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Peripheral Serotonin is an Incomplete Signal for Eliciting Satiety in Sham-Feeding Rats

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SIMANSKY, K. J., J. JAKUBOW, F. C. SISK, A. H. VAIDYA AND K. EBERLE-WANG. *Peripheral serotonin is an incomplete signal for eliciting satiety in sham-feeding rats.* PHARMACOL BIOCHEM BEHAV 43(3) 847–854, 1992.—Peripheral administration of serotonin [5-hydroxytryptamine (5-HT)] to rats equipped with gastric cannulae reduced their 30-min consumption of sweetened milk after overnight deprivation whether the cannulae were closed (real feeding) or open (sham feeding). The anorectic action of 5-HT (1.6, 4.0, and 10.0 μmol/kg, IP) in sham feeding was dose-related, rapid in onset, and persisted during the 30-min testing session. However, 5-HT failed to elicit resting—the terminal behavioral phase of satiety—in sham-feeding rats. Direct comparison of the effects of 4.0 μmol/kg 5-HT under both feeding conditions established that this dose promoted resting only when rats fed with their cannulae closed. The actions of 5-HT on feeding and resting were behaviorally selective because serotonergic treatment did not retard the beginning of feeding, alter sham drinking of water, or reduce investigation by food-deprived rats of a novel object in an open field. Together, the results suggest that 5-HT exerts separate actions to inhibit feeding and accelerate the process of satiation as marked by resting. However, peripheral 5-HT is inadequate as a signal for modulating satiety in the absence of postingestive stimuli.

Serotonin 5-Hydroxytryptamine Peripheral serotonin Feeding Anorexia Satiety Sham feeding Sham drinking Exploration

ENDOCRINE and neuronal cells within the gastrointestinal system synthesize high concentrations of the biogenic amine serotonin [5-hydroxytryptamine (5-HT)] (12,22). Accordingly, a considerable amount of interest has developed for testing whether 5-HT from the gut might provide a signal for the short-term control of feeding. Using systemic administration as a probe for this function, 5-HT (9,21,29) and some of its peripherally acting analogs (13,26,27) reduced food intake by rats under a variety of testing conditions. This anorectic action occurred at doses of 5-HT that did not act as unconditional stimuli for a conditioned taste aversion or impair sensorimotor performance (9,21). Thus, 5-HT apparently reduced food intake by a selective action on feeding rather than by some nonspecific behavioral effect. Analysis of log-survivor functions in deprived rats consuming pellets suggested that 5-HT lowered intake by reducing the size and duration of bouts of feeding (10). In a runway task using food-deprived rats, 5-HT decreased running speed only after some pellets were consumed (8). The slowing of the running paralleled the decay of the cumulative food intake curve. Overall, these data implied that 5-HT decreased the consumption of food by selectively enhancing the satiety effect of ingested food or of feeding.

The influence of 5-HT on the process(es) leading to satiation can be examined more precisely during sham feeding using rats equipped with gastric cannulae. In a previous study (18), 5-HT reduced gastric sham feeding of a sucrose solution without altering sham drinking. This effect on the consumption of liquid diet was consistent with the idea that peripherally administered 5-HT interacted with oropharyngeal stimuli associated with food or feeding to enhance satiety. Additional information about the role of 5-HT in terminating a meal would be revealed, however, by analyzing the nature of the behavioral changes associated with the anorectic action of 5-HT. If exogenous 5-HT probes a function for this indoleamine in satiety, then peripheral serotonergic stimulation should not only reduce food intake but also accelerate the sequence of nonfeeding activities and resting typical of satiation (1). In a recent study using observational analysis (7), rats given 5-HT subcutaneously did stop feeding sooner and displayed behavioral evidence of satiety more quickly than controls. It remains to be determined, however, whether 5-HT would exert a similar action in sham-feeding rats. Comparing the efficacy of 5-HT to produce behavioral satiety during sham and normal feeding would assess the importance of gastrointestinal stimuli for this serotonergic effect.

The present investigation, therefore, determined the ac-

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tions of systemically administered 5-HT on cumulative milk intake and periprandial behaviors during gastric sham feeding and when rats were feeding with the fistulae closed (i.e., "real feeding"). The specificity of the 5-HT-induced anorexia was reassessed in experiments testing serotonergic actions in sham drinking of water and in food-deprived rats that were exploring an open field. We report that peripherally administered 5-HT inhibited both real and sham feeding in a behaviorally specific manner but augmented satiety only when rats fed with their cannulae closed.

EXPERIMENT 1: ANORECTIC ACTION OF 5-HT IN SHAM FEEDING

Previous work (18) demonstrated that intraperitoneal injection of 2 mg/kg 5-HT (approximately 5 μmol/kg) reduced 2-h sham intake of a sucrose solution by 40% in rats that were mildly food deprived. The present experiments determined dose-response relationships for the inhibition of real and sham feeding by this indoleamine under our feeding conditions. The tests used a sweetened milk diet and a 30-min measurement period after overnight food deprivation as described in prior studies from this laboratory that analyzed the effects of serotonergic drugs on normal feeding (27,28).

