Pharmacology Biochemistry and Behavior, Vol. 47, pp. 59–63, 1994
Printed in the U.S.A.

0091-3057/94 $6.00 + .00

Anorectic Response to Amino Acid Imbalance: A Selective Serotonin$_3$ Effect?

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Received 15 March 1993

JIANG, J. C. AND D. W. GIETZEN. *Anorectic response to amino acid imbalance: A selective serotonin$_3$ effect?* PHARMACOL BIOCHEM BEHAV 47(1) 59–63, 1994.—The anorectic responses to imbalanced amino acid diets (IMB) are ameliorated by pretreatment with large (mg/kg) doses of the serotonin antagonists, tropisetron [3-α-tropanyl-1*H*-indole-3-carboxylic acid ester, formerly known as ICS-205,930 (ICS)] and MDL 72,222 [1α*H*,3α,5α-*H*-tropan-3-*yl*-3,5-dichlorobenzoate (MDL)], effects earlier attributed to the 5-hydroxytryptamine$_3$ (5-HT$_3$) receptor. Subsequent identification of the 5-HT$_4$ receptor, and recognition that ICS and MDL also bind to 5-HT$_4$ receptors, led us to question whether the results seen with these drugs were due to activity at the 5-HT$_3$ or 5-HT$_4$ receptor subtype. 1,2,3,9-Tetrahydro-9-methyl-3 [(2-methyl-1*H*-imidazol-1-*yl*) methyl] 4*H*-carbazol-4-one) [ondansetron (OND)], a reportedly 5-HT$_3$-selective receptor antagonist, has been used to block 5-HT$_3$ receptors in demonstrating the 5-HT$_4$ receptor, and so seems securely selective for the 5-HT$_3$ receptor type. Therefore, we tested the effects of OND on the rat's feeding responses to IMB. Pretreatment with 0.1 or 1 μg/kg OND fully restored intake of IMB to > 100% of control between 6 and 12 h after introduction of IMB. We conclude that the previous similar increases in IMB intake seen after ICS and MDL were due to their antagonist activity at the 5-HT$_3$ receptor and that the 5-HT$_3$ receptor may have an important role in mediating the rat's anorectic responses to IMB.

Rat Amino acids Deficiency Imbalance Physiology Feeding behavior Drug effects
Serotonin$_3$ receptor Serotonin Pharmacology Ondansetron Anorexia Dietary choice

RATS rapidly and reliably reduce their food intake when offered a diet that is imbalanced with respect to the essential amino acids (15,21,25,26). The biochemical responses to an amino acid-imbalanced diet (IMB) include a plasma amino acid pattern similar to that seen in severe amino acid deficiency (15). A reduction in food intake is the first behavioral sign of a response to the amino acid deficiency induced after the first meal of IMB (11). Studies of the postingestive effects of IMB have shown that responses to these diets involve, first, recognition of the imbalance and, second, rejection of the diet. The rejection phase is most likely accompanied by development of a learned aversion (2,8,10–12,25,26).

Serotonin [5-hydroxytryptamine (5-HT)] has been implicated in the CNS control of feeding for many years (1,9, 16,19). Peripherally injected 5-HT, which does not cross the blood–brain barrier (22), also affects food intake and has been demonstrated to produce a dose-dependent anorexia (7,23) in 18- and 24-h food-deprived rats. More recent research on 5-HT systems has demonstrated that postsynaptic 5-HT receptor agonists not only cause marked anorexia in rats (9,19) but also exacerbate the anorectic response of rats to IMBs (13). Hammer et al. (14) studied the 5-HT receptor subtype(s) responsible for mediating the anorexigenic effects of IMB. Antagonists selective at the 5-HT$_1$, 5-HT$_2$, dopamine, and α-adrenergic sites, or at combinations of these receptor sites, did not affect intake of IMB. In contrast, the 5-HT$_3$ receptor antagonists, tropisetron [3-α-tropanyl-1*H*-indole-3-carboxylic acid ester, formerly known as ICS-205,930 (ICS)] and MDL 72,222 [1α*H*,3α,5α-*H*-tropan-3-*yl*-3,5-dichlorobenzoate (MDL)], significantly increased intake of both mild and severe IMB (14). Thus, it was concluded that the 5-HT$_3$ receptor subtype was the primary 5-HT mediator in this feeding model.

