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Naltrexone, Serotonin Receptor Subtype Antagonists, and Glucoprivic Intake: 2. Insulin

JAMES E. KOCH,* IWONA W. BECZKOWSKA† AND RICHARD J. BODNAR*†[1]

**Department of Pharmacology, Mount Sinai School of Medicine, New York, NY 10029*
†Department of Psychology and Neuropsychology Doctoral Subprogram
Queens College, City University of New York, Flushing, NY 11367

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KOCH, J. E., I. W. BECZKOWSKA AND R. J. BODNAR. *Naltrexone, serotonin receptor subtype antagonists, and glucoprivic intake: 2. Insulin.* PHARMACOL BIOCHEM BEHAV 42(4) 671-680, 1992. – Opiate antagonist inhibition of deprivation-induced intake and 2-deoxy-D-glucose (2DG) hyperphagia is significantly enhanced by the 5-hydroxytryptamine$_3$ (5-HT$_3$) antagonist, ICS-205,930. Interactions between opiate antagonists and either 5-HT or 5-HT$_2$ antagonists produced smaller effects. The present study evaluated whether insulin (5 U/kg) hyperphagia was affected by methysergide (0.5-5 mg/kg), ritanserin (0.25-2.5 mg/kg), and ICS-205,930 (0.5-5 mg/kg) alone or in combination with naltrexone (2.5-10 mg/kg). Whereas ICS-205,930 stimulated insulin hyperphagia across the 6-h time course, ritanserin and, to a lesser degree, methysergide reduced insulin hyperphagia. Naltrexone marginally (19-33%) reduced insulin hyperphagia. Pairing naltrexone with either ICS-205,930 or ritanserin significantly suppressed insulin hyperphagia after 2 h. Pairing naltrexone with each of the serotonin antagonists significantly enhanced insulin hyperphagia after 4 and 6 h. These data suggest that 5-HT$_2$ and 5-HT$_3$ receptor subtypes interact with opioid systems to modulate insulin hyperphagia. Given that central insulin reduces food intake and body weight, the interaction between serotonergic and opioid systems may occur peripherally.

Insulin hyperphagia Naltrexone Methysergide Ritanserin ICS-205,930 Serotonin receptors

MULTIPLE serotonin (5-HT) receptors [see reviews: (41,42)] have been categorized into 5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{1C}$, 5-HT$_{1D}$, 5-HT$_2$, and 5-HT$_3$ subtypes. 5-HT$_{1A}$ receptor agonists stimulate feeding putatively through activation of 5-HT autoreceptors (14,15,24,25). 5-HT $_{1B}$ and 5-HT $_{1C}$ receptor agonists inhibit intake (23,28,48). 5-HT receptor antagonists have not produced such consistent effects. Methysergide, a nonselective 5-HT antagonist, marginally reduces deprivation-induced feeding (3,16), fails to affect spontaneous feeding during the light cycle (5), but stimulates intake in well-satiated rats (13,17). Whereas the 5-HT$_2$ antagonists ritanserin and ketanserin typically fail to alter spontaneous intake [(5,13,21, 28,34,51), but see (17)], ketanserin but not ritanserin marginally reduces deprivation-induced feeding (3). Whereas the 5-HT$_3$ antagonist ICS-205,930 failed to affect deprivation-induced feeding (3), it significantly stimulated free feeding during the light cycle for up to 4 h (5) and blocked anorexia induced by amino acid imbalance (20). In a companion article (5), our laboratory demonstrated that whereas neither methysergide nor ICS-205,930 affected 2-deoxy-D-glucose (2DG) hyperphagia, ritanserin produced a short-acting and significant reduction in this response.

The hyperphagic responses following acute exposure to 2DG (53) and insulin (8,9,54) dissociate from each other. 2DG but not insulin hyperphagia is reduced following either subdiagphragmatic vagotomy or lesions placed in the ventromedial hypothalamus, zona incerta, medial forebrain bundle, or midbrain tegmentum (7,19,35,36,47,49,59). Opioid control of 2DG hyperphagia has been confirmed by significant reductions following peripheral (33) and central (1) administration of general opioid antagonists, as well as significant reductions following central administration of μ-selective [β-funaltrexamine: (2)] and κ-selective [norbinaltorphamine: (1)] opioid antagonists. In contrast, general opioid receptor antagonists produce only moderate effects upon (4,31,38,46) or fail to affect (33) insulin hyperphagia. Further, insulin hyperphagia is significantly reduced by the μ-selective antagonist β-funaltrexamine but not by antagonists of the μ_1-, κ- and δ-receptor subtypes (4). Indeed, the magnitude of inhibition following either naltrexone or β-funaltrexamine was more pronounced for 2DG hyperphagia relative to insulin hyperphagia.

