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Feeding Elicited by Cholinergic and Adrenergic Hypothalamic Stimulation of Anorectic Tumor-Bearing Rats[1]

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CHANCE, W. T., F. M. VAN LAMMEREN AND J. E. FISCHER. *Feeding elicited by cholinergic and adrenergic hypothalamic stimulation of anorectic tumor-bearing rats.* PHARMACOL BIOCHEM BEHAV 31(1) 209–213, 1988.— Injection of norepinephrine into the hypothalamus of methylcholanthrene sarcoma-bearing rats elicited a normal feeding response both prior to and following the development of anorexia. Feeding elicited by cholinergic stimulation of the hypothalamus of tumor-bearing rats with carbachol was normal prior to the onset of anorexia, but decreased in magnitude as the anorexia became more severe. These data indicate that noradrenergic feeding mechanisms in the hypothalamus of tumor-bearing rats are functioning normally during anorexia. However, cholinergic feeding mechanisms in the hypothalamus of tumor-bearing rats appear to be depressed to the same degree as ad lib intake, possibly through adaptation or depletion of endorphin systems that mediate stress-induced feeding.

Cancer anorexia Hypothalamus Feeding Norepinephrine Carbachol Methylcholanthrene sarcoma

ANOREXIA has been recognized as a common feature of cancer for many years (24). This reduction in food intake contributes to the cachectic response in cancer patients and presents significant therapeutic problems. Thus, radiation or chemotherapy may intensify the anorexia, and a severely cachectic patient is not a good candidate for surgical resection of a tumor. It is paradoxical that a growing tumor should induce anorexia, since the host must provide nutrients for itself and the neoplastic tissue. Therefore, in the absence of direct interference with intake or absorption, cancer anorexia appears to result from a dysfunction of central nervous system (CNS) mechanisms of appetite control. This dysfunction may occur because of depressed activity in neurons that stimulate hunger or hyperactivity in neural circuits that elicit satiety.

Traditionally, hypothalamic areas have been implicated in the control of hunger and satiety. Lesions of the ventromedial hypothalamus (VMH) have been reported to cause hyperphagia and obesity (11), while destruction of the lateral hypothalamic (LH) areas elicits severe anorexia (1). Electric stimulation of these brain areas has produced the opposite results, with VMH stimulation inhibiting feeding (25) and LH stimulation eliciting feeding (16). Although more recent investigations have demonstrated that these effects are due to neural activity in fibers passing through the hypothalamus from more caudal brain areas (10), these studies do illustrate a neuroanatomical specificity for hunger and satiety. A neurochemical specificity has also been demonstrated by several reports showing that alpha-adrenergic stimulation of areas around the VMH elicits feeding, while beta-adrenergic stimulation of LH areas elicits satiety in food-deprived rats (13).

Although previous research suggests that hypothalamic mechanisms which control feeding were normal in anorectic tumor-bearing rats (18), this research was based on gross electrolytic lesions of large areas of the VMH (2) or LH (19). Thus, hyperphagic VMH-lesioned tumor-bearing rats exhibited cancer anorexia, and rats that had recovered from the anorectic effects of LH lesions also showed normal anorexia following tumor induction. To our knowledge, no reports of hypothalamic electrical or chemical stimulation of anorectic tumor-bearing rats have been published. Therefore, in the present experiment we investigated whether anorectic tumor-bearing rats respond normally to the injection of norepinephrine (NE) and carbachol (CARB) into the paraventricular (PVH) and perifornical (PFH) hypothalamic areas. These hypothalamic areas were chosen for these investigations because NE has been reported to be most effective in eliciting feeding in the PVH (12) and CARB readily stimulates feeding when injected into the PFH (3,4). Therefore, if the hypothalamus is functioning normally in anorectic tumor-bearing rats, one should observe similar feeding responses in normal and tumor-bearing rats following the injection of these drugs into the hypothalamus.

