Effects of Hypophysectomy and Hormone Replacement on the Local and Metastatic Growth of Morris Hepatoma 44

Julius Erdstein, Harvey J. Guyda, and Seymour Mishkin

Department of Medicine, Royal Victoria Hospital and McGill University Clinic [J. E., S. M.], and Polypeptide Hormone Laboratories, McGill University [H. J. G.], Montreal H3A 1A1, Canada

ABSTRACT

We have demonstrated recently that the local metastatic growth of Morris hepatoma 44 is thyroid dependent (Mishkin, S., Morris, H. P., Yalovsky, M., and Murthy, P. V. N. Gastroenterology, 77: 547–555, 1979; Mishkin, S. Y., Pollack, R., Morris, H. P., Yalovsky, M., and Mishkin, S. Cancer Res., 41: 3040–3045, 1981) and that exogenous thyroxine (8 µg/kg/day) and prolactin (100 µg/day) significantly stimulated tumor growth, while growth hormone (100 µg/day) failed to do so (Pollack, R., Mishkin, S. Y., Morris, H. P., and Mishkin, S. Hepatology, 2: 836–842, 1982).

In the present study, thyroid ablation (hypothyroidism) and hypophysectomy inhibited tumor growth significantly. These effects were almost totally reversed by administration of exogenous thyroxine to hypothyroid rats. While prolactin or growth hormone or thyroxine alone failed to restore tumor growth in hypophysectomized animals, administration of all three hormones partially but significantly reversed the inhibition of tumor growth. The number and size of pulmonary metastases paralleled local growth in all the above-mentioned conditions. Plasma membrane lactogenic receptors, measured using human growth hormone, were decreased in hypothyroidism and hypophysectomy groups. Binding levels were restored in those groups in which tumor growth was stimulated. In summary, the local and metastatic growth of Morris hepatoma 44 is affected by anterior pituitary hormones. Plasma membrane lactogenic receptors may mediate these effects.

INTRODUCTION

Hepatocellular carcinoma is a hormone-sensitive tumor. Early studies (20) indicated that hepatocarcinogenesis was sensitive to thyroid function. Induction of hepatomas by 2-acetaminofluorene was inhibited by thyroid ablation using thiouracil. More recently, it was demonstrated that induction of hypothyroidism (propylthiouracil, radioactive iodine, or surgical thyroidectomy) significantly inhibited the local growth of an established experimental tumor. The growth of artificially induced hepatic metastases following intraportal injection of Walker tumor cells is inhibited by HYP. This inhibition was reversed by the administration of exogenous ovine prolactin to hypophysectomized rats (7). The role of PRL in tumor growth, however, is complicated by evidence indicating a protective effect of PRL on urethane-induced pulmonary adenomas in mice (12). These results are in contrast to the stimulatory activity of PRL widely demonstrated in mammary tumors (2, 15).

It is now widely accepted that rat liver possesses receptors which specifically bind lactogenic hormones, namely, PRL, placental lactogen, and primate growth hormone. The receptors have been prominent in adult female rats and minimal or undetectable in fetal and immature rats (13, 25). Studies on the binding of human growth hormone to several different rat liver preparations indicate that binding is greatly influenced by the endocrine status of the animal from which the plasma membranes were prepared (10). Both PRL and estrogen have been shown to be important in inducing and regulating lactogenic receptor levels (24). Small amounts of thyroid hormones are necessary for the maintenance of liver lactogenic binding sites. This may occur via mechanisms which involve the pituitary, but also through others which do not, since rats which are both hypophysectomized and thyroidectomized receiving thyroxine replacement have a restoration of lactogenic binding sites without an increase in serum PRL (4).

In the present study, we set out to examine the effects of pituitary ablation and partial hormone replacement on the local and metastatic growth of the Morris hepatoma 44. Plasma membrane receptors for lactogenic hormones were quantitated using human growth hormone. These measurements were performed in an attempt to understand the effects of certain hormones on hepatoma growth, as well as to elucidate their mechanism of action.

MATERIALS AND METHODS

Female Buffalo rats (Simonsen Laboratories, Gilroy, CA) were shipped to Howard University (Washington, DC) for implantation of Morris hepatoma 44; approximately 106 cells were injected into the musculature of each hind limb. Within a few days of inoculation, the animals were transferred to Montreal. Animals were housed in individual wire-bottomed cages, fed Purina laboratory chow, and given water ad libitum. Surgical HYP was carried out via the transphenoidal approach 4 weeks after

---

1 This work was supported by grants from the Medical Research Council of Canada; was presented at the Annual Meeting of the Canadian Society for Clinical Investigation, Quebec City, September 1982 (5); and is dedicated to the memory of Moe Yalovsky.

