Effects of a 5-HT₃ antagonist on peripheral 5-hydroxytryptamine-induced anorexia

ROBIN STEVENS

University of Nottingham, Nottingham, England

and

STEPHEN EDWARDS

University College of Swansea, Swansea, Wales

Since antagonizing peripheral 5-HT₂ receptors incompletely blocks the anorexia induced by systemically administered 5-HT, another 5-HT receptor subtype is implicated in this effect. To determine whether the 5-HT₃ receptor is the additional type involved, the specific, peripheral 5-HT₃ antagonist [[3-(1-Methyl-1H-indol-3-yl)-1,2,4-oxadiazol-5-yl]methyl]trimethyl-ammonium iodide (MIO) was used. After an overnight fast, rats were injected with MIO followed 1 h later by 5 mg/kg of 5-HT. There was a dose-dependent potentiation by MIO of the 5-HT-induced anorexia that peaked at a dose of 2.5 mg/kg of MIO. However, MIO by itself failed to induce an anorexia. Although the 5-HT₃ receptor is implicated in 5-HT-induced anorexia, it is suggested that its activation counters the effects of 5-HT in the mediation of the anorexia.

It has been established that serotonergic mechanisms are involved in the control of eating behavior, and that these mechanisms occur centrally and peripherally. The latter mechanism is less well known and is based on the finding that systemically administered 5-hydroxytryptamine (5-HT), which does not penetrate the blood-brain barrier (Oldendorf, 1971), produces a dose-dependent reduction in food intake (Bray & York, 1972; Clineschmidt, McGuffin, Pflueger, & Totaro, 1978; Edwards & Stevens, 1989; Fletcher & Burton, 1984; Pollock & Rowland, 1981). The anorexia produced by systemically administered 5-HT is not a result of sensorimotor disruption or of malaise (Fletcher & Burton, 1984; Montgomery, Fletcher, & Burton, 1986; Pollock & Rowland, 1981). Moreover, this anorectic effect is large enough to block both insulin- and 2-deoxyglucose-induced hyperphagias (Carruba et al., 1986). 5-HT produces the full behavioral sequence of satiety, and it has been argued that it must be considered a putative peripheral satiety signal (Edwards & Stevens, 1991b), although it has been reported that it fails to provide an adequate signal for complete satiety in the absence of post-ingestive cues in fistulated animals (Simansky, Jakubow, Sisk, Vaidya, & Eberle-Wang, 1992).

The investigation of a peripheral 5-HT mechanism using serotonergic agonists and antagonists is reliant on the availability of suitable drugs. For instance, the finding that peripheral 5-HT-induced anorexia can be blocked by 5-HT

antagonists such as methysergide (Fletcher & Burton, 1984) is difficult to interpret in this context since methysergide acts on both central and peripheral 5-HT receptors. Clearer evidence on the peripheral 5-HT system comes from the effects of 5-HT antagonists that do not pass the blood-brain barrier. Xylamidine does not cross the blood-brain barrier and, moreover, acts specifically on 5-HT₂ receptors. This drug has been found to attenuate the anorexia induced by peripheral 5-HT treatments (Edwards & Stevens, 1989; Fletcher & Burton, 1986).

Further support for the mediation of the peripheral serotonin anorectic effect by the 5-HT₂ receptor subtype comes from the finding that (+)-s- α -methyl-5-HT, a 5-HT₂ agonist that does not cross the blood-brain barrier, produces a dose-dependent reduction in eating in rats (Edwards, 1990). Moreover, this effect is blocked by xylamidine (Edwards & Stevens, 1989). When xylamidine has been used to antagonize 5-HT treatments, it fails to completely block the induced anorectic effect (Edwards & Stevens, 1989), which indicates that a non-5-HT₂ receptor subtype is involved in the suppression effect. Among the plausible candidates is the 5-HT₃ receptor since peripheral 5-HT₃ receptors are thought to be involved in cardiovascular (Saxena, 1989) and gastrointestinal (Engel, Buchheit, & Richardson, 1989) responses to 5-HT. But the evidence on this receptor subtype is confusing. Thus, although the peripheral 5-HT₃ receptor antagonist SDZ 206-608 does not attenuate peripheral 5-HT-induced anorexia, and may potentiate it, the highly selective 5-HT₃ receptor agonist 2-methyl-5HT has no effect on feeding behavior (Edwards, 1990).