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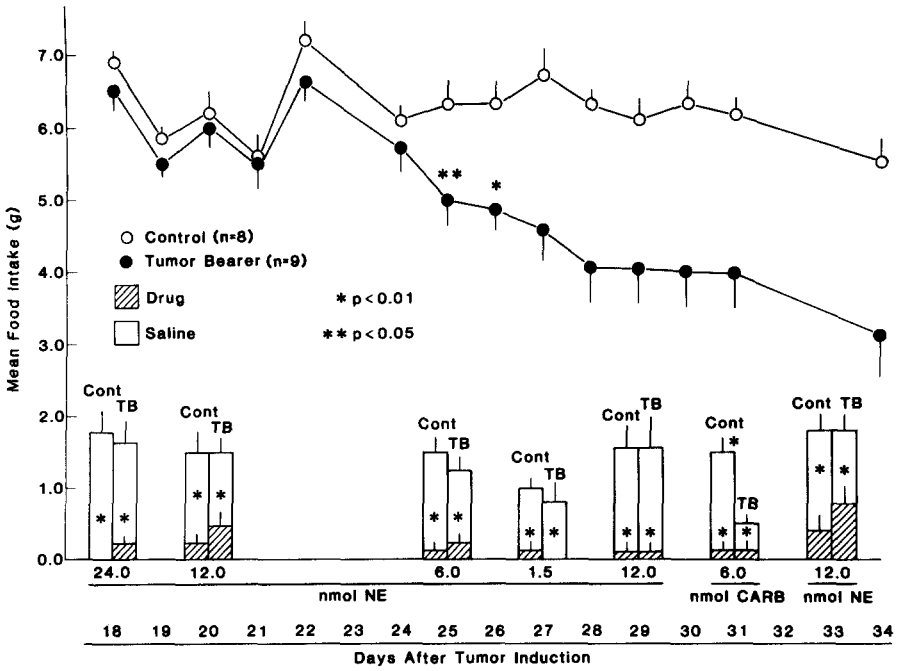

FIG. 1. Mean (±SEM) daily food intake (g/100 g bodyweight) by rats bearing MCA sarcomas (filled circles) and nontumor-bearing control rats (open circles). Bars represent a dose-response analysis of eating (total g) following the injection of norepinephrine (NE, open bars) or saline (cross-hatched bars) into the paraventricular hypothalamus of MCA sarcoma-bearing (TB) and control (C) rats. On day 31, the efficacy of carbachol (CARB) in eliciting eating was assessed in TB and C rats.

METHOD

Forty-four adult, male, Fischer 344 rats (Charles River Laboratories, Wilmington, MA) served as subjects in these experiments. The mean (±SEM) bodyweight of these rats at the time of tumor transplantation was 270±6 g. These animals were housed in suspended wire cages located in a temperature- and humidity-controlled environment under a 12 hr light/dark cycle. All animals were maintained ad lib on water and ground Purina rat chow, provided in spill-resistant food cups.

Each rat was anesthetized with ether, and a 24 ga. cannula was surgically implanted into the PVH or PFH at the respective stereotaxic coordinates (21): Anterior=5.3 or 6.5 mm, Lateral=0.5 or 1.0 mm, Ventral=−7.2 or −8.5 mm below the top of the skull. Two weeks later, methylcholanthrene (MCA) sarcomas were induced in half of these animals by the injection (SC) of a cell suspension (15) containing 2×10^6 viable (by trypan blue exclusion) MCA cells. This tumor system is described in more detail in our previous reports (7,8). The remaining rats received control injections of 0.2 cc normal saline. These tumor-bearing (TB) and control rats were divided into two experiments to assess the effects of injecting NE into the PVH and CARB into the PFH. In each experiment, daily records of bodyweight and food intake were recorded, with food intake values being corrected for any spillage. Tests of drug-induced feeding were conducted during periods of maximum satiation in the morning hours. To provide control data for each drug test, food and water intake were assessed for one hr following the injection of 1 μl of normal saline. Immediately thereafter, 1 μl of NE (1.5 to 24 nmol) or CARB (4 nmol) was injected into the hypothalamus and intake was again assessed for 1 hr. This paradigm of drug administration was employed rather than a randomization of saline and drug treatments because the drug treatment could possibly have residual effects on feeding, which could influence intake during any subsequent control period. These drugs were injected according to previously reported techniques (13) employing a 31 ga. injection needle that terminated flush with the implanted cannulae and a 50 μl Hamilton microliter syringe to control the injection volume. In the first experiment, the effects of 24 nmol of NE were tested 18 days after tumor induction. Since pilot experiments suggested that the feeding response of anorectic TB rats to NE was not decreased, repeated tests with lower doses (12.0, 6.0, 1.5 and 12.0 nmol) of NE were conducted on days 20, 25, 27 and 29 to examine possible differences at lower doses. On day 31 the effects of 6.0 nmol of CARB were assessed. Two days after this test of CARB (day 33), the 12.0 nmol dose of NE was assessed again.

Since the feeding response following the injection of CARB into the PVH was attenuated in TB rats, the second experiment more fully investigated CARB-induced feeding in additional TB (n=14) and control (n=13) rats. In this experiment CARB (4.0 nmol) was injected into the PFH on day 12, prior to the onset of anorexia. Additional tests of CARB-induced feeding were conducted on days 15, 19, 22, 26 and 29. Therefore, comparison of CARB-stimulated feeding throughout the anorectic period was possible.

