Pharmacology Biochemistry & Behavior, Vol. 15, pp. 179–183, 1981. Printed in the U.S.A.

Peripherally Administered Serotonin Decreases Food Intake in Rats

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Received 7 March 1981

POLLOCK, J. D. AND N. ROWLAND. *Peripherally administered serotonin decreases food intake in rats*. PHARMAC. BIOCHEM. BEHAV. 15(2) 179–183, 1981.—We report that intraperitoneal injection of serotonin produces a dose-related decrease in the food intake of hungry rats. The efficacy of serotonin was increased by prior treatment with clorgyline, a type A monoamine oxidase inhibitor. Doses of serotonin which were anorectic did not significantly impair locomotor activity or sensorimotor performance. Further, 2 mg/kg serotonin (ED50 on food intake) did not produce a conditioned taste aversion when paired repeatedly with sucrose ingestion. We conclude that the anorectic effects of serotonin are not secondary to nonspecific effects of the agent, and suggest that peripheral serotonin may play a role in normal satiation.

Serotonin Gut Satiety Food intake Clogyline Taste aversion Sensorimotor performance

PERIPHERAL signals, generated in immediate response to food, are likely to be importantly involved in the termination of ongoing meals. Recently, much interest has been devoted to the rule of prandially-released gut hormones in satiation [12,25]. Such hormones may then act locally to generate afferent nerve impulses, or act at a distal receptor possibly in the central nervous system.

One substance which is released prandially, but has received little attention as a putative hormone of satiation, is serotonin (5-hydroxytryptamine). Thus, increased pressure on the intestinal mucosa [7], acidic conditions in the duodenum [15,21], or intraduodenal infusion of hypertonic glucose [11] produce increased serotonin levels in hepatic portal and peripheral venous blood, and depletions of serotonin stores in the gut.

It is thus of some interest that exogenously-administered serotonin decreases the food intake of hungry rats [5,9], and of free-feeding rats [26] and rabbits [22]. Since peripherally-administered serotonin does not cross the blood-brain barrier [2, 17, 18, 20, 30] the anorectic effect is presumably of peripheral, possibly hepatic, origin. The present study further investigates the effects of intraperitoneally-administered serotonin on food intake. We find that serotonin decreases food intake in a dose-related manner, and that anorectic doses do not produce appreciable sensorimotor dysfunctions; neither do they support a conditioned taste aversion.

EXPERIMENT 1: DOSE-RELATED SUPPRESSION OF FEEDING BY SEROTONIN

The first experiment confirms and extends previous observations which showed that peripherally-administered serotonin decreased feeding of deprived rats [5,9]. We have run a dose-response curve for serotonin on food intake, and have observed the effect of type A monoamine oxidase inhibition (by clorgyline) on serotonin induced anorexia. Clorgyline retards the degradation of serotonin [13,27], and so is expected to increase its effect.

METHOD

Subjects

The experiment was run in two parts using a total of 65 naive Sprague-Dawley rats (Zivic Miller, Allison Park, PA) weighing 280–350 g. Water was present at all times. The singly-housed rats were maintained in a ventilated room with an artificial lighting cycle (lights on 0600–1800 hr). All experiments were started between 1300 and 1500 hr.

Drugs

The drugs used were serotonin creatinine sulfate (Sigma), creatinine (Sigma), and clorgyline (May & Baker). All were dissolved in 0.9% NaCl and injected in a volume of 1 ml/kg. Serotonin doses are expressed in terms of the free base.

Procedure

One day after arrival, the animals were deprived of their standard laboratory chow pellets (Wayne lab blox) for 24 hr.

In the first part of the experiment rats received intraperitoneal (IP) injections of serotonin (0.5, 1.0, 2.0 or 5.0 mg/kg; N=6/group), or the vehicle (N=5). An additional control group (N=6) received creatinine in a dose equal to the amount present in the highest dose of serotonin.

