

# Influence of Prolactin and Growth Hormone on Rat Mammary Tumors Induced by *N*-Nitrosomethylurea<sup>1</sup>

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## ABSTRACT

The effects of hypophysectomy and prolactin-suppressing drugs on the growth of mammary tumors induced in Sprague-Dawley rats by *N*-nitrosomethylurea and dimethylbenz(a)anthracene were compared. The influence of ovine prolactin and growth hormone administration on *N*-nitrosomethylurea-induced tumors was also studied in hypophysectomized animals. After hypophysectomy, all 13 tumors induced in 13 rats by *N*-nitrosomethylurea underwent regression, as did ten of 12 induced by dimethylbenz(a)anthracene. There were no new tumors. Pergolide mesylate, a long-acting ergoline derivative, was given in a dose of 80  $\mu$ g twice daily by s.c. injection for 28 days. Only three of 12 *N*-nitrosomethylurea-induced tumors regressed, while four became static. However, only two new tumors developed in the 12 pergolide-treated rats, compared to 11 in the 12 untreated controls. Bromocriptine mesylate, at ten times the pergolide dose, was even less effective; one of 16 tumors regressed, two became static, and eight new tumors appeared in the 16 rats. In contrast, eight of 12 dimethylbenz(a)anthracene-induced tumors regressed during pergolide therapy, two became static, and there was only one new tumor among the 12 rats. Prolactin, 1 mg twice daily for 7 days by s.c. injection, was given to another eight rats bearing 11 *N*-nitrosomethylurea-induced tumors, commencing 7 days after hypophysectomy. Regression of five tumors borne by four rats was reversed but resumed when treatment was stopped. Regression of five tumors in the other four animals was arrested without regrowth; the sixth became palpable. All of these six grew rapidly when growth hormone, 2 mg twice daily, was administered in addition to prolactin.

## INTRODUCTION

There has been considerable recent interest in the utilization of rat mammary carcinomas induced by NMU<sup>3</sup> as a model for human breast cancer (1, 5, 15, 18, 20). Different dosage schedules of carcinogen administration have been used, and these influence the hormone dependence of the resulting tumors. Thus, when the NMU is given i.v. in 3 doses of 5 mg/100 g body weight at 4 weekly intervals, there is a wide variation in tumor response to ovariectomy or treatment with an antiestrogen (14, 16). In contrast, tumors obtained after exposure to the same dose on 2 occasions 1 week apart are nearly always extremely sensitive to estrogen withdrawal or antiestrogen therapy (16).

In a preliminary study, we found that, unlike DMBA-induced rat mammary tumors, carcinomas obtained with NMU are not stimulated to regrow after ovariectomy by perphenazine-mediated hyperprolactinemia (12). This observation suggested that these tumors require estrogen for their growth, rather than being predominantly prolactin dependent. Further, we showed (15), as did Arafah *et al.* (2), that estradiol replacement frequently prevents tumor regression after hypophysectomy. In this regard, the hormone dependence of NMU-induced rat mammary carcinomas does appear more akin to some human breast cancers than to the DMBA model.

In the study reported here, the responses of tumors induced by NMU and DMBA to hypophysectomy and pharmacological prolactin suppression were compared, and the effects of prolactin and growth hormone replacement on the growth of NMU-induced tumors were examined after pituitary ablation.

## MATERIALS AND METHODS

Mammary tumors were induced in female Sprague-Dawley rats (King Laboratories, Oregon, Wis.) by 2 doses of NMU as described previously (16) or by DMBA. The DMBA (a gift from The Upjohn Company, Kalamazoo, Mich.) was given in a fat emulsion as a single 5-mg dose i.p. when the animals were 50 days old. Tumor size was measured at weekly intervals with a caliper. Measurements of the maximum tumor diameter (*L*) and that at right angles to it (*W*) were made, and the surface area was calculated from the formula  $(L/2) \times (W/2) \times \pi$ . Animals were entered sequentially into the various endocrine treatment groups when one tumor, designated the reference tumor, had achieved a maximum diameter of approximately 2.0 cm. This ensured that there was an even distribution of tumors with different latent periods among the groups.

