Activation of hypothalamic serotonin receptors reduced intake of dietary fat and protein but not carbohydrate

BRENDA K. SMITH,1 DAVID A. YORK,1,2 AND GEORGE A. BRAY1,2

1Obesity, Diabetes and Metabolism Section, Pennington Biomedical Research Center, Louisiana State University, Baton Rouge 70808; and 2Department of Physiology, Louisiana State University School of Medicine, New Orleans, Louisiana 70112

Smith, Brenda K., David A. York, and George A. Bray. Activation of hypothalamic serotonin receptors reduced intake of dietary fat and protein but not carbohydrate. Am. J. Physiol. 277 (Regulatory Integrative Comp. Physiol. 46): R802–R811, 1999.—Systemic treatment with dexfenfluramine (dF), fluoxetine, or serotonin (5-hydroxytryptamine, 5-HT) recently was shown to suppress fat and occasionally protein but not carbohydrate intake in rats when a macronutrient selection paradigm was employed. These reports contrast with the prevailing literature, which for the past decade has described a role for serotonin neurotransmission in the modification of dietary carbohydrate consumption. To test the hypothesis that the suppression of fat selection and/or consumption by systemic serotonin agonists involves stimulation of central 5-HT receptors, a series of experiments was performed in nondeprived rats. In experiment 1, third cerebroventricular (3V) infusion of the nonselective 5-HT antagonist metergoline prevented the reduction in fat but not carbohydrate feeding caused by systemic dF. Furthermore, 3V metergoline alone increased fat intake. In experiments 2 and 3, 3V infusion of 5-HT1B/2C receptor agonists d-norfenfluramine (DNF) or quipazine inhibited fat intake exclusively. Next, the infusion of DNF or 5-HT into the region of the paraventricular nucleus (PVN) reduced both fat and protein intake (experiments 4 and 5). Finally, in experiment 6, when rats were grouped by baseline diet preference, 5-HT infused into the PVN led to a dose-related decrease in fat intake in both carbohydrate- and fat-preferring rats. In contrast, there were no dose effects of 5-HT on carbohydrate or protein intake in either preference group. However, in fat-preferring rats, the highest dose of 5-HT reduced intake of all three macronutrient diets. These results demonstrate a selective effect of exogenous serotonergic drugs in the hypothalamus to reduce fat rather than carbohydrate intake and suggest that higher baseline fat intake enhances responsiveness to serotonergic drugs.

paraventricular nucleus; fenfluramine; quipazine; food intake; preference; 5-hydroxytryptamine

RECENT EVIDENCE supports a selective action of systemically administered serotonin (5-hydroxytryptamine, 5-HT) or its receptor agonists to suppress fat intake both in animal studies designed to allow a concurrent evaluation of the consumption of individual fat, carbohydrate, and protein diets (14, 16, 17, 36, 43) and in human studies where macronutrient content of meals was assessed directly (2, 10, 18). These reports contrast with the once prevailing concept that drugs that increase serotonergic activity selectively reduce carbohydrate intake (24, 26, 47, 48) and point to the strong influence of methodology, e.g., the diet choices available, on the outcomes of studies of diet selection (1). For example, chronic peripheral administration of dexfenfluramine (dF) suppressed the intake of a high-carbohydrate, low-protein diet when rats were given a choice between two diets differing only in protein and carbohydrate content (30) with fat composition held constant. However, in a study employing a diet paradigm in which rats were allowed to choose among three separate macronutrient diets, daily intraperitoneal injections of low-dose dF decreased both absolute and proportional fat intake by 30% and 14%, respectively, compared with controls (43). In another experiment, both fat- and carbohydrate-preferring rats (characterized on the basis of their daily voluntary fat intake) significantly reduced their fat consumption while receiving dF treatment (43). The reduction in fat intake observed in both preference groups indicates that the anorectic effect of systemic dF is not simply to suppress intake of the preferred macronutrient diet.

Intrahypothalamic microinjections of fenfluramine and other 5-HT agonists into the region of the paraventricular nucleus (PVN) have been shown to cause hypophagia (7, 15, 45). A number of other studies have demonstrated that injection of 5-HT or 5-HT agonists such as d-norfenfluramine (DNF) into the PVN decreased the consumption of a carbohydrate-rich diet (23–26, 41, 45), thus providing support for the concept that this brain region plays a role in a carbohydrate-specific satiety mechanism. However, more recently it has been observed that dietary fat but not carbohydrate intake was selectively suppressed in response to systemic treatment with dF (36, 43). To test the hypothesis that the suppression of fat selection and/or consumption by systemic serotonin agonists involves stimulation of central 5-HT receptors, a series of experiments was performed.

