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Effects of Serotonin and the Serotonin Blocker Metergoline on Meal Patterns and Macronutrient Selection

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LEIBOWITZ, S. F., J. T. ALEXANDER, W. K. CHEUNG AND G. F. WEISS. *Effects of serotonin and the serotonin blocker metergoline on meal patterns and macronutrient selection.* PHARMACOL BIOCHEM BEHAV 45(1) 185–194, 1993.—Serotonin [5-hydroxytryptamine(5-HT)] in the paraventricular nucleus (PVN) of rats has a suppressive effect on feeding behavior and causes a selective decrease in carbohydrate ingestion, specifically at the onset of the natural (dark) feeding period. Studies conducted here provide further evidence for this phenomena, showing a similar dose-related decrease in carbohydrate ingestion at dark onset after PVN injection of 5-HT or of the agonists, *d*-norfenfluramine or fluoxetine, which act through endogenous 5-HT. To further characterize the effects of this indoleamine on the macrostructure of feeding, a computer-automated data acquisition system was used to analyze macronutrient feeding patterns in freely feeding animals maintained on the pure diets of protein, carbohydrate, and fat. Results indicate that PVN administration of 5-HT at dark onset decreases intake of the carbohydrate nutrient by decreasing meal size, feeding time, and feeding rate for this nutrient and increasing the satiating effect of carbohydrate. These effects, which occur specifically during the first meal after injection, are opposite those seen after peripheral administration of the 5-HT receptor antagonist, metergoline. This drug stimulates feeding through a selective increase in carbohydrate intake, characterized by an increase in meal size, percent composition, and feeding time for this nutrient and a decrease in the satiety ratio for carbohydrate. These results implicate the serotonergic system in the termination of carbohydrate-rich meals that are prevalent during the early hours of the natural feeding cycle.

Meal patterns *d*-Norfenfluramine Fluoxetine Metergoline Carbohydrate Satiety

SEROTONIN [5-hydroxytryptamine (5-HT)], when administered into the paraventricular nucleus (PVN), suppresses food intake mainly through a selective decrease in carbohydrate ingestion (1,22,23,35). It has been proposed (22) that 5-HT is most active in the initial hours of the natural feeding cycle, when carbohydrate is the preferred nutrient (36,42). This is supported by biochemical evidence showing that endogenous 5-HT in the medial hypothalamus or PVN displays a circadian rhythm with a clear peak at dark onset (25,27,31,41) and that exogenous 5-HT produces its strongest effect on carbohydrate intake at this time (22,25).

Fluoxetine (FLU), a 5-HT reuptake inhibitor (10), and *d*-norfenfluramine (DNF), which releases 5-HT (3,30), have also been shown to suppress food intake. Similar to 5-HT, these drugs specifically decrease intake of carbohydrate (44,45), an effect localized to the medial hypothalamus (24). This effect of 5-HT in the medial hypothalamus is mediated by 5-HT_1 receptors, more specifically 5-HT_{1B}, as opposed to 5-HT_2 or 5-HT_3 receptors (6,22). Blockade of these 5-HT_1 receptors has recently been shown to stimulate food intake (7,9) and particularly carbohydrate ingestion (39).

To further characterize the role of 5-HT and its receptors in feeding behavior, this present study compared the effects of 5-HT and the serotonergic agonists FLU or DNF on macronutrient intake at the onset of the natural feeding cycle. The impact of this indoleamine in the PVN on the macrostructure of feeding was also investigated in rats maintained in a computer-automated apparatus designed to continuously monitor their intake of the three macronutrient diets. Because the effect of 5-HT is believed to be mediated by 5-HT_1 receptors, its role in feeding was further examined after peripheral administration of the serotonin antagonist, metergoline (MTG), which has been shown to block 5-HT_1 as well as 5-HT_2 receptor sites (8,16).

METHOD

Subjects

Adult, male Sprague-Dawley rats (n = 74), weighing 250–300 g at the start of the experiment, were obtained from the Charles River Laboratories (Kingston, NY). Rats were individually housed in stainless steel wire mesh cages (43 ×

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22 × 19 cm). For some of these cages, glass food jars were placed in along the front wall of the cage. For meal pattern experiments, three openings were cut in the front wall of the cage and Plexiglas food jars were placed on scales in front of these openings. All rats were housed in a temperature-controlled room (22°C) with a 12 L : 12 D cycle and lights off at 3:30 p.m.

Diets

Rats were maintained on a self-selection feeding paradigm and provided with separate sources of protein, carbohydrate, and fat freely available to allow them to alter separately intake of each of these nutrients while maintaining proper growth. The protein diet (3.7 kcal/g) consisted of 93% casein (National Casein Co.) mixed with 4% minerals (U.S.P. XIV Salt Mixture Briggs, ICN Pharmaceuticals, Montreal, Quebec), 2.97% (Vitamin Diet Fortification Mixture, ICN), and 0.03% cysteine (L-cysteine HCl, ICN). The carbohydrate diet (3.7 kcal/g) was composed of 28% dextrin, 28% corn starch (ICN), 37% sucrose (Domino) mixed with 4% minerals and 3% vitamins. The fat diet (7.7 kcal/g) consisted of 86% lard (Armour) mixed with 8% minerals and 6% vitamins. The placement of the jars containing these diets were changed daily to prevent position preferences. Water was available ad lib through a water bottle placed at the back of the cage. Introduction of fresh diet and jar rotation were scheduled daily approximately 5 h before the onset of the dark cycle (10:00–11:00 a.m.) to ensure minimal disturbance to the animals at the onset of the active dark cycle.

