Underfeeding-Induced Suppression of Mammary Tumors: Counteraction by Estrogen and Haloperidol (43596)

S. THYAGARAJAN,* J. MEITES,† AND S. K. QUADRI*,[1]

Department of Anatomy and Physiology, Kansas State University, Manhattan, Kansas 66506; and the Department of Physiology,† Michigan State University, East Lansing, Michigan 48824*

Abstract. **The purpose of this study was to investigate the mechanism by which underfeeding induces regression of carcinogen-induced mammary tumors in the rat and to determine if tumor regression in underfed rats could be prevented on a chronic basis by maintaining elevated circulating levels of estrogen and/or prolactin (PRL) by treatment with estradiol benzoate (EB) and a dopamine receptor blocker, haloperidol (HAL). Female rats with 7,12-dimethylbenzanthracene-induced mammary tumors were fed *ad libitum* (full-fed), half-fed (HF), or half-fed and treated with EB (HF+EB), HAL (HF+HAL), or both (HF+EB+HAL) for 15 weeks. Tumor diameter, tumor number, and body weight were determined each week. At the end of the experiment, hypothalamic concentrations of catecholamines, indoleamines, and their metabolites were determined by high performance liquid chromatography.**

Tumor diameter, tumor number, and body weight increased progressively in the full-fed rats, but decreased significantly in the HF rats. Treatment of HF rats with EB, HAL, or both prevented tumor regression, but had no effect on body weight, which declined continuously. In the HF rats, there was an increase in the concentration of dopamine and a decrease in the concentration of serotonin in the hypothalamus, whereas treatment with HAL reversed these effects. EB had no effect on neurotransmitter concentrations in the HF rats, but treatment of HF+EB animals with HAL decreased the dopamine concentration. The changes in dopamine and serotonin observed in HF rats are known to inhibit PRL secretion, whereas HAL, which blocked these changes, is a well established stimulator of PRL secretion. Since the mammary tumors are dependent on PRL for development and growth, it is probable that the regression of these tumors in the HF rats was ultimately due to a decrease in PRL secretion, and the prevention of this regression in HF+HAL rats was ultimately due to an increase in PRL secretion. EB, a potent PRL stimulator, probably blocked tumor regression in HF+EB rats by increasing PRL secretion by a direct effect on the pituitary. [P.S.E.B.M. 1993, Vol 203]

7,12-Dimethylbenzanthracene (DMBA)-induced mammary tumors in the rat depend primarily on prolactin (PRL) for their development and growth (1). Estrogen also stimulates mammary tumor growth by increasing PRL secretion as well as by a direct action on the mammary tissue (2). The mammary tumors regress after ovariectomy and in the absence of sufficient circulatory levels of PRL (2). Underfeeding of tumor-bearing rats also causes tumor regression (3), but the mechanism of this effect is not clear. Underfeeding is known to decrease the secretion of a number of hormones, including PRL and estrogen (4), thus raising the possibility that regression of mammary tumors in underfed rats occurs due to a deficiency of PRL and estrogen.

The purpose of this study was to determine whether tumor regression in underfed rats could be prevented on a chronic basis by maintaining elevated circulating levels of estrogen and/or PRL. For this purpose, the tumor-bearing rats were treated with a dopamine receptor blocker, haloperidol (HAL), estradiol benzoate (EB), or both. HAL is a potent stimulator of PRL secretion in the rat and other species (5, 6), whereas EB stimulates

[1] To whom requests for reprints should be addressed at Department of Anatomy and Physiology, VMS 228, Kansas State University, Manhattan, KS 66506.

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PRL secretion as well as increases serum levels of estrogen. In one acute study, these treatments have been found to prevent tumor regression for a short period of time (3), but it is not known if they would do so on a long-term basis. Since the secretion of PRL and gonadotropic hormones, which affect estrogen production, is regulated to a great extent by hypothalamic catecholamines and indoleamines, changes in these neurotransmitters during underfeeding with or without simultaneous treatment with HAL and EB were also measured.