First we showed that the selective suppression of fat intake by systemic dF (36, 43) can be blocked centrally with metergoline, a 5-HT antagonist. Next, the effects of the centrally administered 5-HT receptor agonists quipazine and DNF on diet selection were investigated to determine whether stimulation of 5-HT1B/2C receptors is sufficient to suppress fat selection and/or consumption. In the present report we demonstrate that these serotonin agonists inhibit fat and not carbohydrate intake in a self-selection paradigm. The presence of both 5-HT1B and 5-HT2C receptors has been demonstrated in the PVN region (3, 46) that is densely innervated by serotonergic fibers from the raphe nuclei.
(39). Although microinjection into the PVN of 5-HT (7, 22, 31) and other serotonergic drugs (15, 31) with affinities for the 5-HT$_{1B}$ and 5-HT$_{2C}$ receptor subtypes has been shown to decrease chow intake, the effective doses were two- to eightfold higher than those reported to selectively decrease carbohydrate consumption in a macronutrient diet paradigm (23, 26). Thus to clarify the dose effects of intrahypothalamic 5-HT on macronutrient diet selection, a dose-response study was undertaken in rats with a range of baseline fat consumption. We hypothesized that local infusion of 5-HT into the PVN would suppress fat consumption independent of baseline diet preferences, as observed previously with systemic fenfluramine treatment (43). The present results show a selective dose effect of 5-HT treatment on fat rather than carbohydrate intake and provide evidence for an effect of individual differences in baseline fat consumption on responsiveness to serotonin stimulation.

**METHODS**

Animals. Adult, male Sprague-Dawley rats (Harlan Sprague-Dawley, Indianapolis, IN) were individually housed in hanging wire mesh cages (35 × 22 × 15 cm) in a temperature-controlled room (21–23°C). Rats received Purina chow (#5001) and tap water ad libitum for several days after they arrived in the facility until the experimental diets were initiated. Before behavioral testing began, the rats were adapted for at least 2 wk to a 12:12-h light-dark cycle with lights on at 2300. All research protocols were approved by the Pennington Biomedical Research Center Institutional Animal Care and Use Committee.

Experimental diets. Rats were placed on the experimental diet ~2 wk before surgery. Only rats maintaining body weight and with a minimum protein intake of 10% of total energy received surgery. A three-choice diet paradigm was used, in which rats self-selected from three food cups, each containing a single macronutrient source supplemented with vitamins and minerals: carbohydrate (cornstarch and powdered sugar), fat (vegetable shortening), and protein (casein) (see Table 1). The fat diet was changed every 24 h to ensure freshness against oxidation. Every 48 h fresh diet was added to the jars containing carbohydrate and protein diets.

Stereotaxic surgery. Rats weighing 325–350 g were anesthetized with ketamine (80 mg/kg) and xylazine (12 mg/kg) and implanted with right unilateral intracranial cannulas from a flat skull position. The tip of the 25-gauge stainless steel guide cannula was either aimed 1 mm above the dorsolateral PVN according to the following stereotaxic coordinates relative to bregma: −0.4 mm lateral, −1.8 mm caudal, and at a depth of −7.0 mm; or aimed into the third ventricle at midline: −2.8 mm caudal and at a depth of −8.1 mm. The guide cannula was anchored to the skull with two stainless steel screws and dental cement and then dosed with a 31-gauge wire obturator. Rats were allowed to recover for at least 7 days before experimental testing began.

Drug administration. The drugs used in this study were dexfenfluramine hydrochloride, metergoline, quipazine, serotonin hydrochloride (Research Biochemicals, Natick, MA), and d-norfenfluramine HCl, the active metabolite of dexfenfluramine (generously supplied by Servier Amerique). All drugs were dissolved in sterile, preservative-free 0.9% NaCl (Fujisawa USA, Deerfield, IL) except metergoline, which was first dissolved in 5% tartaric acid and then diluted in deionized water. Drugs were infused gradually into the cerebroventricle or tissue over a period of 60 s, with the exception of experiment 1, in which metergoline or its vehicle was infused slowly over 2 min. The longer infusion period allowed the vehicle containing tartaric acid (pH 2.0–3.5) to be more evenly diluted by the cerebrospinal fluid and thus minimize adverse effects. PN infusions were made over 60 s with a 31-gauge stainless steel injector (Small Parts, Miami Lakes, FL; 250 µm external diameter), 28-gauge Teflon tubing, and a Harvard pump; the injector (extending 1.0 mm beyond the end of the cannula) was left in place for an additional 60 s to allow diffusion of injectate away from the cannula.

General experimental procedure. During the week before testing began, all rats received several mock injections; this involved manually restraining the rat as if to insert the injector cannula, placing the rat in a plastic bucket for 2 min, and running the infusion pump. In the experiment, infusions were performed in nondeprived rats within 30 min of dark onset, which occurred at 1100. Typically some anticipatory eating occurs before lights out; thus 2 h before the start of each test, food was removed from the home cage to standardized food ingestion proximate to the time of injection. After injection the preweighed jars of fresh food were replaced. Measurements of food intake were corrected for spillage. In experiments 2–6, a within-subject design was used, in which each rat received both drug and vehicle. Naive rats were used for each experiment with the exception of experiment 4.