Interactions between 5-HT and opioid antagonists upon food intake have been observed following food deprivation (3,16) and glucoprivation induced by 2DG (5). Peripheral but

[1] Requests for reprints should be addressed to Dr. R. Bodnar, Department of Psychology, Queens College, CUNY, 65-30 Kissena Blvd., Flushing, NY 11367.

not central cotreatment with 5-hydroxytryptophan significantly potentiated naloxone hypophagia in food-deprived rats (16). Our laboratory found that the 5-HT_3 antagonist ICS-205,930 was particularly potent in potentiating the hypophagic actions of naloxone in food-deprived rats relative to the selective 5-HT_2 antagonists ritanserin and ketanserin, as well as the nonspecific antagonist methysergide (3). Further, the companion article (5) has shown that whereas ICS-205,930 failed to alter 2DG hyperphagia itself, it significantly potentiated the hypophagic effects of naltrexone in rats treated with 2DG. Both methysergide and ritanserin had far milder interactive effects with naltrexone upon 2DG hyperphagia. Given the dissociations between 2DG and insulin hyperphagia, and the differences in pattern and potency of opioid antagonist effects upon 2DG and insulin hyperphagia, the present study attempted to extend the potential interactions between opioid and 5-HT antagonists upon food intake by examining the effects of methysergide (nonspecific antagonist), ritanserin (5-HT_2 antagonist), and ICS-205,930 (5-HT_3 antagonist) upon: a) insulin hyperphagia and b) naltrexone's inhibition of insulin hyperphagia.

METHOD

Adult, male, albino Sprague-Dawley rats (300–550 g; Charles River Laboratories, Wilmington, MA) were maintained individually in wire mesh cages on a 12 L : 12 D cycle with Purina Rat Chow and water available ad lib. In all experiments, rats were initially monitored for daily body weight and food intake over 3 days to establish normal intake patterns. The protocols described in this experiment were approved by the Queens College Institutional Care and Use Committee.

Drugs

Methysergide (Sandoz Labs, East Hanover, NJ) was dissolved in 0.9% normal saline and administered intraperitoneally. Ritanserin (Janssen, Beerse, Belgium) was initially prepared in 100% methanol at a concentration of 10 mg/ml and then titrated with 0.9% normal saline to a concentration of 2.5 mg/ml 0.5 h prior to subcutaneous administration. ICS-205,930 (Sandoz, Basle, Switzerland) was initially prepared in 100% dimethyl sulfoxide (DMSO) at a concentration of 10 mg/ml and then titrated with 0.9% normal saline to a concentration of 5 mg/ml 0.5 h prior to subcutaneous administration. The vehicle injections for each serotonin antagonist group consisted of their respective solute and injection route; significant differences in intake among solutes failed to occur, and the vehicle scores in this and subsequent protocols were pooled. The doses and injection intervals were chosen in this and subsequent protocols on the basis of these serotonin antagonist effects upon deprivation-induced feeding itself and their interaction with naloxone upon deprivation-induced feeding (3). Naltrexone (2.5, 5, and 10 mg/kg, Sigma Chemical Co., St. Louis, MO) was dissolved in 0.9% normal saline and administered subcutaneously. Insulin (5 U/kg bovine pancreatic insulin, Sigma) was dissolved in 0.9% normal saline and kept on ice until subcutaneous administration.

Insulin Feeding

At 1–3 h into the light cycle, rats received subsets of the following injection conditions, summarized in Table 1 (section A). In each test, the food bin was removed from the cage and an interval of 15 min elapsed between injection pairs. Preweighed food pellets were presented and food intake, adjusted for spillage, was measured at 2, 4, and 6 h after the second injection. In this paradigm, rats received a maximum of five pairs of injections according to an incompletely counterbalanced design with a minimum of 1 week elapsing be-

TABLE 1

PROTOCOLS OF SEROTONIN ANTAGONIST EFFECTS AND SEROTONIN ANTAGONIST-OPIOID ANTAGONIST EFFECTS UPON INSULIN HYPERPHAGIA

A. Serotonin Antagonists and Insulin Hyperphagia

First Injection (mg/kg)	Second Injection	n
Vehicle	Vehicle	20
Vehicle	Insulin	20
Methysergide 0.5	Insulin	7
Methysergide 2.5	Insulin	7
Methysergide 5.0	Insulin	6
Ritanserin 0.25	Insulin	7
Ritanserin 1.25	Insulin	7
Ritanserin 2.5	Insulin	7
ICS-205,930 0.5	Insulin	6
ICS-205,930 2.5	Insulin	6
ICS-205,930 5.0	Insulin	6