The involvement of the 5-HT₃ receptor in the peripheral serotonergic mechanism that controls eating needs to be clarified. The experiments reported here used the specific 5-HT₃ antagonist [[3-(1-Methyl-1H-indol-3-yl)-1,2,4-

The authors would like to thank Merck, Sharpe and Dohme for the donation of [[3-(1-Methyl-1H-indol-3-yl)-1,2,4-oxadiazol-5-yl]methyl]trimethyl-ammonium iodide. Thanks are also extended to Kate Jenkins for help with testing and to Teresa Sharpe and Sarah Orchard for technical assistance with the animals. Correspondence should be addressed to R. Stevens, Department of Psychology, University of Nottingham, Nottingham NG7 2RD, U.K. (e-mail: rgs@psychology.nottingham.ac.uk).

oxadiazol-5-yl]methyl]trimethyl-ammonium iodide (MIO), which does not penetrate the blood-brain barrier (Swain et al., 1991). Nomura, Maeda, Yoshino, and Yamaguchi (1994) evaluated several 5-HT₃ antagonists and found that MOI was the most potent of the four tested (the others were GR38032F, ICS205-930, and MDL72222) in its ability to bind to 5-HT₃ receptors as well as in showing several orders of greater binding to 5-HT₃ receptors than to 5-HT₂ or 5-HT_{1A} receptors. In their behavioral tests on the effects of shock-induced stress effects, they used MOI in the dose range of 0.1–10 mg/kg. In the first study, the action of MOI in combination with systemically injected 5-HT was investigated. Based on the previous studies using 5-HT₃ agonists and antagonists, our prediction was that MIO would not antagonize the effects of 5-HT, but instead would potentiate them.

EXPERIMENT 1

Method

Subjects. The subjects were 20 male Wistar rats. They were 105 days old and weighed 270–320 g. The rats were housed individually in plastic cages that had stainless steel tops and removable food hoppers. The ambient temperature was kept at 22°C, and a 12:12-h light:dark cycle was maintained (lights on at 0730). They were fed Pillsbury chow pellets and provided with tap water ad lib.

Drugs. The drugs used were 5-hydroxytryptamine creatinine sulphate (5-HT; Sigma) and the specific peripheral 5-HT₃ antagonist [[3-(1-Methyl-1H-indol-3-yl)-1,2,4-oxadiazol-5-yl]methyl]trimethyl-ammonium iodide (Merck, Sharp and Dohme). Both drugs were dissolved in 0.9% saline and injected at volumes of 1.33 ml/kg (MIO) and 0.86 ml/kg (5-HT). The dosages of MIO were 0.625, 1.25, 2.5, and 5.0 mg/kg. A single dose of 5-HT (5 mg/kg) was used.

Procedure. The rats were habituated to a restricted feeding regime for 10 days before drug testing. Food was provided in a sawdust-free cage at 1100 h, and the rats were returned to their home cages at 1500 h. Mock saline injections, to about the same volume as those used in the experimental treatments, were given before feeding.

Two subcutaneous injections were given for each treatment, one at 0900 h and one at 1000 h. For five of the treatment conditions, the first injection was of one of the four doses of MIO or of saline, and the second injection was of 5-HT. In a sixth treatment, both injections were of saline. All rats received all treatments. The order of treatments was counterbalanced across animals, and 2 or 3 drug-free days were allowed between experimental treatments. Prewedged food (of at least 200 g) was presented at 1100 h. The food was reweighed at 1200, 1300, 1400, and 1500 h. Food crumbs from the cage floor were collected and weighed each time.

