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Serotonin Depletion by 5,7-Dihydroxytryptamine or Para-Chloroamphetamine Does Not Affect Cancer Anorexia[1]

WILLIAM T. CHANCE, MAARTEN VON MEYENFELDT, AND JOSEF E. FISCHER

Department of Surgery, University of Cincinnati Medical Center
231Bethesda Avenue, Cincinnati, OH 45267

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CHANCE, W. T., M. VON MEYENFELDT AND J. E. FISCHER. *Serotonin depletion by 5,7-dihydroxytryptamine or para-chloroamphetamine does not affect cancer anorexia.* PHARMACOL BIOCHEM BEHAV **18**(1) 115–121, 1983.—Anorectic tumor-bearing rats exhibited increased brain levels of the 5-HT precursor, tryptophan, and metabolite, 5-hydroxindoleacetic acid (5-HIAA). In an effort to determine whether indoleamine systems had any role in the etiology of cancer anorexia the anorectic effects of cancer (Walker 256 carcinosarcoma) were investigated in immature female rats that had been depleted of brain serotonin (5-HT) by the intracisternal injection of 5,7-dihydroxytryptamine (5,7-DHT) or the systemic injection of para-chloroamphetamine (PCA). Although both 5,7-DHT and PCA significantly reduced brain concentrations of 5-HT and 5-HIAA by approximately 50%, no effects on the onset or severity of the anorectic response to cancer were observed. Similarly, neither drug affected eating in non-tumor-bearing control animals. Therefore, these data do not support increased brain 5-HT activity as a primary mediator of cancer anorexia.

Cancer anorexia Food intake Serotonin Tryptophan 5-hydroxyindoleacetic acid 5,7-dihydroxytryptamine Parachloroamphetamine Dopamine Norepinephrine Walker 256 carcinosarcoma

THE development of anorexia and cachexia continues to present significant problems in the management of cancer [26]. The appetite of an anorectic patient is likely to be further compromised by radiation therapy or chemotherapy. Furthermore, the cachectic patient is an unlikely candidate for surgical resection of a tumor. Therefore, elucidation of the aberrations in CNS mechanisms of hunger and satiety with cancer anorexia remains a goal with obvious therapeutic benefits.

Although the monoamines, norepinephrine (NE; [1, 22, 25, 28]) and dopamine (DA; [11, 17, 25, 31]) have been related to the control of food intake, a large body of literature suggests that neuronal serotonin (5-HT) activity may function to mediate satiety. Thus a variety of studies employing pharmacological, neurochemical and neuroanatomical procedures have associated increased brain 5-HT activity with anorexia [4, 5, 16, 20, 21, 33] and decreased 5-HT neurotransmission with hyperphagia [2, 7, 10, 30, 32].

Initial observations [19] from this laboratory suggested that brain 5-HT activity was increased in anorectic rats bearing Walker (W) 256 carcinosarcomas. Thus, elevations of the 5-HT precursor, tryptophan (Trp) and metabolite, 5-hydroxyindoleacetic acid (5-HIAA), were suggestive of increased turnover of indoleamines in anorectic tumor-bearing (TB) rats. Additional experimentation [36] continued to support this hypothesis by demonstrating changes in these indoles immediately prior to the onset of significant anorexia. Furthermore, in these studies [19,36] the absence of such changes in pair-fed control rats, that were allowed access only to that amount of food consumed by the anorectic TB rats, suggests that these changes in indoleamines were not the result of undernutrition. Therefore the possibility of increased CNS 5-HT activity mediating cancer anorexia was a plausible and testable hypothesis.

Although acute intraventricular (IVT) injections of large doses of the inhibitor of Trp-hydroxylase, parachlorophenylalanine (PCPA), significantly delayed the onset of anorexia in TB rats [9], this delay was transient and did not parallel the reductions in brain 5-HT and 5-HIAA. However, the disruption of food intake by the trauma associated with the acute surgeries and the IVT administration of high doses of PCPA methyl ester posed additional problems for the assessment of a role of 5-HT in cancer anorexia.