METHOD

Subjects and Diet

Subjects were male Sprague-Dawley rats from AAI, Inc. (Boyertown, PA) that were housed individually in suspended polycarbonate cages with stainless steel mesh floors. The animal colony was an AAALAC-approved vivarium maintained at an ambient temperature of 22 ± 1°C with a 12-h light photoperiod beginning at 0600 h. Rats were given access to food for only 7 h each day (1000–1700 h) with water available continuously. The food was a liquid diet consisting of a 50% dilution of Carnation Sweetened Condensed Milk that contained 0.03% (w/v) formaldehyde as a preservative. The caloric density of this formula was 2.1 kcal/ml with approximately 69% of the calories derived from carbohydrates, 9% from proteins, and 22% from fats. The milk was provided in 100-ml graduated glass bottles fitted with rubber stoppers and stainless steel spouts (Ancare Inc., Manhasset, NY).

Action of 5-HT in Real Feeding

Twenty-nine rats weighing 345–485 g at the time of testing were adapted to the feeding schedule for 3 weeks. They were then assigned randomly to one of four groups to receive IP injections of either vehicle, 2.5, 5.0, or 10.0 μmol/kg 5-HT (5-HT creatinine sulfate, Sigma Chemical Co., St. Louis, MO, MW = 387). Rats were injected 6 min before milk was placed on the cages after the overnight fast and the volume consumed during the first 30-min access to diet was recorded. 5-HT was prepared in nitrogenated distilled-deionized water just before use. All injections were in a volume of 2 ml/kg.

Gastric Cannulation

Twelve rats weighing 287–367 g at the time of surgery were prepared with gastric fistulae for sham feeding. A stainless steel cannula (15.0 mm long, 6.0 mm., i.d., 7.8 mm o.d. with a circular flange at the base that was 15.8 mm in diameter) was implanted in the rumen along the greater curvature of the stomach as described previously (1). Rats were anesthetized with an IP injection of an anesthetic combination containing 32 mg/kg sodium pentobarbital (Sigma) and 140 mg/kg chloral hydrate (Sigma) in a formulation known as Equithesin (3.3 ml/kg). After laparotomy, a small perforation was made in the rumen with iris scissors and the flange of the cannula was inserted via the hole into the stomach. A doughnut-shaped piece of Marlex polypropylene mesh (C. R. Bard, Inc., Billerica, MA) was passed over the shaft of the cannula, pressed against the serosal surface of the stomach, and sutured in place with two loops of 4-0 silk. A small stab wound was made in the abdominal wall and the shaft of the cannula was then exteriorized. The cannula was kept in place with a stainless steel washer that fit around the cannula shaft to anchor it against the external wall of the abdomen. The cannula was closed with a stainless steel screw cap and the midline incision was closed with interrupted 3-0 silk sutures for the muscle and wound clips for the skin. The area around the cannula was cleaned daily and the washer was removed 48–72 h after surgery. Rats were returned to the feeding regimen with 7-h access to milk immediately after surgery.

Action of 5-HT in Sham Feeding

Daily sham feeding began 2 weeks postoperatively. Thirty minutes before milk was provided, the screw caps were removed from the cannulae and the stomachs were flushed with warm 0.9% (w/v) NaCl until the effluent was clear. Rats were then placed in their cages and allowed to sham feed for 30 min. The screw caps were then replaced and rats given access to milk for the ensuing 6.5 h.

After 3 weeks of training, we tested the effects of 4.0 μmol/kg 5-HT on sham feeding. Animals were assigned to two groups (n = 6/group) and tested on 2 successive days in a counterbalanced design, thus allowing each rat to serve as its own control. Milk intakes were recorded every 5 min during the 30-min session. Unobstructed sham feeding was verified by determining that the volume of effluent collected equaled or exceeded the amount consumed.

The dose-response relationship for the anorectic action of 5-HT in sham feeding was determined 3 weeks later (59 days postoperatively). One rat was excluded from the study because its cannula became loose. The 11 remaining rats were administered 0, 1.6, 4.0, and 10.0 μmol/kg 5-HT in random order with 1 day intervening between tests. As previously, milk intakes were recorded every 5 min.

Statistical Analysis

Data were analyzed by the appropriate multifactorial analysis of variance (ANOVA) (14). A posteriori comparisons were made by simple F-tests followed by the Newman-Keuls multiple range test or by orthogonal t-tests, as appropriate. The doses that reduced food intake by 50% (ID_{50}s) were determined by linear regression of the log dose-response functions using the method of least squares (30). A probability level of $\alpha = 0.05$ for Type I errors was used as the threshold for statistical significance.