Later findings by Dumuis et al. (5) demonstrated the existence of a 5-HT$_4$ receptor (17) that is also antagonized by high doses of ICS and MDL, doses comparable to those used by Hammer et al. (14). This led us to question whether the 5-HT receptor responsible for mediating intake of IMB was the 5-HT$_3$ or 5-HT$_4$ subtype. In the present study, we used the selective 5-HT$_3$ receptor antagonist, 1,2,3,9-tetrahydro-9-methyl-3 [(2-methyl-1*H*-imidazol-1-*yl*) methyl] 4*H*-carbazol-4-one) [ondansetron (OND)] [as ondansetron HCl dihydrate, previously known as GR38032F, Glaxo Group Research Ltd. (3,4)]. OND has been used in vitro to block 5-HT$_3$ receptors in the demonstration of 5-HT$_4$ effects (6,24), showing its lack of

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effect at the 5-HT_4 receptor. Therefore, if OND increased intake of IMB to levels previously demonstrated for ICS and MDL (14) we should be able to conclude that the effects of ICS and MDL were indeed due to their activities at the 5-HT_3 and not the 5-HT_4 receptor. This would provide further more conclusive evidence for involvement of the 5-HT_3 receptor in the mechanisms underlying the rat's rejection of IMB.

METHOD

Sixty male albino rats weighing 220–250 g (Simonson Laboratories, Inc., Gilroy, CA) were housed individually in stainless steel hanging wire cages in a vivarium maintained at 22 ± 2°C on a 12 D : 12 L cycle, with lights out at 11:00 a.m. They were maintained on stock diet (chow; Purina 5001, Ralston, St. Louis, MO) and water ad lib for the first few days after arrival to allow for adaptation to the environment. Rats were then switched to a low-protein basal diet with purified L-amino acids as the protein source and isoleucine as the growth-limiting amino acid (BAS). The composition of the diets used in this study has been published elsewhere (14,21). Rats were maintained on the BAS diet for 12 days before the first experimental day. During the last 3 days of adaptation to the BAS diet, baseline food intake measurements were made at 3-, 6-, 9-, 12-, and 24-h intervals and corrected for spillage. On the third baseline day, animals were weighed and assigned by body weight to one of 10 groups in a 5 × 2 factorial design. The drug conditions were 0.1, 1.0, 10, and 100 μg/kg OND or saline vehicle (VEH); the diet conditions were BAS or an isoleucine-imbalanced diet [IMB; (14,21)]. Thus, the groups were: VEH-BAS; VEH-IMB; 0.1 μg/kg OND-BAS; 0.1 μg/kg OND-IMB; 1.0 μg/kg OND-BAS; 1.0 μg/kg OND-IMB; 10 μg/kg OND-BAS; 10 μg/kg OND-IMB; 100 μg/kg OND-BAS; and 100 μg/kg OND-IMB. On the experimental day, IP injections of OND or VEH were begun 60 min prior to lights out. Following completion of injections, animals were presented with a clean, preweighed food cup, containing either IMB or BAS, at the onset of the dark period. Food intake was measured and corrected for spillage at 3-, 6-, 9-, 12-, and 24-h intervals for the first 24 h following injection and daily for the next 2 days. Statistical analysis consisted of analysis of variance (ANOVA) with interactions between drug and diet, using the general linear model and posthoc testing with LS means on PC-SAS version 6.03 (SAS, Cary, NC). Values reported are means ± SE. Significance was assumed at $p < 0.05$.

RESULTS

Pretreatment with OND increased intake of the isoleucine-imbalanced diet. Results similar to those seen by Hammer et al. (14) with ICS and MDL administration were observed on the first experimental day during the 3- to 6-h, 6- to 9-h, 0- to 9-h, and 9-to 12-h time intervals after OND treatment (Fig. 1). The effect of OND during the 3- to 6-h period was to increase intake of IMB in groups treated with the 0.1- and 1.0-μg/kg doses [overall, $F(9, 50) = 10.43$, $p < 0.0001$; effect of OND, $F(4, 50) = 4.36$, $p = 0.004$]. After 0.1- and 1.0-μg/kg OND, intake of IMB was increased to 221 and 224% of that for the VEH-IMB group, respectively ($p < 0.04$ for each dose).

