Pharmacology Biochemistry and Behavior, Vol. 45, pp. 589–592, 1993
Printed in the U.S.A. All rights reserved.

0091-3057/93 $6.00 + .00
Copyright © 1993 Pergamon Press Ltd.

The Selective 5-HT_3 Receptor Antagonist, Ondansetron, Augments the Anorectic Effect of *d*-Amphetamine in Nondeprived Rats

STEVEN J. COOPER,[1] SARAH E. GREENWOOD AND DAVID B. GILBERT

Laboratory of Psychopharmacology, School of Psychology, University of Birmingham, Birmingham B15 2TT, UK

Received 3 August 1992

COOPER, S. J., S. E. GREENWOOD AND D. B. GILBERT. *The selective 5-HT_3 receptor antagonist, ondansetron, augments the anorectic effect of* d*-amphetamine in nondeprived rats.* PHARMACOL BIOCHEM BEHAV **45**(3) 589–592, 1993.—Previous behavioural studies have shown that 5-hydroxytryptamine$_3$ (5-HT_3) receptor antagonists either block or have no effect on amphetamine-induced effects. The present experiments investigated whether or not the highly selective 5-HT_3 receptor antagonist ondansetron would affect the anorectic effect of a small dose (1 mg/kg) of *d*-amphetamine. Nondeprived male rats were tested in two feeding paradigms: consumption of a palatable sweetened mash and ingestion of a 3% sucrose solution. Ondansetron (10–100 μg/kg) did not antagonize amphetamine-induced anorexia; instead, in both paradigms consumption was reduced still further when the 5-HT_3 antagonist was given in conjunction with amphetamine. Ondansetron given alone had significant effects on consumption, but the direction of the effect differed according to the paradigm. Sweetened mash intake was significantly increased at 30 and 100 μg/kg, while sucrose ingestion was significantly reduced at 10 and 30 μg/kg ondansetron. It is suggested that ondansetron has two opposing effects on intake, one of which (hyperphagia) can be masked by *d*-amphetamine, leaving an anorectic effect that augments that of *d*-amphetamine.

Ondansetron *d*-Amphetamine 5-HT_3 receptors Anorexia

THERE is a growing interest in the behavioural effects of selective 5-hydroxytryptamine$_3$ (5-HT_3) receptor antagonists (1,7). In an early published study, Costall and colleagues reported that ondansetron (GR38032F) injected either into the nucleus accumbens or systemically blocked the effect of amphetamine to increase activity when it was injected into the nucleus accumbens (6). In addition, they found that ondansetron inhibited the hyperactivity response to intraaccumbens injection of dopamine. From these and other data, these authors concluded that ondansetron can reduce elevated dopaminergic activity in the mesolimbic system in both the rat and marmoset (6). In support of this idea, Hagan and colleagues (10) found that ondansetron reduced the increase in dopamine turnover in the nucleus accumbens produced by injection of a neurokinin analogue into the ventral tegmental area of rats. Interestingly, Costall et al. noted that ondansetron did not affect the hyperactivity induced by parenteral administration of amphetamine (6), and we confirmed that it did not affect amphetamine-induced hyperlocomotion (16). Subsequently, it has been reported that the 5-HT_3 antagonists ICS-205,930 and MDL 72222 did not affect an amphetamine-induced conditioned place preference and that several 5-HT_3 receptor antagonists, including ondansetron, failed to affect the discriminative stimulus effects of amphetamine (3,12) in rats. There is evidence, nevertheless, that both effects of amphetamine, the conditioned place preference and discriminative stimulus properties, are mediated, at least to some degree, by the nucleus accumbens (4,13).

We have been interested in the possible dopaminergic mediation of amphetamine-induced anorexia in rats (9), and it is a natural extension from this work to ask if a 5-HT_3 receptor antagonist, like ondansetron, should block this effect. A recent human psychopharmacological study by Silverstone and coworkers (15) is relevant to this question. They found that amphetamine decreased ratings of hunger in overnight-fasted subjects, as expected. Ondansetron attenuated amphetamine's effect. In the present study, *d*-amphetamine-induced anorexia was obtained in two groups of nondeprived rats: one trained to consume a highly palatable sweetened mash, while the other was trained to ingest a palatable 3% sucrose solution. In addition, effects of ondansetron alone were examined in the two paradigms, as well as in combination with *d*-amphetamine.