Therefore, in the present experiments, we chose to deplete brain 5-HT by less traumatic methods that would not disrupt feeding behavior at the onset of anorexia. Long-term depletion of brain 5-HT may be accomplished by the intracisternal (ICT) injection of the neurotoxin, 5,7-

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dihydroxytryptamine (5,7-DHT; [6]) or the systemic (IP) injection of para-chloroamphetamine (PCA; [34]). Although neurotransmitter depletion with PCA appears to be specific to 5-HT systems [34], ICT 5,7-DHT will also significantly deplete brain NE unless pretreatments with desipramine (DMI) are administered [6]. An advantage in employing these drugs is that long-term reductions (>50%) in brain 5-HT follow a single treatment. Therefore each of these agents may be administered well before the induction of tumors, and the subsequent onset of anorexia in 5-HT depleted and normal TB rats may be documented.

METHOD

Subjects

Sixty-six immature (40–90 g), female, Sprague-Dawley rats (Harlan Laboratories, Madison, WI) served as subjects in these experiments. These rats were individually housed under a 12-hr light-dark cycle in a temperature- and humidity-controlled environment. Food (Purina rat chow pellets) and water were continuously available to all animals.

Drug Treatments

In the first experiment, 5-HT was depleted employing 5,7-DHT. Each of the 33 rats (40–60 g) was weighed and treated with 30 mg/kg DMI (IP; Merrell Pharmaceuticals, Cincinnati, OH). Thirty minutes later, each rat was anesthetized with ether and either 100 μg (free base) of 5,7-DHT creatinine sulfate (Sigma Chemical Co., St. Louis, MO) contained in 20 μl of normal saline plus 0.5% ascorbic acid (n=19) or vehicle (20 μl saline plus ascorbic acid; N=14) was injected into its cisterna magna (ICT). These ICT injections were administered through a 27-ga hypodermic needle using a Hamilton (Reno, NV) 50 μl syringe.

In the second experiment, 5-HT was depleted employing DL-PCA HCl (Sigma Chemical Co), with 10 mg/kg of the drug being administered (IP) to 16 of the rats (70–90 g). The remaining rats (n=17; 70–90 g) received control injections (IP) of normal saline.

Induction of Tumors

The W 256 tumor was originally obtained from E. G. and G. Mason Research Institute (Worcester, MA). Our tumor stock is maintained *in situ* by harvesting ascites fluid, containing tumor cells, 5 days after the IP injection of 1×10^6 viable (by trypan blue exclusion) cells. In the first experiment, tumors were induced in 10 of the 5,7-DHT-treated rats and seven of the saline-treated rats by the injection of 1×10^5 viable W 256 cells into the thigh muscle. Control injections (0.1 ml, IM) of normal saline were administered to the remaining rats of each group. The weight range of the rats at this time was 100–150 g.

In a second experiment, tumors were induced (5×10^4 cells) in PCA-treated (n=8) and saline-treated (n=8) rats 3 days after the drug treatments. Again, control rats from both depleted and normal groups were given injections (0.1 ml, IM) or normal saline. The weight range of the rats in the PCA experiment at the time of tumor induction was 80–110 g.

Daily records of food intake, water intake and body weight were recorded to the nearest 0.1 g. Food consumption was corrected for any spillage by the rats and for any body weight differences of individual animals. Thus presented values represent daily mean intake per 100 g body weight of the individual groups. The rats in the 5,7-DHT experiment were sacrificed on day 9 (post-tumor induction), while those in the PCA experiment were decapitated on day 8.