Stereotaxic Surgery

After a 2-week adaptation period to the laboratory conditions, animals that were to receive PVN injections were stereotaxically implanted, under metofane anesthesia, with 23-ga stainless steel cannulae aimed at this nucleus. With the incisor bar 3.3 mm below the interaural line, the stereotaxic coordinates used were: +6.8 mm anterior to the interaural line, −0.4 mm lateral to the midsagittal sinus, and −6.9 mm ventral to the skull surface. A 31-ga injector extended 1.5 mm beyond the length of the cannula to reach the dorsomedial surface of the PVN. Subjects were given 1 week to recover from the surgery and animals intended for the meal pattern experiments were given another week to adapt to the apparatus before initiation of testing. Histological analysis of brain tissue showed all injection sites to be in the area of the PVN, at least within 0.3 mm of its border.

Drugs

In Experiment 1, 5-hydroxytryptamine creatinine sulfate complex (5-HT; Sigma Chemical Co., St. Louis, MO) was dissolved in 0.9% bacteriostatic saline at doses ranging from 1.3–20.0 nmol/0.3 μl saline. Fluoxetine HCl (Eli Lilly and Co., Indianapolis, IN) and *d*-norfenfluramine HCl (Servier Amerique) were also dissolved in saline at doses of 3.1–25.0 nmol/0.3 μl, and in Experiment 2 5-HT was injected at a dose of 2.5 nmol/0.3 μl. Metergoline (Hoffmann-La Roche Inc., Nutley, NJ) was dissolved in 1% ascorbic acid at doses of 0.03–1.0 mg/kg in Experiment 3 and 1.0 mg/kg in Experiment 4. Drugs were gradually injected into the brain over a period

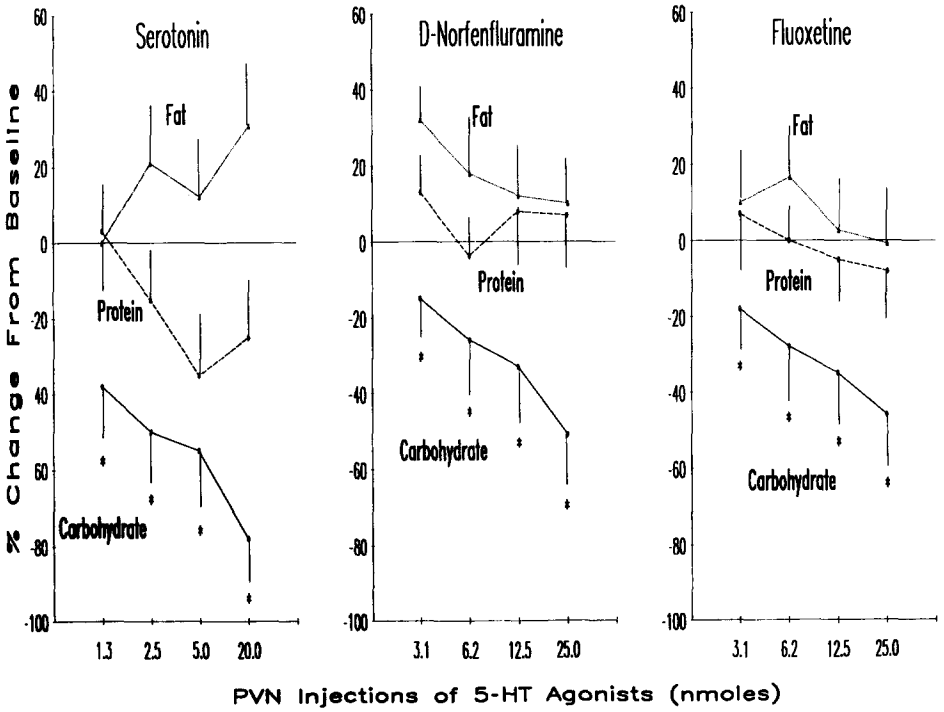

FIG. 1. Dose-response study of paraventricular nucleus (PVN) administration of 5-hydroxytryptamine (5-HT) (0.3–20.0 nmol), *d*-norfenfluramine, and fluoxetine (3.15–50 nmol). Analyses of percent change from baseline 1 h after injections reveal a selective, dose-dependent decrease in carbohydrate kcal intake after all three compounds with little or no change in protein and fat intake. $^{*}p < 0.05$ for direct comparisons between saline and drug scores.

of 10–15 s using a 10 μl Hamilton syringe (Hamilton Co., Reno, NV) and a polyethylene tubing that connects the syringe to the injector.

Test Procedures

For each experiment, measurements of 24-h nutrient intake and body weight were taken three times a week to ensure that the animals had adapted to the diets and were consuming minimum amounts of all three diets. Animals consuming less than 15% (n = 3) or greater than 50% (n = 2) of their total diet in the form of a single nutrient, as well as animals that failed to gain proper body weight (n = 1), were eliminated from the study.

For Experiments 1 (n = 13 for 5-HT; n = 16 for DNF; n = 16 for FLU) and 3 (n = 12), the testing procedures were as follows. Food was removed 10–15 min before dark onset and weighed. Animals were injected immediately prior to dark onset with either saline or different doses of the drug in randomized order. Food was returned, and intake of the three nutrients was recorded for 60–90 min, respectively. For Experiments 2 (n = 8) and 4 (n = 9), animals were maintained in the computer apparatus and injected immediately prior to dark onset with either saline vehicle or drug in counterbalanced order. Each animal was tested until two complete 12-h scorable days were attained. Data were eliminated due to spillage, computer malfunction, or unstable readings.

