Pharmacology Biochemistry & Behavior, Vol 24, pp 1133–1136, 1986 © Ankho International Inc Printed in the U S A 0091-3057/86 $3 00 + 00

BRIEF COMMUNICATION

Microstructural Analysis of the Anorectic Action of Peripherally Administered 5-HT

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Received 23 May 1985

FLETCHER, P J AND M. J BURTON *Microstructural analysis of the anorectic action of peripherally administered 5-HT* PHARMACOL BIOCHEM BEHAV 24(4) 1133–1136, 1986 —The anorectic action of systemically administered 5-HT (1, 2 and 4 mg/kg SC) was investigated in food deprived rats using the technique of microstructural analysis; small food pellets were delivered to a food hopper, and the time and occurrence of each pellet removal was recorded Log-survivor analysis of inter-pellet intervals was used to define feeding bouts, and this was then used to compute measures of bout frequency, bout size, bout duration and eating rate The 5-HT reduced food intake by selectively decreasing bout size and bout duration No effects of 5-HT were observed on any of the other parameters measured. These effects of 5-HT are robust over a range of bout criteria, and replicable Methysergide (3 mg/kg IP) attenuated the anorectic action of 5-HT by a significant increase in bout frequency and an attenuation of the effects of 5-HT on bout size, and bout duration. The results are discussed in terms of a possible role for peripheral 5-HT in the control of satiety, and implications for the mode of action of serotonergic anorectic agents such as fenfluramine

Peripheral 5-HT Microstructural analysis Feeding bouts Eating rate Satiety

IT has been shown that systemically administered serotonin (5-HT) reduces food intake in the food deprived rat [5, 10, 18]. Since 5-HT does not readily cross the blood brain barrier [17], this effect is presumably mediated peripherally We have shown [10] that the anorectic action of 5-HT is reversed by the 5-HT antagonist methysergide, but not metergoline, indicating that 5-HT acts via a particular type of peripheral 5-HT receptor. This receptor site appears to be associated with a peripheral 5-HT neuronal system, since the anorectic action of 5-HT is potentiated by the type A monoamine oxidase inhibitor clorgyline, and the selective serotonergic re-uptake inhibitor LM 5008 [9]. The finding that subdiaphragmatic vagotomy enhances the anorectic action of 5-HT [11] suggests that 5-HT may reduce food intake by interacting with some aspect of vagal function.

These findings indicate that a peripheral serotonergic mechanism may be involved in the control of food intake. The experiments can be criticised on the grounds that the anorectic action of 5-HT was determined by simply weighing the amount of food consumed over a test session. It is possible, therefore, that the anorectic action of 5-HT arises because of some nonspecific disruptive effects of the drug which were not detected in this insensitive test procedure. However, it has been reported that systemically administered 5-HT does not impair sensorimotor performance [18], or possess any aversive properties as measured in a conditioned taste aversion paradigm [10,18]. Experiments in this report examine the behavioural effects of 5-HT in some detail by analysing the action of 5-HT using the microstructural analysis of feeding behaviour. This technique consists of dividing a measure of total food intake into bouts, and then examining the changes in bout frequency, bout size, bout length and eating rate following drug administration [7]. Thus, it is possible to obtain a detailed behavioural profile of the mode of action of a given anorectic agent.

Using this technique it has been found that systemic administration of fenfluramine decreases food intake by reducing bout size and eating rate [4,7]. Drugs which block the re-uptake of 5-HT produce the same effects [4]. The effects of these drugs have usually been interpreted in terms of their actions on brain 5-HT systems (see [2, 3, 12] for reviews), but it is possible that such effects are mediated in part by peripheral 5-HT systems. In keeping with this view it has been suggested that the reduction in bout size and eating rate

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observed with serotonergic agonist drugs are mediated by peripheral and central mechanisms respectively [4] Accordingly it is hypothesized that systemically administered 5-HT decreases food intake by selectively reducing bout size only