2 Recipient of a Canadian Liver Foundation Summer Student Fellowship.

3 To whom requests for reprints should be addressed.

Received June 22, 1983; accepted March 19, 1984.

Evidence for pituitary involvement in tumor growth arose from studies conducted on women with Stage IV breast cancer (22). Results indicated that HYP was effective in inducing remissions in women who previously responded to other types of hormonal manipulation. Animal studies (21) further demonstrate the specific role of the pituitary gland in the growth of an established experimental tumor. The growth of artificially induced hepatic metastases following intraportal injection of Walker tumor cells is inhibited by HYP. This inhibition was reversed by the administration of exogenous ovine prolactin to hypophysectomized rats (7). The role of PRL in tumor growth, however, is complicated by evidence indicating a protective effect of PRL on urethane-induced pulmonary adenomas in mice (12). These results are in contrast to the stimulatory activity of PRL widely demonstrated in mammary tumors (2, 15).

It is now widely accepted that rat liver possesses receptors which specifically bind lactogenic hormones, namely, PRL, placental lactogen, and primate growth hormone. The receptors have been prominent in adult female rats and minimal or undetectable in fetal and immature rats (13, 25). Studies on the binding of human growth hormone to several different rat liver preparations indicate that binding is greatly influenced by the endocrine status of the animal from which the plasma membranes were prepared (10). Both PRL and estrogen have been shown to be important in inducing and regulating lactogenic receptor levels (24). Small amounts of thyroid hormones are necessary for the maintenance of liver lactogenic binding sites. This may occur via mechanisms which involve the pituitary, but also through others which do not, since rats which are both hypophysectomized and thyroidectomized receiving thyroxine replacement have a restoration of lactogenic binding sites without an increase in serum PRL (4).

In the present study, we set out to examine the effects of pituitary ablation and partial hormone replacement on the local and metastatic growth of the Morris hepatoma 44. Plasma membrane receptors for lactogenic hormones were quantitated using human growth hormone. These measurements were performed in an attempt to understand the effects of certain hormones on hepatoma growth, as well as to elucidate their mechanism of action.
tumor inoculation. Hypophysectomized animals were housed in separate cages (plastic), fed Purina laboratory chow, and given 2 water supplies: (a) 5% glucose; and (b) 5% glucose and 5% salt ad libitum. Hypophysectomized animals were randomized into 5 treatment groups as follows: (a) HYP; (b) HYP plus thyroxine; (c) HYP plus PRL; (d) HYP plus growth hormone; and (e) HYP plus thyroxine plus PRL plus growth hormone. The remaining hepatoma-bearing animals were placed in the following groups: (a) control (tumor-bearing); (b) hypothyroid; and (c) hypothyroid plus thyroxine. There were 12 animals in each group, except for untreated controls of which there were 15.

Hypothyroidism was induced using carrier-free potassium [131I]iodide (1 mCi per 100 g body weight) (18). Exogenous thyroxine (Sigma Chemical Co., St. Louis, MO) was administered in a dose of 8 µg per kg of body weight i.p. daily. Ovine PRL and bovine growth hormone (generous gifts of the National Pituitary Agency, Baltimore, MD) were administered in doses of 100 µg i.p. daily (23).

Animals were weighed, and tumor diameter was measured using an electronic caliper once weekly. Animals were sacrificed after 7 weeks of treatment and 4 weeks after their arrival by rapid decapitation, and blood was collected. Livers and tumors were excised, blotted dry, and weighed. Receptor Assay. At time of sacrifice, tumors were removed into cold 0.3% sucrose in which the tissue was rinsed, minced, and homogenized, using a Virtis homogenizer. The homogenate was fractioned by differential centrifugation to yield 15,000 x g and 100,000 x g pellets. The studies were performed with the 100,000 x g pellet, which will be referred to simply as the membrane preparation. Membrane fractions were resuspended by homogenization in Tris-Mg2+ buffer [25 mM TrishCl (pH 7.4), -10 mM MgCl2] (13). An aliquot was solubilized by heating in N NaOH for 30 min at 100°, and the protein was measured by the method of Lowry et al. (14).