RESULTS

Intraperitoneal injection of 5-HT reduced 30-min milk consumption by intact rats in a dose-related manner, $F(3, 25) = 15.73$, $p < 0.01$, with 2.5, 5.0, and 10.0 μmol/kg decreasing intakes to 11.1 ± 2.6 ml ($p < 0.05$), 7.6 ± 1.5 ml ($p < 0.01$), and 1.1 ± 0.4 ml ($p < 0.01$), respectively, compared to the mean for controls (17.2 ± 1.5 ml). The estimated ID_{50} was 3.8 μmol/kg (approximately 1.5 mg/kg of the salt).

TABLE 1

EFFECT OF 5-HT ON MILK CONSUMPTION WITHIN SUCCESSIVE 5-min INTERVALS OF SHAM FEEDING

Treatment	Measurement Interval (min)					
	0-5	5-10	10-15	15-20	20-25	25-30
Vehicle	7.8 ± 0.8	9.1 ± 0.5	8.7 ± 0.6	9.2 ± 0.6	8.8 ± 0.6	9.2 ± 0.5
5-HT	4.2 ± 0.7*	3.8 ± 0.7*	3.0 ± 0.6*	4.5 ± 1.1*	4.6 ± 0.8*	6.1 ± 0.9†

All values represent mean milk intakes ± SE (in ml) for 12 rats tested under each condition in a counterbalanced design. The dose of 5-HT was 4.0 μmol/kg IP.

*†Each mean after 5-HT was smaller than the corresponding mean after vehicle: *$p < 0.01$, except the last time interval, †$p = 0.02$. All comparisons were made by orthogonal t-tests (two tailed) within the time intervals after overall ANOVA.

Based upon these results in normally feeding intact rats, we tested the effects of 4.0 μmol/kg 5-HT in sham feeding. At this dose, 5-HT reduced overall 30 min intake by 50% (26.1 ± 3.6 vs. 52.8 ± 2.8 ml after vehicle injection, $p < 0.01$). Table 1 presents the intakes within each 5-min period of the test. The data were combined over the 2 days of the counterbalanced experiment because the order of administering 5-HT and vehicle did not affect the outcome ($p > 0.10$ for order effect and for all interactions with order). 5-HT reduced milk consumption across all time intervals, $F(1, 11) = 25.28$, $p < 0.01$. Although the magnitude of this anoretic action appeared to vary during the test, the interaction of 5-HT treatment with time did not reach significance, $F(5, 55) = 2.04$, $p = 0.09$.

Given these data, we constructed a dose-response function by determining the effects of 1.6, 4.0, and 10.0 μmol/kg 5-HT on sham feeding. As in real feeding, 5-HT reduced sham feeding in a dose-related manner, $F(3, 30) = 19.1$, $p < 0.01$ (Fig. 1). Total milk consumption increased with time ($p < 0.01$) but 5-HT systematically decreased the slopes of the cumulative intake functions (5-HT × time interaction, $p < 0.01$). Overall, the three doses of 5-HT diminished the amount of milk rats ingested by 12 (n.s.), 42 ($p < 0.01$), and 80% ($p < 0.01$) from the baseline of 52.6 ± 3.5 ml after injection of vehicle (estimated ID_{50} = 4.6 μmol/kg). Despite the orderly anorectic effect of 5-HT in sham feeding, however, superficial observation of the rats during these tests suggested that they never satiated because they were never observed resting.

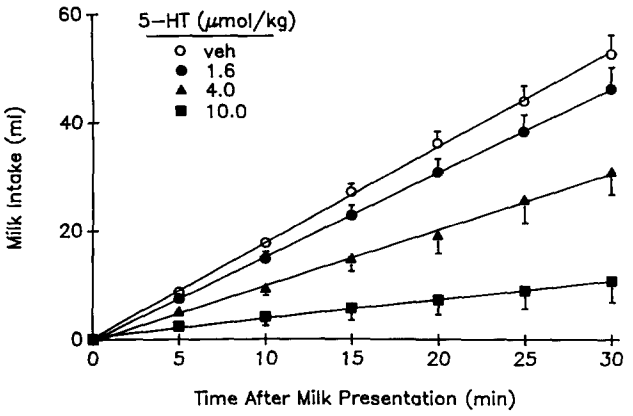

FIG. 1. Time-effect and dose-effect functions for the effect of peripherally administered 5-HT on sham feeding in rats. The data represent the cumulative amounts of sweetened milk consumed at successive 5-min intervals (means ± SE) by 11 rats, all of which were tested under each condition.

DISCUSSION

These results confirmed the previous report that peripherally administered 5-HT reduced sham feeding of a sweetened liquid diet by rats (18). In the earlier study, rats were maintained on pelleted chow but sham fed a single carbohydrate solution after a brief deprivation period. In contrast with this dessert test, rats in the present experiments were tested after a longer period of deprivation with a more complex diet that was their only food throughout the day. Clearly, these differences proved unimportant because, using similar doses of 5-HT, even the time courses of intakes from the two studies were virtually identical.