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In the second part, 30 rats received two injections spaced 10 min apart. Four groups (N's=6) were first injected with clorgyline (5.0 mg/kg) then with vehicle, 0.5, 1.0 or 2.0 mg/kg serotonin. The fifth group (N=6) received two injections of vehicle.

In both parts of the experiment, a preweighed amount of food pellets was placed in the cage 5 min after the last injection. After 60 min the amount eaten was determined (±0.02 g) by subtracting the amount remaining plus the collected spillage from the initial weight.

Statistical Analysis

One-way analysis of variance was used to determine whether any difference existed among the group means. If a treatment effect was revealed, then a Sheffé multiple comparison test was performed to ascertain which means were different from one another. At each dose of serotonin, comparisons were made (*t*-tests) between groups which did and did not receive pretreatment with clorgyline.

RESULTS

When given alone, serotonin produced a dose-related suppression of food intake (Fig. 1). There was a significant difference ($p<0.05$, Sheffé) between the two control groups and the four experimental groups. Among the experimental groups which received serotonin, there was no significant difference ($p>0.05$) between the food intakes of rats given 0.5 and 1.0 mg/kg, and between groups given 2.0 and 5.0 mg/kg. The two higher doses produced greater suppressions of feeding than the two lower doses ($p<0.05$).

Figure 1 also illustrates that food intake is suppressed in a dose-related way in rats treated with clorgyline and serotonin. No significant difference was found between the control groups which received vehicle + vehicle, or clorgyline + vehicle. The Sheffé contrasts showed significant ($p<0.05$) differences in the amount eaten among each of the groups which received serotonin, as well as between the control and experimental groups.

When compared to animals treated with serotonin alone, rats pretreated with clorgyline showed greater suppressions of feeding after 0.5, 1.0 and 2.0 mg/kg serotonin (p's<0.05, *t*-tests).

Observation of the animals revealed no remarkable abnormalities except at the highest dose (5.0 mg/kg serotonin, or 2.0 mg/kg serotonin plus clorgyline) when some reddening of the eyes was observed. Animals in all groups were seen to drink occasionally during the test.

DISCUSSION

We have shown that IP-administered serotonin produces a dose-related suppression of food intake in 24-hr food deprived rats. The efficacy of serotonin was enhanced by clorgyline, which suggests that the anorectic effect was due to the action of serotonin rather than one of its metabolites. These findings extend previous observations of serotonin-provoked anorexia in fed [26] and fasted [5,9] rats. However, intraportally administered serotonin was anorectic in fed, but not fasted, rabbits [22], and this may reflect a route or species difference. Finally, our observation of drinking is consistent with the fact that serotonin induces drinking in water-replete rats [29].

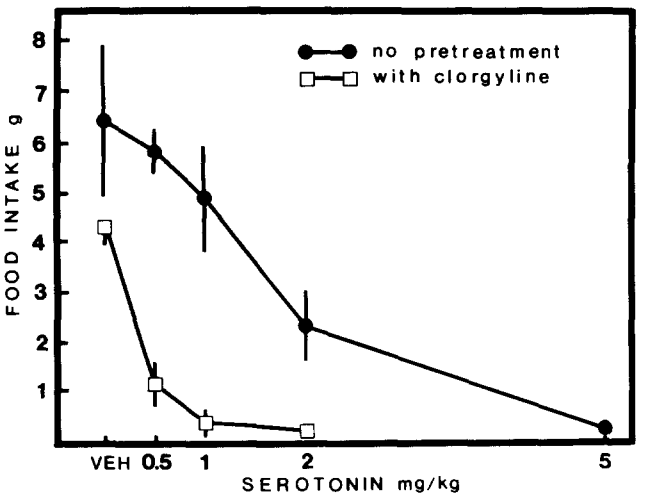

FIG. 1. The amount of chow consumed in a 1-hr test by rats which were deprived of food for 24 hr, then given IP injections of various doses of serotonin alone (●–●) or after pretreatment with type A monoamine oxidase inhibitor, clorgyline (5 mg/kg) (□–□). Each point represents the mean ± SE of 5–6 rats.