**Experiment 1**: effect of third cerebroventricular administration of 5-HT receptor antagonist metergoline on systemic df-induced anorexia. To test whether the suppression of fat intake in response to systemic df (43) can be blocked centrally, metergoline was administered into the third ventricle. To ensure a sufficient baseline fat intake in this experiment, rats with a voluntary fat-to-carbohydrate (kcal) ratio of >0.5 were randomly assigned to four treatment groups (n = 5 or 6/group). Each animal received an intraperitoneal injection of either df (1.5 mg/kg) or sterile 0.9% NaCl, followed 30 min later by a third ventricular infusion of either metergoline (100 nmol/5 µl) or the same volume of vehicle. Food intake was measured 4 h later. The dose of metergoline was selected based on a previous study in which feeding was significantly enhanced after intraventricular infusion (5).

Experiments 2 and 3: effect of third cerebroventricular infusion of serotonin agonists quipazine or DNF on macronutrient selection. Macronutrient and total intakes of freely feeding rats after either quipazine (330 nmol/2.5 µl; n = 12) or DNF (416 nmol/2.5 µl; n = 6) was infused into the third ventricle were compared with food intake after saline infusion. Food intake was measured 2 h after lights out. Each rat received only one drug and one saline infusion in counterbalanced order.

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**Table 1. Composition of macronutrient diets**

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Carbohydrate</th>
<th>Fat</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cornstarch</td>
<td>58.11</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Powdered sugar</td>
<td>29.06</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Casein</td>
<td>0.00</td>
<td>0.00</td>
<td>87.17</td>
</tr>
<tr>
<td>Dl-Methionine</td>
<td>0.11</td>
<td>0.20</td>
<td>0.11</td>
</tr>
<tr>
<td>Vegetable shorten</td>
<td>0.00</td>
<td>75.12</td>
<td>0.00</td>
</tr>
<tr>
<td>AIN-76A vitamin mix*</td>
<td>0.77</td>
<td>1.49</td>
<td>0.77</td>
</tr>
<tr>
<td>AIN-76A mineral mix*</td>
<td>3.07</td>
<td>5.95</td>
<td>3.07</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>0.18</td>
<td>0.34</td>
<td>0.18</td>
</tr>
<tr>
<td>Cellulose (Aphal)</td>
<td>8.72</td>
<td>16.91</td>
<td>8.72</td>
</tr>
<tr>
<td>Energy density, kcal/g</td>
<td>3.53</td>
<td>6.85</td>
<td>3.53</td>
</tr>
</tbody>
</table>

Ingredients are expressed as percentages by weight. *Vitamin and mineral mixes contain 97% and 12% sucrose, respectively.
Experiments 4 and 5: effect of PVN infusion of serotonin agonist dNF or 5-HT on macronutrient selection. dNF (208 nmol/0.3 µl), 5-HT (235 nmol/0.3 µl), or the same volume of saline was infused into the PVN region, and macronutrient and total intakes were measured 2 h after dark onset. Each rat (n = 11) was tested once with drug and once with saline; tests were separated by 2 days in the 5-HT experiments and by at least 4 days in the dNF experiments because of the longer half-life of this drug. Eleven rats tested initially in experiment 4 with dNF along with two additional rats from an unpublished pilot study were tested 2 wk later in experiment 5 (n = 13) with 5-HT. The doses selected were based on the 50% suppression of food intake observed with central administration of dNF (300 nmol or 72 µg) (32, 38) and 5-HT (25–100 nmol or 5–20 µg) (7, 22).

Experiment 6: dose-response effects of PVN infusion of 5-HT on macronutrient selection. The objectives of this experiment were 1) to compare the dose effects of PVN 5-HT (0.3, 3, 30, or 300 nmol/0.3 µl) or the same volume of saline on macronutrient selection and total intake in freely feeding rats, and 2) to examine the influence of baseline preferences on the feeding response to 5-HT. All rats received a saline infusion first, followed by all doses of 5-HT administered in a counterbalanced manner; tests were separated by 2–3 days. A repeat saline injection was performed halfway through the 5-HT dose testing, and the mean of the two saline tests was used for statistical analysis. Food intake was measured at 1 and 2 h after lights out. The range of doses selected was designed to include those previously tested for effects on macronutrient selection (23, 26). Macronutrient preferences were characterized based on the percent of baseline calories consumed as fat, e.g., ≥40% = fat preferring, ≤40% = carbohydrate preferring, but the values were not calculated until the dose-response tests were completed. Under this criterion, daily proportional fat intake, averaged over a 3-day period immediately before the beginning of the experiment, was 26 ± 2% for carbohydrate-prefering rats and 59 ± 4% for fat-prefering rats.