Apparatus

A computer-assisted data acquisition system was used to take measurements of macronutrient intake throughout the 12-h nocturnal cycle. This system, described in detail elsewhere (36), permits continuous recording from multiple inputs, using a personal computer, Ohaus Port-O-Gram electronic balances, commercially available communications hardware, and custom software. The scales were connected to an IBM XT computer housed in a separate room to allow

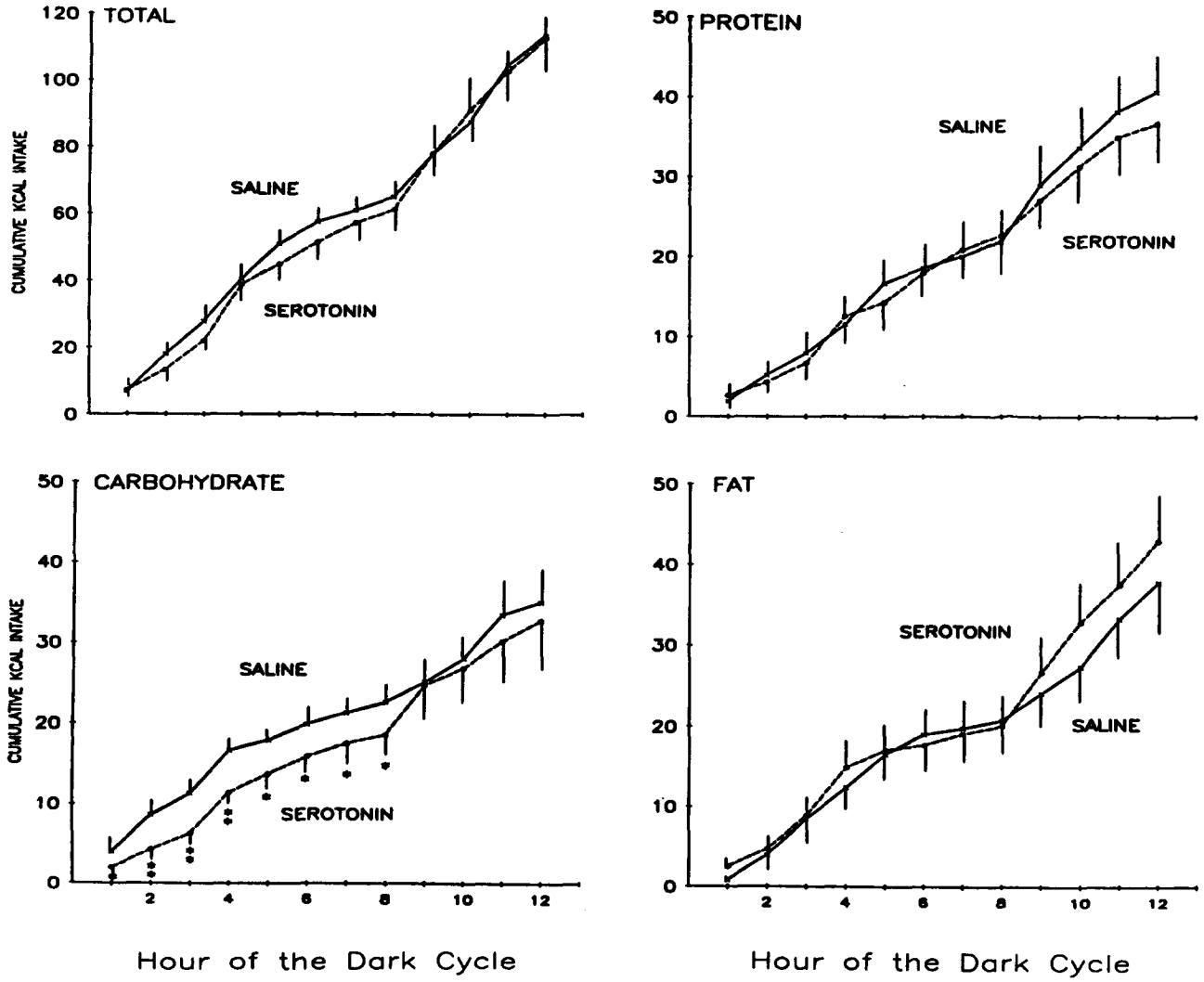

FIG. 2. Analysis of cumulative nutrient intake across the 12-h dark cycle after paraventricular nucleus (PVN) serotonin (5-HT; 2.5 nmol) or saline injections. Analysis of variance revealed a selective suppression of carbohydrate intake, lasting approximately 8 h. Protein, fat, and total caloric intake were unaffected over the 12-h period. *$p < 0.05$, **$p < 0.01$ between saline and 5-HT scores at specific time intervals.

remote monitoring and collection of data with minimal disturbance to the animals. The computer was programmed to record each scale's display every minute throughout the dark cycle. This information was compiled into data files and subsequently organized into hourly intervals. Individual bouts of eating were analyzed and the data organized into complete meals, using published criteria (33). A meal was characterized as a minimum intake of 0.1 g and separated from other meals by an intermeal interval of 10 min. This excluded random responses or noise and did not eliminate actual meals.