Results

A summary of the mean food intakes under each dose of MIO over time and the summed intake for each treatment is presented in Table 1. The intakes were analyzed using a 6 × 4 within-subjects analysis of variance (with treatments and time as factors). There were significant effects of treatments [$F(5,95) = 7.05, p < .0001$], and the rats ate significantly different amounts at the different, preset times [$F(3,57) = 131.48, p < .0001$].

When the effects of treatments were investigated further, using the Newman-Keuls test for pairwise comparisons, it was found that 5-HT alone significantly reduced food intake as compared with the saline-saline treatment ($p < .05$). Moreover, the combination of MIO and 5-HT significantly reduced overall food intake as compared with the saline-saline treatment for all MIO doses ($p < .01$ in each of the four comparisons). The only combined 5-HT-MIO treatment that differed from the 5-HT-saline treatment was the 2.5-mg dose of MIO ($p < .05$).

The time main effect arose primarily because the rats (in all treatment conditions) ate significantly more in the 1st hour of food availability than in any of the other 3 h ($p < .01$ for each pairwise comparison). There was significantly more eaten in Hour 4 than in Hour 3 ($p < .01$) and Hour 2 ($p < .01$).

Discussion

In accordance with previous findings, 5-HT alone suppressed eating behavior. When given in combination with 5-HT, all doses of MIO potentiated this anorectic effect. The peak effect of MIO occurred with the second highest amount (2.5 mg/kg), and at this dose it was found that the combined treatment (MIO and 5-HT) suppressed eating significantly more than 5-HT alone. Although these effects were found in intake over the 4-h feeding period, they reflect a drug effect that occurred primarily in the 1st hour of feeding, the only hour in which a significant treatment effect occurred (according to an analysis of simple effects). Thus, MIO did not prolong (beyond the 1st hour of food availability) the eating-suppression effect of 5-HT. Under all treatment conditions, the amount eaten after the 1st hour was similar, with all groups showing an increase in eating during the last hour. Thus, the process that generates the eating suppression produced by 5-HT alone or in combina-

Table 1
Food Intake (in Grams) for Each Treatment Over Time

Treatment	Hours Post Food Presentation								Sum of	
	1		2		3		4		Hours 1–4	
	<i>M</i>	<i>SEM</i>	<i>M</i>	<i>SEM</i>	<i>M</i>	<i>SEM</i>	<i>M</i>	<i>SEM</i>	<i>M</i>	<i>SEM</i>
Saline/saline	6.85	0.26	2.65	0.17	3.55	0.17	4.05	0.17	17.10	0.32
Saline/5-HT	5.55	0.38	2.65	0.29	3.45	0.28	3.75	0.35	15.40	0.73*
0.625/5-HT	5.25	0.31	2.70	0.28	3.10	0.32	3.60	0.34	14.65	0.45†
1.25/5-HT	4.85	0.28	2.95	0.21	2.75	0.27	3.55	0.22	14.10	0.54†
2.50/5-HT	5.15	0.33	2.25	0.19	2.85	0.22	3.15	0.26	13.40	0.55†
5.00/5-HT	5.10	0.33	2.40	0.23	3.10	0.27	3.30	0.26	13.90	0.54†

Note—The treatments show the dose of MIO and what was given in the second injection. Significance of the comparisons between the saline/saline condition and other treatments: * $p < .05$, † $p < .01$.

tion with MIO is still in effect in the last hour of food availability, otherwise the rats in the drug-treatment conditions would have compensated for their initial poor appetite.

The findings confirm those of Edwards (1990) in showing that peripheral 5-HT₃ antagonists do not block 5-HT-induced anorexia. Therefore, some other 5-HT receptor subtype in addition to the 5-HT₂ receptor has to be involved in peripheral serotonin-induced anorexia.