At the conclusion of this second experiment, the rats were sacrificed by decapitation and the brains were removed and stored in 10% formalin prior to gross histological verification of cannulae placement using a dissecting microscope.

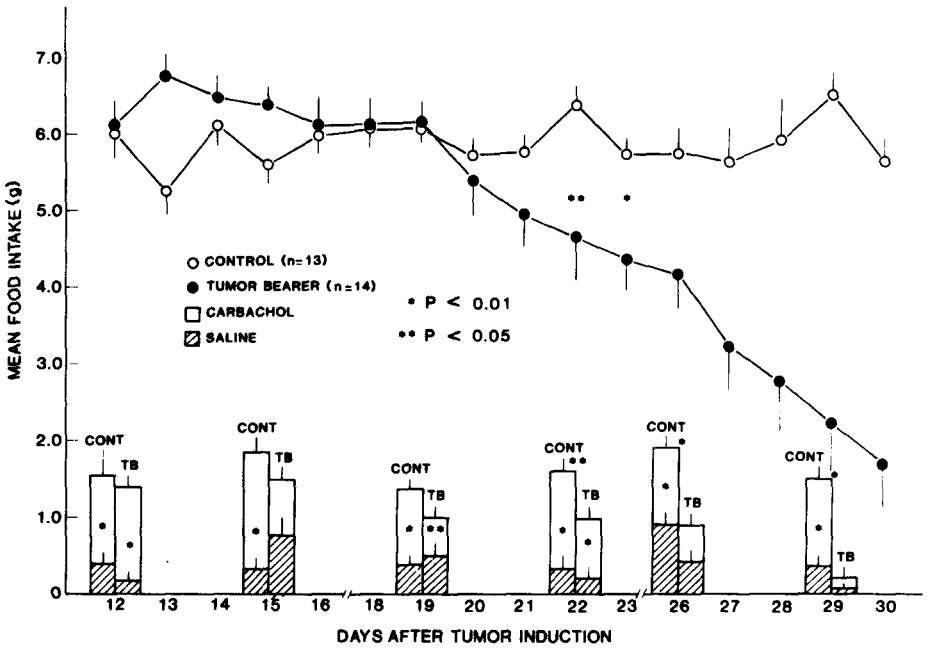

FIG. 2. Mean (±SEM) daily food intake (g/100 g bodyweight) by rats bearing MCA sarcomas (filled circles) and nontumor-bearing control rats (open circles). Bars represent the mean total food intake (g) during the 60 min following the hypothalamic injection of 6.0 nmol of carbachol (open bars) or the preceding 60 min control period initiated by the intrahypothalamic injection of normal saline (cross-hatched bars).

The tumors were also excised and weighed in the second experiment.

All data were statistically analyzed employing analysis of variance (ANOVA) techniques. Individual group means were compared by *t*-tests.

RESULTS

In the first experiment, the TB rats first exhibited significantly ($p<0.05$) reduced food intake by day 25 (Fig. 1). As also indicated in Fig. 1, the intrahypothalamic administration of all doses of NE elicited significant feeding in both TB and control groups. Furthermore, there was no difference between TB and control groups following the administration of any dose of NE. However, the feeding response following the intrahypothalamic administration of 6.0 nmol of CARB was of much less magnitude in TB rats ($p<0.01$) than in control rats. However, when 12.0 nmol of NE was administered on day 33, TB and control rats again exhibited similar feeding responses.

This reduction of CARB-induced feeding in TB rats was further investigated in the second experiment by assessing the feeding response to this drug both prior to and following the development of anorexia. As illustrated in Fig. 2, significant ($p<0.05$) anorexia was first observed in the TB rats on day 22. Although CARB elicited significant feeding in both groups following the first 4 injections (days 12, 15, 19 and 22), TB rats ate significantly less than did the control rats on day 22. This feeding response continued to decrease across the next two drug administrations (days 26 and 29) to the complete loss of the behavior on day 29. Thus, the feeding response following cholinergic stimulation of the hypothalamus of TB rats roughly followed the severity of the anorexia.

Mean (±SEM) tumor weight at the conclusion of this experiment was 43±4 g. Histological localization of cannulae revealed placements to be generally within 1 mm of the PFH, with mean (±SEM) coordinates of the center of the termination of the cannulae tracts being: AP=5.0±0.1, L=0.9±0.1, and V=−3.3±0.1 mm.