METHOD

Experiment 1

Procedure Male Lister hooded rats (239–250 g) were initially housed in standard laboratory cages (North Kent Plastics) under a 12 hour light-dark cycle The rats were trained to consume their daily food ration (Spratt's laboratory chow) in a 6 hour period beginning 2 hours into the light phase of the light-dark cycle Water was freely available at all times

Following 7 days habituation to this deprivation procedure the rats were transferred to the apparatus used to record feeding. Full details of the apparatus have been reported elsewhere [7] The apparatus consisted of 8 aluminum cages equipped with a food hopper, drinking bottle and nest box Food pellets (45 mg Campden pellets) were delivered singly to the food hopper. Removal of each pellet from the hopper activated an infra-red photo beam system, and a further pellet was delivered approximately 1 5 seconds later The time of each pellet removal (since the beginning of data logging) was recorded by a microprocessor system, and stored on a floppy disk This information was later transferred to a PDP 11/40 minicomputer for long-term storage and analysis Thus, a continuous record of feeding for each rat was available

Each rat was housed in one of the cages and allowed access to the pelleted food until food intakes had stabilised On test days all rats were injected (SC) with 1, 2 or 4 mg/kg serotonin creatinine sulfate (Sigma), or saline and returned immediately to the cage Data logging was initiated immediately All rats received saline and every dose of 5-HT in a counterbalanced order, with at least 2 drug free days between successive test days

Experiment 2

A second experiment was conducted to examine the reliability of the effects of 5-HT using rats maintained on a more restricted feeding schedule. In addition the effects of a single dose of methysergide on 5-HT induced anorexia were examined. The dose of methysergide was chosen on the basis of previous work: being the highest dose which attenuated 5-HT anorexia without significantly altering food intake in its own right [10,16] The use of a higher dose affecting food intake would necessarily complicate the interpretation of the results.

Procedure In a second experiment male Lister hooded rats (235–275 g) were initially housed under identical conditions to Experiment 1, except that they were allowed access to food for 4 hours a day After a 7 day habituation period to restricted food access they were transferred to the test cages, and maintained on the 45 mg pellets with water available at all times. When 4 hour food intakes had stabilized all animals were injected with 3 mg/kg methysergide or distilled water (IP), followed thirty minutes later by 2 mg/kg 5-HT or saline (SC) The rats were returned immediately to the test cages and data logging was initiated.

Each rat received every combination of methysergide or vehicle and 5-HT or vehicle in a counterbalanced order with at least two drug-free days between successive test days

Bout analysis A previous report [10] has shown that the maximal effect of the range of doses of 5-HT used in this experiment occurs during the first hour after injection Also the dipsogenic action of 5-HT does not appear to interfere with feeding over this time period [10,16] Therefore, the analysis of the feeding data was confined to the first hour only

TABLE 1

THE EFFECTS OF INCREASING DOSES OF 5-HT ON BOUT PARAMETERS IN A 1-HOUR FEEDING TEST

	5-HT (mg/kg)			
Parameter	0	1	2	4
Total intake (g)	10 45 (0 76)	6 79† (0 84)	5 11† (0 58)	4 02† (0 50)
Total time (min)	25 70 (2 39)	16 9† (2 21)	12 20† (1 71)	8 82† (1 21)
Bout freq	5 00 (0 85)	6 14 (1 18)	5 43 (1 60)	4 86 (0 99)
Bout size (g)	2 69 (0 71)	1 44* (0 35)	1 52 (0 45)	1 14* (0 32)
Bout dura (min)	6 53 (1 62)	3 61* (0 87)	3 61* (1 08)	2 46† (0 65)
Eating rate (g/min)	0 49 (0 03)	0 77 (0 18)	0 54 (0 08)	0 68 (0 08)
Median IPI (sec)	6 02 (0 34)	6 07 (0 52)	6 13 (0 27)	5 04 (0 27)

Values represent mean (and SEM) for 7 rats
*Differs from 0 mg/kg 5-HT, $p<0$ 05
†Differs from 0 mg/kg 5-HT, $p<0$ 01