Data Analysis

One-hour measurements of food intake in Experiments 1 and 3 and cumulative 12-h intake data obtained in Experiments 2 and 4 were statistically evaluated using two-way analysis of variance (ANOVA). The data for the three diets were examined separately, with the main effect of the drug analyzed across the 12 time points. Significant effects were followed by comparisons between meals using Duncan's new multiple-range test. Analyses of specific meal parameters, comparing saline and drug scores, were made using paired Student's *t*-tests. Satiety ratios, defined as postmeal interval divided by meal size, were determined according to previously established criteria (32). In calculating the satiety ratios, data from animals consuming <0.1 kcal of a specific diet in a particular meal were eliminated from this analysis. Percent of total diet for a given nutrient was determined by relating the kcal intake of this nutrient to the total kcal intake.

RESULTS

Experiment 1: Dose-Response Study with PVN 5-HT, DNF, and FLU

This experiment examined and compared, as a function of dose, the short-term feeding responses induced by PVN administration of 5-HT and of the 5-HT agonists, DNF and FLU, during the first hour of the dark period. The saline baseline scores for this interval, to which the drug values were compared, were 10.9 ± 0.8 kcal for total kcal intake, which included 5.2 ± 0.5 kcal of carbohydrate, 1.3 ± 0.3 kcal of protein, and 4.4 ± 0.8 kcal of fat. Figure 1 presents the results obtained with the drugs in terms of percent change from these baseline scores. The data obtained with 5-HT (1.3–20.0 nmol) revealed a dose-dependent, selective suppression of the carbohydrate diet ($p < 0.05$). At the highest dose tested, PVN 5-HT decreased intake of this nutrient by approximately 80% ($p < 0.05$), while protein and fat intake were unaffected. Similar results were obtained with PVN injection of the 5-HT agonists, DNF (3.1–25.0 nmol) and FLU (3.1–25.0 nmol), both of which selectively and dose-dependently sup-

TABLE 1

EFFECTS OF PVN INJECTIONS OF 5-HT (2.5 nmol) ON MEASURES FOR MEAL 1

	Saline	Serotonin	Difference
Meal size (kcal)			
Carbohydrate	5.1 ± 1.9	1.8 ± 0.9	−3.3*
Protein	3.6 ± 1.4	2.5 ± 1.4	−1.1
Fat	1.9 ± 0.8	2.8 ± 0.9	+0.9
Total	10.6 ± 2.2	7.1 ± 1.8	−3.5
Meal composition (% of total diet)			
Carbohdyrate	52.8 ± 9.6	32.3 ± 15.5	−20.5*
Protein	32.2 ± 7.4	23.1 ± 12.0	−9.1
Fat	15.0 ± 6.4	44.6 ± 15.7	+29.6*
Feeding time (min)			
Carbohdyrate	9.3 ± 3.5	2.3 ± 0.9	−7.0*
Protein	6.1 ± 2.9	4.5 ± 2.2	−1.6
Fat	1.5 ± 0.7	1.9 ± 0.7	+0.4
Total	16.9 ± 6.7	8.6 ± 2.8	−8.3*
Feeding rate (kcal/min)			
Carbohydrate	0.62 ± 0.1	0.21 ± 0.1	−0.41*
Protein	1.14 ± 0.7	0.22 ± 0.1	−0.92
Fat	0.66 ± 0.3	1.37 ± 0.5	+0.71
Total	2.42 ± 0.9	1.80 ± 0.5	−0.62
Meal duration (min)	26.5 ± 10.5	15.9 ± 6.0	−10.6
Post meal interval (min)	45.1 ± 11.6	67.6 ± 14.0	+22.5
Satiety ratio (Carb) (post-meal interval/meal size)	9.7 ± 4.1	56.4 ± 24.8	+46.7*

*$p < 0.05$ for direct comparisons between saline and 5-HT.

TABLE 2

EFFECTS OF PVN 5-HT INJECTIONS (2.5 nmol) ON MEASURES FOR MEALS 1-3

	Meal 1	Meal 2	Meal 3
Meal size (kcal)			
Carbohydrate	−3.3*	−3.6*	−0.8
Protein	−1.1	−4.9	−8.9*
Fat	+0.9	−4.4	−3.8
Total	−3.5	−12.8*	−13.4
Meal composition (% of total diet)			
Carbohydrate	−20.5*	−8.7	+8.3
Protein	−9.1	+2.1	−24.5
Fat	+29.6*	+6.5	+16.5*
Feeding time (min)			
Carbohydrate	−7.0*	−3.8*	−1.5
Protein	−1.6	−5.8	−8.4
Fat	+0.4	−4.9	−5.5
Total	−8.3*	−14.3*	−15.4
Feeding rate (kcal/min)			
Carbohydrate	−0.41*	−0.10	+0.10
Protein	−0.92	−0.26	−0.43*
Fat	+0.71	+0.23	+1.14
Total	−0.62	−0.12	+0.80
Meal duration (min)	−10.6	−23.7*	−20.0
Postmeal interval (min)	+22.5	−14.0	+3.1
Satiety ratio (postmeal interval/meal size)	+46.7*	−0.20	−1.1

*$p < 0.05$ for direct comparisons between saline and 5-HT.

pressed intake of the carbohydrate diet ($p < 0.05$), producing approximately a 50% reduction at the highest dose tested. Similar to 5-HT, both DNF and FLU had little effect on protein or fat intake. These results clearly demonstrate that PVN injection of 5-HT itself selectively suppresses carbohydrate intake and that these effects are similarly seen with 5-HT agonists that act through endogenous 5-HT.