Edwards (1990) also found that SDZ 206-608 potentiates the anorectic effect of 5-HT, and the present study shows that MIO has the same effect. A surprising aspect of the present study is that the 2.5-mg/kg dose of MIO was more potent than the largest dose; this was the only dose of MIO and 5-HT that suppressed eating significantly more than 5-HT alone. An examination of the total intakes over the 4 h given in Table 1 shows what seems like a simple dose-response relationship between MIO and eating suppression that peaks at the 2.5-mg dose. It is not clear why the highest dose was less effective, but it may be related to an anomaly that occurred in the two experiments on SDZ 206-608 reported by Edwards (1990). In the first experiment, it was found that a 2-mg/kg dose of SDZ 206-608 potentiated the anorexia induced by 5 mg/kg of systemically injected 5-HT, and that a lower dose did not. In the second experiment, SDZ 206-608 was administered at 2.0, 4.0, and 6.0 mg/kg (in conjunction with the same dose of 5-HT as in the first experiment). However, only the largest dose potentiated the effect of 5-HT. There are no obvious reasons for the anomalous dose-response relationships for peripheral 5-HT₃ antagonist drugs.

Clearly the anorectic response generated by peripheral 5-HT is complex and has multiple components. Agonizing 5-HT₂ receptors partly accounts for the response, but antagonizing 5-HT₃ receptors potentiates the response. Thus, logically, we must predict that if hungry rats are treated just with a 5-HT₃ antagonist, there should be an anorectic response. This was tested in the next experiment by using MIO treatments that were not followed by injections of 5-HT.

EXPERIMENT 2

Method

Subjects. The subjects were 10 male Wistar rats. They were 100 days old and weighed 280–310 g.

Drugs. MIO was prepared as described previously, and the dosages were 0.625, 1.25, and 2.5 mg/kg. The highest dose was omitted because of the small amount of drug that was available, and the 5.0-mg dose in the previous experiment was less potent than the 2.5-mg treatment.

Procedure. Apart from the injection schedule, the procedure was the same as that in Experiment 1. The rats were injected subcutaneously with MIO or saline at 1000 h, 1 h before food was given. The order of treatments was counterbalanced across animals, and 2 or 3 drug-free days were allowed between experimental treatments.

Results

Table 2 presents a summary of the mean food intakes under each dose of MIO. A 4 × 4 within-subjects analysis of variance (with treatments and time as factors) was

used to analyze the feeding data. Although an examination of the treatment means shows that food intake was lower with MIO, the overall effect of treatment [$F(3,27) = 8.77$, $p = .15$] was not significant. Typically for this feeding schedule, it was found that the rats ate significantly different amounts at the different times [$F(3,27) = 77.28$, $p < .0001$]. Pairwise comparisons showed that animals ate more in the 1st hour than in each of the other 3 h ($p < .01$ in each of the three comparisons).

Discussion

The main effect of drug treatment was not significant, so it seems that antagonizing peripheral 5-HT₃ receptors in hungry rats does not produce a predicted anorexia. However, the amount eaten was less under all drug treatments than in the saline condition. Although it is recognized that it is not strictly correct to make comparisons between the treatment conditions when the overall treatment effect failed to reach significance, comparisons were made using the Duncan test. Food intake was significantly less ($p < .05$) under the 2.5-mg/kg dose of MIO than under the saline treatment. It is not being claimed that peripheral 5-HT₃ antagonists are able to induce an anorectic effect, but it seems sensible to remain agnostic on the issue.

There was an unexpected aspect of the time effect. In previous experiments it has been usual to find that food intake across 4 h is a J-shaped function. After an initial heavy eating bout in the 1st hour, animals consume small amounts in the next 2 h and then eat more in the last, 4th, hour. This was the pattern seen in Experiment 1. Here it was found that the smallest amount of food was eaten in the 4th hour. Since this pattern of eating was seen in the saline condition as well as under the three drug treatments, the effect is probably peculiar to this sample of rats and cannot be ascribed to MIO.