Materials and Methods

Animals. Female Sprague-Dawley rats were obtained at the age of 35–40 days (Harlan Sprague-Dawley, Inc., Indianapolis, IN) and housed in air-conditioned, temperature-controlled (23 ± 2°C), animal quarters (photoperiod, 0500–1900 hr). They were provided with feed and water *ad libitum*. At the age of 50–55 days, they were injected through the tail vein with a single dose of 1 ml of a lipid emulsion containing DMBA (Upjohn Co., Kalamazoo, MI). About 2 months after DMBA administration, the rats began to develop mammary tumors. After tumor development, the rats were housed in individual cages and divided randomly into five different groups.

Treatment. Rats in group 1 were fed *ad libitum*, and rats in groups 2–5 were fed 50% of the feed consumed by rats in group 1. The average feed consumption by rats in group 1 was determined every week, and the feed given to groups 2–5 was adjusted accordingly. Rats in group 1 (full-fed [FF]; $n = 7$) and group 2 (half-fed [HF]; $n = 5$) were injected with a mixture of 0.1 ml of 0.3% tartaric acid and 0.1 ml of 0.3% ethanol. This mixture was used as a vehicle for injecting estradiol benzoate (EB) and haloperidol (HAL) to groups 3–5. Rats in group 3 ($n = 6$) were injected with 0.2 μg of EB/rat, dissolved in 0.2 ml of the vehicle described above (HF+EB). Rats in group 4 ($n = 5$) were injected with 120 μg of HAL/rat, dissolved in 0.2 ml of the vehicle (HF + HAL). Rats in group 5 ($n = 6$) were treated with 0.2 μg of EB and 120 μg of HAL/rat (HF+EB+HAL). All injections were given sc every day for 15 weeks. The rats in groups 3–5 were half-fed for a week before beginning treatment with EB and/or HAL. Mammary tumor diameter, mammary tumor number, and body weight were determined every week throughout the treatment period. Tumor size was measured by averaging the two perpendicular diameters measured with vernier calipers.

At the end of the treatment period, the rats were sacrificed, and the medial basal hypothalami were dissected out using the posterior part of the optic chiasm as the anterior limit, the anterior part of the mammillary bodies as the posterior limit, and the lateral hypothalamic sulci as the lateral limits. To prevent oxidation of the neurotransmitters, each medial basal hypothalamic block was put in 200 μl of 0.1 *M* $HClO_4$ (7) and stored at −70°C until analyzed for neurotransmitters by high performance liquid chromatography with electrochemical detection (HPLC-EC).

HPLC-EC. The HPLC-EC procedure has been described in detail previously (7, 8). Briefly, at the time of analysis, the samples were thawed at 60°C for 1 min, then homogenized and centrifuged for the collection of supernatant, which was used for the determination of neurotransmitters and protein. The supernatant (10 μl) from each sample was mixed with 10 μl of the internal standard (isoproterenol) and injected onto a C18, 5-μm particle size, 250-mm long analytical column (Bioanalytical Systems, West Lafayette, IN). The column was kept in a CTO-6A column oven (Shimadzu, Columbia, MD) at a constant temperature of 37°C. The mobile phase, consisting of monochloroacetic acid (14.15 g/liter), octanesulfonic acid (0.25 g/liter), EDTA (0.25 g/liter), sodium hydroxide (4.675 g/liter), acetonitrile (3.5%), and tetrahydrofuran (1.4%), was pumped through the HPLC system with the help of an LC-6A pump (Shimadzu) at a flow rate of 1.6 ml/min. The sensitivity of the LC-4B amperometric detector (Bioanalytical Systems) was 1 namp full scale, and the potential of the working electrode was 0.65 V in channel 1 with respect to an Ag/AgCl reference electrode. The data were analyzed using a C-R4A Chromatopac integrator (Shimadzu). Protein concentrations in supernatants were determined by bicinchoninic acid assay (Pierce, Rockford, IL), and neurotransmitter concentrations were expressed in terms of micrograms of protein. Differences between groups were determined by analysis of variance, followed by Fischer's least significant difference test.