Binding of 125I-labeled human growth hormone was assayed by incubating 100 µl of a 10-µg/ml solution of the labeled hormone with 200 µg of membrane protein in a final volume of 0.5 ml containing Tris-Mg2+ buffer and 400 µg of bovine serum albumin. All incubations were performed in duplicate at 4° for 2 hr. The incubation was terminated by addition of 3 ml of ice-cold 0.1% bovine serum albumin Tris-Mg2+ buffer. Parallel incubations were performed in the presence of excess (1.0 µg) unlabeled human growth hormone. Bound and free 125I-labeled hormone were separated by centrifugation at 3000 rpm for 30 min (8).

Quantitation of Pulmonary Metastasis. At sacrifice, lungs were inflated before removal by intratracheal inflation of 4% formalin. The right lung from each specimen was sectioned transversely at the same levels to yield 3 slices, each having an approximate thickness of 5 mm. The first sample was taken from the top of the superior lobe and embedded to yield 3 slices, each having an approximate thickness of 5 mm. The second and third samples, constituting cross-sections of the intermediate lobe and basal margin of the inferior lobe, respectively, were treated in a similar manner. The tissues were embedded in paraffin blocks and cooled on ice, and 5-µm-thick sections were cut with a rotary microtome. A routine hematoxylin-eosin stain was performed prior to the morphometric analysis. Photomicrographs of the slides were made, and the data were then compiled using a Carl Zeiss Mop III digitizer.

Serum PRL Levels. Serum PRL levels in controls were determined, courtesy of Dr. K. Ruf, according to methods established previously (19).

RESULTS

The growth rate of Morris hepatoma 44 primary hindlimb implants was inhibited to a similar degree in rats subjected to thyroid ablation and HYP (Chart 1). While the simultaneous administration of exogenous PRL, growth hormone, and thyroxine to hypophysectomized rats restored growth rate to the control pattern, the injection of each hormone individually was without significant effect (Chart 2). The growth rates of primary tumor in all experimental groups paralleled each other between Weeks 5 and 9 after implantation. Unlike any other group, the growth rate of tumors in control rats accelerated after Week 9. Primary tumor size in control and hypophysectomized rats receiving triple hormone replacement (HYP plus PRL plus growth hormone plus thyroxine) was always greater than those of HYP rats. Although the growth curve for HYP rats receiving thyroxine was below that of HYP rats, the difference was not significant. These data, which are based on caliper measurements, were confirmed at time of sacrifice 7 weeks after the initiation of treatment (Chart 3). A significant reduction in tumor weight (measured as a percentage of animal weight) was demonstrated using the Student t test (p < 0.05) in the hypophysectomized group relative to controls. Mean values for tumor weight in 15
controls and 12 hypophysectomized animals were 6.83 ± 0.81 (S.E.) and 1.25 ± 0.22 g, respectively. Similar significant reductions in tumor weight were measured in hypophysectomized animals receiving single hormone replacement as well as in hypothyroid rats. However, mean values for tumor weight of hypothyroid animals receiving exogenous thyroxine and hypophysectomized animals receiving a combined daily administration of all 3 hormones did not differ significantly from untreated tumor-bearing controls.

The binding of 125I-labeled human growth hormone to lactogenic receptor sites on the plasma membrane of hepatoma cells was studied (Chart 5). Setting control binding as 100% corresponding to 5.7 ± 1.5% specific binding, it was found that thyroid ablation and hypophysectomy reduced specific binding levels to 38.0% ± 3.6% and 30.4% ± 6.0% of control values, respectively. Binding was restored to near-normal values in the hypophysectomized rats receiving combination hormone replacement and hypothyroid rats given injections of thyroxine. The binding of human growth hormone to plasma membrane correlated well with both local and metastatic tumor growth in the groups studied (Chart 6). Correlation coefficients of 0.938 and 0.907 were obtained when plotting the aforementioned data.

**DISCUSSION**

The previously documented inhibition of tumor growth in rats subsequent to thyroid ablation (18) has been confirmed, and a similar degree of tumor growth inhibition was demonstrated in
hypophysectomized hepatoma-bearing rats. It may have initially been thought that the reduction in growth observed in hypophysectomized rats resulted from secondary hypothyroidism due to the absence of thyroid-stimulating hormone. However, exogenous thyroxine replacement to hypophysectomized rats failed to restore tumor growth (Chart 3) while, as shown previously (18), thyroxine administration reversed the effects of induced hypothyroidism. These results seem to imply a specific role for the pituitary in regulating tumor growth.