EXPERIMENT 2: SENSORIMOTOR PERFORMANCE IN SEROTONIN-TREATED RATS

The reductions in food intake described in the first experiment could have been produced either by a specific effect of serotonin on an ingestion-related system, or by some nonspecific effect of serotonin. The latter might include a reduced sensorimotor performance or arousal and, although this did not appear to be the case in the first experiment, we systematically tested this possibility in the present study.

METHOD

Subjects

The subjects were 36 male Sprague-Dawley rats (Zivic Miller) weighing 280–350 g. They were housed singly with food pellets and tap water available ad lib, with the same lighting cycle as in Experiment 1. All tests were performed in the middle part of the day.

Procedure

In a test of spontaneous locomotor activity, groups of six rats received IP injections of 0.9% saline, 2.0 or 5.0 mg/kg serotonin, and 10 min later were placed in a novel cage. This was a cylindrical arena, 45 cm dia. A small lamp was mounted at floor level on one wall, and a photocell detector was positioned diametrically opposite. Cage crossings interrupted the beam, and these crossings were recorded on an electromechanical counter. The number of crossings was recorded every 10 min for a period of 60 min.

In tests of catalepsy and akinesia, groups of six rats received injections of 0, 2.0 or 5.0 mg/kg serotonin. Other rats received 5 mg/kg clorgyline followed 10 min later by 0 or 1.0 mg/kg serotonin. Ten min after the last injection the animals were placed on a flat surface and the latencies to move one paw, then all four paws, were recorded with a digital timer.

TABLE 1
SENSORIMOTOR PERFORMANCE IN RATS TREATED WITH SEROTONIN

Drug	Latency to move (sec)		Catalepsy (sec)	
	one paw	four paws	front	hind
Vehicle	0.43 ± 0.03	0.82 ± 0.19	0.73 ± 0.15	0.55 ± 0.08
2 mg/kg Serotonin	2.05 ± 1.39	4.75 ± 2.09	0.97 ± 0.19	2.80 ± 2.22
5 mg/kg Serotonin	1.15 ± 0.26	5.37 ± 0.72	23.73 ± 19.37	3.65 ± 1.80
5 mg/kg Clorgyline + 1 mg/kg serotonin	1.20 ± 0.40	2.83 ± 0.89	2.60 ± 0.88	4.62 ± 2.17

Shown are mean ± SE for groups of six animals.
One way ANOVA's for each task gave F(3,20)'s of 0.81, 2.96, 1.33, 0.96 (p's>0.05), for no significant drug dose effects.

Front limb catalepsy was then tested. The rat was gently placed with its front paws on top of a rigid wooden block which was 5 cm high, and with its hind limbs on the floor. The latency to move both paws off the block was recorded. The procedure was then repeated with the hindlimbs on the block. The latencies were subjected to analysis of variance.

RESULTS AND DISCUSSION

The results of the spontaneous locomotor activity test (Fig. 2) revealed no significant ($p>0.05$) differences among the groups at any time point. However, it should be noted that the activity of the rats treated with 5.0 mg/kg serotonin was considerably reduced, especially early in the hour, and one of the six animals in this group was observed to be dragging its hind limbs. No other rat exhibited this or any other symptoms such as snake tail, head weaving, or forelimb treading. These latter are thought to be reliable indices of central serotonin receptor activation [3, 13, 14, 24, 27].

The data from the sensorimotor tests are summarized in Table 1. All of the doses of serotonin tended to increase the latencies to respond, but those increases were not statistically significant. Further, the maximum latencies that we observed were considerably shorter than those typically obtained in these tests using rats treated with classical sedatives.

Collectively, these data indicate that rats treated with serotonin in anorectic doses (ED 50–90) are not suffering from sensorimotor disorders of the magnitude which would be needed to account for the degree of anorexia observed in the first experiment.