B. Naltrexone, Serotonin Antagonists, and Insulin Hyperphagia

First Injection (mg/kg)	Second Injection (mg/kg)	Third Injection	n
Vehicle	Vehicle	Vehicle	30
Vehicle	Vehicle	Insulin	30
Vehicle	Naltrexone 2.5	Insulin	24
Vehicle	Naltrexone 5.0	Insulin	24
Vehicle	Naltrexone 10	Insulin	24
Methysergide 0.5	Naltrexone 2.5	Insulin	7
Methysergide 2.5	Naltrexone 2.5	Insulin	7
Methysergide 5.0	Naltrexone 2.5	Insulin	7
Methysergide 0.5	Naltrexone 5.0	Insulin	7
Methysergide 2.5	Naltrexone 5.0	Insulin	7
Methysergide 5.0	Naltrexone 5.0	Insulin	7
Methysergide 0.5	Naltrexone 10	Insulin	7
Methysergide 2.5	Naltrexone 10	Insulin	7
Methysergide 5.0	Naltrexone 10	Insulin	7
Ritanserin 0.25	Naltrexone 2.5	Insulin	7
Ritanserin 1.25	Naltrexone 2.5	Insulin	7
Ritanserin 2.5	Naltrexone 2.5	Insulin	7
Ritanserin 0.25	Naltrexone 5.0	Insulin	7
Ritanserin 1.25	Naltrexone 5.0	Insulin	7
Ritanserin 2.5	Naltrexone 5.0	Insulin	7
Ritanserin 0.25	Naltrexone 10	Insulin	7
Ritanserin 1.25	Naltrexone 10	Insulin	7
Ritanserin 2.5	Naltrexone 10	Insulin	7
ICS-205,930 0.5	Naltrexone 2.5	Insulin	7
ICS-205,930 2.5	Naltrexone 2.5	Insulin	7
ICS-205,930 5.0	Naltrexone 2.5	Insulin	7
ICS-205,930 0.5	Naltrexone 5.0	Insulin	7
ICS-205,930 2.5	Naltrexone 5.0	Insulin	7
ICS-205,930 5.0	Naltrexone 5.0	Insulin	7
ICS-205,930 0.5	Naltrexone 10	Insulin	7
ICS-205,930 2.5	Naltrexone 10	Insulin	7
ICS-205,930 5.0	Naltrexone 10	Insulin	7

tween different experimental conditions. The insulin dose was chosen to elicit a significant, although not maximal, increase in intake so as to optimally observe potential increases and decreases in insulin hyperphagia.

Serotonin Antagonist/Naltrexone Interactions upon Insulin Feeding

Rats received subsets of the following injection conditions, summarized in Table 1 (section B), at 1-3 h into the light cycle. In each test, the food bin was removed from the cage and intervals of 15 min elapsed between the first and second injections and the second and third injections. Preweighed food pellets were presented and food intake, as previously described, was measured at 2, 4, and 6 h after the third injection. Rats received a maximum of six triads of injections according to an incompletely counterbalanced design with a minimum of 1 week elapsing between different experimental conditions. A previous study (3) indicated that long-term changes in intake over a 10-week interval does not occur in young, mature, rats. The wide range of naltrexone doses was chosen because insulin hyperphagia is blocked only in part by opioid antagonists and only at relatively high doses (4, 31,33,38,46).

Statistical Analyses

Split-plot analyses of variance (ANOVAs) assessed significant effects upon individual intake points. Dunnett comparisons were used to discern differences between vehicle and drug treatments. Dunn comparisons were used to discern differences between serotonin receptor subtype antagonists and either insulin or naltrexone/insulin conditions.

RESULTS

Methysergide and Insulin Hyperphagia

Significant differences in intake were observed among groups after 2, $F(4, 55) = 14.77$, $p < 0.0001$, 4, $F = 15.10$, $p < 0.0001$, and 6, $F = 6.70$, $p < 0.0002$, h. Insulin significantly increased food intake over the 6-h time course (Fig. 1A). Methysergide failed to alter insulin hyperphagia except for the high (5 mg/kg) dose, preventing significant increases in insulin intake after 6 h.

Ritanserin and Insulin Hyperphagia

Significant differences in intake were observed among groups after 2, $F(4, 56) = 11.48$, $p < 0.0001$, 4, $F = 16.89$, $p < 0.0001$, and 6, $F = 9.19$, $p < 0.0001$, h. Insulin hyperphagia was significantly reduced by 51% after 2 and 4 h following pretreatment with the intermediate (1.25 mg/kg) dose of ritanserin (Fig. 1B). Neither the lower (0.25 mg/kg) nor higher (2.5 mg/kg) doses of ritanserin altered insulin hyperphagia.

ICS-205,930 and Insulin Hyperphagia

Significant differences in intake were observed among groups after 2, $F(4, 53) = 36.64$, $p < 0.0001$, 4, $F = 39.83$, $p < 0.0001$, and 6, $F = 34.12$, $p < 0.0001$, h. Insulin hyperphagia was significantly potentiated by all ICS-205,930 doses at 2 (142-172%), 4 (105-117%), and 6 (90%) h (Fig. 1C).

Naltrexone and Insulin Hyperphagia

Naltrexone failed to significantly alter insulin hyperphagia across the time course and across the antagonist's dose range. Nonsignificant reductions in insulin hyperphagia were observed after 2 h following the 2.5 (33%)-, 5 (25%)-, and 10 (19%)-mg/kg doses.

Methysergide, Insulin Hyperphagia, and Naltrexone (2.5 mg/kg)

Significant differences in intake were observed among groups after 2, $F(5, 97) = 7.59$, $p < 0.0001$, 4, $F = 11.42$, $p < 0.0001$, and 6, $F = 7.68$, $p < 0.0001$, h. Neither naltrexone itself nor naltrexone paired with methysergide significantly altered insulin hyperphagia across the 6-h time course (Fig. 2A).

Naltrexone (5 mg/kg). Significant differences in intake were observed among groups after 2, $F(5, 99) = 13.26$, $p < 0.0001$, 4, $F = 19.72$, $p < 0.0001$, and 6, $F = 14.56$, $p < 0.0001$, h. Insulin hyperphagia at 2 h was significantly enhanced (92%) following pretreatment with naltrexone and methysergide (5 mg/kg) (Fig. 2B). Naltrexone alone or naltrexone paired with methysergide failed to affect insulin hyperphagia at other doses and at other intervals.