The anorectic action of 5-HT in sham feeding persisted for the duration of the 30-min test. Nonetheless, using a counterbalanced design demonstrated that rats recovered their normal baseline sham feeding intakes on the day following injection with 5-HT. The lack of a carryover effect suggested that 5-HT did not inhibit sham feeding by producing overt toxicity. Furthermore, rats given 5-HT continued to sham feed throughout the testing session although seemingly at a lower rate. This inhibitory effect on consumption was dose related, but despite a marked reduction in intake by a rather large dose of 5-HT rats were not observed resting.

EXPERIMENT 2: BEHAVIORAL COMPARISON OF 5-HT IN REAL AND SHAM FEEDING

If 5-HT normally provides a peripheral signal for satiation, then it might be expected to promote behaviors during feeding that are characteristic of postprandial satiety. As just noted, 5-HT did not appear to elicit resting, which is the terminal behavioral phase of satiety, in sham-feeding rats. In this experiment, a method based upon that of Antin et al. (1) was used for documenting more precisely whether and how 5-HT influenced periprandial behavior. Using this method, 5-HT reportedly enhanced the appearance of the complete behavioral sequence of satiety in rats that were eating solid food after 20 h of food deprivation (7). Accordingly, we compared directly the effects of 5-HT during real and sham feeding in the same rats.

METHOD

Two weeks after the dose-response study in Experiment 1, we compared the effects of 4.0 μmol/kg 5-HT on the feeding

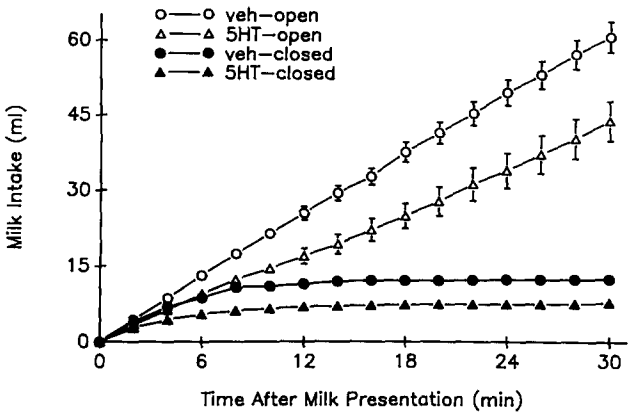

FIG. 2. Time courses for the effects of intraperitoneal injections of 4.0 μmol/kg 5-HT or its vehicle on the intakes of sweetened milk during sham feeding (cannulae open) and real feeding (cannulae closed). The data represent cumulative intakes (means ± SE) at successive 2-min intervals for 11 rats, all of which were tested under each condition.

of rats with the cannulae opened (sham feeding) and closed (real feeding). The 11 rats were tested under each of the four conditions (vehicle–open, 5-HT–open, vehicle–closed, 5-HT–closed). During these experiments, milk intakes were recorded every 2 min. In addition, an observer who was unaware of the drug treatments of the individual rats recorded whether each rat fed, drank water, engaged in nonfeeding activities (e.g., locomotion, grooming, sniffing), or rested. Within each of the 1-min intervals of the test, the occurrence rather than the duration of these behaviors was noted for each rat (after [1]). The data were analyzed as in Experiment 1.

RESULTS

Figure 2 shows the cumulative intake functions comparing the effects of 5-HT on sham and real feeding. An overall ANOVA revealed that 5-HT reduced feeding, $F(1, 10) = 58.40$, $p < 0.01$. Not surprisingly, the cumulative intakes increased with time, were lower for real feeding than for sham feeding, and all interactions were significant (all p values < 0.01). Inspection of Fig. 2 suggests that these statistical interactions occurred because real-feeding but not sham-feeding rats satiated during the 30 min test, and 5-HT appeared to accelerate this satiety.

Observational analysis clearly dissociated the effects of 5-HT on periprandial behavior between the two feeding conditions. In either condition, rats began feeding immediately when the milk was placed on the cage. With the cannulae closed, rats engaged in a sustained bout of feeding that was gradually replaced by other nonfeeding activities (i.e., locomotion, sniffing, rearing, licking at the cage, grooming) and then eventually by resting. We defined the terminal phase of satiety as the occurrence of resting within three successive observation intervals (28). Using this criterion, rats injected with vehicle took an average of 18.3 ± 1.3 min before satiating completely. 5-HT shortened the time until resting to 12.1 ± 0.9 min ($p < 0.01$). Most notably, when the cannulae were closed rats were seen resting during 11.8 ± 1.1 of the 30 observation intervals after injection of vehicle and 5-HT increased this to 17.5 ± 0.7 intervals ($p < 0.01$). By contrast, no rat was seen resting after vehicle treatment during sham feeding, and one rat was observed resting only once after 5-HT in this condition.