During the 6- to 9-h period (Fig. 2A), OND at the 0.1-μg/kg dose increased IMB intake to 188% of the VEH-IMB group ($p < 0.02$) (or 112% of BAS intake by the VEH-BAS

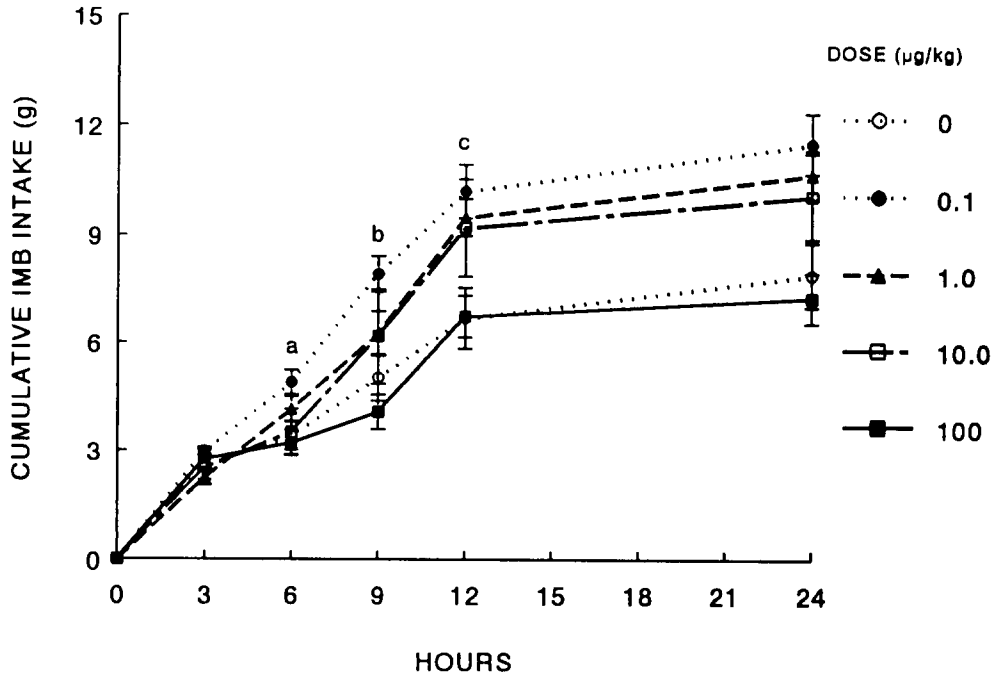

FIG. 1. Effect of ondansetron preinjections on cumulative intake of an isoleucine-imbalanced diet (IMB) over the first 24 h after introduction of the test diets at the onset of the dark cycle. Markers, mean intake (in g); error bars, SE; a, 0.1- and 1.0-μg/kg doses significantly greater than VEH; b, 0.1 μg/kg dose greater than VEH; c, 1.0- and 10-μg doses greater than VEH; all $p < 0.05$. Doses indicated in the legend are in μg per kg body weight.