Naltrexone (10 mg/kg). Significant differences in intake were observed among groups after 2, $F(5, 108) = 11.70$, $p < 0.0001$, 4, $F = 19.05$, $p < 0.0001$, and 6, $F = 16.38$, $p < 0.0001$, h. Insulin hyperphagia was significantly enhanced following pretreatment with naltrexone and methysergide (5 mg/kg) after 2 h (64%) and following pretreatment with naltrexone and methysergide (0.5 mg/kg) after 6 h (56%) (Fig. 2C). Other naltrexone and methysergide dose combinations failed to affect insulin hyperphagia at other intervals.

Ritanserin, Insulin Hyperphagia, and Naltrexone (2.5 mg/kg)

Significant differences in intake were observed among groups after 2, $F(5, 97) = 6.57$, $p < 0.0001$, 4, $F = 15.29$, $p < 0.0001$, and 6, $F = 10.75$, $p < 0.0001$, h. Insulin hyperphagia was significantly inhibited (55%) following pretreatment with ritanserin (0.25 mg/kg) and naltrexone (Fig. 3A). Neither naltrexone alone nor naltrexone paired with ritanserin affected insulin hyperphagia at other doses and at other intervals.

Naltrexone (5 mg/kg). Significant differences in intake were observed among groups after 2, $F(5, 99) = 6.59$, $p < 0.0001$, 4, $F = 17.14$, $p < 0.0001$, and 6, $F = 12.96$, $p < 0.0001$, h. Neither naltrexone alone nor naltrexone paired with ritanserin altered insulin hyperphagia across the 6-h time course (Fig. 3B).

Naltrexone (10 mg/kg). Significant differences in intake were observed among groups after 2, $F(5, 108) = 7.53$, $p < 0.0001$, 4, $F = 21.24$, $p < 0.0001$, and 6, $F = 17.68$, $p < 0.0001$, h. Insulin hyperphagia was significantly inhibited after 2 h following pretreatment with naltrexone and ritanserin doses of 0.25 (76%) and 1.25 (72%) mg/kg (Fig. 3C). In contrast, insulin hyperphagia was significantly potentiated after 4 h following pretreatment of naltrexone and ritanserin doses of 1.25 (56%) and 2.5 (65%) mg/kg. Insulin hyperphagia was significantly potentiated after 6 h following pretreatment of naltrexone and the high (2.5 mg/kg) ritanserin dose (68%). All other combinations of naltrexone and ritanserin failed to alter insulin hyperphagia.