[1] To whom requests for reprints should be addressed.

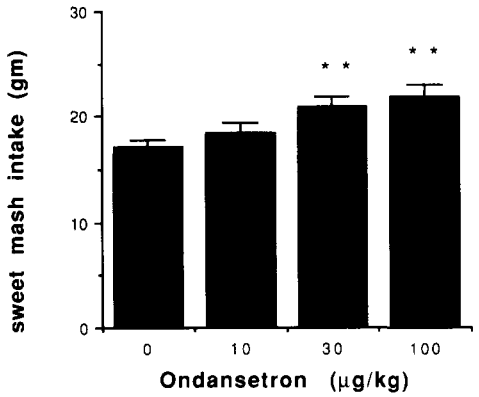

FIG. 1. The selective 5-hydroxytryptamine$_3$ (5-HT$_3$) receptor antagonist ondansetron (10–100 μg/kg) significantly increased the consumption of a sweetened mash in nondeprived rats over a 30-min test. The results are shown as mean ± SEM intake (g) (n = 20). Levels of significance: *$p < 0.05$; **$p < 0.01$ (Dunnett's test).

METHOD

Subjects

Animals were 40 adult, male hooded rats (General strain) bred in the School of Psychology. They were housed in pairs in plastic cages with ad lib access to Pilbury's rat and mouse breeding diet and water. They were maintained at a room temperature of 22°C with a 12 L : 12 D cycle (light on at 7:00 a.m.). Animals were weighed regularly before the tests to accustom them to handling. They weighed 300–400 g at the start of testing.

Drug Administration

Each animal served as its own control and was tested under eight conditions. In the first four conditions, rats received 0, 10, 30, or 100 μg/kg ondansetron together with an injection of *d*-amphetamine vehicle. In the second four conditions, they received 0, 10, 30, or 100 μg/kg ondansetron in combination with 1.0 mg/kg *d*-amphetamine. The vehicle was isotonic saline, and both drugs were injected 20 min before the test session. The IP route of administration was used in both cases. The order of injections was counterbalanced across animals, and at least 48 h was left between consecutive injections.

Test Procedures

Animals were divided into two groups. The first group was familiarised with the palatable sweetened mash over a period of 5 days. The diet consisted of 100 ml sweetened condensed milk, 300 ml ground maintenance diet No.1 (Special Diet Services Ltd, Essex, UK), and 400 ml distilled water. After thorough mixing, this sets to a firm consistency and each rat was given a 30- to 50-g portion in daily 30-min tests. For the feeding test, each animal was transferred to a stainless steel cage (24 × 24 × 27 cm). By the end of the familiarisation period, the latency to start eating was minimal. Consumption of the diet was measured to the nearest 0.1 g with corrections made for any spillage. The second group were familiarised with drinking a 3% sucrose solution presented in a 100-ml graduated tube over a 5-day period. The test period was 30 min and the volume of sucrose ingested was measured to the nearest 0.5 ml. The procedures were maintained for the two groups through the drug testing phase of the experiments.

Data Analysis

A repeated-measures analysis of variance was used to evaluate the drug effects on the consumption of sweet mash and sucrose. Dunnett's t-test was used to compare individual injection conditions with the vehicle control. The data for ondansetron alone are considered first in each case, followed by the data for the *d*-amphetamine–ondansetron combinations.

RESULTS

Sweet Mash Intake

Under control conditions, nondeprived rats consumed about 17 g of the sweetened mash in the 30-min test period.