Results

Mammary Tumor Size. Tumor size initially was in the range of 1.3–1.6 cm in various groups, with no significant differences among the different groups. As shown in Figure 1, during the 15-week treatment period tumor diameter increased by 213.8 ± 86% (mean ± standard error) in the FF rats, but decreased by 81.8 ± 13% in the HF rats ($P < 0.001$). The decrease in the HF rats was apparent from the second week of treatment. In contrast to those in HF rats, there were significant ($P < 0.05$) increases in tumor diameter in HF+EB (83.7 ± 36%), HF+HAL (42.1 ± 23%), and HF+EB+HAL (43.3 ± 11%) rats.

Mammary Tumor Number. In the FF group, the average number of tumors/rat increased from 1.2 ± 0.1 to 2.1 ± 0.2 during the 15-week treatment period (Fig. 2). In the HF rats, a significant ($P < 0.01$) reduction occurred in the number of tumors, from 1.6 ± 0.3 at the beginning of the treatment to 0.4 ± 0.2 at the end of the treatment period. In contrast to that in the HF

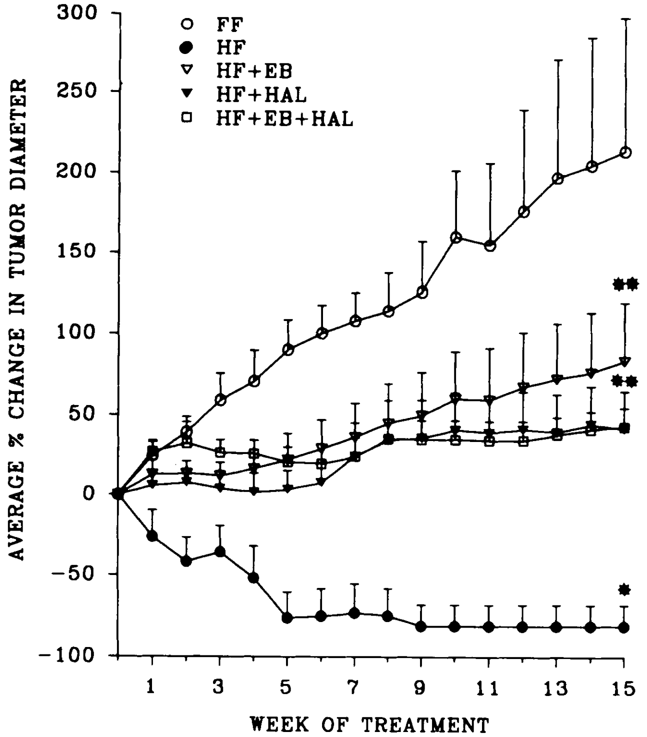

Figure 1. Effects of feed restriction alone or in combination with EB and HAL on average tumor diameter in rats. The control (FF) animals had free access to food. The animals in all other groups were given half of the feed (HF) consumed by the control animals. EB (0.2 μg/rat) and HAL (120 μg/rat) were injected subcutaneously every day during the 15-week treatment period. * Significantly ($P < 0.05$) different from FF. ** Significantly ($P < 0.05$) different from HF.

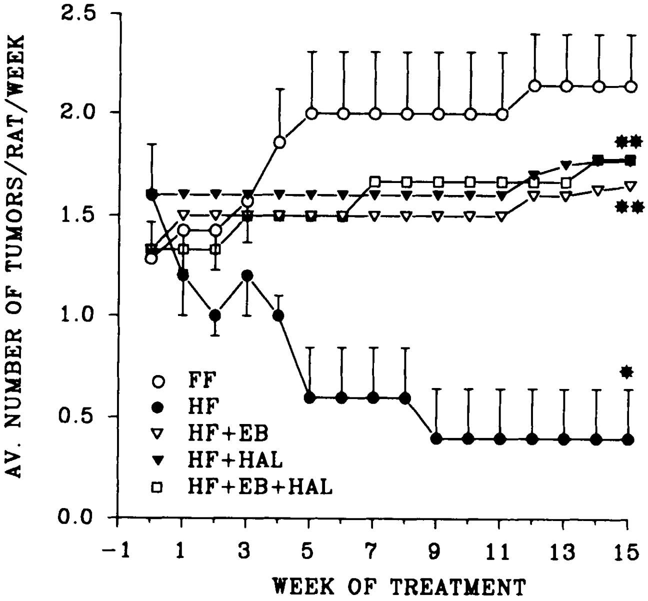

Figure 2. Effects of feed restriction alone or in combination with EB and HAL on the number of tumors/rat/week. See Figure 1 for details. The standard error ranged from 0.21 to 0.22 in HF+EB, from 0.24 to 0.25 in HF+HAL, and from 0.21 to 0.33 in HF+EB+HAL groups. The standard errors for FF and HF are shown in the figure. * Significantly ($P < 0.05$) different from FF. ** Significantly ($P < 0.05$) different from HF.