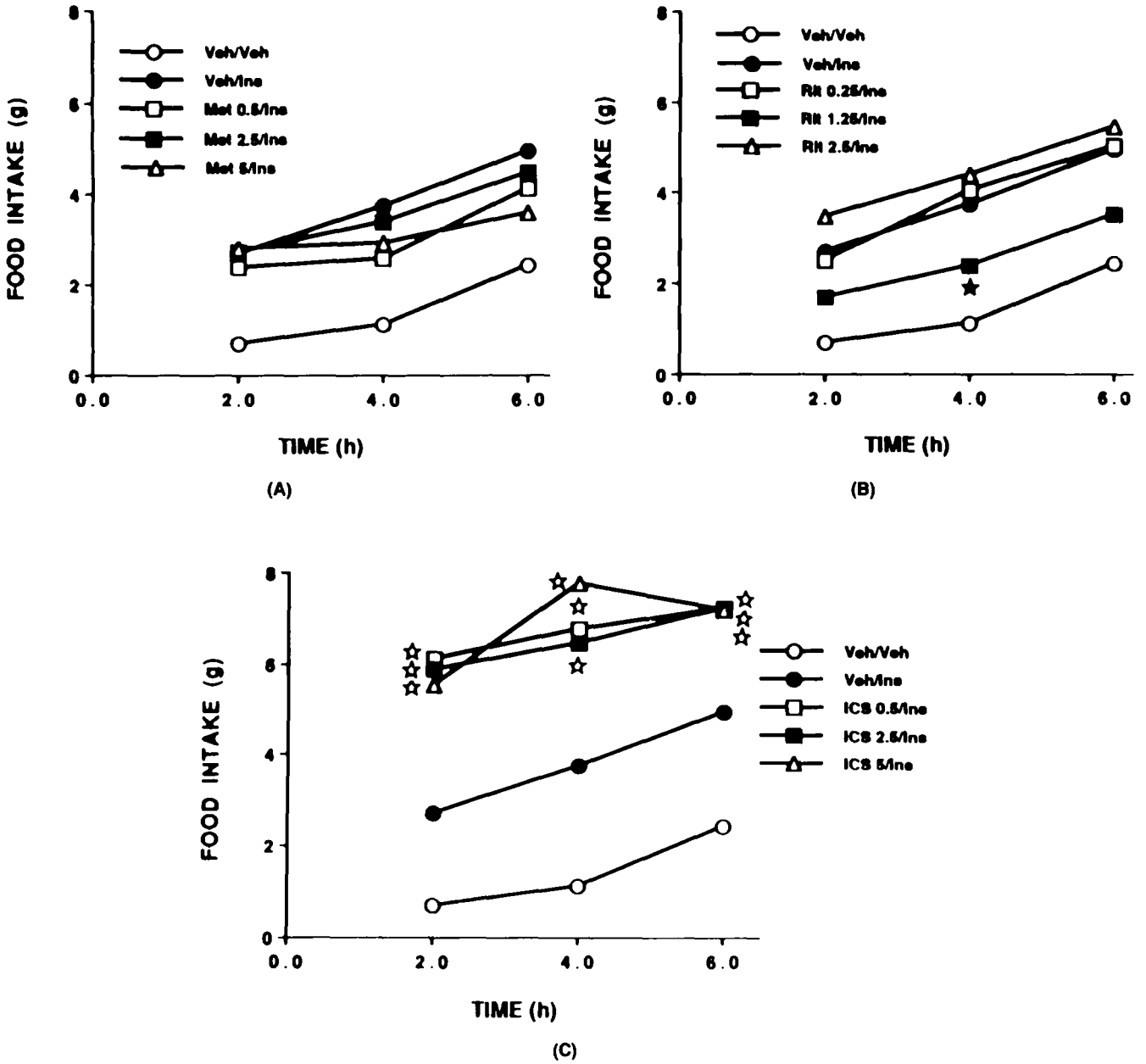

FIG. 1. Alterations in food intake in vehicle (Veh)- and insulin (Ins)-treated rats following pretreatment with methysergide (Met: 0.5-5 mg/kg, A), ritanserin (Rit: 0.25-2.5 mg/kg, B), and ICS-205,930 (ICS: 0.5-5 mg/kg, C). Insulin produced significant hyperphagia across the 6-h time course. (★, ☆) significant respective reductions and increases in insulin hyperphagia relative to the corresponding Veh pretreatment (Dunnett comparisons, $p < 0.05$).

ICS-205,930, Insulin Hyperphagia, and Naltrexone (2.5 mg/kg)

Significant differences in intake were observed among groups after 2, $F(5, 103) = 9.26$, $p < 0.0001$, 4, $F = 25.61$, $p < 0.0001$, and 6, $F = 17.85$, $p < 0.0001$, h. Insulin hyperphagia was significantly inhibited after 2 h following pretreatment of naltrexone and the high (5 mg/kg) dose of ICS-205,930 (61%) (Fig. 4A). In contrast, insulin hyperphagia was significantly enhanced after 4 h following pretreatment of naltrexone and ICS-205,930 doses of 0.5 (68%) and 2.5 (80%) mg/kg. Insulin hyperphagia was significantly enhanced after 6 h following pretreatment of naltrexone and ICS-205,930 doses of 0.5 (60%) and 5 (56%) mg/kg.

Naltrexone (5 mg/kg). Significant differences in intake were observed among groups after 2, $F(5, 99) = 7.91$, $p < 0.0001$, 4, $F = 20.56$, $p < 0.0001$, and 6, $F = 18.86$, $p < 0.0001$, h. Insulin hyperphagia was significantly inhibited after 2 h following pretreatment of naltrexone and ICS-205,930 doses of 2.5 (58%) and 5 (76%) mg/kg (Fig. 4B). Naltrexone and ICS-205,930 pretreatment failed to alter insulin hyperphagia after 4 h. Insulin hyperphagia was significantly enhanced after 6 h following pretreatment of naltrexone and ICS-205,930 doses of 2.5 (71%) and 5 (76%) mg/kg.