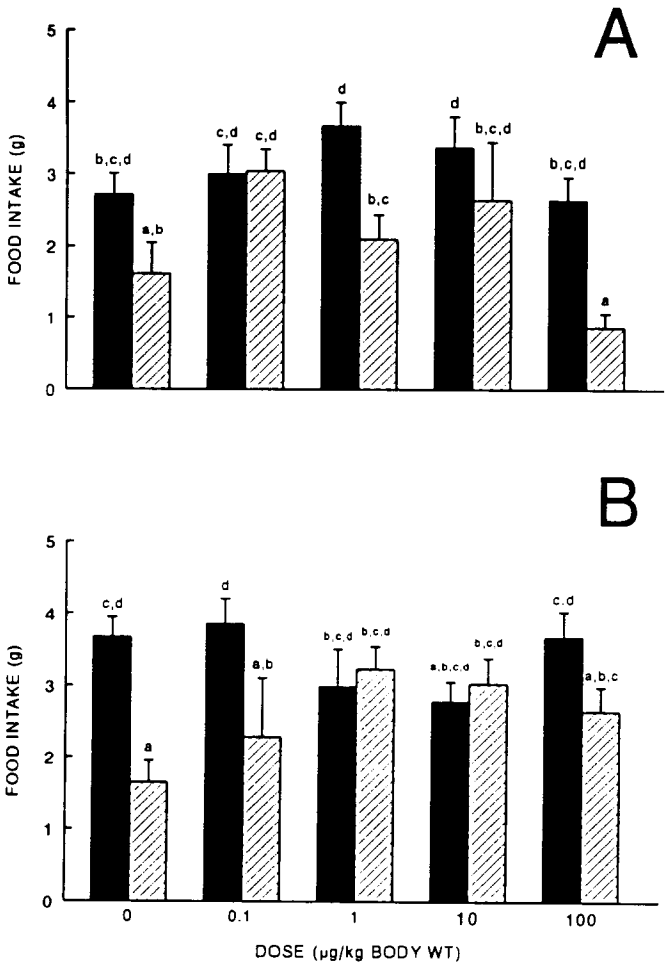

FIG. 2. Effect of ondansetron preinjections on food intake measured (A) between 6 and 9 h or (B) between 9 and 12 h after introduction of the test diets. Bars represent mean intake of the diets (in g): black bars, basal diet (BAS); hatched bars, isoleucine-imbalanced diet (IMB); error bars, SE. Values with different letters are significantly different at $p < 0.05$.

group). Overall, $F(9, 50) = 4.07$, $p < 0.001$. For the effect of OND, $F(4, 50) = 3.83$, $p < 0.01$. Cumulative intake was still increased during the 0- to 9-h period in the OND-IMB group treated with 0.1 μg/kg OND, reaching 157% of the intake of the VEH-IMB group. For the 0-to 9-h period, overall, $F(9, 50) = 18.57$, $p < 0.0001$. For the effect of OND, $F(4, 50) = 3.53$, $p < 0.02$. The dose × diet interaction was significant ($p = 0.02$). Significance of the increase in food intake for the IMB group given 0.1 μg/kg OND vs. the VEH-IMB group was $p < 0.01$. There was also a significant ($p < 0.04$) increase in intake of BAS diet in the 1.0-μg/kg group vs. the VEH-BAS group during the 6- to 9-h period only.

The significant effect of OND during the 9- to 12-h period was due to increased IMB intakes in the 1.0- and 10-μg/kg groups, which were increased over intake of the VEH-IMB group by 195% ($p = 0.01$) and 184% ($p < 0.03$), respectively (Fig. 2B). Intake of the 100-μg/kg OND-IMB group was intermediate between the VEH-BAS and VEH-IMB groups and was therefore not statistically different from either VEH-treated group ($p = 0.09$ and $p = 0.1$ for the BAS and IMB comparisons, respectively).

For the 0- to 24-h period, intakes of the 0.1- and 1.0-μg/kg OND-IMB groups were intermediate between the VEH-BAS and VEH-IMB groups and were therefore not statistically different from either VEH group ($p = 0.46$ and $p = 0.51$ for the 0.1-μg/kg OND-IMB group vs. the VEH-BAS and VEH-IMB groups, respectively, and $p = 0.71$ and 0.77 for the 1.0-μg/kg OND-IMB group vs. the VEH-BAS and VEH-IMB groups, respectively). For the 0- to 24-h period, overall, $F(9, 50) = 0.41$, $p = 0.92$. For the effect of OND, $F(4, 50) = 0.74$, $p = 0.57$. As expected, the IMB diet reduced intake in the vehicle-treated control group at each time period ($p < 0.0001$).

On the second and third days after the single OND treatment on day 1, the only significant differences among groups were due to the IMB diet. There was no significance for drug or drug × diet interaction (all $p > 0.1$).

More recently, in a second study using an imbalanced diet with threonine [see diet composition in (14)], rather than isoleucine as the limiting amino acid in an otherwise identical protocol, similar results were found. For example, intake of the threonine imbalanced diet by the 10-μg/kg OND-IMB group was 164% of the vehicle-treated group during the 9- to 12-h period (other data not shown).

