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Peripheral Serotonin Administration Selectively Reduces Fat Intake in Rats

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KANAREK, R. B. AND H. DUSHKIN. *Peripheral serotonin administration selectively reduces fat intake in rats.* PHARMACOL BIOCHEM BEHAV **31**(1) 113–122, 1988.—Recent research has led to the hypothesis that serotonergic mechanisms may be involved in both the control of energy intake and appetites for specific nutrients. Most of this research has focused on serotonin (5-HT) within the central nervous system. However, there is evidence which suggests that peripheral 5-HT also may be involved in the control of energy intake and nutrient selection. To further assess this suggestion, the effects of peripheral 5-HT administration on energy consumption and nutrient intakes were examined in adult male Sprague-Dawley rats given separate sorces of protein, fat and carbohydrate. Administration of 5-HT (doses ranging from 2–6 mg/kg) led to significant dose-related decreases in total energy intake in both freely feeding and food-restricted rats. Examination of individual nutrient intakes revealed that following 5-HT, fat intake was more suppressed than either carbohydrate or protein intakes. Administration of the 5-HT antagonist, methysergide, blocked the suppressive effects of 5-HT on both total energy intake and fat intake. The present data support the proposal that peripheral serotonergic mechanisms play a role in ingestive behaviors.

Food intake Diet selection Serotonin Protein Fat Carbohydrate Ingestive behaviors

IT recently has been hypothesized that the neurotransmitter serotonin (5-HT) may play an important role in the regulation of food intake and appetites for specific nutrients. The basis for this hypothesis comes from experiments demonstrating that alterations in 5-HT activity within the central nervous system (CNS) can produce changes in feeding behavior [e.g., (2, 9, 18, 21)]. In general, experimental manipulations which increase serotonergic activity suppress feeding behavior. For example, administration of a variety of 5-HT agonists leads to a reduction in food intake [e.g., (2, 3, 5, 21, 29, 38, 39, 45, 46)]. Conversely, although the data are not as consistent, decreases in serotonergic activity have been associated with increases in food consumption (12,43).

With respect to appetites for specific nutrients, it has been proposed that an increase in 5-HT activity within the CNS is associated with a selective reduction in carbohydrate consumption (45,46). Data to support this proposal comes from experiments investigating the effects of the drug fenfluramine (FEN) on diet selection. FEN is an anorectic agent whose primary action within the CNS is to promote 5-HT release and block its reuptake into presynaptic neurons. In early studies examining the effects of dl-FEN on diet selection, rats were given a choice of two composite diets: a low protein-high carbohydrate diet and a high protein-low carbohydrate diet. Fat content of the two diets was identical. In this situation, FEN decreased total caloric intake primarily by reducing intake of the low protein-high carbohydrate diet (45). The selective reduction in intake of this diet led to a decrease in carbohydrate intake while protein intake remained relatively unaltered. Subsequent studies using other drugs which increase brain 5-HT also demonstrated selective reductions in carbohydrate consumption (46).

Although the preceding data suggest that elevations in 5-HT are associated with a decreased appetite for carbohydrate, this association has not been universally observed (2, 3, 28, 29). There are a number of variables, such as feeding schedule and diet composition, which interact with the effects of FEN on diet selection. For example, Blundell and McArthur (3) observed that FEN selectively decreased carbohydrate intake in food-restricted rats, however, this effect was not seen in animals with unrestricted access to food. Using separate sources of the three macronutrients, which permits evaluation of the effects of FEN on fat intake as well as protein and carbohydrate intakes, no evidence of a selective reduction in carbohydrate intake was observed following FEN administration (29). The preceding data make it evident that caution must be exercised in evaluating the effects of manipulations in 5HT activity on nutrient choice.

Most investigators have concluded that the relationship between 5-HT and feeding behavior is mediated in the CNS. However, there is evidence that peripheral serotonergic mechanisms also may contribute to the control of feeding

TABLE 1

SELF-SELECTION COMPONENTS

Protein Component (3.76 kcal/g)
960 g casein (ICN Pharmaceuticals, Cleveland, OH)
40 g AIN Mineral Mix (ICN Pharmaceuticals)
20 g Vitamin Diet Fortification Mix (ICN Pharmaceuticals)
Carbohydrate Component (3.76 kcal/g)
575 g corn starch (Teklad Test Diets, Madison, WI)
275 g dextrin (Teklad Test Diets)
100 g commercial grade sucrose
10 g Solka-floc (BW-200, James River Corp., Berlin, NH)
40 g AIN Mineral Mix
20 g Vitamin Diet Fortification Mix
Fat Component (7.85 kcal/g)
912 g Crisco (Proctor and Gamble, Cincinnati, OH)
48 g Safflower Oil (Hollywood Health Foods, Los Angeles, CA)
90 g AIN Mineral Mix
50 g Vitamin Diet Fortification Mix

Vitamin and minerals were added to the components so that the three dietary rations contained equal amounts of these micronutrients on a per kilocalorie basis.