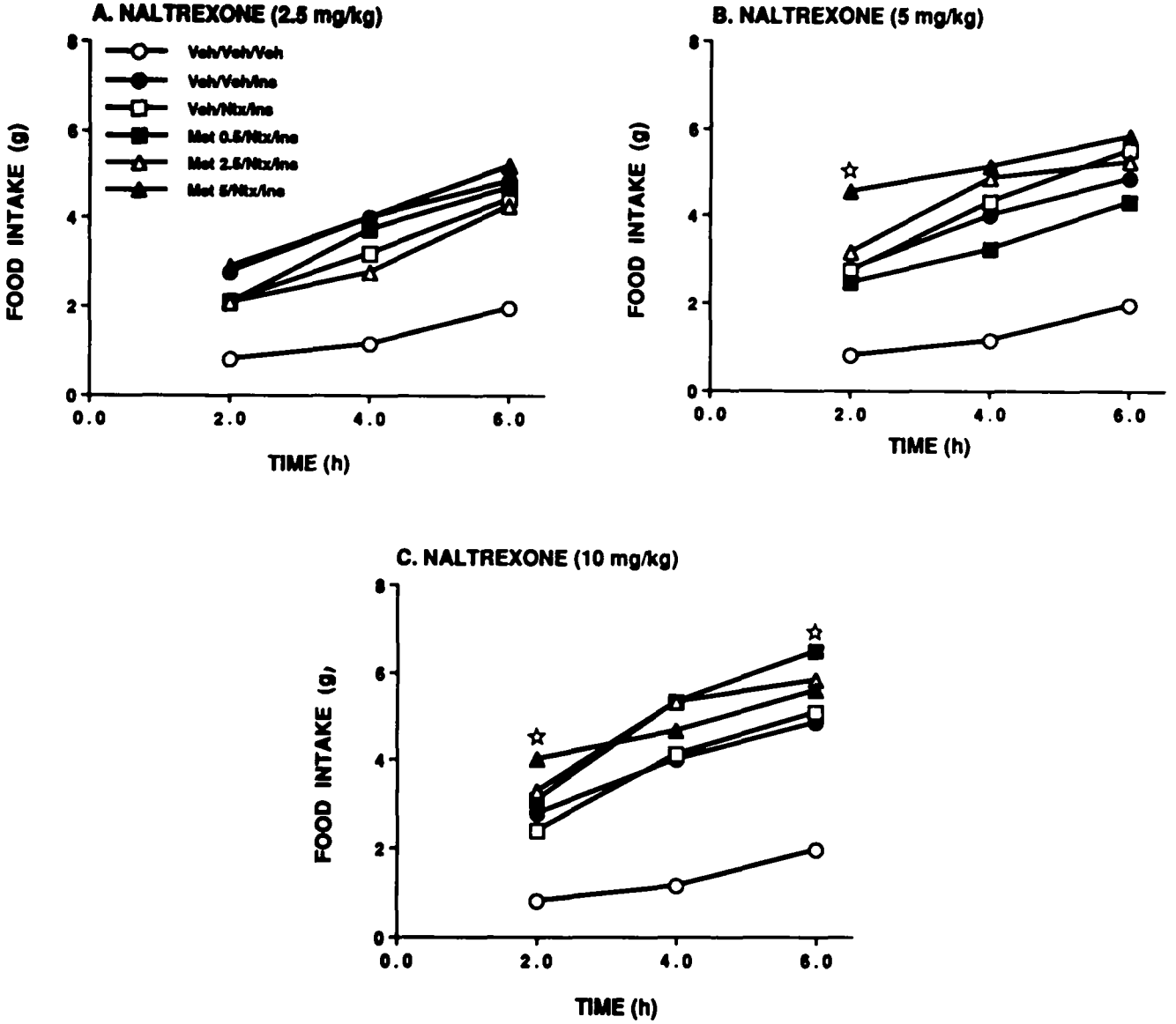

FIG. 2. Alterations in food intake in insulin-treated rats following paired pretreatment with naltrexone (Ntx) and methysergide (Met: 0.5–5 mg/kg). Insulin produced significant hyperphagia across the 6-h time course, Naltrexone doses of 2.5 mg/kg (A), 5 mg/kg (B), and 10 mg/kg (C) marginally (19–33%) inhibited insulin hyperphagia. (☆) significant increases in insulin hyperphagia h following naltrexone paired with 5-HT receptor subtype antagonists in this and subsequent figures (Dunnett comparisons, $p < 0.05$).

Naltrexone (10 mg/kg). Significant differences in intake were observed among groups after 2, $F(5, 108) = 9.49$, $p < 0.0001$, 4, $F = 16.91$, $p < 0.0001$, and 6, $F = 13.82$, $p < 0.0001$, h. Insulin hyperphagia was eliminated after 2 h following pretreatment of naltrexone and the high (5 mg/kg) dose of ICS-205,930 (Fig. 4C). In contrast, insulin hyperphagia was significantly enhanced following pretreatment of naltrexone and ICS-205,930 (0.5 mg/kg) at 4 (89%) and 6 (60%) h.

DISCUSSION

The following major findings were observed in the present study. First, serotonin receptor subtype antagonists differentially altered insulin hyperphagia. Whereas insulin hyperphagia was significantly inhibited for up to 4 h following the intermediate dose of the 5-HT_2 antagonist ritanserin, the entire dose range of the 5-HT_3 antagonist ICS-205,930 significantly and potently potentiated insulin hyperphagia across the 6-h time course. Only the high dose of methysergide reduced the duration of insulin hyperphagia to 4 h. Second, a wide dose range (2.5–10 mg/kg) of naltrexone produced marginal, nonsignificant reductions (19–33%) in insulin hyperphagia. Third, combination of serotonin receptor subtype antagonists with naltrexone produced differential effects upon insulin hyperphagia that varied as a function of the antagonist. The pairing of the high dose of ICS-205,930 with the dose range of naltrexone significantly reduced insulin hyperphagia after