rats, there was no significant decline in tumor number in the rats treated with EB and/or HAL. The average numbers of tumors at the beginning and end of the treatment were 1.3 ± 0.2 and 1.6 ± 0.2 in the HF+EB group, 1.6 ± 0.2 and 1.7 ± 0.2 in the HF+HAL group, and 1.3 ± 0.2 and 1.7 ± 0.3 in the HF+EB+HAL group.

Body Weight. The average body weight of the rats in the FF group increased throughout the treatment period and was 39.7 ± 17% ($P < 0.05$) above the pretreatment level by the end of the treatment period (Fig. 3). In contrast to the FF rats, the average body weight in the HF rats decreased by 18.4 ± 6% ($P < 0.05$). Similar decreases in body weight were observed in the other groups: 15.9 ± 4% in the HF+EB group, 20.9 ± 1% in the HF+HAL group, and 23.6 ± 2% in the HF+EB+HAL group. Differences in body weight between FF (group 1) and the half-fed rats (groups 2–5) began to be significant from the second week of treatment.

Neurotransmitters. As shown in Figure 4, the hypothalamic dopamine (DA) concentration in the HF group (21.5 ± 0.5 pg/μg protein) was higher ($P < 0.01$) than that in the FF group (19.1 ± 0.4 pg/μg protein). Compared to those in the HF rats, DA concentrations were significantly ($P < 0.01$) lower in the HF+HAL (18.5 ± 0.5 pg/μg protein) and the HF+EB+HAL rats (15.7 ± 0.5 pg/μg protein). The DA concentration in the HF+EB rats (22.5 ± 2.9 pg/μg protein) was not different from that in the HF rats. Norepinephrine and dihydroxyphenylacetic acid concentrations did not differ in the various groups.

The concentration of serotonin (5-HT) in the HF rats (78.2 ± 10.7 pg/μg protein) was lower ($P < 0.05$) than that in the FF rats (133.4 ± 17.6 pg/μg protein; Fig. 5). In contrast to that in the HF rats, the 5-HT concentration was higher ($P < 0.05$) in the HF+HAL rats (120.2 ± 12.2 pg/μg protein). 5-HT concentrations in HF+EB (96.8 ± 19 pg/μg protein) and HF+EB+HAL rats (96.2 ± 12 pg/μg protein) were not different from those in HF rats. No significant differences occurred in the concentration of 5-hydroxyindoleacetic acid among the various treatment groups.

Discussion

These results demonstrate that the regression of carcinogen-induced mammary tumors in severely underfed rats can be prevented on a chronic basis by treatment with estrogen and haloperidol. This effect becomes even more impressive when it is noted that these treatments prevented tumor regression and maintained tumor size and number in the face of a body weight that declined continuously throughout the almost 4-month long period of treatment and was more than 60% below that in the full-fed controls near the end of the treatment period.