GENERAL DISCUSSION

Taken together, the two experiments reported here raise some problems about the role of 5-HT₃ receptors in peripheral 5-HT satiation mechanisms. Thus, we have the anomalous position where 5-HT-induced anorexia is potentiated when 5-HT₃ receptors are antagonized, but the antagonism of 5-HT₃ receptors in the absence of exogenous 5-HT fails to induce an anorectic response. Moreover, there is the reported failure of the 5-HT₃ agonist

Table 2
Food Intake (in Grams) for Each Treatment Over Time

Treatment	Hours Post Food Presentation							
	1		2		3		4	
	<i>M</i>	<i>SEM</i>	<i>M</i>	<i>SEM</i>	<i>M</i>	<i>SEM</i>	<i>M</i>	<i>SEM</i>
Saline	8.0	0.45	3.2	0.47	2.5	0.22	2.5	0.48
0.625 MIO	7.5	0.54	3.1	0.38	2.5	0.40	1.9	0.35
1.25 MIO	7.2	0.33	2.2	0.63	2.7	0.30	3.1	0.35
2.50 MIO	7.1	0.61	2.3	0.42	2.7	0.47	2.5	0.56
Means	7.5		2.70		2.6		2.5	

2-methyl-5HT to produce an increase in food intake (Edwards, 1990). The latter finding may be a result of 2-methyl-5HT's acting centrally as well as peripherally and the central effect's possibly opposing its peripheral action. 5-HT₃ receptors have been discovered in the central nervous system (Tyers, Costall, & Naylor, 1989), and there are high concentrations of these receptors, in various species, in the nucleus of the solitary tract, where they are present on vagal afferent terminals (Pratt & Bowerly, 1989). But there is doubt about the involvement of 5-HT₃ receptors in *d*-fenfluramine-induced anorexia that is primarily a central effect (Lawton & Blundell, 1993; Neill & Cooper, 1989). To resolve the question properly, we need to determine what effect a peripherally active and specific 5-HT₃ receptor agonist would have on eating behavior.

In summary, according to the results of Experiment 1, 5-HT₃ receptors are not the non-5-HT₂ receptors that act in conjunction with 5-HT₂ receptors (Edwards & Stevens, 1991a, 1991b) to produce peripheral 5-HT-induced anorexia. Although there is some doubt raised because of the failure to find an anorectic effect from the antagonism of peripheral 5-HT₃ receptors in the absence of exogenous 5-HT, activation of 5-HT₃ receptors may play a part in masking 5-HT-induced anorexia.