To divide the feeding records of the rats into the various bout parameters, a bout criterion was selected by transforming the frequency distribution of inter-pellet intervals into a log-survivor curve Briefly, the gradient of the curve represents the rate of occurrence of responses, in this case pellet removals When responses occur in bouts, defined as a number of short inter-response intervals followed by a long interval, a sudden change in the gradient of the log-survivor curve marks the transition from a high rate of responding (within bouts) to a low rate of responding (between bouts) [19] The interval length at this gradient change provides an estimate of the length of interval which can be used to distinguish between intervals occurring within or between bouts, this 'breakpoint' can be used to define a bout. This technique has been shown to be a reliable method of defining bouts in free feeding [7] and deprived rats [21]

In food deprived rats treated with an anorectic agent it is sometimes difficult to identify the breakpoint because of the relatively small number of responses generated Therefore, in this experiment a breakpoint was chosen from a log-survivor curve generated from the pooled data for all rats treated with saline

The various bout parameters were derived using this criterion, and the effects of 5-HT were analysed by one-way analysis of variance, followed by Dunnett's test for comparisons against a control mean In the second experiment data were analysed by two-way analysis of variance using pretreatment (vehicle or methysergide) and drug treatment (vehicle or 5-HT) as factors, and t-tests where appropriate. Parameters calculated were bout frequency (number of bouts within the test session), bout size (g), bout duration (the total time a bout lasted), mean eating rate (bout size divided by bout length) and the median inter-pellet interval (sec)

RESULTS

Experiment 1

Table 1 shows the effects of increasing doses of 5-HT on the various bout parameters derived from a criterion of 25 seconds. Total food intake was significantly reduced by 5-HT over the hour test, $F(3,18)=18.9$, $p<0.001$. The anorectic action of 5-HT was characterised by reductions in bout size, $F(3,18)=3\ 66$, $p<0.05$, and bout duration, $F(3,18)=4\ 22$, $p<0\ 05$ Comparisons using Dunnett's test showed that the reduction in bout size caused by 1 and 4 mg/kg was significantly different from control, but the effect of 2 mg/kg just failed to reach significance. Since bout frequency was not changed by 5-HT, $F(3,18)=0.78$, $p>0.01$, the total time spent eating was significantly reduced, $F(3,18)=16.77$, $p<0.01$ Inspection of Table 1 shows that across doses of 5-HT the reduction in bout size was paralleled by reduction in bout duration, implying that the rate of eating was not altered by 5-HT This is confirmed by the finding that 5-HT did not alter the mean rate of eating, $F(3,18)=1.38$, $p>0.05$, or the median inter-pellet interval, $F(3,18)=3.09$, $p>0.05$ A similar profile of changes was observed when the data were analysed using criteria providing an underestimate and an overestimate of the breakpoint on the log-survivor curve (results not shown).

Experiment 2

A longer median IPI was found for the rats used in this experiment and this resulted in the generation of a larger overall bout criterion (55 sec). As in Experiment 1, 5-HT reduced total food intake, $F(1,7)=39.01$, $p<0.001$, by decreasing only bout size and bout duration, $F(1,7)=12.3$ and 12.9, respectively, both $p<0.01$, to approximately 40% of control values. A significant main effect of pretreatment, $F(1,7)=10\ 2$, $p<0\ 025$, and a significant pretreatment drug interaction, $F(1,7)=8\ 02$, $p<0.05$, indicated that methysergide attenuated the anorectic action of 5-HT. This was characterised by an attenuation of the effects of 5-HT on bout size and bout duration Mean values for bout size were 1 34 (±0 27) g (5-HT alone) and 1 75 (±0.32) g (methysergide and 5-HT) The mean bout durations were 3 7 (±0.6) min and 5.4 (±0 99) min respectively In addition, a small but significant effect of methysergide was observed on bout frequency, $F(1,7)=5\ 7$, $p<0.05$, reflecting the fact that methysergide increased this measure when administered alone, and in combination with 5-HT