TABLE 2

MEAN (±SEM) CUMULATIVE 1, 3 AND 6 HR CALORIC INTAKES (kcal) FOLLOWING IP INJECTIONS OF SALINE OR 5-HT FOR FREELY FEEDING RATS FED EITHER GROUND PURINA CHOW OR A SELF-SELECTION DIET

		Dose of 5-HT (mg/kg body weight)		
	Time	0	2	4
Purina Chow	1 hr	12.6	16.5	10.9
(N=7)		2.2	2.3	2.9
	3 hr	26.3	38.0*	26.5
		2.5	4.9	3.6
	6 hr	42.8	49.9	41.1
		2.7	4.7	4.8
Self-Selection	1 hr	13.3	14.3	11.9
(N=7)		4.0	1.8	2.9
	3 hr	47.2	42.9	26.2*
		5.1	2.6	3.9
	6 hr	62.8	56.5	50.2
		5.0	4.0	8.0

Each animal received each dose of the drug with injections separated by a minimum of 6 days. *Significantly different from saline ($p<0.05$).

behavior (4, 7, 11, 14, 15, 24, 25, 32, 34, 37). Systemic administration of 5-HT leads to dose-related decreases in caloric intake in both rats (7, 9, 23, 32) and rabbits (34). As 5-HT does not readily cross the blood-brain barrier (27), its anorectic effects are presumed to be associated with peripheral mechanisms. The present experiments further assessed the role of peripheral 5-HT in feeding behavior. The first experiment examined the effects of 5-HT on caloric intake and nutrient selection in freely feeding and food restricted rats offered separate sources of protein, fat and carbohydrate. The second experiment determined the ability of the serotonergic antagonist, methysergide, to block the effects of 5-HT on feeding behavior.

EXPERIMENT 1

METHOD

Animals and Diets

Fourteen drug-naive male Sprague-Dawley rats (CD outbred, Charles River Breeding Laboratories, Wilmington, MA), weighing between 300 and 340 g at the beginning of the experiment, were used. Animals were housed individually in standard stainless steel cages in a temperature-controlled room (21±1°C) with a reverse 12-12 hr light-dark cycle (lights on: 2200–1000 hr).

Animals were divided into two groups matched on the basis of body weight. Animals in the first group (N=7) were fed a self-selection regime with separate sources of protein, fat and carbohydrate (Table 1). Animals in the second group (N=7) were fed ground Purina Rodent Chow No. 5001 (caloric density=3.6 kcal/g). Purina chow and the protein and carbohydrate rations were presented in Wahmann (Timonium, MD) LC-306A nonspill food cups. The fat ration was presented in 75 ml glass cups. All animals had ad lib access to water throughout the experiment.

Drugs

Serotonin creatinine sulfate (Sigma Chemical Co., St. Louis, MO) was dissolved in physiological saline to concentrations that allowed selected doses to be administered in a volume of 1 ml/kg.

Procedure—Part A

To allow for adjustment to the dietary conditions, all animals received ad lib access to their respective diets with body weights and nutrient intakes measured daily for two weeks. Following this adjustment period, drug injections were begun. Two days preceding each drug injection day, each rat received an intraperitoneal (IP) injection of physiological saline at 1000 hr. On test days, animals received IP injections of either 2 mg/kg or 4 mg/kg 5-HT at 1000 hr. Nutrient intakes were measured at 1, 3 and 6 hrs following administration of saline or 5-HT. Drug injections were separated by six days.

Procedure—Part B

Three days after the completion of Part A, both groups of animals were transferred to a six-hour feeding schedule with food available from 1000 to 1600 hr. Ten days were allowed for adjustment to the new feeding schedule with body weights and nutrient intakes measured daily. Following this adjustment period, drug injections were begun. Three doses of serotonin were used: 2 mg/kg, 4 mg/kg and 6 mg/kg (IP). Drug doses were given in a random order to animals. Two days preceding each drug injection day, each animal was given an IP injection of physiological saline.