Biochemical Analyses

In the first experiment the rats' brains were removed immediately following decapitation, dissected into right and left hemispheres, and frozen in liquid nitrogen. In one brain-half, Trp, 5-HT and 5-HIAA were determined fluorometrically according to the method of Curzon and Green [14] following acid-butanol extraction (10 volumes), with 5-HT and 5-HIAA being complexed with o-phthalaldehyde (Sigma Chemical Co.) and read on an Aminco Bowman fluorometer. Brain Trp was extracted in the same fraction as 5-HT and assayed fluorometrically according to the method of Bloxam and Warren [3]. In the contralateral hemispheres, NE and DA were determined using high performance liquid chromatography with electrochemical detection [24]. The brain halves were homogenized in five volumes of 0.4 M $HClO_4$ solution, containing (per 100 ml) 50 mg EDTA, 100 mg $Na_2S_2O_5$ and 10 μg of alpha-methylNE (as an internal standard). After centrifugation (31,000×g, 15 min, 4°C), shaking (10–15 min) with activated alumina (100 mg) and washing (three times with 10 ml H_2O), each sample was eluted from the alumina with 1.0 ml of 0.1 N HCl solution (containing 10^{-5} M $Na_2S_2O_5$) and centrifuged (1000×g, 10 min). Supernatants (20 μl aliquots) were injected into the HPLC system (Beckman Model 110A pump, Altex reverse phase C-18 column, Palo Alto, CA; Bioanalytical Systems, Inc., Model LC-4 electrochemical detector, W. Lafayette, IN), with NE eluting from the column in 1.6 min and DA in 5.5 min after the solvent front of the buffer (10% methanol; 90% 0.1 M KH_2PO_4, 0.15 mM sodium octyl sulfate, 0.1 mM Na_2 EDTA; pH=3.0).

In the second experiment the brains were rapidly removed and dissected into three gross regions in order to better establish the effects of PCA on areas known to be rich in 5-HT. Each brain was placed on its dorsal surface and the hypothalamic area was defined as tissue dissected to a depth of approximately 2.5 mm, extending from the optic chiasm to the posterior mamillary area and bounded laterally by the choroid fissure. The mesencephalon was next identified and dissected by vertical cuts just anterior to the superior colliculus and posterior to the inferior colliculus, following removal of the cerebellum and cerebral cortex. Thus the hypothalamus, mesencephalon and remainder of the brain were each frozen in liquid nitrogen prior to biochemical assay. In each of these sections, levels of Trp, 5-HT and 5-HIAA were determined fluoremetrically, according to the method of Curzon and Green [14].

Statistical Evaluation

The data of these experiments were evaluated employing analysis of variance (ANOVA) techniques, with post hoc comparisons of individual means being made by *t* tests and Duncan's Multiple Range test. Except as indicated, statistical significance refers to probabilities less than 0.01.

RESULTS

As may be observed in Fig. 1, the 5,7-DHT treatment did not delay the onset of anorexia in TB rats or lessen its severity. Since five of the TB animals died prior to sacrifice on day 9, the statistical evaluations were conducted over days 4

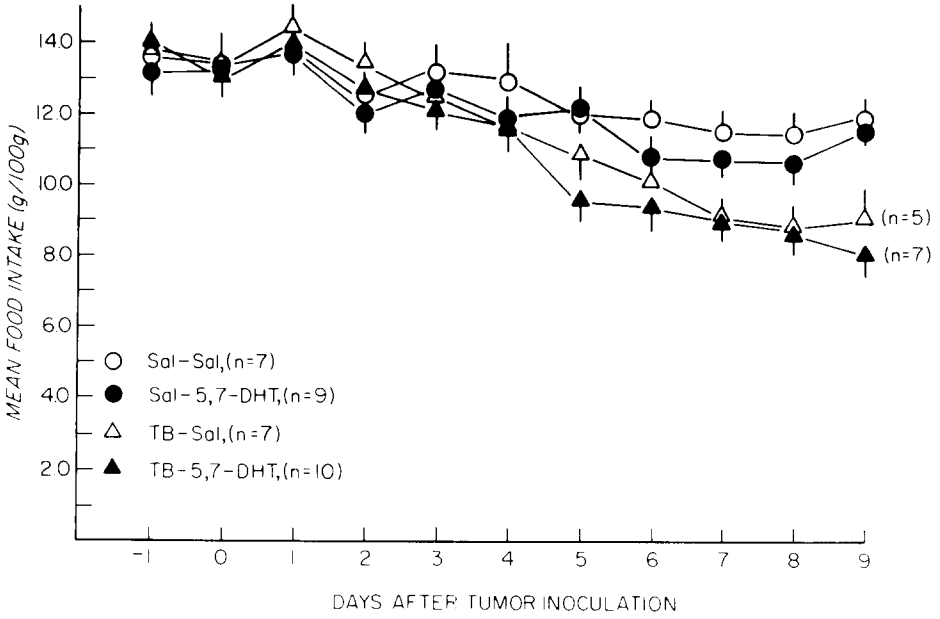

FIG. 1. Mean (±SEM) daily food intake by control (Sal) and tumor-bearing (TB) rats following the intracisternal injection of 5,7-dihydroxytryptamine (5,7-DHT) or control injection of normal saline.

