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Counteraction of Underfeeding-Induced Inhibition of Mammary Tumor Growth in Rats by Prolactin and Estrogen Administration (41625)

F. C. LEUNG,[1] C. F. AYLSWORTH,[2] AND J. MEITES[3]

Department of Physiology, Neuroendocrine Research Laboratory, Michigan State University, East Lansing, Michigan 48824

Abstract. The purpose of this study was to determine whether administration of estrogen or/and haloperidol (HAL), a drug that increases prolactin (PRL) secretion, could counteract the inhibitory effects of underfeeding on growth of established carcinogen-induced mammary cancers in rats. Sprague–Dawley female rats were fed half of the complete diet consumed daily by *ad libitum* fed controls, beginning 1 week before daily injection of estradiol benzoate (EB) or/and HAL for 3 weeks. Body weight, mammary tumor number, and mammary tumor diameter were measured at weekly intervals, and at the end of the 3 weeks blood was collected for assay of serum PRL. Full-fed (FF) control rats showed an increase by the end of 3 weeks in average body weight, mammary tumor number, and mammary tumor size whereas half-fed (HF) rats showed a significant decrease in average body weight, tumor number, and tumor size, and serum PRL concentrations when compared with these parameters in FF rats. Administration of EB to the HF rats partially prevented loss of mammary tumor size and significantly increased serum PRL levels over those of HF or FF control rats. Injection of HAL or the combination of HAL and EB completely prevented the decrease in mammary tumor number and size in the HF rats. These results suggest that regression in number and size of established mammary tumors in HF rats during a 3-week period is due primarily to a decrease in secretion of estrogen and PRL, the two hormones essential for mammary tumor growth in rats.

The effects of caloric restriction on development and growth of tumors, including mammary cancers, have been widely investigated. In general, rats subjected to underfeeding showed a significant inhibition in development and growth of carcinogen-induced mammary cancers (1). The incidence of spontaneous mammary tumors in mice subjected to underfeeding also was significantly decreased (2). The inhibitory effects of underfeeding could be due to lack of nutrients available to the tumor tissue, but also to reduced secretion of hormones by the anterior pituitary. In a recent study, rats subjected to underfeeding showed reduced blood levels of prolactin (PRL), luteinizing hormone, follicle-stimulating hormone, thyroid-stimulating hormone, and growth hormone, as compared to values in full-fed rats (3). Development of carcinogen-induced mammary tumors in underfed rats was significantly enhanced by administration of estrogen and/or haloperidol, a drug that promotes PRL secretion (1). Estrogen and PRL have been shown to be essential for mammary tumor development and growth in the rat (4).

The purpose of this investigation was to determine if the reduced growth of mammary tumors established by underfeeding could be due to decreased secretion of PRL and and/or estrogen. It also was of interest to determine whether administration of estradiol benzoate (EB) or haloperidol (HAL) (a dopamine receptor blocker that increases serum PRL secretion), or both, could counteract inhibition of growth of carcinogen-induced mammary tumors produced by underfeeding.

Materials and Methods. Virgin female Sprague–Dawley rats (Harlan Research Animals, Indianapolis, Ind.), 50–55 days of age, were given a single iv injection of 1 ml lipid emulsion containing 5 mg of 7,12-dimethylbenz(a)anthracene (DMBA), obtained through the courtesy of Dr. Paul Schurr, The Upjohn Co., Kalamazoo, Michigan. The rats were

[1] Present address: Merck Sharp & Dohme Research Laboratories, P.O. Box 2000 (R80-152), Rahway, N.J. 07065.

[2] Present address: Department of Anatomy, Michigan State University, East Lansing, Mich. 48824.

[3] Aided in part by NIH research Grant CA 10771 from the National Cancer Institute. To whom reprint requests and correspondence should be addressed.