Data Analysis

Data were analyzed using one-way ANOVAs for repeated

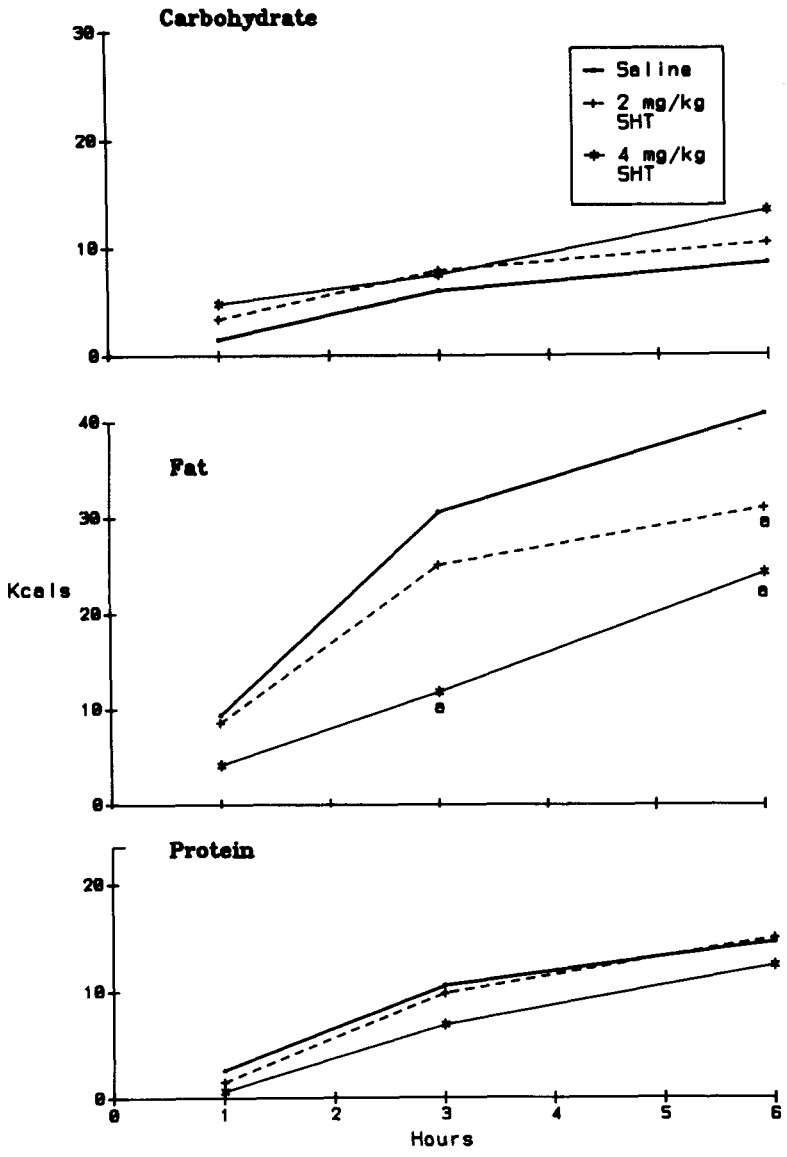

FIG. 1. Carbohydrate, fat and protein intakes in freely fed rats at 1, 3 and 6 hours following the administration of saline, 2 mg/kg, and 4 mg/kg 5-HT. At 3 hours postinjection, a=intake significantly different ($p<0.05$) from intakes following saline and 2 mg/kg 5-HT; at 6 hours postinjection, a=intakes significantly different ($p<0.05$) from intake following saline.

measures. Post hoc comparisons between conditions employed the Bonferroni t-statistic (26). Data reported as significant have a p value of 0.05 or less. As there were no significant differences among saline injections, saline values were averaged for comparison with 5-HT injections.

RESULTS

Experiment 1A

Animals maintained on Purina chow. At 3 hours postinjection, animals consumed significantly more Chow after injections of 2 mg/kg 5-HT than after injections of either saline or 4 mg/kg 5-HT, $F(2,18)=15.4$, $p<0.01$ (Table 2). No other differences were observed as a function of drug administration in animals allowed ad lib access to Chow.

Animals maintained on the self-selection regime. At 3 hours postinjection, administration of 5-HT resulted in a dose-related reduction in total caloric intake, calculated as the sum of caloric intake from each of the three macronutrients, $F(2,18)=15.3$, $p<0.01$ (Table 2). Animals given 4 mg/kg 5-HT consumed significantly less food at 3 hours postinjection than animals given saline. Although similar trends were observed at the other measurement points, these failed to reach statistical significance.

Examination of individual macronutrient intakes indicated that 5-HT administration led to dose-related reductions

TABLE 3

MEAN (±SEM) CUMULATIVE 1, 3 AND 6 HR CALORIC INTAKES (kcal) FOLLOWING IP INJECTIONS OF SALINE OR 5-HT FOR FOOD RESTRICTED RATS FED EITHER GROUND PURINA CHOW OR

		Dose of HT (mg/kg body weight)			
	Time	0	2	4	6
Purina Chow (N=7)	1 hr	46.1 2.9	31.7 3.9	27.0* 6.4	16.6* 4.0
	3 hr	69.5 6.1	49.3* 4.4	45.5* 7.2	39.4* 6.4
	6 hr	91.4 6.8	78.2 4.7	79.7 10.0	72.7 12.5
Self-Selection (N=7)	1 hr	52.2 7.1	33.5* 4.6	28.9* 6.2	14.0* 2.5
	3 hr	77.2 6.6	59.6* 6.9	60.8 9.8	44.6* 8.8
	6 hr	93.0 5.9	102.5 12.4	86.8 7.5	63.1† 9.4

Each animal received each dose of the drug with injections separated by a minimum of 4 days. *Significantly different from saline ($p<0.05$). †Significantly different from 2 mg/kg 5-HT.

in fat intake. At 3 hr postinjection, animals consumed significantly less fat after injections of 4 mg/kg 5-HT than after injections of either saline or 2 mg/kg 5-HT, $F(2,12)=9.78$, $p<0.01$ (Fig. 1—middle). At 6 hr after injections, animals consumed significantly less fat after both the 2 and 4 mg/kg dose of 5-HT than after saline injections, $F(2,12)=7.79$, $p<0.01$.

Neither carbohydrate nor protein intake varied as a function of 5-HT administration (Fig. 1—top and bottom).

Experiment 1B

Animals maintained on Purina chow. When animals were placed on the 6-hr feeding schedule, caloric intake initially decreased. However, within approximately one week, animals adapted to the feeding schedule by increasing food intake. At the time of testing, animals were consuming essentially the same number of calories per day during the 6-hr period and were gaining weight at the same rate as they had preceding food restriction.

