; p = no significant difference, d/e, d/f, e/f.

Table 2

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Mean body wt (g)</th>
<th>Mean ± SE liver wt (g)</th>
<th>Mean ± SE spleen wt (g)</th>
<th>No. of surviving rats (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Controls</td>
<td>217</td>
<td>9.33 ± 0.24 (a)</td>
<td>0.91 ± 0.23 (a)</td>
<td>25 (62.5) (a)</td>
</tr>
<tr>
<td>2</td>
<td>Pituitary grafts (high prolactin)</td>
<td>224</td>
<td>10.49 ± 0.42 (b)</td>
<td>1.14 ± 0.26 (b)</td>
<td>19 (47.5) (b)</td>
</tr>
<tr>
<td>3</td>
<td>CB-154-treated (low prolactin)</td>
<td>231</td>
<td>9.76 ± 0.25 (c)</td>
<td>0.74 ± 0.12 (c)</td>
<td>25 (61.0) (c)</td>
</tr>
</tbody>
</table>

* Mean ± S.E. p = no significant difference a/b, a/c, b/c.
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Table 3

Influence of prolactin on DMBA-induced leukemogenesis in male Long-Evans rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>No. of rats (beginning of study)</th>
<th>Meana serum prolactin (ng/ml)</th>
<th>No. of rats with leukemia (%)</th>
<th>Meanb latency period of leukemia appearance (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Controls</td>
<td>38</td>
<td>12.4 ± 1.8 (a)</td>
<td>10 (26.3) (d)</td>
<td>139.6 ± 22.9 (g)</td>
</tr>
<tr>
<td>2</td>
<td>Pituitary grafts (high prolactin)</td>
<td>38</td>
<td>40.1 ± 8.3 (b)</td>
<td>5 (13.2) (e)</td>
<td>208.6 ± 25.2 (h)</td>
</tr>
<tr>
<td>3</td>
<td>CB-154-treated (low prolactin)</td>
<td>38</td>
<td>2.3 ± 0.3 (c)</td>
<td>8 (21.1) (f)</td>
<td>137.0 ± 19.3 (i)</td>
</tr>
</tbody>
</table>

*a Mean ± S.E. p < 0.001, a/b, a/c, b/c; p < 0.05, h/i; p = no significant difference, d/e, d/f, e/f, g/h, g/i.

Table 4

Influence of prolactin on body, liver, and spleen weights, and mortality of male Long-Evans rats treated with DMBA

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Meana body wt (g)</th>
<th>Meana liver wt (g)</th>
<th>Meana spleen wt (g)</th>
<th>No. of surviving rats (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Controls</td>
<td>390</td>
<td>12.00 ± 0.40 (a)</td>
<td>0.77 ± 0.05 (a)</td>
<td>14 (36.9) (a)</td>
</tr>
<tr>
<td>2</td>
<td>Pituitary grafts (high prolactin)</td>
<td>396</td>
<td>13.70 ± 0.52 (b)</td>
<td>1.37 ± 0.14 (b)</td>
<td>12 (31.6) (b)</td>
</tr>
<tr>
<td>3</td>
<td>CB-154-treated (low prolactin)</td>
<td>402</td>
<td>12.20 ± 0.64 (c)</td>
<td>1.35 ± 0.32 (c)</td>
<td>14 (36.9) (c)</td>
</tr>
</tbody>
</table>

*a Mean ± S.E. p = no significant difference, a/b, a/c, b/c.

when compared with the CB-154-treated rats. Mean serum prolactin levels were found to be significantly (p < 0.001) elevated in the pituitary-grafted rats and significantly (p < 0.001) reduced in the CB-154-treated rats.

DISCUSSION

Despite nearly a 10-fold difference in mean serum prolactin levels in female rats and nearly a 20-fold difference in the level of the hormone in male rats, no significant difference in the magnitude of this leukemogenic process could be detected in this study. These results demonstrate that there is little difference in the efficiency of the induction of leukemia with DMBA between rats secreting large amounts of prolactin and rats barely secreting this hormone. The only distinction between these groups occurred in males and was a slight delayed appearance of the leukemia in the hyperprolactinemia group when compared to the hypoprolactinemia group. This difference just made the 5% level of significance and was not statistically detected when comparing either of these 2 groups with the control group. It appears, therefore, that striking changes in the secretion of prolactin do not significantly influence either the development or growth of this experimental leukemia.

These results are interesting in view of a number of reports implicating prolactin as an influential hormone in normal hemopoiesis. For example, the administration of prolactin has been shown to increase erythropoiesis in normal (11, 12), polycythemic (10, 12), orchidectomized (10), and testosterone-treated mice (12). In lactating rats, total blood volume and RBC mass are significantly increased above control levels (2). Lactation in these rats is a physiological condition of hyperprolactinemia comparable to the pituitary-grafted female rats used in this study. In hypophysectomized rats, the administration of prolactin has been reported to increase RBC and hemoglobin levels over preinjection values (14). Whether or not the stem cell of erythropoiesis and the target cell of DMBA-induced leukemogenesis are identical or very closely related is currently unknown.

Although prolactin has been reported to be a critical hormone in spontaneous (17-19, 21) and DMBA-induced (15, 16) murine mammary tumorigenesis and has been implicated in a number of other tumor systems (4-6), the results of this study provide evidence that this hormone is not important in DMBA-induced leukemogenesis in the Long-Evans rat. Since hypophysectomy causes a prompt and prolonged regression of this leukemia (13), our search for the other anterior pituitary hormone or hormonal combination prerequisite for this leukemogenic process continues.

ACKNOWLEDGMENTS

CB-154 was supplied through the courtesy of Dr. Richard L. Elton, Sandoz Pharmaceuticals, E. Hanover, N. J. The rat prolactin radioimmunoassay kit was supplied through the courtesy of the National Institute of Arthritis, Metabolic and Digestive Diseases, NIH. We thank Carol Adams, Donna Fox, Clare Hassett, Georgia Louks, Jane Underhill, and Charles Brooks for their technical assistance in this study.

REFERENCES

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