Experiment 2: Meal Pattern Analysis After PVN 5-HT Injections

This experiment was conducted to analyze the macrostructure of macronutrient feeding after PVN injections of 5-HT at a dose (2.5 nmol) found to be effective in the previous experiment.

Twelve-hour cumulative intake. Cumulative intake measurements over the 12-h nocturnal feeding cycle after PVN 5-HT injections (Fig. 2) revealed a highly selective suppression of carbohydrate intake relative to saline baseline scores ($p < 0.01$). Protein, fat, and total kcal intake remained unaffected over the entire 12-h period. No significant time × diet interactions were seen for any of the three nutrients or for total intake. Direct comparisons between the saline and 5-HT scores at specific time points showed that the decrease in carbohydrate intake was strongest during the first 2 h of the dark cycle (−50%, $p < 0.01$) and no longer apparent after the eighth hour of the cycle. Intake of the protein and fat diets was unaffected at any specific time points or over the entire 12-h period.

Meal pattern analysis. Meal pattern analyses demonstrated that the main effects of 5-HT injections were limited to the first three meals, occurring approximately during the first 3–4 h of the dark cycle (Tables 1 and 2). The total number of meals consumed over the 12-h period (8.1 after saline and 8.9 after 5-HT) and the average meal size over this period (15.5 after saline and 12.7 after 5-HT) remained unchanged.

Meal 1. The first meal of the feeding cycle (Table 1), generally occurring within the first hour of the dark, showed a selective suppression of carbohydrate intake (−65%, $p < 0.05$), the time spent consuming this diet (−75%, $p < 0.05$), and the rate of feeding this nutrient (−66%, $p < 0.05$). Consequently, a reliable decrease in the percent composition of this nutrient in the meal (from 53 to 32%, $p < 0.05$) was obtained. Fat and protein kcal intake remained unaffected in this meal; however, the percent of fat in the total diet increased from 15 to 45% ($p < 0.05$), presumably an indirect consequence of the decrease in the carbohydrate component. A small increase in the postmeal interval following this first meal, accompanied by the decrease in carbohydrate kcal intake, resulted in a significant increase in the satiety ratio (postmeal interval/meal size) of this nutrient after 5-HT ($p < 0.05$). Satiety ratios for the other two nutrients, protein and

fat, remained unaffected during this meal (data not shown), indicating that 5-HT is selective in potentiating satiety specifically for carbohydrate.

Meals 2 and 3. Patterns for the second meal of the dark cycle (Table 2) showed, once again, the carbohydrate component to be decreased after 5-HT injection both in terms of kcal intake and feeding time for carbohydrate and, consequently, for total feeding time and meal duration. Analysis of the third meal of the dark cycle (Table 2) revealed a significant suppression in protein kcal intake (from 13.4 to 4.5 kcal, $p < 0.05$) and in the rate of protein consumption (from 0.94 to 0.51 kcal/min, $p < 0.05$), while meal patterns for the carbohydrate and fat components were unaffected. Besides a significant enhancement in the percent fat composition in the third meal, no further changes were seen either in this meal or in subsequent meals, indicating a return to normal baseline feeding patterns.

Experiment 3: Dose-Response Study of IP MTG

This experiment (Fig. 3) was conducted to examine and compare, as a function of dose, the feeding responses to IP injections of the general 5-HT antagonist, MTG, 90 min after injection at dark onset. At doses ranging from 0.03–1.0 mg/kg, MTG produced a selective, dose-dependent increase in carbohydrate intake. One-way analysis of variance (ANOVA) revealed that carbohydrate intake ($p < 0.05$) and total intake ($p < 0.05$) were significantly enhanced while protein and fat intake were unaffected. A maximum effect was seen at the highest dose tested (1 mg/kg), which essentially doubled the amount of carbohydrate consumed and consequently increased total intake by 46%.

Experiment 4: Meal Pattern Analysis After IP MTG Injection

This experiment was conducted to analyze the macrostructure of nutrient intake after IP injections of MTG at the dose (1.0 mg/kg) found to be the most effective in Experiment 3.

Twelve-hour cumulative intake. Analysis of cumulative 12-h intake patterns after MTG injection at dark onset (Fig. 4) revealed an increase in intake of carbohydrate ($p < 0.001$) and protein ($p < 0.001$) over time, resulting in an increase in total 12-h kcal intake ($p < 0.001$). Fat intake remained unaffected, and no diet × time interactions were seen for any of the nutrients or for total intake. Direct comparisons between saline vs. drug scores at specific time points showed that the stimulatory effect on carbohydrate intake was apparent during the first hour after injection (+4.0 kcal, $p < 0.05$) and gradually declined to an increase of +1.5 kcal ($p > 0.05$)