Recent studies have demonstrated that administration of either PRL or thyroxine to tumor-bearing rats significantly stimulated tumor growth, whereas growth hormone failed to elicit any significant effect (23). The present set of experiments has shown that, while the replacement of any of these 3 hormones alone to hypophysectomized rats failed to restore tumor growth, the simultaneous administration of all 3 hormones produced growth levels more closely approximating those typical of tumor-bearing control rats (Chart 3). Furthermore, metastatic growth pattern paralleled local growth rate as was noted in previous studies involving thyroid status (18, 23). It is of particular interest to note that combination hormone replacement resulted in a 3-fold increase in tumor growth relative to hypophysectomized animals (Chart 3). This cannot be considered simply an additive effect of the individual hormones since, given singly, each failed to appreciably increase tumor growth above post-HYP base-line levels. Thus, it appears that the interaction of these hormones provides significant restoration of tumor growth. It must be emphasized, however, that tumor growth was not fully restored, thus implying the involvement of additional factors.

The relationship between PRL and thyroid status is complex. Primary hypothyroidism has been shown to result in elevated serum PRL concentrations (6). In other studies, basal PRL levels were either in the normal range or slightly elevated in the hypothyroid state (27). However, it has been shown that chronic hypothyroidism in the rat results in decreased serum PRL levels, as well as increased release of dopamine into the pituitary portal system (11). Furthermore, in vitro studies on cultured pituitary cells indicate that both thyroxine and triiodothyronine directly stimulate PRL release (1). This evidence raises the possibility that the inhibition of hepatoma growth observed in the hypothyroid state may at least in part be due to a decreased serum PRL level. Finally, in view of the need of thyroid hormones for the maintenance of PRL receptors (4, 9), it seems possible that, if PRL is stimulatory to hepatoma growth, then the decreased tumor growth in the hypothyroid state may involve a concomitant decreased sensitivity of the tumor itself to PRL.

Lactogenic hormone receptors on hepatoma cell membranes seem to be crucial in mediating the effects of the hormonal environment. Both local and metastatic tumor growth correlate well with lactogenic receptor levels (Chart 6). The restoration of tumor growth following 3-hormone combination replacement to hypophysectomized rats is accompanied by an increase in receptor levels. Conversely, failure of PRL replacement to stimulate tumor growth in hypophysectomized rats seems to be attributable to a maintained low level of lactogenic receptors. Thus, in addition to the requirement of a proper hormonal environment, plasma membrane lactogenic receptors must be present in appropriate levels for optimal growth.

It has been reported that replacement therapy with ovine PRL partially restores lost hepatic lactogenic receptors in hypophysectomized female rats (3). However, other investigators showed that PRL in combination with various hormones failed to prevent the decrease in hepatic binding in females subsequent to HYP (24).

Although all the hormonal factors involved in regulating hepatoma growth have yet to be elucidated, it seems clear that the pituitary hormones play a major role. In addition to the documented importance of thyroid hormones, PRL can now be proposed as a key regulator of hepatoma growth. However, before concluding that Morris hepatoma 44 is a PRL dependent tumor, it will be necessary to demonstrate that selective reduction of serum PRL by bromocryptine (8) or pergolide mesylate (25) will inhibit the growth of this tumor. Studies to delineate the effects of various combinations of PRL growth hormone and thyroxine on the growth of Morris hepatoma 44 would further our understanding of the hormonal dependency of this tumor.

ACKNOWLEDGMENTS

The authors would like to thank Dr. K. Ruf, Department of Obstetrics and Gynecology, McGill University, for serum PRL determinations; Dr. L. Slaugher, Department of Pathology, Howard University, Washington, DC for reviewing pathologic sections; Jean Parado for her skilled technical assistance; and Jeffrey Tyberg and Miles Reid for their help in administering injections to animals.

REFERENCES


Fig. 1. Pulmonary metastases at time of sacrifice in lung tissue from untreated tumor-bearing controls (A); hypophysectomized rats (B); hypophysectomized rats receiving exogenous hormones (PRL plus growth hormone plus thyroxine) (C). Sections were prepared in a standardized manner from inflated lung tissue as described in "Materials and Methods." × 3.5.
Effects of Hypophysectomy and Hormone Replacement on the Local and Metastatic Growth of Morris Hepatoma 44

Julius Erdstein, Harvey J. Guyda and Seymour Mishkin

Cancer Res 1984;44:2936-2941.

Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/44/7/2936

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.