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housed in a temperature-controlled (24 ± 0.5°C) and light-controlled (14 hr L:10 hr D) room, and fed rat chow (Ralston–Purina Co., St. Louis, Mo.) and water *ad libitum*. About 8–10 weeks after DMBA administration, when each rat had at least one tumor measuring more than 1 cm in diameter, the rats were placed in individual cages and treated as follows: rats in group 1 (full fed, FF) were given four large pellets of rat chow per rat per day, and half fed (HF) rats in groups 2–5 received two rat chow pellets per rat per day. It was initially determined that *ad libitum* fed rats each consumed about four pellets daily. Rats in groups 1 and 2 were given daily injections of 0.1 ml of 0.3% tartaric acid and 0.1 ml of 0.3% ethanol (control vehicles). Rats in group 3 were injected with 0.2 μg estradiol benzoate (EB) in 0.3% ethanol, and 0.3% tartaric acid. Rats in group 4 received a daily injection of 0.1 ml of 120 μg haloperidol (HAL) dissolved in 0.3% tartaric acid and 0.1 ml of 0.3% ethanol. Rats in group 5 were given a daily injection of 0.2 μg EB and HAL prepared as above.

Rats were placed on their respective feeding regimens 1 week before EB and/or HAL administration, and tumor measurements and body weights were recorded at weekly intervals after initiation of drug treatment for a 3-week period. Average tumor diameter for each palpable tumor was determined by using the mean of the two largest perpendicular diamters, as measured with vernier calipers. Tumor growth was expressed as the percentage change in average tumor diameter when compared with the initial measurements made prior to drug treatment. All blood samples were collected by decapitation at the end of 3 weeks between 9 and 10 AM to avoid the surge of PRL on the afternoon of proestrus. Serum was separated by centrifugation and stored at −20°C until assayed for PRL. Serum PRL was measured by a standard radioimmunoassay (RIA) method (5) with materials supplied by NIAMD. The PRL antibody was generously provided by Dr. C. L. Chen, College of Veterinary Medicine, University of Florida, Gainesville, Florida. Statistical differences between treatment groups were determined by analysis of variance. A difference of $P < 0.05$ was considered to be significant.

Results. The effects of the different treatments on mean body weights are shown in Table I. FF rats (group 1) showed a small but statistically insignificant increase of body weight at the end of 3 weeks when compared with mean body weight at the start of the experiment. Rats (groups 2–5) fed half of the rat chow consumed by the FF controls showed considerable losses in average body weight which were significantly below those of the FF controls by the end of 3 weeks. Table II shows the effects of underfeeding with or without injections of EB and HAL on average tumor number per rat. HF rats (group 2) showed a significant decrease in average tumor number as compared with FF rats (group 1) at the end of 3 weeks. HF rats given injections of EB (group 3) showed no loss in average tumor number at the end of 3 weeks as compared to average initial number, and a small but insignificant increase in number over the HF controls (group 2). HF rats given HAL (group 4) or E and HAL (group 5) showed a significant increase in average tu-

TABLE I. CHANGES IN AVERAGE BODY WEIGHT (gm) PER WEEK DURING TREATMENT

Group and treatment	No.	Week				
		−1	0	1	2	3
1. Controls, FF	7	285.1	316.5	318.7	303.0	332.5
2. Controls, HF	5	282.0	252.3	260.1	226.0	236.4[a]
3. HF + EB	4	270.1	255.8	252.6	220.1	237.7[a]
4. HF + HAL	6	280.7	262.5	249.8	246.1	250.3[a]
5. HF + EB + HAL	7	284.3	258.4	242.5	231.7	245.8[a]

Note. No. = number of rats per group. Abbreviations used: FF, full-fed; HF, half-fed; EB, estradiol benzoate; HAL, Haloperidol.

[a] Significantly different from FF controls ($P < 0.05$).