Table 2 summarizes the effects of 5-HT on the major categories of behavior displayed by rats with closed cannulae up until the beginning of the terminal resting phase of satiety. 5-HT reduced the number of intervals in which rats fed before resting but the indoleamine did not appear to delay the onset of feeding. As feeding waned, 5-HT accelerated the emergence of exploratory behaviors without changing their apparent incidence. Conversely, the indole did not alter the latency until grooming started, but rats injected with 5-HT displayed less grooming before they began resting. Overall, therefore, 5-HT generally enhanced the progression of behavioral events involved in satiation and specifically promoted the earlier onset of resting in rats that fed with their cannulae closed.

As noted above, sham-feeding rats did not rest whether treated with 5-HT or its vehicle. After 5-HT, however, 6 of the 11 rats did not sham feed during at least one observational interval. (One rat stopped sham feeding for one interval after injection of vehicle.) The pause in sham feeding after 5-HT occurred primarily between 11 and 13 min into the 30-min session. During this pause, rats engaged in various exploratory activities and occasionally stood in place but displayed little grooming. They then resumed feeding continuously until the end of the test.

The completion of the satiety process by 5-HT-treated rats approximately 12 min after real feeding began, and the pause in sham feeding at the same time, prompted us to analyze the milk intakes for the first 12 min and remaining 18 min of the test (Table 3). 5-HT decreased real feeding in the first 12 min. Rats essentially ceased feeding after 12 min. Thus, it was not possible to detect an anorectic effect of the indole during the second measurement period. 5-HT also reduced initial sham feeding, and this anorexia persisted for the last 18 min of the test. Notably, although serotonergic treatment lowered sham intakes compared to the respective baselines after vehicle, rats injected with 5-HT always consumed more with their cannulae opened than they did after either vehicle or 5-HT with their cannulae closed.

DISCUSSION

These results demonstrated that peripherally administered 5-HT can reduce the consumption of milk by sham-feeding rats without eliciting complete satiation as marked by the onset of resting. By comparison, 5-HT did seem to enhance satiation and especially promoted resting in the same rats when they fed with their cannulae closed. Although serotonergic treatment failed to elicit resting when the cannulae were open, rats administered 5-HT did pause during sham feeding and briefly displayed behaviors associated with satiation before they resumed feeding. 5-HT reduced the sham consumption of milk even after this transient break in feeding. Therefore, either a direct action of 5-HT or an inhibitory signal from the physiological cascade initiated by 5-HT persisted throughout the 30-min testing period.

Activating peripheral serotonergic receptors increases consumption of water besides inhibiting feeding in rats (9). Thus, it might be questioned whether sham-feeding rats ingested less milk and failed to rest because they were stimulated to drink. However, rats were not observed sipping at the water tubes during either the sham-feeding or real-feeding tests. Furthermore, we have determined that doses larger than those used in the present experiments are required for dipsogenesis when 5-HT is given intraperitoneally rather than subcutaneously

TABLE 2

PERIPRANDIAL BEHAVIORS DISPLAYED BY REAL-FEEDING RATS BEFORE THE ONSET OF RESTING

	Feeding*		Exploration†		Grooming	
Treatment	Initial	Total	Initial	Total	Initial	Total
Vehicle	1.0 ± 0.0	8.7 ± 0.9	7.5 ± 0.9	5.4 ± 0.6	6.8 ± 0.8	7.3 ± 1.4
5-HT	1.1 ± 0.1	6.5 ± 0.6‡	3.2 ± 0.3§	5.7 ± 0.9	7.4 ± 1.1	2.5 ± 0.8§

The onset of resting was defined as the first of three successive intervals in which resting was observed. This was the 19th interval (18.3 ± 1.3) after vehicle injection and the 13th after 5-HT (12.1 ± 0.9). *Initial* refers to the first of the 1-min observation intervals in which the specified behavior was recorded before the rat began resting. *Total* refers to the total number of intervals in which the behavior was observed before the rat began resting. These behaviors did not necessarily occur in successive intervals. All values represent means ± SE for all 11 rats that were tested after each treatment (vehicle and 4.0 μmol/kg 5-HT).

*All rats were observed feeding during the first interval except one rat given 5-HT (second interval).

†Exploration indicates the occurrence of locomotion, rearing, sniffing, and/or licking at the cage surface.

‡§Differs from mean for vehicle: ‡$p < 0.05$, §$p < 0.01$, Student's t-test, two tailed.

(unpublished data). Unlike the present experiments, previous studies of peripheral 5-HT and drinking used the latter route of administration (9). Overall, we consider it unlikely that sham-feeding rats failed to rest and satiate completely because they were motivated to drink.