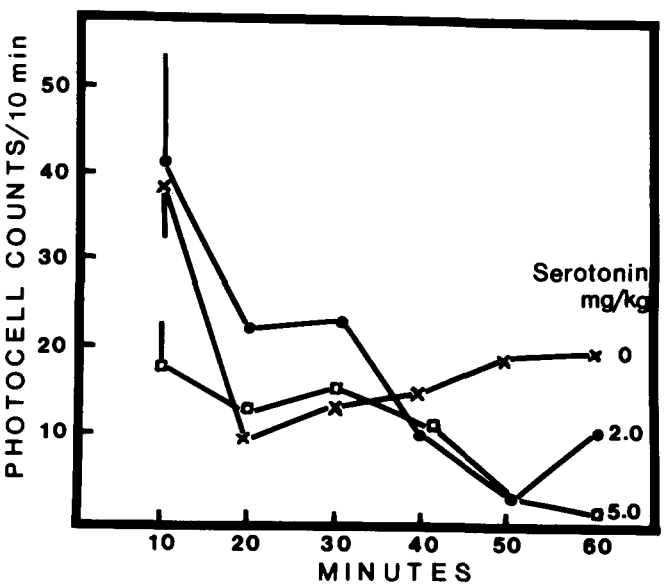

FIG. 2. Locomotor activity, measured as number of breaks of a photobeam in a novel cage, of undeprived rats treated with 0 (saline), 2.0 or 5.0 mg/kg serotonin, IP. Each point is the mean 10 min total counts for 6 rats. For clarity, representative SE's are shown for the first 10 min only. There was no significant difference in locomotor activity between the three groups (ANOVA: $p>0.05$).

EXPERIMENT 3: DOES SEROTONIN SUPPORT A CONDITIONED TASTE AVERSION?

In a final experiment we tested whether the injections of serotonin might be anorectic because the animals feel sick. We have assessed this possibility by investigating whether an anorectic dose of serotonin will serve as the unconditional stimulus for a taste aversion paradigm (e.g. [4, 8, 16, 19]).

METHOD

Subjects

The subjects were 24 male Sprague-Dawley rats (Zivic Miller) housed individually in a 12 hr day lighting cycle as before. Food was available throughout (Wayne lab blox).

Procedure

The animals were placed on a water deprivation schedule on which water was presented for 15 min/day only. The water was presented in graduated burettes with drinking spouts, and the intakes were measured to the nearest 0.1 ml. After 10 days, when the daily intakes were stable, the conditioned taste aversion phase was begun.

On the first day of the experimental phase all animals were given 15% (w/v) sucrose solution instead of water during the 15 min drinking period. Immediately afterward, groups of eight rats were injected with either serotonin (2.0