through 8. A repeated measures ANOVA verified the absence of drug effect, while revealing significant tumor, days and tumor × days effects. Thus food intake by both TB groups was progressively decreased as the tumors grew. As illustrated in Fig. 2, there was a significant drug effect on body weight, with 5,7-DHT-treated rats weighing less than controls at the time of tumor induction (123±2 g vs. 137±4 g) as well as 8 days later (147±4 g vs. 171±12 g). However, weight gain by both groups over the days of the ANOVA is indicated by the significant days effect and absence of interaction effects. There were also significant drug ($p<0.05$), tumor ($p<0.05$), days ($p<0.01$), and tumor × days ($p<0.01$) effects on water intake. As indicated in Fig. 3, water intake was lowest in the 5,7-DHT-TB rats. Additional ANOVA (2×5) conducted between groups revealed intake by the 5,7-DHT-TB group to be significantly lower than the intake

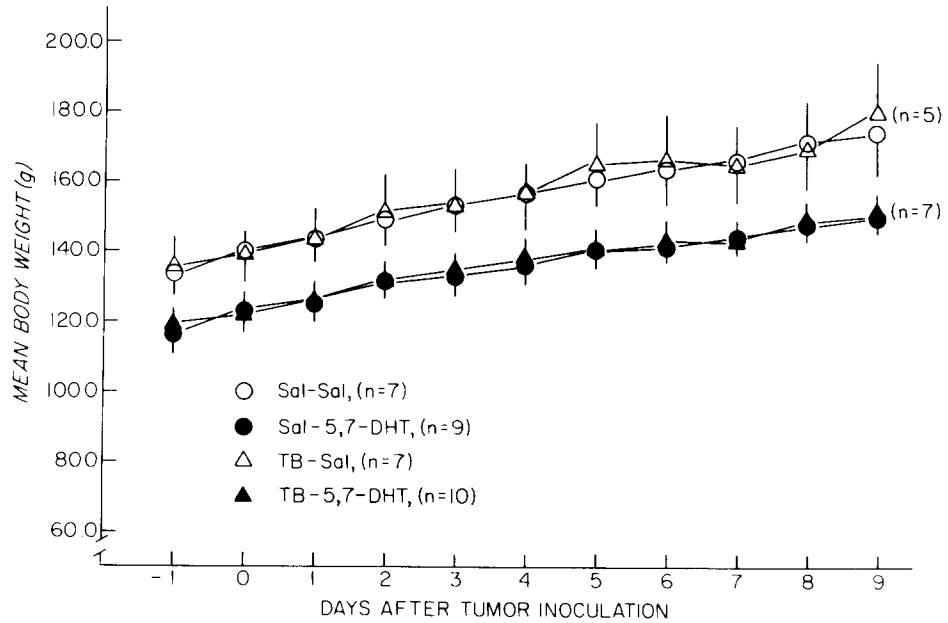

FIG. 2. Mean (±SEM) daily body weight changes in control (Sal) and tumor-bearing (TB) rats that were treated with 5,7-DHT or saline.