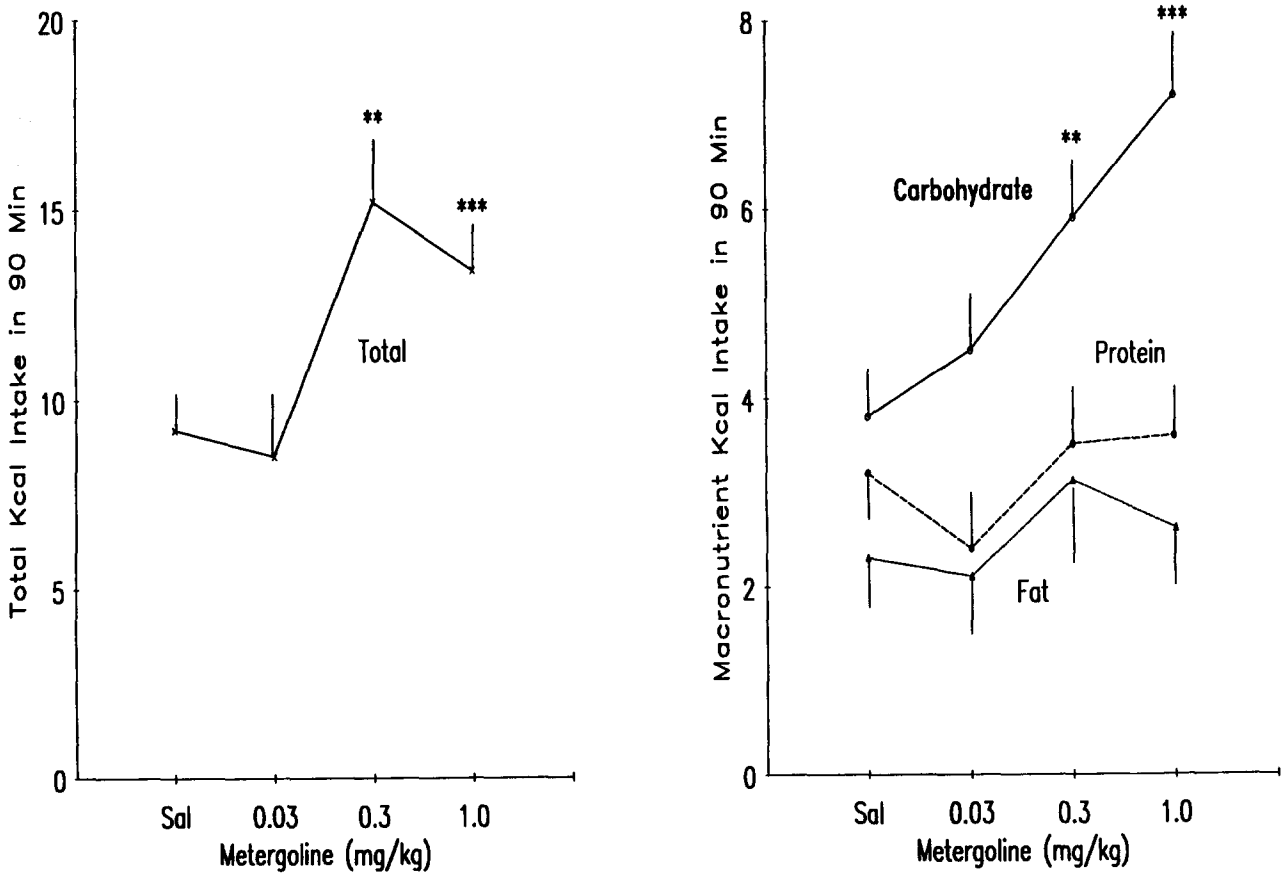

FIG. 3. Peripheral administration of metergoline (MTG), 90 min after injection, produced an increase in total kcal intake due to a selective enhancement of carbohydrate intake with no changes in protein or fat intake. $^{**}p < 0.01$, $^{***}p < 0.001$ for direct comparisons between saline (Sal) and MTG scores.