The nature of the inhibition of feeding by 5-HT requires further definition. Although anorectic when compared to their sham feeding baselines, rats injected with 5-HT still consumed large amounts of milk during the test. These sham-feeding intakes after 5-HT actually exceeded the intakes measured after vehicle when the cannulae were closed. Thus, the anorectic effect of 5-HT in sham feeding was probably a selective action on ingestion rather than some nonspecific debilitation of motor function. If so, then 5-HT presumably interacts with gastrointestinal stimuli that are missing during sham feeding to stimulate the process of satiation because serotonergic treatment did promote resting after real feeding. The following two experiments address further the behavioral specificity of 5-HT in decreasing ingestion.

EXPERIMENT 3: EFFECT OF 5-HT ON SHAM DRINKING OF WATER

In a previous study, a dose of 5-HT that inhibited sham feeding of sucrose did not reduce sham drinking of water (18). These results argued for a specific anorectic effect of 5-HT rather than a nonspecific action of the indole to disrupt oral motor function. This experiment assessed the effects of 5-HT on sham drinking in our rats.

METHOD

Six of the rats that had been tested in the previous sham-feeding experiments were placed on a maintenance schedule with water available for only 6 h each day (1100–1700 h) but with unlimited access to dry pelleted chow (Prolab, Agway, Inc., Syracuse, NY) except during the drinking test. At 1000 h each day, the cannulae were opened and the stomachs flushed with warm saline. The stomachs were flushed again 30 min later to ensure their complete evacuation, and water was pro-

TABLE 3

ANORECTIC ACTION OF 5-HT IN REAL AND SHAM FEEDING

		Milk Intake (ml)	
Feeding Condition	Treatment	0–12 min	12–30 min
Real feeding	Vehicle	11.5 ± 0.8	1.0 ± 0.5
	5-HT	6.9 ± 0.8*	0.9 ± 0.4
Sham feeding	Vehicle	26.1 ± 1.4†	34.9 ± 1.8†
	5-HT	17.0 ± 1.6*†	26.9 ± 2.9*†

All values represent means ± SE for the same 11 rats tested with vehicle and with 5-HT (4.0 μmol/kg, IP) under each feeding condition. Intakes were calculated from the cumulative data for the first 12 and last 18 min of the feeding tests (see Fig. 1). All comparisons were made by Newman-Keuls test after ANOVA on data within the time interval.

*Differs from respective baseline mean after vehicle treatment ($p < 0.01$).

†Differs from means for real feeding in the same measurement interval after injection of vehicle or 5-HT ($p < 0.01$).

vided at 1100 h for the 30-min sham-drinking test. Testing began after 5 days on this schedule. On the first day of testing, rats were injected IP with vehicle 6 min before water was presented, and water intakes were measured every 2 min thereafter for 30 min. The following day, the procedure was repeated except 4.0 μmol/kg 5-HT was administered. On each day, rats were observed for their behavioral responses during the test as in the sham-feeding experiments.

RESULTS AND DISCUSSION

Whether treated with 5-HT or its vehicle, rats began sham drinking immediately upon presentation of water and displayed a common behavioral pattern without resting. Figure 3 shows that 5-HT had no effect on either the total amount of water consumed or on the time course for sham drinking. The six rats drank 52.8 ± 4.7 ml during the entire test period after injection with vehicle and 52.3 ± 2.6 ml after 5-HT. The 30-min baseline intake for sham drinking was comparable to the average milk intakes during the sham feeding after injection of vehicle in Experiments 1 and 2. Thus, these results confirm the previously published demonstration that 5-HT selectively inhibited sham feeding of a liquid diet after food deprivation but not sham drinking of water after water deprivation (18).

EXPERIMENT 4: EFFECT OF 5-HT ON EXPLORATION IN FOOD-DEPRIVED RATS

Together, the results of the first three experiments suggested that peripherally administered 5-HT reduced food intake by a selective behavioral action rather than by interfering generally with motor responses. This effect on feeding may have derived from the ability of serotonergic stimulation to augment some aspects of satiation. The influence of this indole on satiety may be related only to feeding because 4.0 μmol/kg 5-HT reduced sham ingestion of liquid diet but not of water.

Experiment 4 further assessed the specificity of the behavioral effects of peripheral 5-HT by determining whether 4.0 μmol/kg of this agent would decrease exploration of a novel environment. As an index of exploration, we measured the number of times rats touched a novel object in the center of an unfamiliar open-field apparatus (4). To better compare these results with those of Experiments 1 and 2, rats were maintained on a milk diet using the same regimen as employed in the feeding studies. The 16-min duration of the test was chosen because it exceeded the 12-min interval before the onset of resting in real-feeding rats in Experiment 2.