5-HT administration was associated with dose-related decreases in Purina chow intake (Table 3). At 1 hr postinjection, both 4 and 6 mg/kg of 5-HT led to significant reductions in chow intake, $F(3,18)=14.27$, $p<0.01$. At 3 hr after injections, all three doses of 5-HT were associated with significant decreases in intake, $F(3,18)=13.85$, $p<0.01$. No significant differences in chow intake were observed 6 hr postinjection.

Animals maintained on self-selection regime. Total caloric intake initially decreased when animals were placed on the 6-hr feeding schedule. Animals adapted to the schedule in approximately one week at which time caloric intake equalled 93% of ad lib intake. Patterns of nutrient selection were modified slightly when animals were placed on the 6-hr feeding schedule. When given ad lib access to food, animals consumed 52% of their calories as fat, 10.5% as carbohydrate, and 37.5% as protein. When restricted to 6 hr access to food, fat represented 56.4%, carbohydrate 23.2% and protein, 20.4% of total daily caloric intake.

Total caloric intake decreased in a dose-related manner as a function of 5-HT administration (Table 3). At 1 hr postinjection, rats consumed significantly less calories following all doses of 5-HT than after saline injections, $F(3,18)=22.08$, $p<0.01$. Additionally, at 1 hr, rats consumed significantly less calories after receiving 6 mg/kg 5-HT than after receiving 2 mg/kg 5-HT. At 3 hr after injections, rats consumed significantly less calories after receiving 2 mg/kg and 6 mg/kg 5-HT than after saline, $F(3,18)=6.70$, $p<0.01$. Six hr postinjection rats consumed significantly less calories when given 6 mg/kg 5-HT than when given 2 mg/kg of the drug, $F(3,18)=6.06$, $p<0.01$.

Examination of individual macronutrient intake indicated that 5-HT administration was associated with dose related decreases in fat intake (Fig. 2—middle). At 1 hr postinjection, relative to saline intake, animals significantly suppressed fat intake following administration of both 4 and 6 mg/kg 5-HT, $F(3,18)=12.51$, $p<0.01$. At 3 hr postinjection, fat intake continued to be significantly suppressed after 6 mg/kg 5-HT, $F(3,18)=4.22$, $p<0.02$. Fat intake did not vary as a function of 5-HT administration at 6 hr postinjection.

At 1 hr postinjection, animals consumed less carbohydrate following administration of 6 mg/kg 5-HT than following saline, $F(3,18)=7.07$, $p<0.01$. No other differences in carbohydrate intake were observed as a function of 5-HT (Fig. 2—top).

At 3 hr $F(3,18)=3.73$, $p<0.05$ and at 6 hr, $F(3,18)=5.86$, $p<0.01$, postinjection, animals consumed significantly less protein following administration of 6 mg/kg 5-HT than following saline. No other differences in protein intake were observed as a function of 5-HT (Fig. 2—bottom).

EXPERIMENT 2

METHOD

Animals and Diets

Eighteen drug-naive male Sprague-Dawley rats (CD outbred, Charles River Laboratories), weighing between 230 and 260 g at the beginning of the experiment, were used. Animals were housed as in Experiment 1.

Animals were divided into two groups. The first group (N=8) was fed ground Purina chow. The second group of animals (N=10) was fed the self-selection regime described in Experiment 1. Diets were presented as in Experiment 1. All animals had ad lib access to water throughout the experiment.

Drugs

Serotonin creatinine sulfate was dissolved in physiological saline to a concentration of 5 mg/ml and administered at a dose of 5 mg/kg. Methysergide maleate (Sandoz Pharmaceuticals, Hanover, NJ) was dissolved in physiological saline to a concentration of 2.5 mg/ml. Two doses of methysergide were used: 2.5 mg/kg and 5.0 mg/kg.

Procedure

All animals were placed on a 6-hr feeding schedule. Each group received access to its respective diet from 1000 to 1600