REFERENCES

- BRAY, G. A., & YORK, D. A. (1972). Studies on food intake of genetically obese rats. *American Journal of Physiology*, **223**, 176-179.
- CARRUBA, M. O., MANTEGAZZA, P., MEMO, M., MISSALE, C., PIZZI, M., & SPANO, P. F. (1986). Peripheral and central mechanisms of action of serotonergic anorectic drugs. *Appetite*, **7**(Suppl.), 105-113.
- CLINESCHMIDT, B. V., MCGUFFIN, J. C., PELUGER, A. B., & TOTARO, J. A. (1978). A 5-hydroxytryptamine-like mode of anorectic action for 6-chloro-2-[1-piperazinyl]-pyrazine (MK-212). *British Journal of Pharmacology*, **62**, 579-589.
- EDWARDS, S. (1990). *The behavioural pharmacology of peripheral 5-hydroxytryptamine-induced anorexia*. Unpublished doctoral dissertation, University of Nottingham.
- EDWARDS, S., & STEVENS, R. (1989). Effects of xylamidine on peripheral 5-hydroxytryptamine-induced anorexia. *Pharmacology, Biochemistry & Behavior*, **34**, 711-720.
- EDWARDS, S., & STEVENS, R. (1991a). Effects of the peripheral 5-HT₂ antagonist xylamidine on consummatory behaviors. *Psychobiology*, **19**, 243-246.
- EDWARDS, S., & STEVENS, R. (1991b). Peripherally administered 5-hydroxytryptamine elicits the full behavioral sequence of satiety. *Pharmacology, Biochemistry & Behavior*, **50**, 1075-1077.
- ENGEL, G., BUCHHEIT, K. H., & RICHARDSON, B. P. (1989). 5-HT₃ receptors in the gastrointestinal tract. In E. J. Mylencharane, J. A. Angus, I. S. de la Lande, & P. P. A. Humphrey (Eds.), *Serotonin: Actions, receptors, pathophysiology* (pp. 241-248). London: Macmillan.
- FLETCHER, P. J., & BURTON, M. J. (1984). Effects of manipulations of peripheral serotonin on feeding and drinking in the rat. *Pharmacology, Biochemistry & Behavior*, **20**, 835-840.
- FLETCHER, P. J., & BURTON, M. J. (1986). Dissociation of the anorectic effect of 5-HTP and fenfluramine. *Psychopharmacology*, **89**, 216-220.
- LAWTON, C. L., & BLUNDELL, J. E. (1993). 5-HT and carbohydrate suppression: Effects of 5-HT antagonists on the action of *d*-fenfluramine and DOI. *Pharmacology, Biochemistry & Behavior*, **46**, 349-360.
- MONTGOMERY, A. M. H., FLETCHER, P. J., & BURTON, M. J. (1986). Behavioral and pharmacological investigations of 5-HT hypophagia and hypodipsia. *Pharmacology, Biochemistry & Behavior*, **25**, 23-28.
- NEILL, J. C., & COOPER, S. J. (1989). Effects of 5-hydroxytryptamine and *d*-fenfluramine on sham feeding and sham drinking in the gastric-fistulated rat. *Physiology & Behavior*, **46**, 949-953.
- NOMURA, K., MAEDA, N., YOSHINO, T., & YAMAGUCHI, I. (1994). A mechanism of 5-HT₃ receptor mediation is involved etiologically in the psychological stress lesion in the stomach of the mouse. *Journal of Pharmacology & Experimental Therapeutics*, **271**, 100-106.
- OLDENDORF, W. H. (1971). Brain uptake of radiolabelled amino acids, amines, and hexoses after arterial injection. *American Journal of Physiology*, **221**, 1629-1639.
- POLLOCK, J. D., & ROWLAND, N. (1981). Peripherally administered serotonin decreases food intake in rats. *Pharmacology, Biochemistry & Behavior*, **15**, 179-183.
- PRATT, G. D., & BOWERLY, N. G. (1989). The 5-HT₃ receptor ligand [3H]BRL 43694 binds to presynaptic sites in the nucleus solitarius of the rat. *Neuropharmacology*, **28**, 1367-1376.
- PRATT, G. D., BOWERLY, N. G., KILPATRICK, G. J., LESLIE, R. A., BARNES, N. M., NAYLOR, R. J., JONES, B. J., NELSON, D. R., PALACIOS, J. M., SLATER, P., & REYNOLDS, D. J. M. (1990). Consensus meeting agrees the distribution of 5-HT₃ receptors in mammalian hindbrain. *Trends in Pharmacological Sciences*, **11**, 135-137.
- SAXENA, P. R. (1989). Cardiac action of 5-hydroxytryptamine. In E. J. Mylencharane, J. A. Angus, I. S. de la Lande, & P. P. A. Humphrey (Eds.), *Serotonin: Actions, receptors, pathophysiology* (pp. 115-122). London: Macmillan.
- SIMANSKY, K. J., JAKUBOW, J., SISK, F. C., VAIDYA, A. H., & EBERLE-WANG, K. (1992). Peripheral serotonin is an incomplete signal for satiety in sham feeding rats. *Pharmacology, Biochemistry & Behavior*, **43**, 847-854.
- SWAIN, C. J., BAKER, R., KNEEN, C., MOSELEY, J., SAUNDERS, J., SEWARD, E. M., STEVENSON, G., BEER, M., STANTON, J., & WATLING, K. (1991). Novel 5-HT₃ antagonists: Indole oxadiazoles. *Journal of Medicinal Chemistry*, **34**, 140-151.
- TYERS, M. B., COSTALL, B., & NAYLOR, R. J. (1989). 5-HT₃ receptors in the central nervous system. In E. J. Mylencharane, J. A. Angus, I. S. de la Lande, & P. P. A. Humphrey (Eds.), *Serotonin: Actions, receptors, pathophysiology* (pp. 95-100). London: Macmillan.

(Manuscript received February 2, 1995;

revision accepted for publication June 16, 1995.)