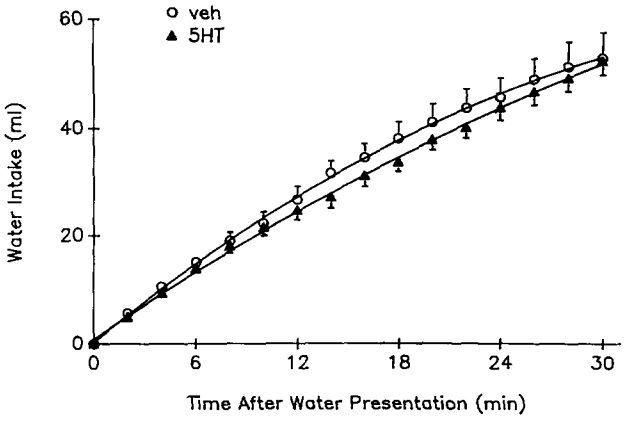

FIG. 3. Cumulative intakes of water at successive 2-min intervals after injection of 5-HT (4.0 μmol/kg, IP) or its vehicle. The data represent means ± SE for six rats tested under each condition.

METHOD

Eighteen male Sprague-Dawley rats weighing 310–385 g at the time of the experiments were obtained from AAI, Inc. and housed individually in suspended wire mesh cages in the vivarium. They were maintained exactly as described for rats used for determining the anorectic action of 5-HT in Experiments 1 and 2.

Rats were tested using a black open-field apparatus (60 cm square with walls 45 cm high) with white lines on the floor that divided the arena into nine squares (20 × 20 cm). A metal spool (7.0 cm in diameter × 3.4 cm high) was attached to the center of the field. The apparatus was located in the room in which rats were housed. Testing was conducted during the light before rats had access to milk after overnight food deprivation. For the test, rats were injected IP with either vehicle or 4.0 μmol/kg 5-HT and 6 min later placed in a corner of the field with the animal facing the center. The behavior of rats was recorded using an Ikegami ICD200 camera (Ikegami Electronics, Maywood, NJ) equipped with a Pentax 31204 12.5- to 75-mm zoom lens and a Panasonic AG2500 videocassette recorder (VHS format). An observer who was unaware of the treatments of individual rats rated the coded videotapes for: a) the latency until the rat first contacted the spool in the center of the field; b) the number of times the rat contacted the spool with its paws, snout, or vibrissae for at least 2 s; c) the number of squares crossed (i.e., locomotion); and d) the frequency of rearing. The sessions lasted 16 min. Rats were tested only once, with either vehicle or 5-HT (n = 9/group), and were naive to the open field.

RESULTS

Table 4 gives the data for the rats' investigation of the novel object. 5-HT did not affect either the latency for rats to initiate contact with the spool, the number of contacts made within successive 4-min intervals of the test, or the total number of contacts made during the 16-min session [vehicle 15.3 ± 2.2 vs. 5-HT 13.4 ± 2.7, $t(16) = 0.54$, n.s.]. Furthermore, ANOVA of the cumulative number of contacts made revealed the expected increase with time for both groups, $F(3, 48) = 62.4$, $p < 0.01$, without any interaction between drug treatment and time, $F(3, 48) = 1.03$, $p > 0.10$, and, as noted, without any main effect of 5-HT. Thus, 5-HT altered neither the time course nor the overall exploratory behavior of rats. Similarly, 5-HT failed to alter the time course for locomotion, $F(3, 48) = 1.40$, $p > 0.10$, or the total number of squares crossed (vehicle 118 ± 13 vs. 5-HT 94 ± 10), $F(1, 16) = 2.63$, $p > 0.10$, and did not affect significantly the time course for rearing, $F(3, 48) = 1.11$, $p > 0.10$, or the total number of rears (vehicle 51 ± 5 vs. 5-HT 40 ± 4), $F(1, 16) = 3.11$, $p = 0.09$.

DISCUSSION

The failure of 4.0 μmol/kg 5-HT to decrease exploratory behavior in food-deprived rats contrasted with the ability of this dose to reduce food intake by 30–50% during real and sham feeding. Thus, the results provided further evidence that 5-HT inhibited feeding without producing general deficits in arousal or motor ability.

TABLE 4

CONTACTS WITH A NOVEL OBJECT DURING EXPLORATION OF AN OPEN FIELD

Treatment	Latency to First Contact (min)*	Time Interval After Start of Test (min)†			
		0–4	4–8	8–12	12–16
Vehicle	1.7 ± 0.4	3.3 ± 0.7	5.9 ± 0.7	3.8 ± 0.6	2.3 ± 0.5
5-HT	1.1 ± 0.4	3.8 ± 1.0	3.8 ± 0.8	3.2 ± 0.6	2.7 ± 0.6

All values represent means ± SE for separate groups of nine rats that were injected with either 5-HT (4.0 μmol/kg, IP) or its vehicle 6 min before being placed in a corner of the open field (see the Method Section of Experiment 4).

*Time elapsed before the rat made its first physical contact with the novel object in the center of the field.

†Number of contacts made within each 4-min interval.