Histological verification of the infusion site. At the end of the experiment, deeply anesthetized rats were perfused transcardially with PBS followed by 10% paraformaldehyde in PBS. Brains of all rats were removed and hypothalamic sections (50 µm) were prepared for histological localization of the cannula and injection site by means of a cresyl violet stain. Data from rats with injection sites further than 1.0 mm from the cannula and injection site by means of a cresyl violet stain. Data from rats with injection sites further than 1.0 mm from the cannula and injection site by means of a cresyl violet stain. Of the 23 rats (n = 2, experiments 3 and 4; n = 3, experiment 5). Data analysis. Macronutrient intakes were converted to kilocalories according to the energy density of the macronutrient diets (see Table 1). In experiment 1, treatment effects were evaluated by macronutrient diet by means of one-way ANOVA; planned comparisons between treatment groups were adjusted by Bonferroni test. In experiments 2–6, macronutrient intakes were analyzed with multivariate, repeated-measures ANOVA with respect to treatment, diets, and drug doses. The SAS System version 6.12 was used for analyses. Data are presented as means ± SE. The Pearson r was used to calculate correlations between baseline proportional fat intake (% kcal measured over 3 days) and percent change from saline in kilocalories (carbohydrate, fat, protein, total) after PVN infusion of 5-HT.

RESULTS

Experiment 1: effect of intracerebroventricular administration of the 5-HT antagonist metergoline on systemic dF anorexia. There were no differences between treatment groups in mean body weight [F(3,18) = 0.76, NS] or in 3-day mean fat intake [F(3,18) = 1.45, NS] measured before the day of the experiment (data not shown). As shown in Fig. 1, there were significant effects of treatment group on fat [F(3,18) = 14.35, P < 0.0001], protein [F(3,18) = 3.67, P < 0.05], and total [F(3,18) = 18.65, P < 0.0001] caloric intake. There was no effect of treatment group on carbohydrate consumption [F(3,18) = 1.42, NS]. When treatment effects were evaluated by macronutrient diet, the same results were observed when the data were analyzed as calories or gram weight of food consumed (Fig. 1; Table 2). Specifically, dF significantly reduced fat intake by ∼90% (P < 0.05) when measured 4 h after dark onset. Smaller reductions in carbohydrate (P = 0.25) and protein (P = 0.54) consumption were not significantly different.

Thus dF reduced both total gram (P < 0.05) and total caloric (P < 0.005) intake by >70% after intraperitoneal injection. The nonspecific 5-HT antagonist metergoline administered into the third ventricle attenuated the decrease in fat intake as well as the decrease in total intake caused by systemic dF (see Fig. 1). Metergoline alone, when administered into the third ventricle, significantly increased the fat intake of nondeprived
Table 2. Effect of intracerebroventricular injection of metergoline to block hypophagic effects of intraperitoneal DNF on mean 4-h dietary intakes

<table>
<thead>
<tr>
<th></th>
<th>Saline + Vehicle</th>
<th>Dexfenfluramine + Vehicle</th>
<th>Saline + Metergoline</th>
<th>Dexfenfluramine + Metergoline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate kcal</td>
<td>13.5 ± 4.3</td>
<td>4.8 ± 2.0</td>
<td>11.3 ± 3.7</td>
<td>6.8 ± 2.6</td>
</tr>
<tr>
<td>g</td>
<td>3.9 ± 1.2</td>
<td>1.4 ± 0.6</td>
<td>3.2 ± 1.0</td>
<td>1.9 ± 0.8</td>
</tr>
<tr>
<td>Fat kcal</td>
<td>14.0 ± 2.7</td>
<td>1.5 ± 1.3*</td>
<td>31.3 ± 4.3*</td>
<td>10.8 ± 3.4</td>
</tr>
<tr>
<td>g</td>
<td>2.1 ± 0.4</td>
<td>0.2 ± 0.2*</td>
<td>4.6 ± 0.6*</td>
<td>1.6 ± 0.5</td>
</tr>
<tr>
<td>Protein kcal</td>
<td>1.1 ± 0.4</td>
<td>0.0 ± 0.0</td>
<td>2.5 ± 1.0</td>
<td>0.4 ± 0.2</td>
</tr>
<tr>
<td>g</td>
<td>0.3 ± 0.1</td>
<td>0.0 ± 0.0</td>
<td>0.7 ± 0.3</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
<td>Total kcal</td>
<td>28.6 ± 5.2</td>
<td>6.3 ± 1.6*</td>
<td>45.0 ± 3.8*</td>
<td>18.0 ± 2.3</td>
</tr>
<tr>
<td>g</td>
<td>6.2 ± 1.4</td>
<td>1.6 ± 0.5*</td>
<td>8.5 ± 0.8</td>
<td>3.7 ± 0.5</td>
</tr>
</tbody>
</table>

Values are means ± SE from self-selected intake in 3-choice, macronutrient diet protocol by nondeprived rats during early dark (4 h) feeding period. *Significantly different from saline + vehicle (P < 0.05, Bonferroni). DNF, Dexfenfluramine.

rats by 55% compared with the control group (P < 0.005) but did not significantly enhance either carbohydrate or protein. The stimulation of food intake by metergoline resulted in a 36% increase in total caloric intake (P < 0.05). Expressed as gram weight of food consumed, the increase in total intake (27%) was not reliably different (Table 2), because it did not reflect the higher energy density of the enhanced dietary fat intake.