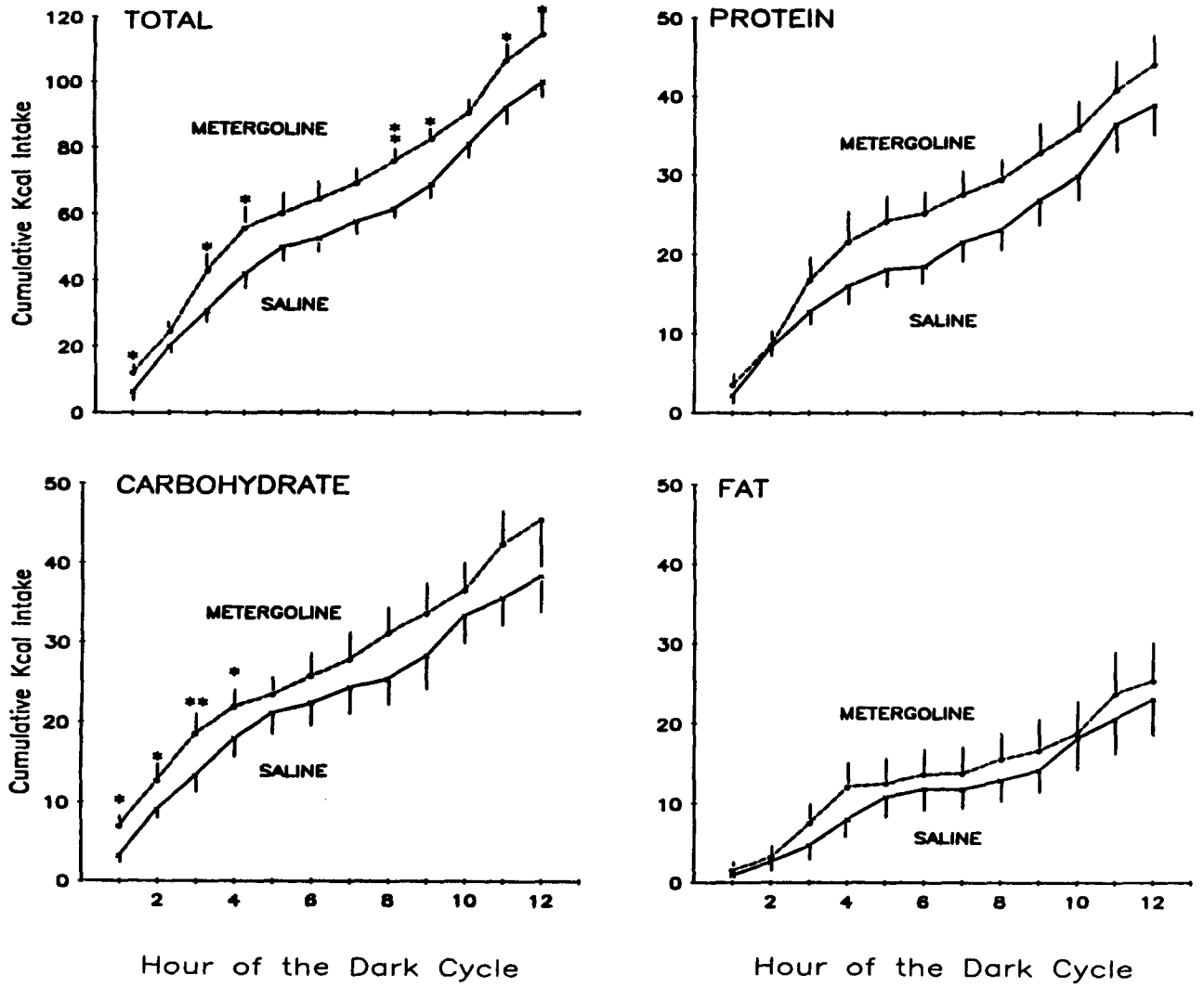

FIG. 4. Analysis of cumulative intake over the 12-h period after peripheral injections of metergoline (MTG; 1.0 mg/kg). Analyses of variance revealed a selective increase in carbohydrate intake, while fat intake remained unaffected over the 12-h period. While protein intake was unaffected during the initial hour of the dark, MTG produced a significant increase in intake of this nutrient across the 12-h period but not at any specific hour. $*p < 0.05$, $**p < 0.01$ for direct comparisons between saline and MTG scores at specific time intervals.

by the fifth hour. Although protein intake was significantly enhanced over the entire 12-h period, a reliable effect on ingestion of this nutrient was not statistically detected at any particular hour or meal. Total intake was increased at specific intervals, and fat intake remained unaffected at all times.

Meal patterns analysis. Meal pattern analyses over the 12-h nocturnal cycle after IP MTG administration showed no changes in total meal number (9.6 after saline vs. 8.8 after MTG), although a reliable increase in the average meal size over the 12 h (10.7 kcal after saline and 14.2 kcal after MTG, $p < 0.01$) was seen.

Meal 1. The saline baseline scores for these MTG tests were similar to the saline scores reported for the PVN 5-HT scores in Table 1. Thus, in Table 3 only difference scores, between MTG and saline, are reported for the first three meals after injection. Analyses of the first meal revealed a selective enhancement of carbohydrate intake relative to saline baseline. This increase was reflected by an enhancement of kcal intake (+49%, $p < 0.05$) and feeding rate (+72%, $p < 0.05$) for this nutrient. Protein and fat intake remained unaffected, resulting in an insignificant rise in total kcal intake. A small decrease in the postmeal interval following this first meal, accompanied by the increase in the meal size for carbohydrate, significantly reduced the satiety ratio (postmeal interval/meal size) for this nutrient. The satiating effect of the protein and fat diets (data not shown) was not altered.

Meals 2 and 3. Patterns for meal 2 (Table 3) revealed no changes for any of the meal parameters. Meal 3, however, showed significant changes in carbohydrate intake. Kilocalorie intake (+91%, $p < 0.05$), percent composition in the total diet (+50%, $p < 0.05$), and feeding time (+101%, $p < 0.05$) for this nutrient were significantly enhanced. Although protein and fat intake remained generally unaffected during this meal, protein feeding rate was reliably increased. No other

TABLE 3
EFFECTS OF IP MTG INJECTIONS (1 mg/kg) ON MEASURES FOR MEALS 1-3

	Meal 1	Meal 2	Meal 3
Meal size (kcal)			
Carbohydrate	+1.7*	−0.2	+3.9*
Protein	−0.3	+1.2	+2.4
Fat	+0.7	+0.4	+0.1
Total	+2.3	+1.4	+6.4*
Meal composition (% of total diet)			
Carbohydrate	+2.0	−13.1	+14.0*
Protein	+0.7	+8.7	−10.2
Fat	−2.6	+4.3	−3.8
Feeding time (min)			
Carbohydrate	+1.1	+1.6	+7.0*
Protein	−1.4	+1.0	+2.7
Fat	−0.2	0.0	−0.1
Total	−0.5	+2.4	+9.6*
Feeding rate (kcal/min)			
Carbohdyrate	+0.40*	−0.23*	+0.22
Protein	+0.10	+0.18	+0.15*
Fat	+0.52*	+0.06	+0.14
Total	+1.03†	0.00	+0.50
Meal duration (min)	−1.4	+3.6	+10.2
Postmeal interval (min)	−20.6	+4.3	+40.3*
Satiety ratio (Carb) (postmeal interval/meal size)	−14.8*	−0.90	+4.5

*$p < 0.05$, †$p < 0.01$ for direct comparisons between saline and MTG.

changes were seen in this meal or in subsequent meals, demonstrating that the effects of MTG were relatively short-lived, with rats displaying normal baseline feeding after the third meal of the dark cycle.