Hypophysectomies were performed by the parapharyngeal route, and tumor responses were evaluated over a 28-day period. Completeness of the ablative procedure was confirmed by arrest of body growth, uterine, ovarian, and adrenal atrophy, and cessation of the estrous cycle. Pergolide mesylate, a potent and long-acting ergoline derivative (8), was a gift from Lilly Research Laboratories, Indianapolis, Ind. It was administered by 2 s.c. injections at 8:00 a.m. and 4:00 p.m. each in a dose of 80  $\mu$ g dissolved in distilled water. Preliminary experiments determined that this schedule maintained the serum prolactin below levels detectable by radioimmunoassay (2.5 ng/ml) in samples obtained immediately before the morning dose. Another group of 16 rats with NMU-induced tumors was treated with bromocriptine mesylate (a gift from Sandoz Pharmaceuticals, Inc., East Hanover, N. J.) in 2 daily doses of 0.8 mg each given by s.c. injection at 8:00 a.m. and 4:00 p.m.

OPRL and OGH were provided by the National Pituitary Agency, NIH; the biopotencies of the 2 preparations were 31.0 and 0.56 IU/mg, respectively. The OGH had been determined to have less than 0.5 IU/mg prolactin activity. For s.c. injection, the peptides were dissolved in 0.9% NaCl solution adjusted to pH 9.0. Preliminary experiments showed that effective doses of OPRL and OGH for influencing tumor growth after hypophysectomy were, respectively, 1 and 2 mg given twice a day.

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<sup>3</sup> The abbreviations used are: NMU, *N*-nitrosomethylurea; DMBA, dimethylbenz(a)anthracene; OPRL, ovine prolactin; OGH, ovine growth hormone.

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Table 1  
Overall responses of NMU- and DMBA-induced rat mammary tumors to hypophysectomy and ergoline derivatives

Treatment group	No. of rats/ group	Tumor area (sq cm)		Classification of responses to treatment			
		Pretreatment	Posttreatment	Regressions	Static	Progressions	New tumors
NMU-induced controls	12	2.08 ± 0.03 <sup>a</sup>	5.36 ± 3.11 <sup>b</sup>	0 (0) <sup>c</sup>	3	9	11
NMU induced, hypophysectomy	13	2.38 ± 0.33	0.07 ± 0.19 <sup>d</sup>	13 (11)	0	0	0
NMU induced, pergolide treated	12	2.14 ± 0.50	3.52 ± 3.02 <sup>b</sup>	3 (2)	4	5	2
NMU induced, bromocriptine treated	16	2.14 ± 0.40	5.32 ± 3.82 <sup>b</sup>	1 (0)	2	13	8
DMBA-induced controls	13	2.20 ± 0.31	5.91 ± 3.70 <sup>b</sup>	1 (1)	3	9	11
DMBA induced, hypophysectomy	12	2.41 ± 0.64	0.45 ± 0.82 <sup>d</sup>	10 (8)	2	0	0
DMBA induced, pergolide treated	12	2.26 ± 0.51	1.70 ± 2.88	8 (5)	2	2	1

<sup>a</sup> Mean ± S.D.

<sup>b</sup>  $p < 0.01$ .

<sup>c</sup> Numbers in parentheses, complete regressions.

<sup>d</sup> Significantly different from corresponding pretreatment values by Student's paired  $t$  test ( $p < 0.001$ ).

Tumor responses to hypophysectomy or prolactin suppression were classified as: regression, a decrease in surface area of 50% or more; progression, an increase of 50% or more; or static. A tumor which ceased to be palpable was designated a complete regression.

## RESULTS

Table 1 summarizes the responses of NMU- and DMBA-induced mammary tumors to hypophysectomy and treatment with prolactin-suppression drugs. The posttreatment surface area refers to the measurement 28 days after initiating therapy or observing the controls. The mean tumor area of the control reference tumors induced by either carcinogen increased approximately 2.5-fold during this period (paired Student's  $t$  test,  $p < 0.01$ ). There were 11 new tumors in each control group. All of the reference tumors borne by 13 rats exposed to NMU underwent regressions after hypophysectomy; in 11, these were complete. The responses occurred rapidly, an average reduction of 63% in tumor surface area being achieved by 7 days posthypophysectomy. No new tumors appeared. Hypophysectomy also caused regression of 10 of the 12 DMBA-induced tumors, 2 showed arrest of growth, and there were no progressions. Again, no new tumors developed during the observation period after hypophysectomy.