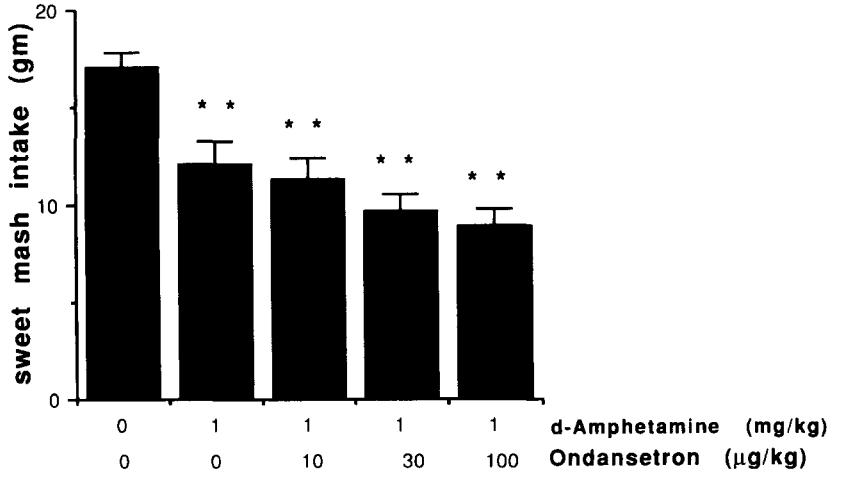

FIG. 2. The anorectic effect of 1.0 mg/kg *d*-amphetamine was enhanced by ondansetron (10–100 μg/kg) in rats consuming a sweetened mash over a 30-min test. Other details are as described in the Fig. 1 legend.

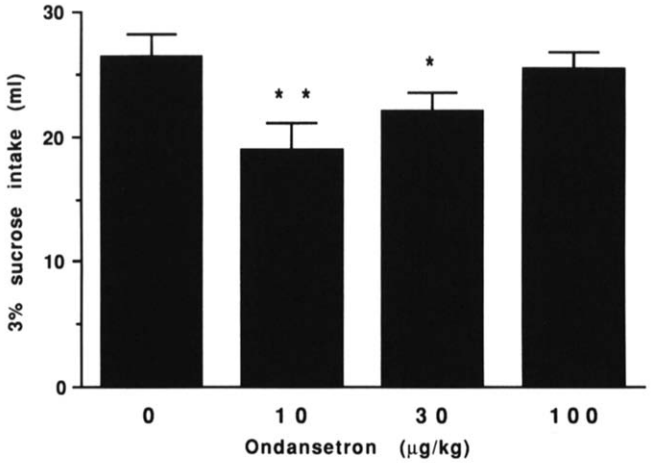

FIG. 3. Ondansetron significantly decreased the consumption of a 3% sucrose solution in a nonmonotonic manner. The most effective dose tested was 10 μg/kg. Other details are as described in the Fig. 1 legend.

Ondansetron (10–100 μg/kg) had a significant effect on intake, $F(3, 57) = 11.79$, $p < 0.001$. This was due to a hyperphagic effect, significant at both 30 and 100 μg/kg (Fig. 1). Intake after 100 μg/kg ondansetron was almost 22 g.

d-Amphetamine (1.0 mg/kg) produced an expected anorectic effect, significantly reducing the intake of food to 12 g in the 30-min test (Fig. 2). Ondansetron enhanced the anorectic effect so that in the condition when *d*-amphetamine was given in combination with 100 μg/kg ondansetron intake was reduced to less than 9 g (Fig. 2).

3% Sucrose Intake

Under control conditions, nondeprived rats consumed nearly 27 ml of the sucrose solution in the 30-min test period. Ondansetron (10–100 μg/kg) had a significant effect on intake, $F(3, 57) = 7.45$, $p < 0.001$. However, in this paradigm ondansetron exerted significant anorectic effects at 10 and 30 μg/kg (Fig. 3). Intake after 10 μg/kg ondansetron was reduced to less than 20 ml.

When *d*-amphetamine was coadministered with ondansetron, the result (Fig. 4) was surprisingly similar to the results for the sweetened mash. The *d*-amphetamine-induced anorexia was enhanced, in a dose-dependent way, by ondansetron. Comparisons with the effects of ondansetron, alone, on 3% sucrose intake indicate that there was no simple additive effect of the two drugs on intake. Thus, ondansetron (100 μg/kg) did not significantly affect sucrose intake when given alone but did produce the largest change in *d*-amphetamine-induced anorexia. Conversely, ondansetron (10 μg/kg) had the greatest effect when given alone but had relatively little impact on *d*-amphetamine-induced anorexia (Figs. 3 and 4).