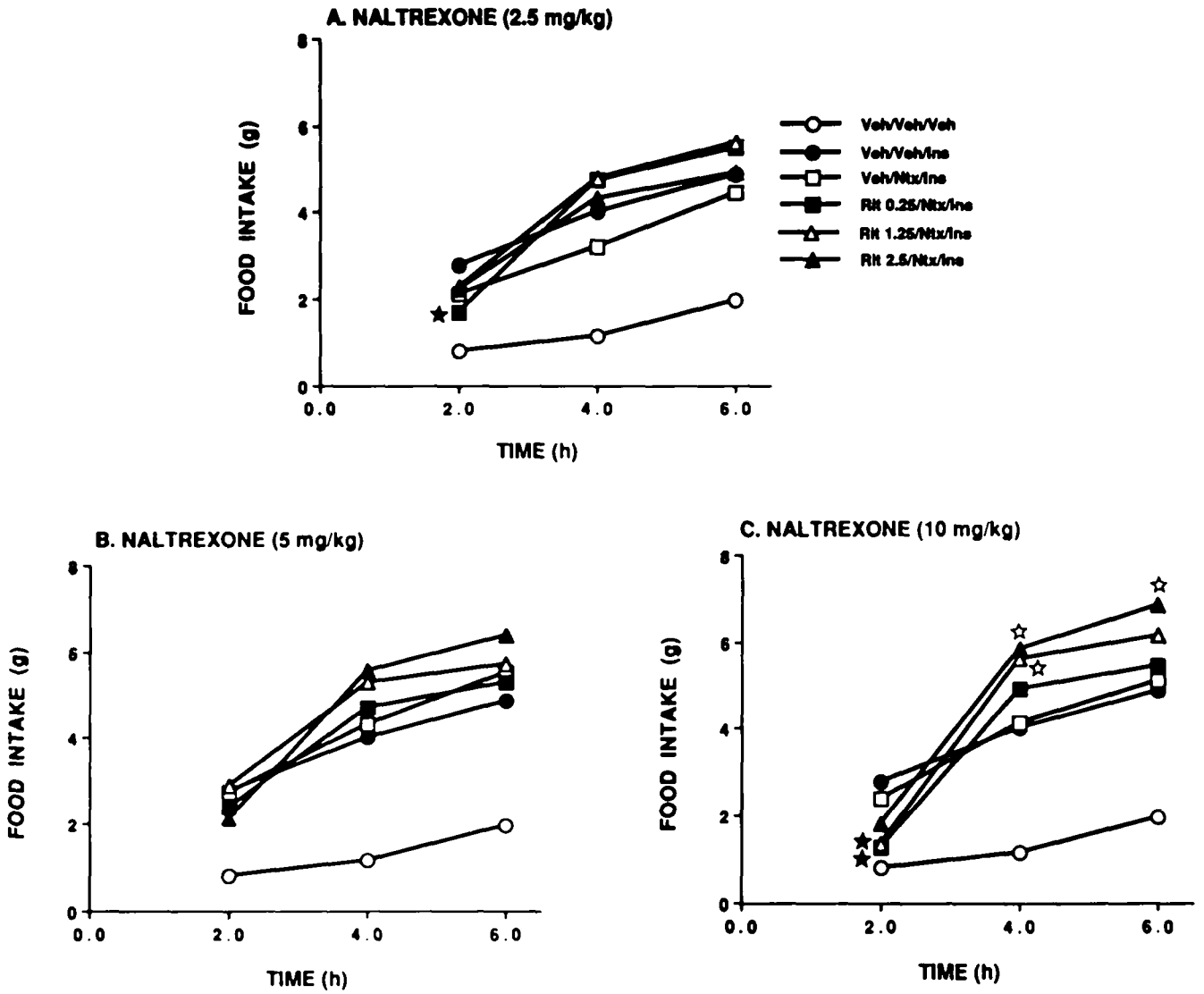

FIG. 3. Alterations in food intake in insulin-treated rats following paired pretreatment with naltrexone (Ntx) and ritanserin (Rit: 0.25–2.5 mg/kg). Ritanserin paired with naltrexone inhibited insulin hyperphagia after 2 h and potentiated insulin hyperphagia after 4–6 h. (★) significant decreases in insulin hyperphagia following naltrexone paired with 5-HT receptor subtype antagonists in this and the subsequent figure (Dunnett comparisons, $p < 0.05$). (☆) defined in Fig. 2.

2 h. The pairing of the low dose of ritanserin with naltrexone also significantly reduced insulin hyperphagia after 2 h. In contrast, insulin hyperphagia after 2 h was significantly potentiated by pairing methysergide and naltrexone. Fourth, combination of serotonin receptor subtype antagonists with naltrexone altered insulin hyperphagia as a function of intake interval. In contrast to reductions in insulin hyperphagia after 2 h induced by pairing naltrexone and either ICS-205,930 or ritanserin, the pairing of these antagonists and naltrexone significantly potentiated insulin hyperphagia after 4 and 6 h. Methysergide produced milder potentiations in insulin hyperphagia when paired with naltrexone. The inability of naltrexone to significantly alter insulin hyperphagia is in general agreement with previous reports. Systemic administration of general opiate antagonists produced either small, significant reductions in insulin hyperphagia (31,38,46) or failed to affect this response (33). Indeed, central administration of naltrexone (20–50 μg) only reduced insulin hyperphagia by 30% after 2 h (4), which is similar to the peak effects observed in the present study. Further, naltrexone is at least 40 times more effective in inhibiting 2DG hyperphagia [0.25 mg/kg: (36%)] (5) relative to insulin hyperphagia [10 mg/kg: (19%)], effects similar to that reported previously (33). Moreover, whereas opioid control of 2DG hyperphagia appears to be mediated by μ_2- and κ-receptors (1,2,52), opioid control of insulin hyperphagia appears to be mediated by the μ_2 opioid binding site (4). Again, the ability of the μ-selective antagonist β-funaltrexamine to inhibit 2DG hyperphagia is far more pronounced than its ability to inhibit insulin hyperphagia (2,4). Thus, it appears that the endogenous opioid system plays a far lesser