TABLE II. EFFECT OF UNDERFEEDING WITH OR WITHOUT INJECTIONS OF ESTRADIOL BENZOATE (EB), OR/AND HALOPERIDOL (HAL) ON AVERAGE MAMMARY TUMOR NUMBER (NUMBER OF TUMORS PER RAT PER WEEK)

Group and treatment	No.	Week 0	1	2	3
1. Controls, FF	7	2.4 ± 0.7†	2.4 ± 0.7	3.2 ± 0.6	3.4 ± 0.8
2. Controls, HF	5	2.4 ± 0.6	2.4 ± 0.6	1.8 ± 0.3	1.0 ± 0.0[a]
3. HF + EB	4	2.0 ± 0.7	2.0 ± 0.7	1.7 ± 0.4	1.7 ± 0.4
4. HF + HAL	6	2.0 ± 0.4	2.0 ± 0.4	3.5 ± 0.9[b]	3.1 ± 0.8[b]
5. HF + EB + HAL	7	2.4 ± 0.4	2.1 ± 0.4	2.8 ± 0.6[b]	3.0 ± 0.5[b]

Note. No. = number of rats per group. † = standard error of mean.
[a] Significantly different from FF controls ($P < 0.05$).
[b] Significantly different from HF controls ($P < 0.05$).

mor number over HF controls (group 2), and average tumor number equalled that of FF controls (group 1).

Figure 1 shows the effects of underfeeding with or without injections of EB and HAL on mammary tumor growth, expressed as percentage change in average tumor diameter when compared with pretreatment values. FF rats showed a steady rise in average tumor diameter, which increased by about 14% by 1 week, by 23% by 2 weeks, and 38% by the end of 3 weeks. HF rats showed a significant reduction in average tumor size, decreasing by about 26% by 1 week, 30% by 2 weeks, and 42% by the end of 3 weeks. HF rats given injections of EB and/or HAL showed a reduction in average tumor size as compared with FF control rats at the end of 1 week, but by the end of 2 weeks these rats showed a significant increase in average tumor diameter over that of HF control rats. HF rats given HAL showed average tumor diameter at the end of 3 weeks equal to that of FF controls, whereas average tumor diameter in rats given both EB and HAL was significantly greater than that of FF controls at the end of 3 weeks.

Serum PRL levels are shown in Table III. HF rats showed a significant reduction in serum PRL as compared with FF rats. HF rats injected daily with EB (group 3) for 3 weeks showed a many-fold increase in average serum PRL concentration over that of HF (group 2) or FF (group 1) controls. HF rats given HAL (group 4) exhibited a greater rise in serum PRL levels than in the estrogen-treated rats (group 3), whereas in rats given both EB and HAL (group 5) serum PRL concentrations were significantly higher than in any other group.

Discussion. The present results show that a reduction in food intake to half of that consumed daily by *ad libitum* fed rats, resulted in a significant decrease in average tumor number and size, and a significant reduction in serum PRL levels. Attempts to measure serum estradiol levels in these rats were not successful. However, in a previous study, when Sprague–Dawley female rats were HF, ces-

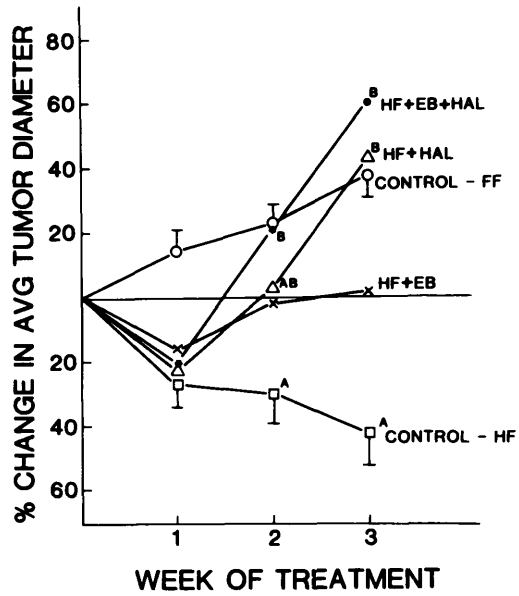

FIG. 1. Effects of underfeeding with or without EB and HAL on percentage change in average tumor diameter. (A) Significant difference from FF control rats ($P < 0.05$). (B) Significant difference from HF control rats ($P < 0.05$).