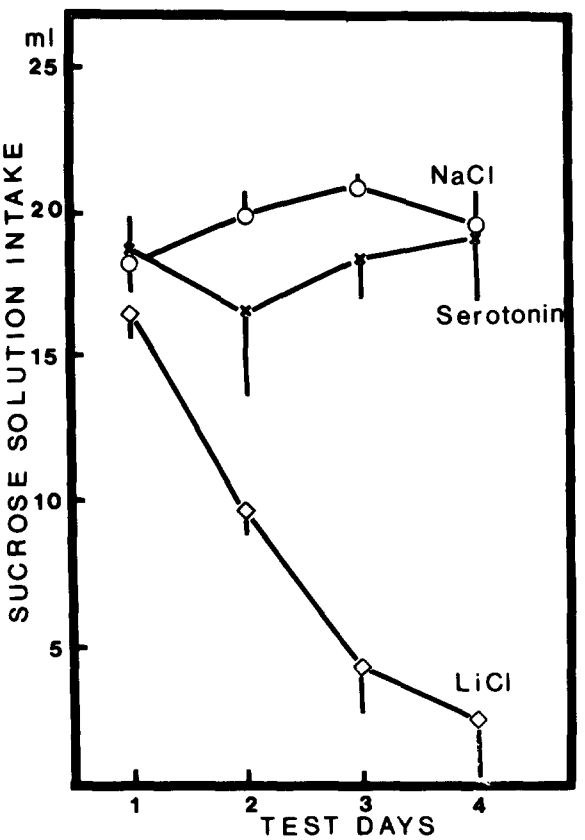

FIG. 3. The amount of 15% sucrose solution consumed on test days by rats maintained on a 15 min/day watering schedule. Each test was followed immediately by injection of saline (○−○), 2 mg/kg serotonin (x−x) or 0.15 M lithium chloride (◇−◇). Shown are the mean ± SE for groups of 8 rats. Analysis of Covariance for trial 4 and trial 1 as covariate shows significant differences among the means, $F(2,21)=41.9, p<0.005$, and Sheffé contrasts show significant differences between the LiCl group and the saline and serotonin groups (p's<0.005). The serotonin group did not differ from the saline controls ($p>0.05$).

mg/kg), lithium chloride (1.8 mEq/kg of 0.15 M solution), or 0.15 M NaCl control. On days two and three all animals received water as usual. On the fourth day the sucrose solution was again presented and was again followed by the injection of serotonin, LiCl, or NaCl. The procedure was repeated a total of four times. The intake of sucrose on each test was used to evaluate the development of a conditioned taste aversion.

RESULTS AND DISCUSSION

The results are shown in Fig. 3. Animals treated with NaCl showed stable sucrose intakes across the four test sessions. Likewise, the animals injected with serotonin (2.0 mg/kg) after each sucrose trial maintained stable intakes. In contrast, and as expected, animals given repeated contiguous pairings of the sucrose solution with LiCl showed a progressive decrease of intake across treatments ($p<0.05$, analysis of covariance). Thus, in a paradigm in which LiCl produces a clear taste aversion, there is no evidence that an anorectic dose of serotonin produced systemic sickness.

These results must, however, be regarded with some caution. Putative satiety factors such as cholecystokinin have been found to support conditioned taste aversion learning in some paradigms [10] but not in others [25]. It has been argued by Deutsch [10] that a procedure such as that used in the present work is relatively insensitive, and would reveal only severe gastrointestinal distress. However, Smith and Gibbs [25] have countered that the conditioned taste aversion paradigm may be inadequate as a test of malaise in either direction. For example, among many unexpected findings using taste aversion paradigms are the facts that rodenticides do not uniformly produce conditioned taste aversions, even when the animals are visibly sick [19]. On the other hand, agents which are generally regarded as producing feelings of well-being such as amphetamine, morphine, and loxapam, have yielded aversions in paradigms similar to the present (e.g. [4, 8, 16]).

GENERAL DISCUSSION

The present data indicate that peripherally-administered serotonin may reduce food consumption by acting upon receptors in the periphery. This belief is predicated upon reports that serotonin does not cross the blood brain barrier in a number of different paradigms [2, 17, 18, 20, 30]. Also, the doses of serotonin used in the present experiments did not elicit any behavioral symptoms characteristic of central serotonergic activation [3, 13, 14, 24, 27]. These doses did not markedly impair sensorimotor performance, and neither did they appear to make the animals sick. It is of interest that some gut tumors are serotonin-secreting, and in these cases the extreme hyperserotoninemia may produce side effects including diarrhea which are characteristic of the carcinoid syndrome [6,23].

Our data may also indicate that serotonin in the periphery is normally involved in satiety. In order to be considered a putative satiety factor it must be released in temporal association with food intake. There is some evidence that this may be the case, since serotonin is liberated by increased intraluminal pressure [7] and by glucose infusions [11]. Furthermore, the hydrochloric acid released in the gut during digestion may evoke serotonin release, since infusions of HCl into the duodenum elevate blood levels of immunoreactive serotonin [15] and decrease gut stores of serotonin [21]. There are very large amounts of serotonin stored in nerve terminals in the gut [28] and one role of the released serotonin may be in peristalsis [28,31]. However, the released serotonin will also gain access to the liver where it may initiate afferent nerve impulses [1]. These could then be putative satiety signals at a central integrative site.

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