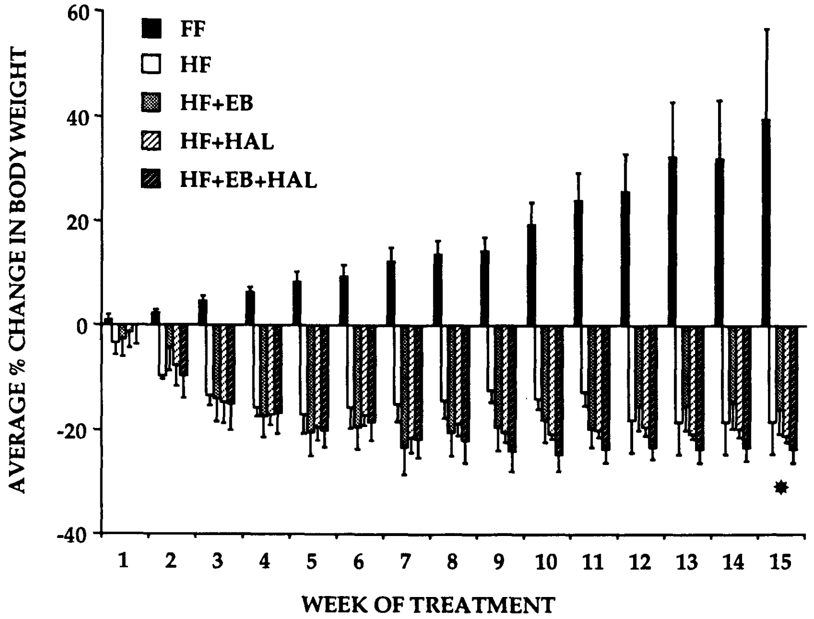

Figure 3. Effects of feed restriction alone or in combination with EB and HAL on the average body weight. See Figure 1 for details. * Significantly ($P < 0.05$) different from pretreatment within respective groups and from week 15 in FF.

DMBA-induced mammary tumors in the rat depend on PRL for their development and growth. We and others have demonstrated in a number of studies that procedures which increase serum PRL, such as administration of PRL (9) or pituitary grafts in the kidney capsule (10), stimulate, whereas procedures that decrease serum PRL levels, such as hypophysectomy (11) or treatment with ergot derivatives (12), inhibit tumor growth. In the present study, underfeeding produced an increase in the DA concentration and a more than 50% decrease in the 5-HT concentration in the hypothalamus. Both of these changes would lead to a decrease in PRL secretion, since DA inhibits and 5-HT increases PRL secretion. There were no significant effects of underfeeding on the dihydroyphenylacetic acid and 5-hydroxyindoleacetic acid levels, indicating that changes in DA and 5-HT were due to changes in the synthesis, rather than in the degradation, of amines. Norepinephrine, which is not known to be involved in the control of PRL secretion to any significant extent, was not affected by underfeeding. Although PRL was not measured in this study, the same level of underfeeding as in the present study has been shown to decrease serum PRL levels by more than 70% in a relatively short period of 3 weeks (3). A number of drugs, such as L-dopa and pargyline, which increase DA activity and thereby decrease PRL release, are also potent inhibitors of tumor growth (13, 14). Thus, the results of the present study indicate that the mechanism by which underfeeding inhibits tumor growth involves changes in hypothalamic DA and 5-HT activities that are known to decrease PRL release. This conclusion is further supported by the fact that when the underfeeding-induced increase in DA concentration and decrease in 5-HT concentration were blocked in half-fed rats by HAL treatment, it resulted in prevention of tumor regression, no doubt due to stimulation of PRL secretion. HAL is a powerful stimulator of PRL secretion in several species (5, 6) and has been shown to produce a more than 30-fold increase in serum PRL levels in rats that were underfed and had mammary tumors, just as they were in the present study (3).

Estrogen is a powerful stimulator of PRL release in the rat and other species (15). The same level of EB treatment as that used in the present study has been shown to produce an increase of more than 1000% in serum PRL levels in underfed rats with mammary tumors (3). Estrogen stimulates PRL release by acting in the hypothalamus, in which it decreases dopaminergic activity (16, 17), and also by a direct action on the pituitary (15). In the present study, estrogen was unable to affect the DA or 5-HT concentrations in the hypothalamus, probably due to underfeeding. Therefore, the marked increase in PRL release that occurs in EB-treated underfed rats with mammary tumors (3) must be due to a direct action of estrogen on the pituitary.