Pergolide mesylate was also effective in suppressing growth of tumors induced by DMBA; after 28 days, the mean tumor surface area was reduced significantly compared to the control group ( $p < 0.01$ ). Eight of the 12 reference tumors underwent regressions, and in 5, these were complete (Table 1). There was only one new tumor (0.08/rat). In contrast, only 3 of 12 NMU-induced tumors regressed during pergolide therapy while another 4 became static. There was no significant difference between the mean tumor surface area of the control and pergolide-treated groups; in the treatment group, this increased significantly during the 28 days of pergolide therapy ( $p < 0.01$ ). However, the prolactin-suppressing drug did prevent the appearance of new tumors; only 2 (0.17/rat) developed during the treatment period.

Bromocriptine, at 10 times the dose of pergolide, was even less effective against NMU-induced mammary tumors. There was a significant increase in tumor surface area during administration of the drug ( $p < 0.01$ ). Only one tumor underwent regression, 13 of the 16 continued to grow, and there were 8 new tumors (0.5/rat).

Another 8 rats bearing 11 NMU-induced mammary carcinomas were subjected to hypophysectomy. Seven days later, all

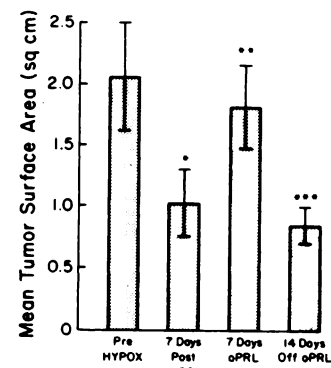


Chart 1. Mean surface areas of 5 NMU-induced tumors immediately before hypophysectomy (HYPOX), with partial regressions 7 days later, stimulation of regrowth by OPRL (oPRL), 1 mg twice a day, and second regressions 14 days after discontinuing hormone replacement. \*, significantly smaller than prehypophysectomy surface areas; \*\*, greater than 7-day posthypophysectomy values; \*\*\*, smaller than values after OPRL (Student's paired  $t$  test, all  $p < 0.001$ ). Bars, S.D.

of the tumors had undergone partial regression, at which time OPRL was given for a further 7 days. Chart 1 shows the hypophysectomy-induced regressions of 5 tumors borne by 4 rats, the subsequent stimulation of growth by OPRL, and the second regressions which occurred when hormone treatment was discontinued. While administration of OPRL to the other 4 rats arrested the regression of 5 tumors after hypophysectomy, regrowth did not occur; a sixth tumor was no longer palpable despite 7 days of OPRL replacement (Chart 2). When OGH, 2 mg twice a day, was given in addition to OPRL, all 6 of these tumors grew rapidly, although the one rat bearing 2 tumors died after 9 days of combined hormone administration (Chart 2).

## DISCUSSION

The study reported here showed that rat mammary tumors induced by NMU and DMBA were equally responsive to hypophysectomy. It should be stressed, however, that the 2-dose schedule of NMU administration was used in these experiments. We have found that the method of induction originally described by Gullino *et al.* (5), in which 3 doses of the carcinogen are given at monthly intervals, yields tumors only 65% of which regress after hypophysectomy.<sup>4</sup> This difference in re-

<sup>4</sup> D. P. Rose and J. J. Noonan, unpublished data.