DISCUSSION

In the two experiments, there was no indication that the selective 5-HT_3 receptor antagonist, ondansetron, antagonised amphetamine-induced anorexia in rats. Instead, the effect of amphetamine was enhanced in both cases by ondansetron. These results contrast with other behavioural results that indicate that under some circumstances 5-HT_3 receptor antagonists reduce amphetamine-induced effects (6) while under others they fail to affect amphetamine-induced effects (3,12). There is at present no single explanation for this diversity of results, so the original suggestion that ondansetron's effects can be attributed to a reduction in mesolimbic dopamine activity (6), if true, can only account for a restricted set of the behavioural data. An alternative possibility is that, under certain experimental conditions, 5-HT_3 receptor antagonists like ondansetron may enhance dopaminergic activity, and if this were to be the case then negative results (i.e., no apparent effect of 5-HT_3 antagonists on amphetamine-induced behavioural effects) are ambiguous.

Enhancement of amphetamine-induced anorexia by a selective 5-HT_3 receptor antagonist is an interesting finding and implies that serotonergic agonist activity at 5-HT_3 receptors may also modulate amphetamine's anorectic effect. The data suggest that more attention might be directed to possible sero-

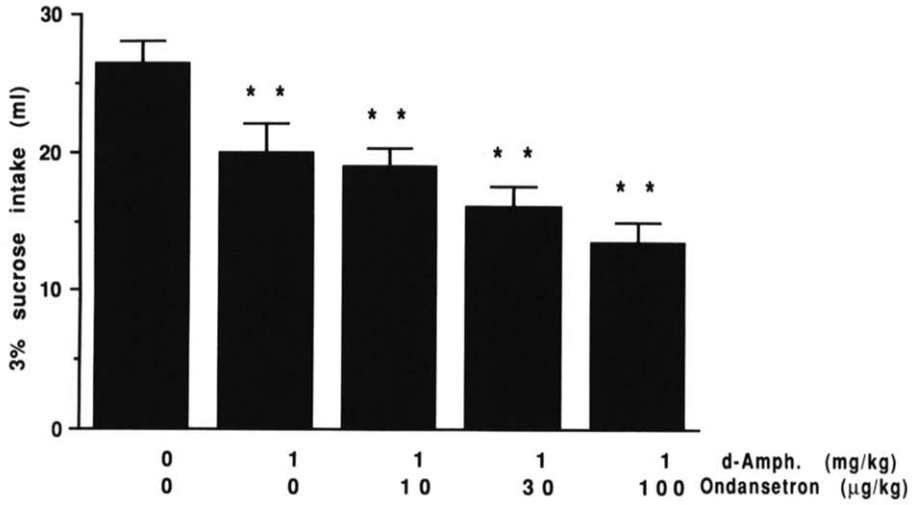

FIG. 4. The anorectic effect of 1.0 mg/kg *d*-amphetamine was enhanced by ondansetron (10–100 μg/kg) in rats ingesting a 3% sucrose solution over a 30-min test. Other details are as described in the Fig. 1 legend.

tonergic-dopaminergic interactions underlying amphetamine-induced anorexia and, more generally, of other drugs that affect appetite.

A further interesting outcome of these studies is that ondansetron exhibited either hyperphagic or anorectic effects when it was administered alone. Thus, there were significant increases in sweet mash consumption at 30 and 100 μg/kg ondansetron but significant decreases in 3% sucrose consumption at 10 and 30 μg/kg ondansetron. These data appear to point to two opposing effects of the 5-HT_3 receptor antagonists on feeding responses. In the first case, ondansetron may suppress feeding, the effect being present at relatively small doses. In the second, ondansetron may stimulate feeding, an effect that becomes more evident at higher doses. An experimental outcome would clearly depend upon the relative weights of the two opposing tendencies, which may vary according to the nature of the test paradigm. As a further proposal, we might add that *d*-amphetamine masks the hyperphagic tendency of ondansetron. This would leave the anorectic tendency of ondansetron unopposed, with the result that the feeding-suppressant effect of *d*-amphetamine would be reliably augmented.

Earlier findings for 5-HT_3 receptor antagonists and feeding have been recently reviewed (5). Shepherd and Rodgers detected an increase in the time spent feeding when ondansetron was administered to free-feeding male mice (14). In contrast, Fletcher and Davies found that ICS-205,930 significantly increased the latency to eat and reduced feeding duration in rats (8). ICS-205,930 has been found to potentiate naloxone's anorectic effect on food-deprived rats (2), while ICS-205,930 and MDL 72222 reversed the anorectic response to imbalanced amino acid diets in rats (11). The present data are the first to demonstrate that ondansetron, given alone, significantly either increased or decreased consumption depending upon the type of food available. Further research should help clarify the factors that lead one effect, or the other, to predominate and this should throw light on the nature of the modulatory effects of 5-HT_3 receptors antagonists on feeding responses.