The present findings contrast also with reports in which the peptidergic satiety factors cholecystokinin (CCK) and bombesin decreased investigation of a novel object in an open field (4,5). The overall pattern of exploratory behavior after CCK, in particular, mimicked that produced by consumption of food (4). On this basis, it was proposed that CCK elicited a general state of satiety that was responsible for accelerating habituation to environmental stimuli as well as for inhibiting feeding (4,5). By comparison, our data implied that 5-HT enhanced aspects of satiation that were related more specifically to feeding. It remains possible that 5-HT would potentiate the ability of feeding to reduce investigation of an open field under our conditions. Demonstrating that 5-HT reduces exploration after feeding but not in deprived rats might argue that stimuli related to feeding play a critical role in determining serotonergic modulation of satiety. As discussed under Experiment 2, the results of the sham-feeding experiments supported this hypothesis.

GENERAL DISCUSSION

The results of these experiments confirmed previous reports in which peripherally administered 5-HT selectively decreased real (7–10,21,29) and sham feeding (18) without producing behavioral toxicity in rats. Our data extended those observations by establishing an immediate and persistent dose-related anorectic action of 5-HT in sham feeding. More importantly, the present findings also demonstrated that 5-HT did not stimulate the full spectrum of satiety-related behaviors in sham feeding because rats given this indole did not rest when they fed with their gastric cannulae open. By contrast, serotonergic treatment did accelerate resting, the terminal behavioral phase of satiation, when rats fed with closed cannulae. The major implication of this study, therefore, is that peripherally administered 5-HT does not provide an adequate signal for eliciting complete satiety in the absence of postingestive cues in rats.

The inadequacy of 5-HT as a stimulus for satiety in fistulated rats differed from previous demonstrations in which the peptidergic satiety factors CCK and bombesin stimulated appropriate nonfeeding activities, terminating in resting after sham feeding (1,2,16,17). CCK and bombesin more potently reduced food intake than elicited resting. Nonetheless, doses of these peptides that inhibited sham feeding by 25–50% clearly produced such behavioral evidence of satiety (16,17). In our study, a dose of 5-HT that decreased food intake by approximately 30% enhanced resting only when the cannulae were closed. Indeed, rats were not observed resting during sham feeding even after a large dose that decreased intake by 80%. Thus, unlike these peptides, 5-HT apparently requires a background of food-related stimulation that is missing during sham feeding to modulate satiety.

The requisite stimulus with which 5-HT must interact to promote resting remains to be identified. Sham feeding may interfere with a critical metabolic change that primes serotonergic effects, but the extent to which gastric fistulation alters absorption of nutrients is controversial (25). The failure of the stomach to fill when the cannula is open is a more obvious difference between sham and real feeding. Thus, gastric distension may provide a signal that is necessary for 5-HT to influence satiation. Recent evidence implies that CCK acts synergistically with cues from gastric loading in reducing feeding (23,24), but the role of the stomach in 5-HT's action has not been explored. Alternatively, 5-HT may require the presence of intestinal stimuli to elicit resting. If so, then our testing of overnight-deprived rather than nondeprived rats would certainly have mitigated against a more comprehensive effect of 5-HT on satiety during sham feeding. Peptides, such as CCK and glucagon, could serve as hormonal or paracrine factors from the intestines or other viscera that interact with 5-HT. An analogous functional interaction occurs between glucagon and CCK in sham feeding (15).

Further studies are needed to clarify the mechanisms and physiological relevance of the effects of peripheral serotonergic stimulation on feeding and postprandial behavior. As noted above, 5-HT reduced the apparent rate of intake virtually immediately after sham feeding commenced. This action differed markedly from the delayed effect of peptides in feeding (11,15). The rapid onset of anorexia might suggest a toxic action of 5-HT. However, 5-HT did not retard the beginning of feeding and did not alter the ingestion of water even though sham drinking was sustained at the same level as sham feeding. A more interesting explanation than toxicity is the possibility that peripheral 5-HT diminished the palatability of the tastant in a manner known to reduce the rate of intake in fistulated rats (6,31). The effects of peripheral 5-HT on the rate of intake, rate of licking, and distribution of licks (6) have yet to be examined.

The action of 5-HT to promote resting in the presence of postingestive cues may operate via an entirely separate mechanism from the reduction of intake or may reflect a complex interaction of oropharyngeal, visceral, and serotonergic stimulation. Certainly, feeding (19) and peripheral injections of some satiety agents (3) have been known to modify the electro-

encephalogram and elicit resting or sleep. Serotonergic processes appear to be involved in regulating electrical activity in viscero receptive areas of the brainstem (20) but it is unknown whether peripheral 5-HT can recruit such effects. Studies using structural analogs of 5-HT have implicated multiple 5-HT receptors in the reduction of food intake produced by peripheral serotonergic activity (27). A similar pharmacological approach may help elucidate whether separate mechanisms underlie the effects of peripheral 5-HT on feeding and on the terminal behavioral phase of satiety.

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