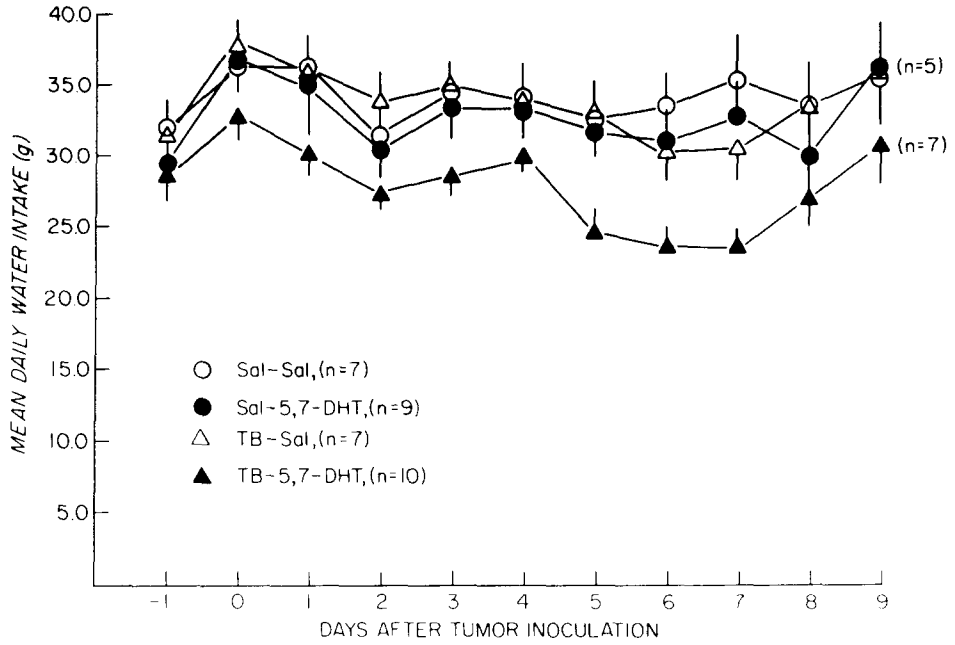

FIG. 3. Mean (±SEM) daily water intake by tumor-bearing (TB) and control (Sal) rats treated with 5,7-DHT or saline.

of the Sal-TB rats or the 5,7-DHT-treated nontumor-bearing (NTB) rats. The significant days effects for both of these comparisons as well as the significant tumor × days interaction are indicative of decreased drinking by both TB groups.

The results of the biochemical analyses for the first experiment are presented in Table 1. There were significant tumor effects for Trp, 5-HIAA and NE, with Trp and 5-HIAA being increased and NE decreased in TB rats. The drug treatment was significant for 5-HT and 5-HIAA, with 5,7-DHT reducing both of the indoles. The group × drug interaction was also significant for 5-HIAA, due primarily to the increase in 5-HIAA in the Sal-TB group.

The effects of PCA on food intake are presented in Fig. 4. As in the preceding experiment, this drug had no effect on the anorectic response of TB animals. The main effects for tumor and days were significant as was the tumor × days interaction, reflecting the progressively decreased eating by the TB groups. Although PCA had no effect on body weight, there were slight, but significant, differences between groups (Fig. 5), with body weight being reduced in TB rats. As suggested by the significant days effect, all groups gained weight across the 5 days of the ANOVA. However, the rate of weight gain was slower in TB rats, as indicated by the significant days × tumor interaction. There was no significant effect of the drug treatment on water intake (Fig. 6). However, a significant tumor effect is reflective of decreased drinking by the TB rats. Water intake did tend to increase across the 5 days of the ANOVA, resulting primarily from elevated drinking by NTB rats (Fig. 6).

The results of the biochemical analyses in the second experiment are presented in Table 2. Although Trp was not affected by the PCA treatment in any of the brain areas, significant tumor effects were observed in the mesencephalon and remainder of the brain, due to increased concentra-

TABLE 1

MEAN (±SEM) WHOLE BRAIN LEVELS OF TRP, 5-HT, 5-HIAA, NE AND DA IN W 256 SARCOMA-BEARING (TB) AND CONTROL (C) RATS FOLLOWING DEPLETION OF INDOLES BY ICT. ADMINISTRATON OF 5,7-DHT OR CONTROL INJECTIONS OF NORMAL SALINE (SAL)

Group	N	Trp (μg/g)	5-HT (ng/g)	5-HIAA (ng/g)	NE (ng/g)	DA (ng/g)
Sal-C	7	2.61 ± 0.28	711 ± 36	474 ± 21	484 ± 16	841 ± 35
5,7-DHT-C	9	2.13 ± 0.02	405 ± 31*	266 ± 21*	503 ± 19	911 ± 24
Sal-TB	5	3.35 ± 0.43	712 ± 33	676 ± 22†	424 ± 38	838 ± 67
5,7-DHT-TB	7	3.24 ± 0.50	356 ± 41*	315 ± 34*	416 ± 34‡	867 ± 48

*$p<0.01$ vs non-depleted controls.
†$p<0.01$ vs Sal-C.
‡$p<0.01$ vs C groups.