Although drug-vehicle comparisons were made within the context of the vehicle condition in experiment 1, there may have been undue effects by the acidic vehicle alone or by its interaction with metergoline. Thus the central saline-infusion intakes of rats in experiment 1 were compared with the central vehicle-infusion intakes of those in experiments 2 and 3 combined, at the same time point (4 h). The results showed that there were no differences between the saline-vehicle and saline rats, respectively, for carbohydrate (13.5 ± 4.3 vs. 15.2 ± 2.1 kcal, P = 0.69), fat (14.0 ± 2.7 vs. 12.5 ± 3.4 kcal, P = 0.78), protein (1.1 ± 0.4 vs. 1.6 ± 0.4 kcal, P = 0.42), or total (28.7 ± 5.2 vs. 29.3 ± 3.8, P = 0.93) intake.

Experiments 2 and 3: effect of intracerebroventricular infusion of the serotonin agonists quipazine or DNF on macronutrient selection. Quipazine infused into the third ventricle of freely feeding rats significantly reduced total food intake [F(1,11) = 10.51, P < 0.01; Fig. 2]. There was a main effect of quipazine treatment [F(1,55) = 9.05, P < 0.005] on macronutrient intake, whereas the interaction of drug by diet bordered on significance [F(2,55) = 3.16, P = 0.05]. Quipazine reliably inhibited fat [F(1,55) = 6.36, P < 0.05] and protein intake [F(1,55) = 17.77, P < 0.0001], but carbohydrate intake was not different from saline control (P = 0.65). In a separate experiment, DNF infused into the third ventricle reduced total food intake [F(1,25) = 24.75, P < 0.0001; Fig. 3]. There was a significant interaction of drug and diet on macronutrient consumption [F(2, 25) = 5.86, P < 0.01] as a result of the exclusive inhibition of fat intake by DNF [F(1,25) = 26.29, P < 0.001].

Experiments 4 and 5: effect of PVN infusion of DNF or 5-HT on macronutrient selection. DNF infused into the PVN region significantly reduced total caloric intake [F(1,30) = 38.41, P < 0.0001; Fig. 4]. There were significant drug-by-macronutrient diet interactions affecting macronutrient intake [F(2,50) = 18.41, P < 0.0001]. Specifically, fat and protein consumption were significantly reduced by 76% [F(1,50) = 23.10, P < 0.0001] and 84% [F(1,50) = 8.29, P < 0.01], respectively (Fig. 4), but the decrease in carbohydrate intake was marginal [23%; F(1,50) = 3.15, P = 0.08].

A 50-µg dose of 5-HT infused into the PVN region significantly reduced total caloric intake [F(1,60) = 25.18, P < 0.0001; Fig. 5]. ANOVA revealed an interaction of drug by diet affecting macronutrient intake [F(2,60) = 15.05, P < 0.0001]. Specifically, fat and protein consumption were significantly decreased by 69% [F(1,60) = 14.36, P < 0.001] and 68% [F(1,60) = 4.03, P < 0.05], respectively, but the reduction in carbohydrate intake (30%) did not reach statistical significance [F(1,60) = 3.00, P = 0.09; Fig. 5].

Experiment 6: effects of PVN infusion of 5-HT, with respect to dose, on macronutrient selection in carbohydrate and fat-prefering rats. In rats with a strong carbohydrate preference, 5-HT resulted in a weak, dose-related suppression of food intake [F(4,140) = 2.53, P < 0.05] as shown in Fig. 6A, as well as a dose-by-diet interaction affecting macronutrient intake [F(8,140) = 3.55, P < 0.001]. Two doses of 5-HT, 30

Fig. 2. Effects of 3V injection of quipazine (150 µg or 330 nmol; n = 12) on mean (±SE) carbohydrate (carb), fat, protein, and total intake at 2 h after dark onset. Results are expressed as absolute kilocalories (A) and grams (B). *P < 0.05 compared with saline.
nmol (P < 0.05) and 300 nmol (P < 0.005), led to significant reductions in fat intake (63 and 83%, respectively) relative to saline intake at 60 min [F(4,40) = 5.45, P < 0.005]. The unreliable effect of a single dose (3 nmol; P = 0.05) contributed to a dose-related suppression of protein intake [F(4,40) = 3.32, P < 0.05; Fig. 6A]. Neither carbohydrate consumption [F(4,40) = 1.67, P = 0.18] nor total caloric intake [F(4,40) = 1.39, P = 0.26] varied significantly as a function of 5-HT dose administration (Fig. 6A).