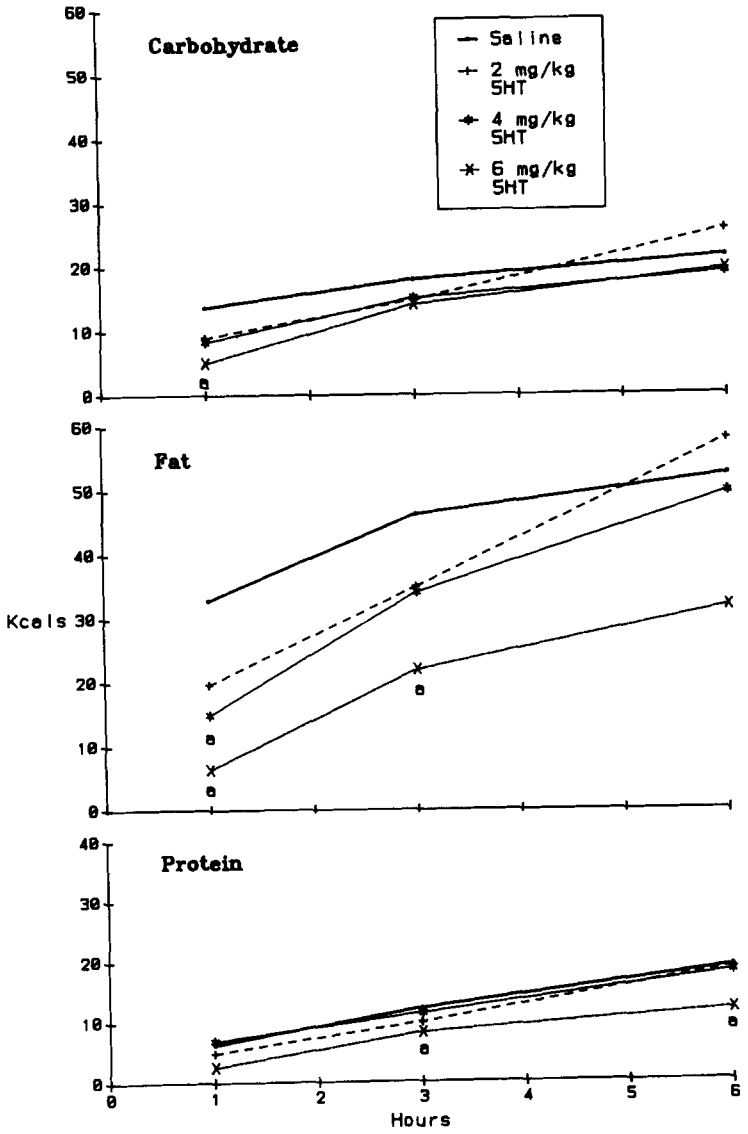

FIG. 2. Carbohydrate, fat and protein intakes in food-restricted rats at 1, 3 and 6 hours following the administration of saline, 2 mg/kg, 4 mg/kg and 6 mg/kg 5-HT. a=intakes significantly different ($p<0.05$) from intakes following saline injections.

TABLE 4
PAIRS OF INJECTIONS GIVEN TO RATS

Injection 1	Injection 2
0.9% saline	0.9% saline
0.9% saline	5 mg/kg 5-HT
2.5 mg/kg methysergide	0.9% saline
2.5 mg/kg methysergide	5 mg/kg 5-HT
5.0 mg/kg methysergide	0.9% saline
5.0 mg/kg methysergide	5 mg/kg 5-HT

Injection 1 was given 30 min preceding injection 2. Each rat received each of the six pairs of injections once.

hr. To allow for adjustment to the dietary conditions, body weights and nutrient intakes were measured daily for a 2-week period. Following this adjustment period, drug injections were begun. On each injection day, animals were given two injections, 30 minutes apart, beginning at 0930 hr. Physiological saline or methyseride were administered first, followed by either physiological saline or 5 mg/kg serotonin (see Table 4 for exact injection schedule). Nutrient intakes were measured at 1, 3 and 6 hr following the second drug injection. Drug injections were separated by at least two days.

Data Analysis

Data were analyzed by one-way ANOVAs for repeated measures. Post hoc comparisons were done using the Bon-

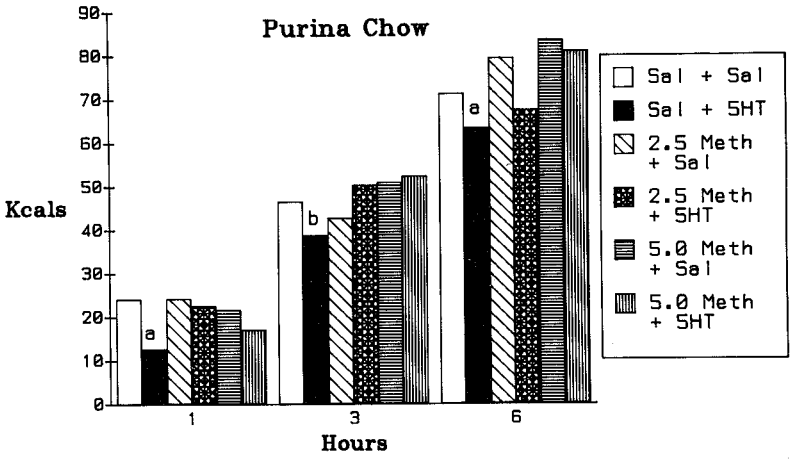

FIG. 3. Purina chow intakes in food-restricted rats at 1, 3 and 6 hours following the administration of saline, saline and 5-HT, methysergide and saline, and methysergide and 5-HT. a=intakes significantly different ($p<0.05$) from intakes following saline, b=intake significantly different ($p<0.05$) from intakes following 2.5 methysergide and 5-HT, 5.0 methysergide and saline, and 5.0 methysergide and 5-HT.