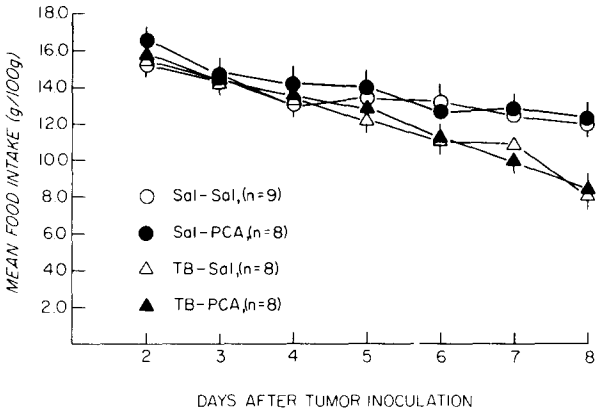

FIG. 4. Mean (±SEM) daily food intake by control (Sal) and tumor-bearing (TB) rats following the systemic (IP) injection of para-chloroamphetamine (PCA) or control injection of normal saline.

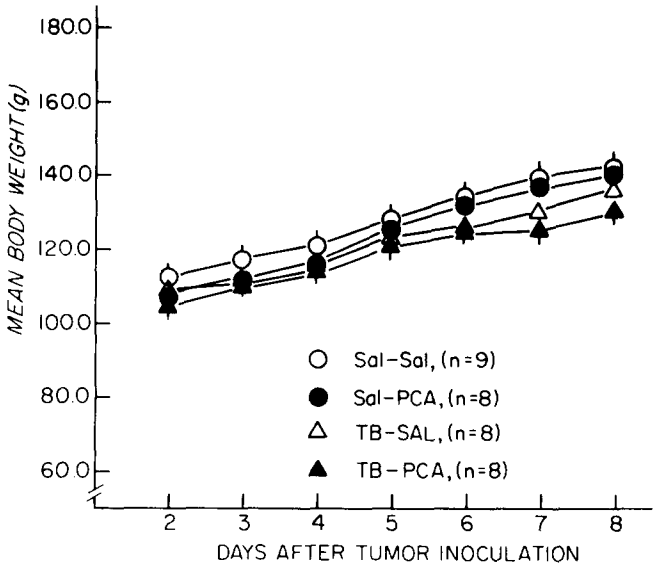

FIG. 5. Mean (±SEM) daily body weight changes in control (Sal) and tumor-bearing (TB) rats that were treated with PCA or saline.

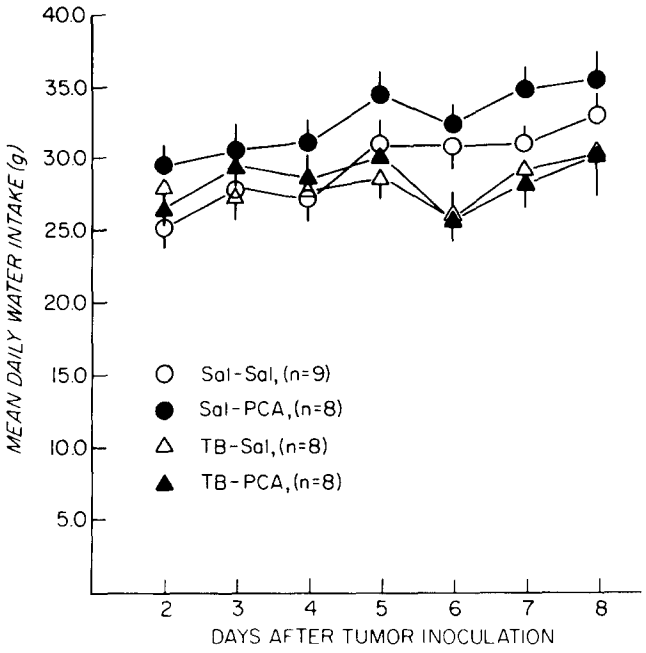

FIG. 6. Mean (±SEM) daily water intake by tumor-bearing (TB) and control (Sal) rats following the injection of PCA or saline.