In fat-preferring rats (Fig. 6B), PVN infusion of 5-HT led to a dose-related decrease in food intake [F(4,40) = 20.81, P < 0.0001]. A dose-by-diet interaction [F(8,140) = 8.52, P < 0.0001] indicated a differential effect of 5-HT depending on diet. In particular, ANOVA by diet showed that fat intake was suppressed by all doses of 5-HT [F(4,40) = 16.03, P < 0.0001] ranging from 40% (P < 0.05) at the 0.3 nmol dose to 89% (P < 0.00001) at the 300 nmol dose (Fig. 6B). However, only the 300 nmol dose of 5-HT resulted in a significant decrease in both carbohydrate (70%; P < 0.0001) and protein (92%; P < 0.005) intake compared with saline, despite evidence for weak dose effects of 5-HT on these macronutrient diets [carbohydrate, F(4,40) = 6.09, P < 0.001; protein, F(4,40) = 3.45, P < 0.05]. In contrast to carbohydrate-preferring rats, total caloric intake in fat-preferring rats was decreased in a dose-related manner [F(4,40) = 18.32, P < 0.0001] with 5-HT administration. An examination of data during the time interval from 1 to 2 h after dark onset revealed that no differences in macronutrient diet or total intake were observed in either preference group as a function of 5-HT (data not shown).

The percent suppression of both carbohydrate (P < 0.05) and total caloric (P < 0.05) intake by the highest dose of 5-HT (300 nmol) was positively correlated with baseline proportional fat intake as measured before the experiment began (Fig. 7, A and D). However, there were no significant relationships between baseline fat intake and percent inhibition of fat or protein consumption after 5-HT injection (Fig. 7, B and C).

**DISCUSSION**

In a series of experiments employing a macronutrient diet selection paradigm, we provide support for hypotheses that the selective suppression of fat intake observed previously with systemic dF involves central 5-HT receptors and that activation of 5-HT1B/2C receptors by centrally administered serotonin receptor agonists leads to a selective reduction in fat intake. An examination of the response to PVN 5-HT administration in rats with low or high baseline fat intakes revealed a dose-related suppression of fat consumption in both preference groups. A significant inhibition of carbohydrate or protein intake was found only in fat-preferring rats after receiving the highest dose of 5-HT. Furthermore, our results indicate an association between baseline fat intake and percent inhibition of carbohydrate consumption at the highest dose of 5-HT.
In previous work the authors (43) and others (2, 14, 16) using the macronutrient selection paradigm have demonstrated a reduction in both fat and protein intake after systemic injection of serotonin or its agonists. In addition, attenuation of fenfluramine anorexia by the nonselective 5-HT receptor antagonist metergoline has been shown (11, 29). The results of experiment 1 extend those findings in preselected, fat-preferring rats by showing that the suppression of fat intake by systemic dF was antagonized by intraventricular metergoline, a nonspecific 5-HT antagonist, thus supporting a central mechanism of action on fat appetite by this anorectic drug. Moreover, rats that received central metergoline alone responded with a striking increase in fat intake compared with vehicle, indicating that metergoline may have inhibited some tonic serotonergic activity in the brain that was effectively restraining fat intake. This observation is consistent with evidence that both systemic and central nervous system injections of metergoline can elicit feeding in satiated rats (5, 6) but stands in contrast to reports of a selective, stimulatory effect of metergoline on carbohydrate consumption (23, 44). Finally, it is possible that the effects of fenfluramine and metergoline administered in tandem were simply additive because the dose of metergoline used in this experiment induced a significant increase in fat intake when given alone. This possibility could be tested by determining whether there is an intracerebroventricular dose of metergoline that attenuates fenfluramine anorexia without stimulating food intake.

The 5-HT agonist quipazine has been shown previously to induce hypophagia when administered via central (38) or systemic (41) routes. In experiment 2 of the present study, employing a macronutrient diet paradigm, the decrease in food intake after quipazine infusion was limited specifically to fat consumption. It is not clear which specific receptor subtype might be responsible for this reduction in fat intake, because quipazine has equal potency at 5-HT1B and 5-HT2C receptors (40). It is possible that this compound may induce some of its anorectic effect through dopamine, as evidenced by the demonstration that quipazine-induced anorexia was significantly reduced by the dopamine receptor antagonist pimozide (42). Although the anorectic effects of quipazine may occur in part through a dopaminergic pathway (13), the effect on macronutrient selection of centrally administered dopamine has not been investigated.