DISCUSSION

Previous research has focused upon hypothalamic mechanisms of cancer anorexia. On the basis of lack of differential effects of electrolytic lesions of VMH (2) and LH (19) areas of TB rats, these studies concluded that cancer anorexia was mediated by extrahypothalamic mechanisms (18). Supporting this position are data indicating that TB rats exhibit a normal feeding response to exogenously-administered insulin (17), which is thought to be mediated by the hypothalamus. The present data illustrate that the hypothalamus of anorectic TB rats may respond normally to some chemical stimuli and abnormally to others. Even during moderately severe anorexia, essentially normal feeding was observed following the intrahypothalamic injection of NE. The general absence of dose-response relations, at least for the larger doses of NE, may suggest the increased sensitivity of this brain area to NE (12), with near maximum feeding responses being elicited by lower doses of the compound.

Several studies have linked hypothalamic NE to both feeding and satiety. Injection of NE into the PVN elicits feeding in satiated rats, while its application to the LH inhibits feeding in food-deprived rats (12,13). Hypothalamic concentrations of NE increase with food deprivation (9), and feeding food-deprived rats increases the release of NE in the hypothalamus (22). Direct injection of nutrients into the gut

has also been reported to stimulate the release of NE in the medial hypothalamus (20). Therefore, one might expect hypothalamic concentrations of NE to be elevated in anorectic rats. In our examinations of cancer anorexia, we have not yet detected consistent alterations in hypothalamic NE levels in anorectic TB rats (7,8). However, it should be emphasized that regional hypothalamic amine changes in these animals could easily be obscured in our whole hypothalamic assays. Nonetheless, the hypothalamus does appear to be functioning normally in these animals with respect to NE-induced feeding and exogenous concentrations of this amine.

A somewhat different pattern of feeding was observed in TB rats following the injection of CARB into the PVH or PFH. Prior to the onset of anorexia, similar amounts of food were consumed by both control and TB rats. Although there was not a significant effect of CARB in TB rats on day 15, this lack of effect was due primarily to increased intake and variability during the control period by TB rats. Assessment of overall differences on day 15 by ANOVA revealed no difference between TB and control rats. As the anorexia developed and became more severe, CARB was less effective in eliciting feeding in TB animals. This diminution in food intake continued to the point where CARB no longer elicited significant feeding.

There is a striking parallel in the reduction of ad lib food intake in TB rats and the decrease of CARB-stimulated feeding. At present, we do not know whether both feeding responses are being suppressed by similar mechanisms or whether the cholinergic link in feeding may be a critical focus of cancer anorexia. Although little is known concerning the mechanisms subserving cholinergically-induced feeding, it does appear to be mediated through the muscarinic-cholinergic receptor (3). Additional research has also suggested an endorphin link in this behavior, since pretreatment with the opiate antagonist, naltrexone, blocks the cholinergic feeding response at much lower doses than it blocks feeding elicited by NE (3). Thus, cholinergically-induced feeding may be an example of stress-induced eating (14), and the chronic stress of cancer may result in adaptation or depletion of endorphin mechanisms that maintain it. Supporting this hypothesis is the observation suggesting that rats bearing Walker (W) 256 carcinosarcomas have abnormal diurnal variations in plasma β-endorphin (27) and exhibit significantly reduced concentrations of dynorphin-A in pituitary neurointermediate lobe (26). In addition, stress-induced feeding elicited by 2-D-oxy-D-glucose is also reduced in these anorectic rats (28). Therefore, hypothalamic mechanisms of feeding involving cholinergic-endorphinergic interaction may not be functioning normally during the physiologic stress of cancer.

We have observed additional biochemical parameters that are altered in the hypothalamus of anorectic TB rats. Our research concerning the role of indoleamines in cancer anorexia suggested that hypothalamic concentrations of the serotonin metabolite, 5-hydroxyindoleacetic acid (5-HIAA), may be elevated in both the W 256 carcinosarcoma and the methylcholanthrene-induced sarcoma (7,23). More recent research (8), employing a more sensitive HPLC assay, has demonstrated significant elevations of hypothalamic 5-HIAA in anorectic TB rats, suggesting increased turnover of serotonin in the hypothalamus. Currently, we are uncertain as to the role of CNS serotonin in cancer anorexia, since depleting this amine does not affect the anorectic response (6) and treatment with serotonin receptor blockers had no effect on the anorectic response (unpublished observations). Thus, elevated serotonin turnover may reflect the fact that an animal is anorectic but not be a primary cause of it. We have also observed a significant decrease in levels of immunoreactive cholecystokinin (CCK) in the hypothalamus of anorectic TB rats (5). Since this peptide has been linked to the mediation of satiety, it appears that TB rats may be down-regulating the production of CCK in order to counteract the anorexia.

In conclusion, several complex alterations of hypothalamic mechanisms that may be involved in the regulation of food intake have been observed in anorectic TB rats. Unfortunately, none of these alterations appear to be a direct cause of the anorexia. However, the decline of cholinergically-induced feeding in parallel with the onset and severity of anorexia suggests that this phenomenon be studied in more detail.

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