TABLE 2

MEAN (±SEM) LEVELS OF TRP, 5-HT AND 5-HIAA IN THE HYPOTHALAMUS, MESENCEPHALON AND REMAINDER OF THE BRAIN IN W 256 TUMOR-BEARING (TB) AND CONTROL (C) RATS FOLLOWING DEPLETION OF BRAIN INDOLES WITH PARA-CHLOROAMPHETAMINE (PCA) OR THE CONTROL INJECTION OF NORMAL SALINE (SAL)

Group	N	Trp (μg/g)	5-HT (ng/g)	5-HIAA (ng/g)
Hypothalamus				
Sal-C	9	3.54 ± 0.20	1502 ± 38	862 ± 29
PCA-C	8	3.70 ± 0.26	898 ± 53*	449 ± 23*
Sal-TB	8	3.81 ± 0.38	1326 ± 62	1016 ± 53†
PCA-TB	8	4.40 ± 0.24	839 ± 24*	489 ± 13*
Mesencephalon				
Sal-C	9	2.50 ± 0.15	1336 ± 39	985 ± 28
PCA-C	8	2.64 ± 0.13	761 ± 34*	530 ± 14*
Sal-TB	8	3.23 ± 0.38	1372 ± 30	1369 ± 77†
PCA-TB	8	3.32 ± 0.29	784 ± 35*	665 ± 36*
Remainder of Brain				
Sal-C	9	3.24 ± 0.36	656 ± 25	433 ± 12
PCA-C	8	2.99 ± 0.29	357 ± 17*	188 ± 7*
Sal-TB	8	4.04 ± 0.50	656 ± 12	582 ± 25†
PCA-TB	8	3.91 ± 0.50	321 ± 19*	237 ± 15*

*$p<0.01$ vs non-depleted controls.
†$p<0.01$ vs Sal-C.

tions of this amino acid in TB groups. Levels of 5-HT were significantly increased in TB rats in the hypothalamus, while 5-HIAA concentrations were elevated in TB rats in the hypothalamus, mesencephalon and remainder of the brain. Treatment with PCA was highly effective in reducing 5-HT and 5-HIAA in each of these areas, with all F ratios being greater than 100 and probabilities being much less than 0.01. The complete absence of interaction indicates that the drug was equally effective in reducing concentrations of these indoles in TB and NTB rats.

DISCUSSION

The results of these experiments replicate our previous reports [9, 19, 36] demonstrating elevated levels of Trp and 5-HIAA in the brains of anorectic rats bearing W 256 carcinosarcomas. Thus these data are suggestive of increased turnover of indoleamines in TB rats. However, the functional significance of this increase in indoleamine activity to the mediation of cancer anorexia remains obscure, since depletion of brain levels of 5-HT and 5-HIAA in TB rats did not delay the onset of anorexia nor lessen its severity. Thus,

following treatment with 5,7-DHT brain 5-HT and 5-HIAA were reduced by over 50% in TB rats. Following PCA treatment similar significant reductions in indoles in TB rats were observed in areas rich in 5-HT cell bodies (mesencephalon) and nerve terminals (hypothalamus) as well as in the remainder of the brain. Despite these biochemical changes, essentially no differences in the anorectic effects of cancer were observed in these animals.

Although several reports [4, 7, 21, 30, 33] suggest a role of 5-HT in mediating satiety, no support for this hypothesis was obtained from the present data. Thus NTB rats treated with 5,7-DHT or PCA exhibited no increase in feeding even though 5-HT and 5-HIAA levels were reduced by 40–50% in all of the assays. These experiments also failed to replicate the previous report [30] that 5-HT depletion following the IVT administration of 5,7-DHT is associated with hyperphagia and obesity. In fact, the lowered body weights of 5,7-DHT-treated rats at the time of tumor inoculations is indicative of reduced food intake following this treatment. Similar observations of reduced body weight following 5,7-DHT have been published by other investigators [12]. Although the magnitude of 5-HT depletion in the treated rats of the present experiment do not appear to be as severe as those reported by Saller and Stricker [30], it should be noted that other investigators [12,18] have been unable to demonstrate 5,7-DHT-induced hyperphagia following reduction of 5-HT by over 80%. Therefore, it has been suggested that the 5,7-DHT-induced hyperphagia and obesity originally observed may have been artefactual in nature [12]. However, the possibility remains that much more severe depletion of brain 5-HT is required to affect food intake.