The results from experiments 3 and 4 indicate that the site of application may be important in determining the effects of centrally administered dNF on macronutrient selection. Although fat intake was strongly suppressed by both third ventricular (3V) and PVN infusion of 5-HT into the PVN of rats, the decrease in food intake after quipazine infusion was limited specifically to fat consumption. It is not clear which specific receptor subtype might be responsible for this reduction in fat intake, because quipazine has equal potency at 5-HT1B and 5-HT2C receptors (40). It is possible that this compound may induce some of its anorectic effect through dopamine, as evidenced by the demonstration that quipazine-induced anorexia was significantly reduced by the dopamine receptor antagonist pimozide (42). Although the anorectic effects of quipazine may occur in part through a dopaminergic pathway (13), the effect on macronutrient selection of centrally administered dopamine has not been investigated.

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sions, protein intake was suppressed only when dNF was administered onto the PVN. There was clearly no effect of the drug on carbohydrate consumption at either injection site. The PVN infusion may have produced a different pattern of receptor activation than the 3V route of administration because the flow of cerebrospinal fluid is rostral to caudal; i.e., the “downstream” effects of a serotonin agonist such as DNF may have been more potent if sufficient quantity reached the brain stem. For example, the anorectic dose of a 5-HT agonist injected into the parabrachial nucleus is 1/50 of that required when the drug is infused into the PVN (21).

DNF is the active metabolite of dF; both compounds inhibit 5-HT reuptake and stimulate its release, whereas DNF also acts as an agonist on 5-HT 2C receptors (8, 9). Although earlier reports indicated that dF and DNF bind only weakly to 5-HT 1B receptors (33), the antagonism of dF hypophagia by the 5-HT 1B antagonist cyanopindolol (11, 12) and the loss of fenfluramine’s hypophagic effect in 5-HT 1B receptor knockout mice (29) indicate that the 5-HT 1B receptor is critical in fenfluramine-induced anorexia. Thus the action of DNF in the PVN to suppress food intake is thought to occur through activation of both 5-HT 1B and 5-HT 2C receptors (29), each of which may have different effects on feeding microstructure (11). The possible function of these receptor subtypes in macronutrient-specific satiety remains to be determined.

In experiment 5, the effects of a single dose of 5-HT (50 µg) injected into the PVN showed a 50% suppression of fat intake and a smaller reduction in carbohydrate intake (30%). An assessment of baseline macronutrient intakes revealed that, although the majority of the rats were consuming ~40% energy from fat, there were too few animals to examine the effects of preference on response to 5-HT.

In experiment 6, 5-HT infused into the region of the hypothalamic PVN of carbohydrate-preferring rats led overall to a dose-related reduction in fat intake, although only with the two highest doses. Neither carbohydrate nor protein intake was significantly decreased by 5-HT administration; thus total caloric intake was unchanged. In contrast, 5-HT administered to fat-preferring rats inhibited fat intake across all doses tested. Furthermore, carbohydrate intake was reliably suppressed by 5-HT in fat-preferring rats, although only at the highest dose, as was protein intake. Thus in rats with a high baseline fat intake, 300 nmol 5-HT inhibited the intake of all three macronutrient diets as well as total calories. The suppressed intake of all three macronutrient diets, observed only in fat-preferring rats, suggests an interaction of the PVN 5-HT system with baseline fat consumption.

Fig. 7. Relationship between baseline dietary fat intake (% total kcal) and intake of carbohydrate (A), fat (B), protein (C), or total calories (D) expressed as percent change from saline after infusion of 5-HT (300 nmol) into PVN region in carbohydrate (○) - and fat-preferring (●) rats. *Significant correlation (Pearson r, 2-tailed, P < 0.05; n = 22).
led to a preferential decrease in carbohydrate intake across diets; meal pattern analysis showed that this effect was limited to the first two meals and occurred during the first 1–2 h of the dark cycle (23). Our present results do not agree with these earlier data, although a meal pattern analysis was not performed. Rather, we measured food intake at 1 and 2 h after dark onset under similar experimental conditions and found that none of the 5-HT doses tested (0.3–300 nmol) were effective in suppressing carbohydrate intake, with the exception of the highest dose in fat-preferring rats only. In contrast, fat intake was inhibited by all doses of 5-HT in fat-preferring rats and by the two highest doses in carbohydrate-preferring rats.