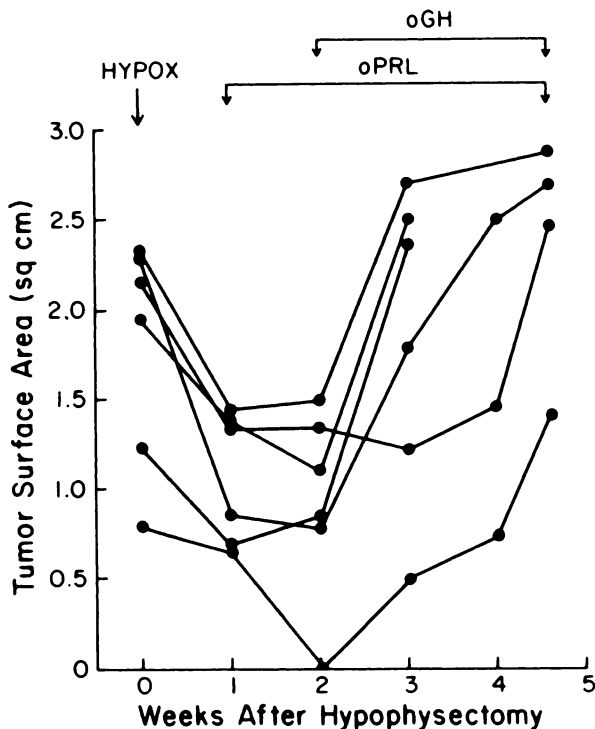


Chart 2. Response of 6 NMU-induced tumors, borne by 4 rats, to hypophysectomy (HYPOX) and hormone replacement with OPRL (oPRL), 1 mg twice daily for 7 days, followed by OPRL plus OGH (oGH), 2 mg twice daily. One rat with 2 rapidly growing tumors died after 9 days of combined hormone replacement.

response to pituitary ablation is consistent with our previous observation that the mode of induction with NMU determines the subsequent hormone dependence of rat mammary tumors (16).

Both estrogens and prolactin influence the growth of DMBA-induced rat mammary tumors (9), although the dominating hormone appears to be prolactin (10). After hypophysectomy, growth of these tumors is stimulated by prolactin administration, while estrogen replacement is without effect (11, 17). Estrogens reactivate tumor growth after ovariectomy, but this may be mediated by way of prolactin release from the adenohypophysis (11).

The majority of DMBA-induced mammary tumors regress or stop growing during treatment with drugs which suppress serum prolactin (6, 13). Our study showed that pergolide mesylate, a new ergoline derivative which is a potent and long-acting suppressor of serum prolactin levels, was generally effective against DMBA-induced mammary tumors. However, neither pergolide nor bromocriptine at 10 times the dose consistently brought about regression of NMU-induced mammary carcinomas. Pergolide did, however, slow the rate of growth and prevent the emergence of new tumors. The dose of bromocriptine used was above that which previous investigators found to be effective in causing significant reduction in rat serum prolactin and in producing regression of DMBA-induced mammary tumors (6).

This relative inefficacy of prolactin-suppressing ergoline derivatives against established NMU-induced tumors contrasts with the effect of bromocriptine on the developmental phase of tumorigenesis. Welsch *et al.* (19) reported a 50% reduction in the number of rats with mammary tumors and a considerable decline in the total number of tumors in animals treated with

the drug shortly after exposure to NMU. A somewhat similar situation holds, but at a later stage, for tumors induced by DMBA. Here, response to ovariectomy (4), tamoxifen (7), and ergoline derivatives (3, 7) diminishes with increasing latent period and size of the tumors.

Although the NMU-induced tumors exhibited only a limited response to pergolide and bromocriptine, regressions after hypophysectomy were either reversed or halted by prolactin administration. In some cases, both prolactin and growth hormone replacement were necessary to obtain complete regrowth of tumors. These results suggest that at least some tumors are dependent on the 2 hormones and that elimination of both is necessary for a full therapeutic response. The failure of ergoline derivatives to induce regressions may be due, therefore, to the fact that these compounds do not suppress serum growth hormone levels (21).

The endocrinology of NMU-induced mammary carcinomas is complicated further by the effects of estrogen on these tumors in hypophysectomized rats. Replacement doses of estradiol inhibited regression when commenced immediately after surgery (15) and arrested its continuation when given 5 days later (2). Usually, estrogen alone did not support tumor regrowth, but this did occur with the addition of prolactin (2). Thus, the available data indicate that prolactin, growth hormone, and estrogens are all involved in the maintenance of optimal growth of rat mammary tumors when these are induced by the 2-dose NMU induction schedule as used in the present and earlier (2, 15, 16) studies.

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