DISCUSSION

Previous studies investigating the anorectic action of systemically administered 5-HT have simply involved determining the amount of food consumed by 5-HT treated animals, and have not directly investigated the behavioural effects of this compound. The present study has addressed this issue and has revealed that in food-deprived rats feeding on a solid diet, 5-HT reduces food intake by selectively reducing bout size and duration. These effects were attenuated by methysergide confirming that the action of 5-HT is mediated by 5-HT receptors. Methysergide also slightly increased bout frequency and this contributed to the attenuation of the anorectic action of 5-HT. Since treatment with 5-HT failed to alter bout frequency or eating rate, this indicates that 5-HT probably does not induce sedation, or interfere with the animal's ability to produce the motor actions necessary for feeding Further evidence against this type of explanation derives from the observation that 5-HT increases consumption of water [14], and certain flavoured solutions [15,16]. It can be concluded that the reduction in food intake caused by 5-HT is not the result of a general disruption of behaviour, such as that produced by amphetamine [4,21].

It is possible that the dipsogenic [14] action of 5-HT causes the rats to stop eating in order to drink. A previous report [10] has suggested that the magnitude of the drinking response to 5-HT is insufficient to account for 5-HT anorexia. It has also been shown that the time courses of 5-HT anorexia and hyperdipsia differ, with the onset of the anorectic response occurring earlier than the hyperdipsic response [16]. Therefore, it is unlikely that the reductions in bout size and duration can be explained in terms of response competition from drinking.

Since 5-HT reduces bout duration and bout size the 5-HT treated rat appears to feed in the same manner as an untreated rat, except that it ceases feeding earlier. It is tempting to speculate that 5-HT is acting to enhance the onset of satiety, which prevents the animal from eating further, and by implication that a peripheral 5-HT system is involved in the neural control of satiety. The cessation of feeding is not a satisfactory indication that satiety has occurred, since many non-specific factors may cause an animal to stop feeding. It has been reported [1] that untreated rats which have ceased eating, display a characteristic sequence of behaviours involving exploration, grooming, resting and sleep. This behavioural sequence is claimed to be an unambiguous marker of satiety [20]. Satiety is dependent upon some post-ingestional consequence of food intake since rats equipped with an open gastric fistula, which allows food to drain away from the stomach immediately after ingestion, consume excessive amounts of a liquid diet and fail to show the behavioural sequence of satiety [1]. The putative satiety hormone cholecystokinin (CCK) terminates feeding in sham-feeding and control rats and elicits the complete behavioural sequence of satiety [1] In order to claim that 5-HT induces satiety it will be necessary to show that 5-HT abolishes sham-feeding, and that 5-HT treated animals do not differ from control rats in terms of post-prandial behaviours.

Evidence from other sources is consistent with the notion that a peripheral serotonergic system is involved in the control of satiety. Increased levels of 5-HT in the blood, coupled with depleted stores of 5-HT in the gut have been demonstrated following increased pressure on the intestinal mucosa [6], acidic conditions in the gut [13] and intraduodenal infusions of hypertonic glucose [8]. However, it remains to be determined whether an increased release of peripheral 5-HT is temporally associated with the termination of feeding bouts

The observation that peripherally administered 5-HT decreases bout duration and bout size may indicate that the reduction in bout size caused by fenfluramine and other indirect serotonergic agonists is mediated peripherally as suggested previously [4] Recent evidence suggests that 5-HT and fenfluramine act via independent mechanisms. Rats pretreated with metergoline [10], or with subdiaphragmatic vagotomy [11] respond differentially to doses of 5-HT and fenfluramine which produce equivalent reductions in food intake Therefore, caution must be used when interpreting the present results in terms of a possible peripheral action of fenfluramine Experiments are currently underway in our laboratory using the microstructural analysis technique to directly assess the possible peripheral action of fenfluramine, and the 5-HT precursor 5-hydroxytryptophan