The reason for the disparity between our results and those of earlier studies is not clear. However, the low doses of 5-HT (2.5–20 nmol) previously reported to suppress carbohydrate ingestion in the early dark photoperiod often did not change total caloric intake (23, 25, 26), whereas in some cases an increase in protein or fat intake was also shown (26). Earlier reports of a selective reduction in carbohydrate consumption with PVN application of DNF (45) also employed lower doses (3–25 nmol) than those used in the present study. These results are in contrast to the reduction in fat, not carbohydrate, intake observed in the current study after hypothalamic administration of DNF at doses of 208 (PVN) or 416 (3V) nmol. Although it has been suggested that higher doses of serotonergic agonists are less specific in their effect on macronutrient selection (25), the doses of DNF and quipazine used in the current study were found to be selective in depressing fat and protein intake but not carbohydrate. Also, these higher doses did not abolish feeding but induced moderate hypophagia by reducing total intake by ≈50%. Furthermore, the highest dose of 5-HT produced differential effects depending on baseline preference; e.g., in carbohydrate-preferring rats, 300 nmol effectively inhibited only fat consumption. Thus the current study provides evidence for diet selectivity in the satiating effects of serotonin and serotonergic agonists in a higher dose range than previously proposed. Finally, concern about possible sedative effects also has influenced the selection of doses in previous studies (25). Although in the current study brief periods of depressed activity were occasionally observed with the two highest doses, these effects were no longer apparent by 15 min after injection. Thus it appears unlikely that the observed differences in diet selection across macronutrients and preference groups could be accounted for by this transient effect of 5-HT administration.

The reports of a preferential suppression of carbohydrate consumption (and not fat) by serotonergic drugs in some studies may be explained by differences in the experimental model, e.g., the choices and composition of test diets employed (19, 24, 36). Although the only apparent difference between the macronutrient diets used in the current serotonin studies and previous ones (23, 25, 26) is the fat source (plant vs. animal origin, respectively), it remains possible that the amount of saturated fat in the diet may differentially affect feeding responses to exogenously administered serotonin or its receptor agonists. For example, the anorectic effect of the 5-HT receptor agonist fenfluramine was found to be greater in rats fed diets containing tallow compared with those fed corn oil (35), indicating a possible interaction of dietary fat source and serotonin on feeding behavior. Specifically, the presence of higher insulin levels in tallow-fed rats may allow more tryptophan to be transported into the brain, resulting in higher levels of serotonin and thus a greater response to the serotonin-releasing effects of fenfluramine (35).

Previous investigations of the role of baseline preference in the outcomes of centrally administered serotonin on macronutrient selection are limited to a single report and failed to reveal an association between saline control macronutrient intakes and the effects of 5-HT on carbohydrate or fat (26). In the present study, rats with both high and low baseline fat consumption significantly reduced their fat intake after microinjection of 5-HT. Notably, the high carbohydrate intake of carbohydrate-preferring rats was not affected by 5-HT. Thus the present results (Fig. 6) confirm our previous observation (43) that the effect of serotonin agonists to suppress fat consumption in nondeprived rats occurs independently of baseline macronutrient preferences.

In the current study there was a significant positive association between baseline proportional fat intake and the percent suppression of carbohydrate intake by the 300 nmol dose of 5-HT (Fig. 7). Thus the ability of 5-HT to induce carbohydrate satiety across all rats (regardless of preference) occurred as a function of baseline fat intake whereas the suppression of fat intake was independent of baseline (Fig. 7). Moreover, a greater responsiveness to the highest dose of 5-HT was observed in fat-preferring rats by their reduced consumption of all three macronutrient diets. This finding indicates that a higher consumption of fat may result in a greater sensitivity to the hypophagic effects of 5-HT and perhaps a lack of macronutrient-specific satiety. It suggests that dietary fat level may alter serotonergic activity. For example, prolactin response to fenfluramine challenge, used clinically as an index of serotonergic activity in the central nervous system (37), was significantly higher in monkeys fed a high-fat diet than those fed a low-fat diet (34). Similarly, the chronic ingestion of a high-fat diet is required to observe the anorectic effect of central enterostatin infusion (28), a pathway for which there is evidence of a serotonergic component (27, 49). The mechanism by which dietary fat could affect central serotonergic activity is unknown but may involve alterations in neuronal membrane composition (4).

In summary, increasing central serotonergic neurotransmission through the use of serotonin agonists resulted primarily in the suppression of fat and protein intake. Microinjection of 5-HT into the PVN led to a reduction in fat intake independent of macronutrient preference. The stimulation of 5-HT activity in the PVN resulted in an inhibition of carbohydrate intake in fat-preferring rats only. Finally, the suppressed intake
of all three macronutrient diets, observed exclusively with the highest dose of 5-HT in fat-prefering rats, suggests that a high baseline fat intake enhances responsivity to exogenous 5-HT.

Perspectives

The results from this study add to the growing number of human and animal studies demonstrating that stimulation by 5-HT agonists leads to a reduction in dietary fat intake and therefore suggest a broader role for 5-HT in appetite than the traditional concept of centrally administered serotonergic drugs.

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[Address for reprint requests and other correspondence: B. K. Smith, Pennington Biomedical Research Center, 6400 Perkins Road, Baton Rouge, LA 70808-4124 (E-mail: smithbk@mhs.psrc.edu).]

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