The present experiments also do not provide support for 5-HT mediation of a delay of cancer anorexia following IVT administration of PCPA [9]. The reductions of 5-HT and 5-HIAA in the present report are much greater than those previously observed [9] following PCPA treatment. Therefore the transient delay of cancer anorexia following IVT PCPA may be due to factors other than reduced brain indoleamine concentrations. This conclusion is further supported by its recent observation that hyperphagia following the acute IVT administration of PCPA is not specific to 5-HT depletion [23], with IVT injections of tryptophan-methyl ester or leucine-methyl ester also facilitating food intake.

Although 5,7-DHT did affect drinking, this effect was most pronounced in the TB rats, since the post hoc 2×5 ANOVA indicated no difference between 5,7-DHT and Sal treated NTB rats. However, a similar effect was not observed following PCA treatment, suggesting the reduction of drinking by 5,7-DHT is not specific to 5-HT systems. Tumor induction was also effective in reducing water intake in both experiments. This reduction in water intake may represent the modest effects that are secondary to the reduction in feeding. Although Morrison [27] has reported increased drinking by TB rats at early stages of anorexia, his data are not directly comparable to the present data due to several differences in experimental variables.

The methods employed to deplete brain 5-HT were specific to that neurotransmitter, with no significant drug effects being observed in either NE or DA concentrations. Although we did not assay catecholamines in the second experiment, we [8] and others [34] have observed PCA treatment not to have long-term effects on brain levels of NE or DA. Concentrations of NE were reduced in TB rats, while CNS levels of DA remained unchanged. It is difficult to assess the significance of decreased NE levels in the absence of metabolite data, since synthesis may be decreased or metabolism increased in TB rats. The absence of changes in NE levels in TB rats sacrificed 6 days after tumor inoculation [9] in a previous report presents further difficulties in interpreting this effect. However, we have observed decreased concentrations of NE in rats bearing W 256 carcinosarcomas in other experiments [35] and have suggested a nutritional etiology of such effects. Data from the same experiment [35] also suggested changes in DA metabolites in anorectic TB rats, which did not appear to result from undernutrition.

Although elevation of indoleamine activity may not be the primary cause of anorexia associated with cancer, neither does it appear to result only from malnutrition. Food deprivation for 24–48 has been reported to cause increases in brain Trp and 5-HIAA [15,29]. These changes, induced by acute food deprivation, may be the result of displaced binding of Trp to albumin by the deprivation-induced increase in free fatty acid levels [13]. Although similar changes in free fatty acids may account for some of the changes in Trp metabolism in TB rats [19], we have not observed significant elevations in plasma-free Trp in pair-fed control rats in acute (9-day W 256 carcinosarcoma) or chronic (33-day methylcholanthrene-induced sarcoma) experiments of cancer anorexia [36]. Thus the pair-fed control rats in the chronic experiment gained only 86% of the body weight of freely-feeding NTB rats and yet exhibited no increases in plasma-free Trp, brain Trp, brain 5-HT or brain 5-HIAA. Additionally, we have observed elevated brain 5-HIAA in rats bearing W 256 carcinosarcomas on the day prior to significantly reduced food intake [36] and have suggested that these changes result from aberrant Trp metabolism secondary to reduced albumin levels in TB rats.

It is foolish to assume that any behavior is controlled exclusively by one neurochemical system. Therefore, rather than being mediated by 5-HT, NE or DA alone, a role for each of these monoamines may eventually be established for cancer anorexia. Thus critical examination of the phenomenon may require the simultaneous manipulation of two or more of these putative mediators of hunger and satiety. Several additional biochemical factors including aberrant amino acid patterns, gamma-amino-butyric acid or changes in endorphins or other peptides [25] may also have a role in the mediation of the anorexia associated with cancer. Nevertheless, elucidation of the specific neurochemical mechanisms of cancer anorexia remains a goal of high priority, due to the potential therapeutic benefits that correction of this abnormality would provide.

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