TABLE III. EFFECTS OF UNDERFEEDING WITH OR WITHOUT EB AND HAL ON AVERAGE SERUM PROLACTIN CONCENTRATIONS

Group and treatment	No.	Serum PRL (ng/ml)
1. Controls, FF	7	55.6 ± 8.9†
2. Controls, HF	5	17.2 ± 1.8[a]
3. HF + EB	4	189.0 ± 39.0[a,b]
4. HF + HAL	6	580.9 ± 66.3[a,b]
5. HF + EB + HAL	7	1555.6 ± 274.0[a,b]

Note. No. = number of rats per group. † = Standard error of mean.

[a] Significantly different from FF controls ($P < 0.05$).

[b] + significantly different from HF controls ($P < 0.05$).

sation of estrus cycles occurred in 2 to 3 weeks and a significant decrease in uterine weight was observed (6). This suggests that estrogen secretion is reduced in HF rats. When HF rats were injected daily with EB, this prevented any reduction in average tumor number and partially prevented loss in average tumor diameter, despite an increase in serum PRL values even above those of FF controls. This failure of EB treatment alone to induce mammary tumor growth in the HF rats equal to that seen in the FF rats may be due to the dose of EB used, since relatively high doses of EB have been shown to reduce the number of specific PRL receptors in the mammary tumor tissue and to inhibit tumor growth even though serum PRL levels were elevated (7). In the present study, the observation that serum PRL values in the rats given EB alone were significantly higher than in the FF controls indicates that the dose of EB used was above physiological levels.

When HF rats were injected daily with HAL, average tumor number and size equaled that of FF controls at the end of 3 weeks, and serum PRL levels were greater than in FF or HF control rats or in HF rats given EB. This suggests that PRL is more important than estrogen for promoting mammary tumor growth in rats, in agreement with similar conclusions by others (8). The HF rats given both EB and HAL showed average tumor number at the end of 3 weeks equal to that of FF rats, average tumor diameter greater than in FF rats, and serum PRL levels far greater than in any other group. The greater average tumor diameter in the HF animals given both EB and HAL than in the FF controls, although not significant, probably can be attributed mainly to the high serum PRL levels, since an increase in PRL secretion has been reported to promote growth of established mammary tumors in rats (4, 7).

We recently reported results similar to those of the present study on *development* of mammary tumors in rats given half-normal food intake for 1 week before and 7 or 30 days after DMBA treatment (1). These rats showed a significant decrease in incidence and size of mammary tumors. However, when rats were injected with EB and HAL during the first week after DMBA treatment, the number and size of the mammary tumors that developed equaled those of the FF controls. Thus mammary tumor *development* as well as mammary tumor *growth* in DMBA-treated female Sprague–Dawley rats depends on adequate secretion of estrogen and PRL. Other anterior pituitary hormones also show a decline in secretion as a result of underfeeding, including growth hormone (GH), gonadotropins, and thyrotropin (TSH) (3). However, GH and thyrotropin have not been shown to be essential for mammary tumor growth in Sprague–Dawley rats (4). Severe underfeeding also can produce enhanced ACTH and adrenal-cortical hormone secretion (3), and glucocorticoid hormones can inhibit mammary tumor growth (9). It is possible that increased secretion of adrenal cortical hormones may have occurred in the HF control rats used in the present study and contributed to the reduction in tumor growth seen in these rats. However, it is clear that if this were so, administration of EB and/or HAL were sufficient to overcome part or all of any inhibitory effects of underfeeding exerted via the adrenal cortex on mammary tumor growth.

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