Besides promoting tumor growth through stimulation of PRL secretion, estrogen by itself is known to stimulate mammary tissue (2). In the present study, although both HAL and EB treatments prevented tumor regression in underfed rats, neither HAL, which produces a marked increase in serum PRL (3), nor the combination of HAL and EB, which increases serum PRL levels by as much as 9000% (3) and also raises serum estrogen levels, were able to promote tumor

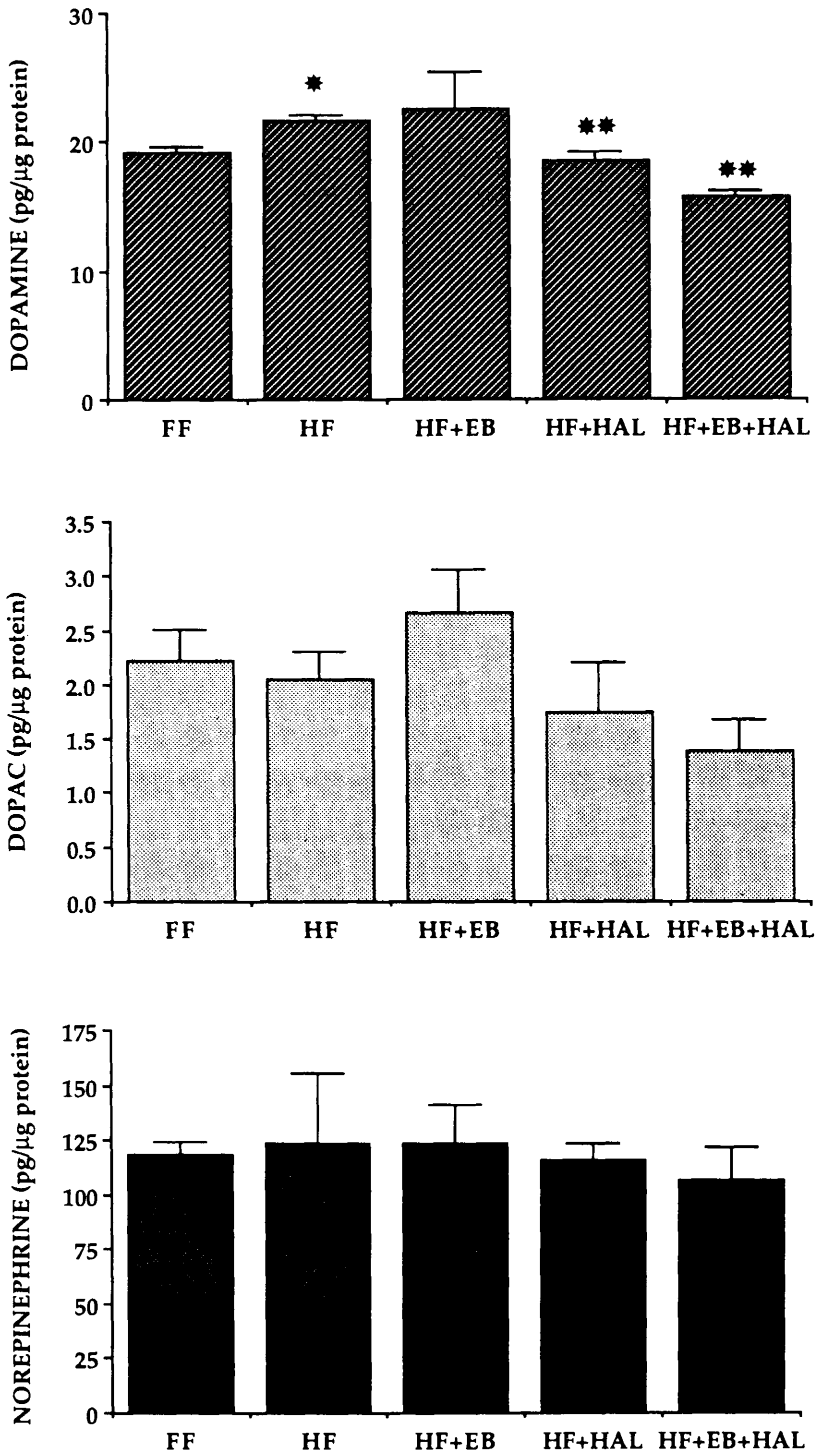

Figure 4. Effects of feed restriction alone or in combination with EB and HAL on the concentrations of hypothalamic dopamine, dihydroxyphenylacetic acid (DOPAC), and norepinephrine. See Figure 1 for details. * Significantly ($P < 0.05$) different from FF. ** Significantly ($P < 0.05$) different from HF.

growth in the half-fed rats to the same extent as in the FF rats. This indicates that besides PRL, there are other factors that are required to sustain maximum tumor growth on a chronic basis in underfed rats. This is in sharp contrast to the situation in rats that are underfed for a short period. In these rats, elevations in serum PRL levels by treatment with HAL alone or in combination with EB are sufficient to promote tumor growth for 3 weeks (3).