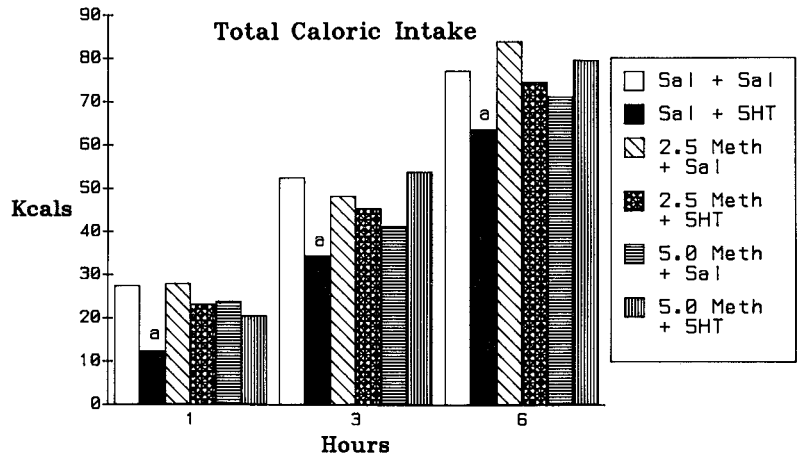

FIG. 4. Total caloric intake for food-restricted rats on the self-selection regime at 1, 3 and 6 hours following the administration of saline, saline and 5-HT, methysergide and saline, and methysergide and 5-HT. a=intakes significantly different ($p<0.05$) from intakes following saline.

ferroni t-statistic. Data reported as significant have a p value of 0.05 or less.

RESULTS

Animals Maintained on Purina Chow

At 1 hr postinjection, administration of 5 mg/kg 5-HT led to a significant suppression in chow intake relative to saline administration, $F(5,35)=4.95$, $p<0.01$ (Fig. 3). Administration of either dose of methysergide in conjuction with 5-HT blocked the suppressive effects of 5-HT on chow intake. At 3 hr postinjection, chow intake following 5-HT administration was not different from intake following saline. However, animals consumed significantly less food when given 5-HT alone than when given 2.5 mg/kg methysergide and 5-HT, 5 mg/kg methysergide alone, or 5 mg/kg methysergide and 5-HT. At 6 hr postinjection, animals ate less chow following 5-HT than following saline, $F(5,35)=11.92$, $p<0.01$. Both doses of methysergide blocked the effects of 5-HT on chow intake.

Animals Maintained on the Self-Selection Regime

Total caloric intake following drug adminsitration was similar to that observed for Purina chow intake (Fig. 4). At all three time points, rat consumed significantly less food following 5-HT administration than after saline injections [1 hr: $F(5,45)=6.98$, $p<0.01$; 3 hr: $F(5,45)=5.06$, $p<0.01$; 6 hr:

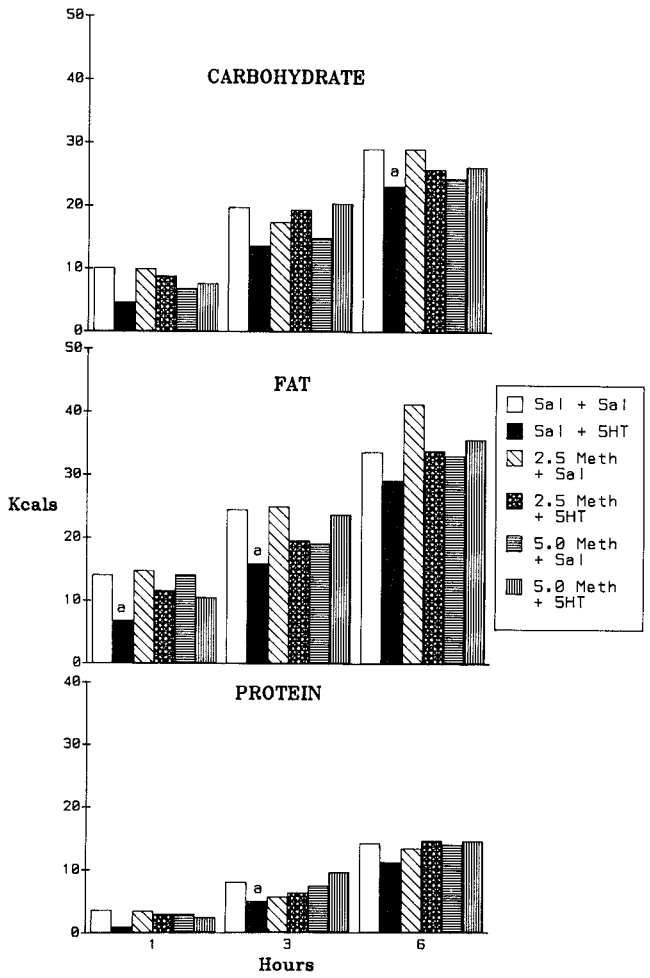

FIG. 5. Carbohydrate, fat and protein intakes for food-restricted rats on the self-selection regime at 1, 3 and 6 hours following the administration of saline, saline and 5-HT, methysergide and saline, and methysergide and 5-HT. a=intakes significantly different ($p<0.05$) from intakes following saline.

$F(5,45)=3.62$, $p<0.01$]. Administration of either dose of methysergide in conjunction with 5-HT blocked the inhibitory effects of 5-HT on total caloric intake.