ACKNOWLEDGEMENTS

This research was supported by a grant from the MRC to M J B and was carried out whilst P J F was in receipt of a research studentship from the SERC (U K) Methysergide was a generous gift from Sandoz We would like to thank Sara Hill for assistance in carrying out Experiment 2, and Rosalee James for typing the manuscript

REFERENCES

1 Antin, J , J Gibbs, J Holt, R C Young and G P Smith Cholecystokinin elicits the complete behavioural sequence of satiety in rats *J Comp Physiol Psychol* **89**: 784–790, 1975
2 Blundell, J E Is there a role for serotonin (5-hydroxytryptamine) in feeding? *Int J Obes* **1**: 15–42, 1977
3 Blundell, J E Serotonin and feeding In *Serotonin in Health and Disease, Vol 5 Clinical Applications*, edited by W B Essman New York Spectrum Pub , 1979, pp 403–450
4 Blundell, J E and C J Latham Pharmacological manipulation of feeding possible influence of serotonin and dopamine on food intake In *Central Mechanisms of Anorectic Drugs*, edited by S Garattini and R Samanin New York Raven Press, 1978, pp 83–109
5 Bray, G A and D A York Studies on food intake in genetically obese rats *Am J Physiol* **223**: 176–179, 1972
6 Bulbring, E and A Crema The release of 5-hydroxytryptamine in relation to pressure on the intestinal mucosa *J Physiol* **146**: 18–28, 1959
7 Burton, M J , S J Cooper and D A Popplewell The effect of fenfluramine on the microstructure of feeding and drinking in the rat *Br J Pharmacol* **72**: 621–633, 1981
8 Drapanas, T , J C McDonald and J D Stewart Serotonin release following instillation of hypertonic glucose into the proximal intestine *Ann Surg* **156**: 528–536, 1962
9 Fletcher, P J Serotonin in the control of feeding behaviour Unpublished D Phil thesis, University of Sussex, 1984
10 Fletcher, P J and M J. Burton Effects of manipulation of peripheral serotonin on feeding and drinking in the rat *Pharmacol Biochem Behav* **20**: 835–840, 1984
11 Fletcher, P J and M J Burton The anorectic action of peripherally administered 5-HT is enhanced by vagotomy *Physiol Behav* **34**: 861–866, 1985
12 Hoebel, B G Pharmacologic control of feeding *Annu Rev Pharmacol Toxicol* **17**: 605–621, 1977
13 Kellum, J M and B M Jaffe Release of immunoreactive serotonin following acid perfusion of the duodenum *Ann Surg* **184**: 633–636, 1976
14 Kikta, D C , R M Threatte, C C Barney, M J Fregly and J E Greenleaf Peripheral conversion of L-5-hydroxytryptophan to 5-HT induces drinking in rats *Pharmacol Biochem Behav* **14**: 889–893, 1981
15 Montgomery, A M J and M J Burton The effects of flavour on 5-HT induced dipsogenesis *Neurosci Lett [Suppl]* **14**: S253, 1983
16 Montgomery, A M J and M J Burton Effects of peripheral 5-HT on consumption of flavoured solutions *Psychopharmacology (Berlin)*, in press, 1985
17 Oldendorf, W H Brain uptake of radiolabelled amino acids, amines and hexoses after arterial injection *Am J Physiol* **221**: 1629–1639, 1971
18 Pollock, J D and N Rowland Peripherally administered serotonin decreases food intake in the rat *Pharmacol Biochem Behav* **15**: 179–183, 1981
19 Slater, P J B The temporal pattern of feeding in the zebra finch *Anim Behav* **22**: 506–515, 1974
20 Smith, G P and J Gibbs Post-prandial satiety In *Progress in Psychobiology and Physiological Psychology Vol 8*, edited by J M Sprague and A N Epstein New York Academic Press, 1979, pp 179–242
21 Willner, P and A Towell Microstructural analysis of the involvement of *beta*-receptors in amphetamine anorexia *Pharmacol Biochem Behav* **17**: 255–262, 1982