DISCUSSION

These results provide clear evidence for a selective and dose-dependent effect of hypothalamic serotonergic stimulation on carbohydrate intake, accompanied by little change in protein or fat ingestion. In the first experiment, which measured feeding 1 h after injection, this was demonstrated by the dose-dependent effects of PVN injection of 5-HT or of the serotonergic agonists, DNF or FLU, which act through endogenous 5-HT. This change in macronutrient intake, which confirms previous reports with peripheral as well as central administration of these agents (2,22,26,43–45,47), was also seen in Experiment 2, in which measurements were taken over the 12-h feeding period. In this test, PVN 5-HT produced an immediate reduction in carbohydrate ingestion that was still apparent up to 8 h after injection; however, no change in protein or fat intake occurred at any time across the 12-h period. This clearly contrasts with the effect of peripherally administered 5-HT, which was predominantly associated with a decrease in fat intake (17).

Analyses of the animals' meal patterns over the 12-h cycle after PVN 5-HT showed that the impact of PVN serotonergic stimulation was restricted primarily to the first two meals of the cycle, with recovery from the injection occurring over the next one to two meals. This initial effect of 5-HT was characterized by a decrease in the size of the meal in association with a reduction in the feeding time and feeding rate and an increase in the satiety ratio specifically for the carbohydrate diet. There was no change in the number of meals consumed, supporting the hypothesis that 5-HT acts on the process of satiation (2,35). This specific alteration in meal size as opposed to meal frequency, and an enhancement of postprandial satiety, is similar to that seen in animals on a single mixed diet after PVN administration of 5-HT (35) or peripheral injection of the 5-HT-releasing compound fenfluramine (4,13, 14) or various 5-HT uptake blockers (5,12,28,29,38,46).

It has been proposed that the control of feeding by hypothalamic 5-HT is expressed phasically, activated primarily at the onset of the natural feeding cycle after an inactive period of little eating behavior (22). This is based upon evidence that this indoleamine is particularly effective at this time, producing little change in carbohydrate intake at other periods of the feeding cycle (25). The present findings support this evidence, demonstrating that 5-HT's effect on meal patterns is relatively short-lived and focused on the first one to two meals of the

nocturnal feeding cycle. Studies of natural meal patterns show that these initial meals are particularly rich in carbohydrate (36,42). Thus, 5-HT synthesis may be activated specifically during these meals and released within the PVN to terminate these meals. This proposal is consistent with biochemical studies showing a sharp peak in PVN levels of the 5-HT metabolite, 5-hydroxyindoleacetic acid, specifically at the onset of the dark cycle and a rapid decline in these levels over the next 1-2 h (41).

This suppressive effect of 5-HT on food intake appears to be mediated by the 5-HT_{1B} receptors in the medial hypothalamus (6). The results obtained here with peripheral injection of the antagonist MTG, which blocks 5-HT_1 receptors (6,11), support this proposal. In tests with 90-min measurements of food intake at dark onset, MTG produced a dose-dependent increase in total kcal intake. This agrees with the results of other studies in animals on a single mixed diet (7,9,39) and with the finding that MTG blocks the suppression of food intake induced by PVN 5-HT injection (43) or peripheral fenfluramine administration (34). The finding here, that MTG produces a selective and dose-dependent stimulatory effect on carbohydrate intake with no change in protein or fat intake, supports a recent investigation in animals given mixed diets containing high, medium, and low concentrations of carbohydrate (39).

With meal pattern analyses, the selective increase in carbohydrate intake at dark onset after peripheral MTG administration was detected until the third meal, which generally occurred in the third and fourth hour of the feeding cycle. This stimulatory effect was characterized by an increase in kcal intake, feeding time, and feeding rate and a decrease in the satiety ratio for the carbohydrate nutrient, rather than an increase in meal frequency. These effects of peripheral MTG are opposite to those seen with PVN administration of 5-HT. While fat intake was not affected in any of the meal parameters, MTG produced a delayed stimulatory effect on protein intake, starting around the fourth hour after injection. This effect on protein intake may reflect the action of MTG on nonserotonergic receptors, possibly dopamine receptors (15, 37), which have been linked to the ingestion of protein (20).

Together, these results obtained with 5-HT agonists in the PVN and a 5-HT antagonist peripherally administered support the existence of medial hypothalamic 5-HT receptors that control feeding behavior. This receptor system appears to be physiologically active in the rat in modulating ingestion of a specific nutrient, namely, carbohydrate, and in controlling the temporal rhythms of carbohydrate ingestion, specifically at the beginning of the natural feeding cycle (22). The possibility that this hypothalamic serotonergic system may be functional in humans is supported by clinical evidence indicating that serotonergic agonists reduce food intake, particularly of carbohydrate (37), while MTG selectively enhances food intake and intake of "sweet high-carbohydrate" foods (37). Moreover, in bulimic patients who consume large amounts of carbohydrate there is evidence for a deficiency in the activity of the serotonergic system in the brain (18).

It has been proposed that 5-HT and NE act antagonistically to maintain proper nutrient balance specifically at the onset of the dark cycle (22). Comparisons of feeding behavior after injection of these compounds at this time show that they have opposite effects specifically on carbohydrate intake, altering meal size and meal duration, and that NE increases and 5-HT decreases carbohydrate ingestion by affecting the satiating value of this nutrient (21). In agreement with this proposal are central injection mapping studies, showing these monoamines to act within the same hypothalamic area (19,25), and biochemical studies, showing their peak endogenous activity to occur specifically at the onset of the dark period (40,41).

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