In summary, it appears reasonable to conclude that the ultimate cause of tumor regression in the underfed rats was probably a decrease in PRL secretion due to

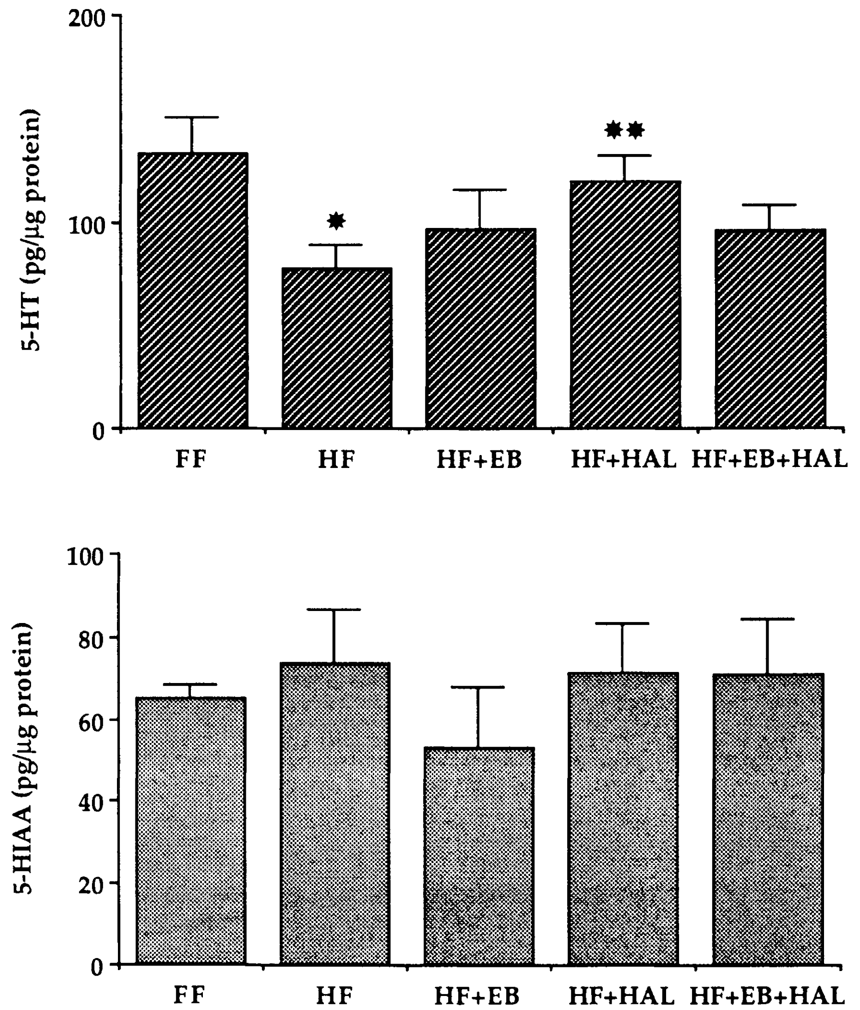

Figure 5. Effects of feed restriction alone or in combination with EB and HAL on the concentrations of hypothalamic 5-HT and 5-hydroxyindoleacetic acid (5-HIAA). See Figure 1 for details. * Significantly ($P < 0.05$) different from FF. ** Significantly ($P < 0.05$) different from HF.

underfeeding-induced changes in the hypothalamic dopaminergic and serotonergic activities, and that elevations in serum PRL that are known to occur by treatment with HAL, which prevents underfeeding-induced changes in hypothalamic neurotransmitters, or by EB, which can act directly on the pituitary to stimulate PRL release, can prevent tumor regression on a chronic basis, but cannot promote maximum tumor growth.

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