ACKNOWLEDGEMENTS

The authors thank Glaxo Group Research for support and for the supply of ondansetron and Dorothy Trinder for preparing the manuscript.

REFERENCES

1. Barnes, J. M.; Barnes, N. M.; Cooper, S. J. Behavioural pharmacology of 5-HT_3 receptor ligands. Neurosci. Biobehav. Rev. 16: 107–113; 1992.
2. Beczkowska, I. W.; Bodnar, R. J. Naloxone and serotonin receptor subtype antagonists: Interactive effects upon deprivation induced intake. Pharmacol. Biochem. Behav. 38:605–610; 1991.
3. Carboni, E.; Acquas, E.; Leone, P.; DiChiara, G. 5-HT_3 receptor antagonists block morphine- and nicotine- but not amphetamine-induced reward. Psychopharmacology (Berl.) 97:175–178; 1989.
4. Carr, G. D.; White, N. M. Conditioned place preference from intra-accumbens but not intra-caudate amphetamine injections. Life Sci. 33:2551–2557; 1983.
5. Cooper, S. J. Ingestive responses following benzodiazepine receptor ligands, selective 5-HT_{1A} agonists and selective 5-HT_3 receptor antagonists. In: Rodgers, R. J.; Cooper, S. J., eds. 5-HT_{1A} agonists, 5-HT_3 antagonists and benzodiazepines: Their comparative behavioural pharmacology. Chichester, UK: John Wiley; 1991: 233–265.
6. Costall, B.; Domeney, A. M.; Naylor, R. J.; Tyers, M. B. Effects of the 5-HT_3 receptor antagonist GR 38032F on raised dopaminergic activity in the mesolimbic system of the rat and marmoset brain. Br. J. Pharmacol. 92:881–894; 1987.
7. Costall, B.; Naylor, R. J.; Tyers, M. B. Recent advances in the neuropharmacology of 5-HT_3 agonists and antagonists. Rev. Neurosci. 2:41–66; 1988.
8. Fletcher, P. J.; Davies, M. Effects of 8-OH-DPAT, buspirone and ICS 205-930 on feeding in a novel environment: Comparisons with chlordiazepoxide and FG 7142. Psychopharmacology (Berl.) 102:301–308; 1990.
9. Gilbert, D. B.; Cooper, S. J. Analysis of dopamine D_1 and D_2 receptor involvement in *d*- and *l*-amphetamine induced anorexia. Brain Res. Bull. 15:385–389; 1985.
10. Hagan, R. M.; Butler, A.; Hill, J. M.; Jordan, C. C.; Ireland, S. J.; Tyers, M. B. Effect of the 5-HT_3 receptor antagonist, GR 38032F, on responses to injection of a neurokinin agonist into the ventral tegmental area of the rat brain. Eur. J. Pharmacol. 138:303–305; 1987.
11. Hammer, V. A.; Gietzen, D. W., Beverly, J. L.; Rogers, Q. R. Serotonin$_3$ receptor antagonists block anorectic responses to amino acid imbalance. Am. J. Physiol. 259:R627–R636; 1990.
12. Moser, P. C. The effect of 5-HT_3 receptor antagonists on the discriminative stimulus effects of amphetamine. Eur. J. Pharmacol. 212:271–274; 1992.
13. Nielsen, E. B.; Scheel-Krüger, J. Cueing effect of amphetamine and LSD: elicitation by direct microinjection of the drugs into the nucleus accumbens. Eur. J. Pharmacol. 125:85–92; 1986.
14. Shepherd, J. K.; Rodgers, R. J. 8-OH-DPAT specifically enhances feeding behaviour in mice: Evidence from behavioural competition. Psychopharmacology (Berl.) 101:408–413; 1990.
15. Silverstone, P. H.; Johnson, B.; Cowen, P. J. Does ondansetron attenuate amphetamine-induced behaviour in human volunteers? Psychopharmacology (Berl.) 107:140–141; 1992.
16. Van der Hoek, G. A.; Cooper, S. J. Antagonism of amphetamine-induced sniffing, but not hyperlocomotion, by the selective 5-HT_3 antagonist, ondansetron. Br. J. Pharmacol. 100:414P; 1990.