The results for OND were compared with our earlier data for ICS and MDL, each at its most effective time point [ICS and MDL data were taken from (14)], and are presented in Fig. 3.

DISCUSSION

As with ICS, a 5-HT_3 receptor antagonist that crosses over to the 5-HT_4 receptor at high doses, OND, a selective 5-HT_3 antagonist, at much lower (μg/kg) doses, restored IMB intake to over 100% of BAS intake (Figs. 1 and 2). By the 6- to 9- or 9- to 12-h intervals, OND (0.1 μg/kg or 1.0 and 10 μg/kg) had restored intake of IMB to 102%, or 108 and 109% of the intake by respective control groups that were fed BAS. In comparing the efficacy of the three antagonists, OND, ICS, and MDL, in restoring intake of IMB, data for the ICS and MDL groups were taken from previous work (14) and therefore statistical comparisons were not made across these groups. Still, it is clear that OND in μg/kg doses was at least as effective as ICS (mg/kg doses) and more effective than MDL (mg/kg doses) in ameliorating the anorexigenic effects of IMB (Fig. 3). Receptor affinities for these three compounds are similar: In rat vagus, pK_B values are MDL 7.9, OND 8.6; in rat brain, pK_i values are MDL 7.5, OND 8.5, and ICS 8.7 (20). However, behavioral tests have yielded a variety of response profiles, for example, some antagonists have no effect in certain tests of behavioral suppression (20). Thus, the high potency of OND in this study, relative to ICS and MDL, should not be considered unusual, but may lead to further work in understanding these dose–response effects.

At the low dose, decreasing efficacy of the 0.1-μg/kg dose with time indicates that a smaller dose of OND would not increase intake of IMB above levels already observed in this study. In addition, a dose of 0.01 μg/kg OND was used in a separate study. There were no changes in intake of BAS or IMB after this low dose at any time period (data not shown). The increased efficacy of the larger doses (1.0 and 10 μg/kg) of OND at the later time interval (i.e., at the 9-to 12-h interval compared with the 6-to 9-h interval) is consistent with the pharmacokinetics and short half-life of OND (27). In addi-

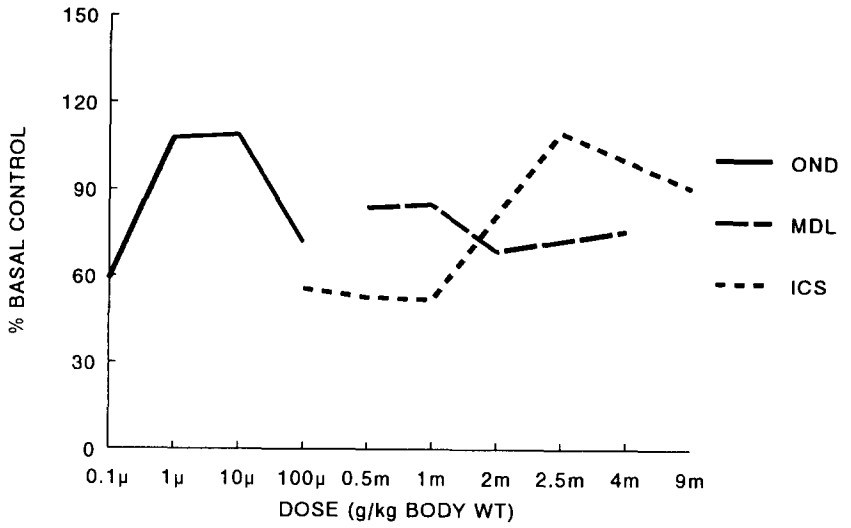

FIG. 3. Comparison of the efficacy of ondansetron (OND), ICS,205-930 (ICS), and MDL 72,222 (MDL) preinjections on intake of an isoleucine-imbalanced diet (IMB) as a percent of each animal's control basal diet intake. Data for each drug are represented for the time period in which the greatest increase in IMB intake was recorded. ICS, 6–12 h; MDL, 0–24 h; OND, 9–12 h. Data for ICS and MDL are taken from (14). Food intake given on the *Y*-axis is intake of IMB as a percent of BAS (control) intake. Doses indicated on the *X*-axis are either μg or mg per kg body weight, as indicated.