Fat intake was significantly suppressed by 5-HT administration at 1 hr, $F(5,45)=5.00$, $p<0.01$ and 3 hr, $F(5,45)=3.49$, $p<0.01$, after injections (Fig. 5—middle). At both time points, methysergide (2.5 and 5.0 mg/kg) given prior to 5-HT reversed the effects of 5-HT on fat intake. Carbohydrate intake was significantly reduced by 5-HT administration relative to saline at 6 hr postinjection, $F(5,45)=2.74$, $p<0.05$ (Fig. 5—top). Administration of 2.5 mg/kg methysergide, prior to 5-HT, returned carbohydrate intake to control saline values. However, 5.0 mg/kg methysergide failed to block the suppressive effects of 5-HT.

Protein intake was significantly reduced by 5-HT administration relative to the control saline condition at 3 hr postinjection, $F(5,45)=2.38$, $p<0.05$ (Fig. 5—bottom). Administration of 5 mg/kg methysergide prior to 5-HT blocked the suppressive effects of 5-HT on protein intake.

GENERAL DISCUSSION

Peripheral administration of 5-HT resulted in dose-related decreases in food intake in food-restricted rats fed Purina chow. The effects of 5-HT on chow intake were most evident at 1 and 3 hours following injections with food intake returning toward saline levels during the last 3 hours of the feeding period. These results concur with those of previous experiments demonstrating reductions in food consumption as a function of systemic 5-HT administration (5, 7, 8, 14, 15, 25, 32, 34). The present studies further demonstrated that 5-HT leads to similar reductions in total energy intake in rats maintained on a dietary self-selection regime. Again, effects of 5-HT on total intake were most pronounced 1 to 3 hours after drug administration. In both dietary conditions, pretreatment with the serotonergic receptor blocker, methysergide [which appears to act primarily on peripheral serotonergic systems (16)] counteracted 5-HT-induced hypophagia. As previously suggested (25), these findings indicate that the reduction in energy intake seen following 5-HT administration is dependent upon postsynaptic peripheral 5-HT receptor stimulation. In contrast to earlier work (25), peripheral administration of 5-HT did not lead to reductions in food intake in rats given ad lib access to chow. However, significant decreases in total caloric intake were observed in rats given ad lib access to the self-selection regime.

Examination of individual nutrient intakes revealed that in both free-feeding and food-restricted animals, peripheral 5-HT administration was associated with greater decreases in fat intake than in either protein or carbohydrate intake. In freely-feeding animals, only fat intake was suppressed following 5-HT administration. When the animals were food-restricted, 5-HT led to a greater reduction in fat intake than in either carbohydrate or protein intake. For example, 1 hr after injections of 6 mg/kg 5-HT, fat intake was reduced to 19% of saline values, carbohydrate to 36% of saline values, and protein to 40% of saline values. Similar patterns of nutrient intakes were observed with other doses of 5-HT and at other measurement points. These differential alterations in nutrient intakes obviously led to variations in the proportion of total energy intake obtained from fat, carbohydrate and protein following 5-HT administration. At all time points, as the dose of 5-HT increased, the proportion of total calories chosen as fat decreased, while the proportion of calories chosen as carbohydrate increased. For example, in food-restricted rats, at 1 hr postinjection, calories from fat represented 63% of total energy intake following saline injections, 59%, after 2 mg/kg 5-HT, 51%, after 4 mg/kg 5-HT and 45% after 6 mg/kg 5-HT. In comparison, at 1 hr postinjection, calories from carbohydrate represented 26% of total energy intake after saline injections, 27%, after 2 mg/kg 5-HT, 29%, after 4 mg/kg 5-HT, and 36% after 6 mg/kg 5-HT. Percent protein intake did not vary systematically as a function of 5-HT administration. The suppressive effects of 5-HT on fat intake were blocked by administration of methysergide.

It is possible that peripheral administration of 5-HT has nonspecific effects on food intake. For example, peripheral 5-HT could interfere with locomotor behavior. However, evidence against this interpretation comes from experiments demonstrating that the doses of 5-HT used in this and previous experiments do not impair locomotor activity (32). In the present studies, the fact that fat intake was suppressed to a greater degree than either protein or carbohydrate intake following 5-HT injections suggest a specific effect for 5-HT on feeding behavor. Additionally, Montgomery and Burton

(24) recently reported that consumption of "food-like" solutions (e.g., sucrose and saccharin) was suppressed by 5-HT while consumption of "water-like" solutions (e.g., water and saline) was enhanced by the same dose of 5-HT. Taking this information together with observations that 5-HT administration does not lead to a conditioned taste aversion (32) argues for a specific effect of peripheral 5-HT on feeding behavior.

The selective decrease in fat observed following 5-HT injections correlates well with results of previous experiments examining the effects of serotonergic agents on diet choice (29,40). For example, using separate sources of the three macronutrients, we observed a substantial decrease in fat intake, smaller decrease in protein intake and sparing of carbohydrate intake following administration of the 5-HT agonist fenfluramine (29). Additionally, Shor-Posner and colleagues (40) recently reported that administration of fenfluramine and quipazine, another 5-HT agonist, led to a greater reduction in fat intake than in either protein or carbohydrate intake. In contrast, administration of the 5-HT receptor antagonist, cyproheptadine, stimulated appetite and produced a greater increase in fat intake than either protein or carbohydrate intake (40).

It should be mentioned that in the present experiments and our previous work examining the effects of fenfluramine on diet selection, rats consumed a larger portion of their calories from fat than from protein or carbohydrate. Thus, it is possible that 5-HT and serotonergic agents act to decrease intake of the most consumed dietary item. Unfortunately, many of the previous studies examining the effects of serotonergic agents on nutrient selection have failed to provide absolute consumption data [e.g., (40, 45, 46)], making this possibility difficult to evaluate at this time.

The physiological basis of 5-HT-induced hypophagia remains to be elucidated. However, several pieces of information suggest that the anorectic effects of 5-HT may be related to its actions on gastric motility. Administration of 5-HT and 5-HT agonists, such as fenfluramine, can lead to a decrease in rate of gastric emptying [e.g., (4, 11, 15, 36, 37)]. It has been suggested that a decrease in gastric emptying can serve as a satiety signal (4,36). 5-HT may produce its anorectic effects by delaying gastric emptying.

The selective effect of 5-HT on fat intake also may be associated with gastric emptying. Fat, itself, prolongs gastric emptying relative to carbohydrate and protein. Consumption of a small quantity of fat in association with 5-HT administration could have an additive effect on gastric emptying. The slowing of gastric emptying produced by these two stimuli could result in the rat's selectively avoiding consumption of additional fat. Data suggesting that rats select dietary items, at least partially, on the basis of nutrient composition of the food in the gastrointestinal tract comes from studies examining the effects of intragastric loads on nutrient selection. In these studies, intragastric loads of pure fat led to selective decreases in fat consumption in rats maintained on a self-selection regime [(1,30); Kanarek and Ho, unpublished data]. In comparison, intragastric loads of carbohydrate selectively suppressed subsequent carbohydrate intake (1,30). These data suggest that rats can detect the nutrient content of previously ingested food and may make subsequent nutrient choices on the basis of this information.

Previous work has demonstrated that immediately following food deprivation rats selectively consume fat [(31); Kanarek, unpublished data]. With respect to this finding, it is intersting to note that in Experiment 1, food-restricted animals suppressed fat intake during the first hour following 5-HT administration. In comparison, significant reductions in fat intake were not observed until 3-hr postinjections in freely-feeding animals. Comparison of nutrient intakes following saline injections revealed that food-restricted rats consumed fat more rapidly than freely feeding animals. It may be that some critical value of fat intake must be achieved before the suppressive effects of 5-HT on subsequent fat intake are observed.

A second alternative for the anorectic actions of peripherally-administered 5-HT has been posited. It has been hypothesized that 5-HT may reduce the incentive value of food-related stimuli by inhibition of cephalic phase insulin secretion (24,25). Insulin is secreted almost immediately in response to food ingestion (22,33). This early phase of insulin secretion occurs prior to food absorption from the gut and has been termed cephalic phase of insulin secretion (33). It has been proposed that the palatability of a food item is directly related to the level of cephalic phase insulin secretion (22). Previous research has demonstrated that 5-HT administration can inhibit insulin secretion (41). Montgomery and colleagues have suggested that 5-HT decreases cephalic phase insulin and thereby, the relative palatability of food stimuli (24,25). In support of this idea, these researchers observed that 5-HT administration reduced the intake of solutions (e.g., sucrose and saccharin) which elicit increases in plasma insulin while increasing consumption of solutions (e.g., saline and quinine) which do not increase insulin secretion.

5-HT-induced alterations in cephalic phase insulin secretion also may play a role in the selective decrease in fat observed in the present experiments. Previous studies have shown that on a long-term basis, insulin deficiency in diabetic animals is associated with a selective decrease in carbohydrate intake (19, 35, 42). However, on a short-term basis, decreases in insulin may have different consequences. High-fat diets are very palatable to rats [e.g., (10,17)]. A number of researchers have found that when given a choice between a high-fat diet and a low-fat diet, rats consistently consume more of the high-fat diet. Indeed, in the present experiments, rats consumed well over 50% of their total calories from fat in control conditions. It may be that if the cephalic phase of insulin secretion is reduced, animals decrease consumption of palatable foods more readily than intake of less palatable items. Thus, in the present experiment, a decrease in cephalic phase insulin secretion would be associated with a reduction in fat intake.

To more fully assess the effects of peripheral serotonin on the regulation of food intake and to determine which specific types of 5-HT receptors are involved, experiments employing antagonists other than methysergide should be conducted. Methysergide acts as an antagonist at both 5-HT_1-like and 5-HT_2 receptors and at high doses can display agonist activity (6). Thus, methysergide provides only rather general information about serotonergic activity.

Finally, while it is clear that exogenously administered serotonin can decrease energy consumption and alter nutrient selection, the role of endogenous serotonin as a satiety signal remains an important question. For serotonin to serve as a satiety signal, there must be a relationship between food intake and serotonin release (32). There is evidence suggestive of this relationship. Serotonergic neurons are numerous in the gastrointestinal region (44). Additionally, elevated levels of serotonin are found in the hepatic portal and peripheral venous blood supply after intraduodenal infusions of hypertonic glucose (13), increased pressure on the intestinal

mucosa (8), and acidic conditions in the duodenum (20). As these events typically are associated with normal food intake, it can be hypothesized that the resulting increase in serotonin serves as a signal to reduce further feeding behavior.

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