tion, these observations, along with the observations that the 100-μg/kg dose reduced BAS intake and did not restore feeding of IMB to BAS control levels, also suggest that further increases in the dose of OND would not increase the efficacy of OND in this feeding model. While benzodiapezines can cause sedation at high doses, 5-HT_3 antagonists have not been reported to have this effect. A decline in responsiveness has, however, frequently been reported at higher doses in other behavioral models [reviewed in (20)]. Because the highest dose of OND reduced intake of the BAS control diet, a general anorectic effect of this largest dose could have blocked the more specific antianorectic effect of OND with respect to the IMB diet. Thus, the 0.1-, 1.0-, and 10-μg/kg doses used in the present study can be seen to be appropriate.

Intake of the control (BAS) diet following OND treatment was increased over intake of the VEH-injected control group in only one OND-treated group (1.0 μg/kg) during only one time period, the 0- to 9-h interval, and, therefore, the present results are not due to a generalized increase in feeding by OND-treated animals. Also, replication of the present findings with a different limiting essential amino acid (threonine) show that the results with isoleucine imbalanced are not specific to isoleucine. Similar results were seen with ICS using both isoleucine and threonine as the limiting amino acid (14).

ICS has been reported to act peripherally in the IMB model (18), perhaps acting at the level of the vagus (30). In the present work, OND was given IP, as were ICS and MDL in the previous studies (14). Thus, while it is possible that OND may also be acting peripherally, a locus of action has not been determined for OND in this model.

Antagonism of the 5-HT_3 receptor has been shown to block chemotherapy-induced vomiting in the range of doses used in the present work (4). Tyers (29) has suggested that nausea such as that seen after chemotherapy may induce conditioned taste aversions (CTAs) via vagally associated 5-HT_3 receptors. In studies designed to determine the mechanism of 5-HT's action in the responses to IMB, we have used ICS in CTA paradigms, including the LiCl-induced CTA to saccharin solutions. In several trials, the CTA to a saccharin solution was blocked by prior treatment with ICS (12). Unfortunately, more recently, we have been unable to replicate these findings using saccharin in solution (Gietzen, unpublished). While we currently have no explanation for this discrepancy, it may be understandable because the CTA responses to a variety of pharmacological agents have been reported to vary depending on the route, dose, pretreatment, and many other experimental variables (9). Nonetheless, after both ICS and MDL we have repeatedly observed blockade of CTA to saccharin when it was included in powdered form in an IMB diet (28). Thus, although further clarification of this issue is necessary, the 5-HT_3 receptor-mediated mechanism in the IMB model may be associated with CTA.

In summary, low doses (0.1 and 1.0 μg/kg) of OND, a selective 5-HT_3 antagonist with no activity at the 5-HT_4 receptor, fully restored feeding of IMB to control levels. These results are consistent with the hypothesis that serotonin acts selectively at the 5-HT_3 receptor in mediating the anorectic responses to the amino acid deficiency induced by IMB. We conclude that (unless, in the future, OND proves not to be selective for the 5-HT_3 receptor) the 5-HT_3, rather than the 5-HT_4, receptor is involved in ameliorating the anorectic response to IMB. Likewise, the previously reported effects of ICS in our aminoprivic model (14) may even more confidently be attributed to action at the 5-HT_3 receptor. Further investigation is clearly necessary for a full understanding of the mechanisms and the locus of 5-HT_3 receptor action in this model.

ACKNOWLEDGEMENTS

The excellent assistance of Jennifer Mueller, Jay King, and Kimberly Dixon in this study is greatly appreciated. This work was supported by USDA Grant CRCR 90-37200-5440 and NIH Grants

DK42274-01 and DK35747-04. Ondansetron HCl dihydrate was a gift of Glaxo Group Research Ltd. Amino acids were a gift from Nutri-Quest. Portions of these data were submitted by J.C.J. in partial satisfaction of the requirements for the masters degree in nutrition at the University of California, Davis, and have appeared in abstract form (FASEB J. 6:A1099; 1992).

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