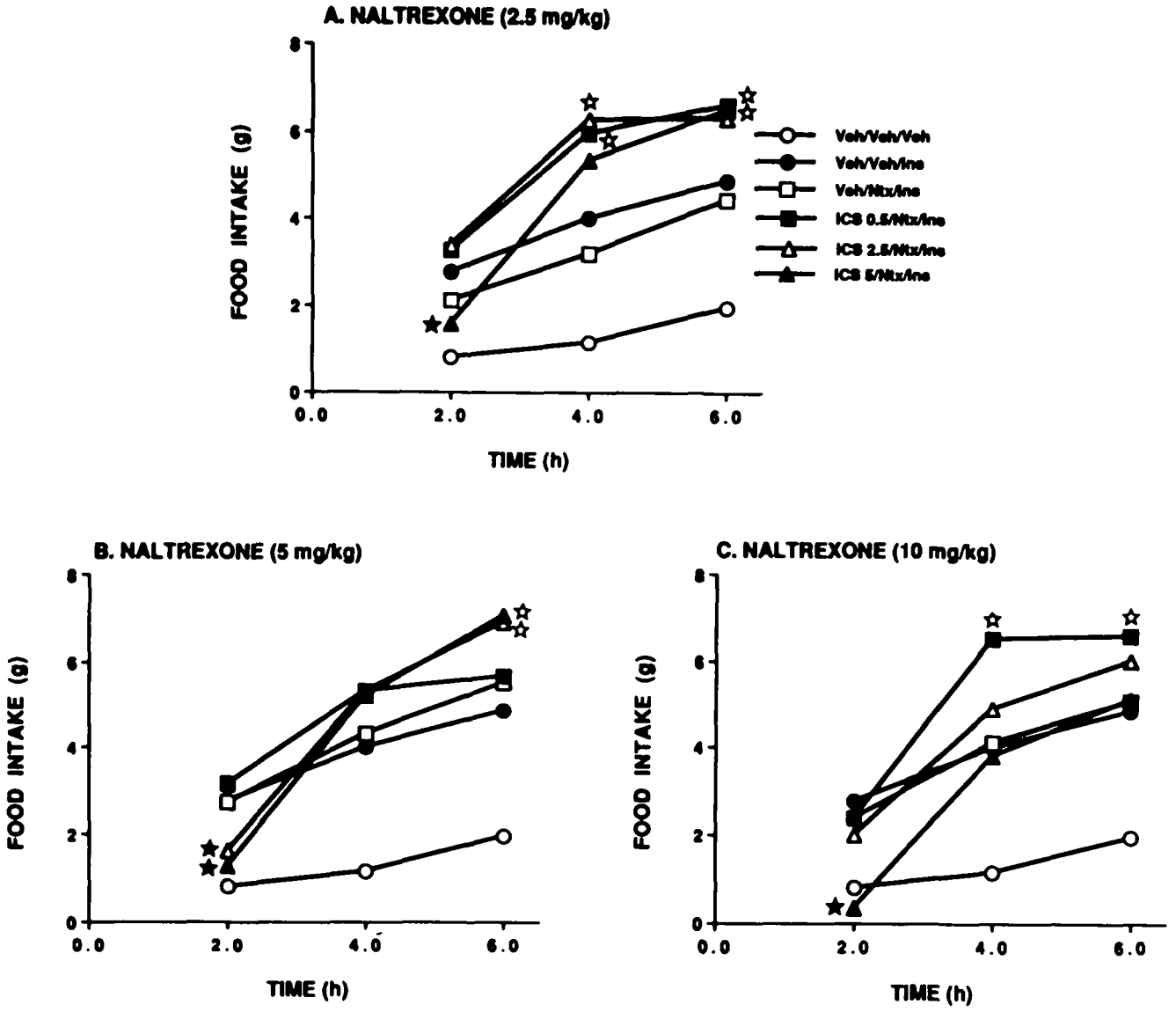

FIG. 4. Alterations in food intake in insulin-treated rats following paired pretreatment with naltrexone (Ntx) and ICS-205,930 (ICS: 0.5-5 mg/kg). ICS-205,930 paired with naltrexone inhibited insulin hyperphagia after 2 h and potentiated insulin hyperphagia after 4-6 h. (★ and ☆) defined in Fig. 2.

role in the mediation of insulin hyperphagia relative to its mediation of 2DG hyperphagia.

Insulin hyperphagia was significantly potentiated across its 6-h time course by the entire dose range (0.5-5 mg/kg) of the 5-HT_3 antagonist ICS-205,930. ICS-205,930 failed to alter hyperphagia induced by either deprivation or 2DG, but increased free feeding in the light cycle, as well as intake of an imbalanced amino acid diet (3,5,20). The 5-HT_2 antagonist ritanserin significantly reduced insulin hyperphagia for up to 4 h. Ritanserin also transiently reduced 2DG hyperphagia (5), but failed to alter spontaneous and deprivation intake (3,34,51). The high dose of methysergide shortened the duration of insulin hyperphagia, an effect similar to its marginal reduction in deprivation intake (3). However, methysergide fails to alter 2DG hyperphagia, and actually stimulates intake in well-satiated rats (13,17). Thus, it appears that whereas ICS-205,930 produces potent increases in insulin hyperphagia ritanserin and, to a lesser degree, methysergide inhibit this ingestive response.

The combination of naltrexone with either of the serotonin receptor subtype antagonists, ICS-205,930 and ritanserin, significantly inhibited insulin hyperphagia after 2 h. ICS-205,930 paired with naltrexone produced the most consistent inhibition with reductions occurring when the two higher ICS-205,930 doses were paired with each of the naltrexone doses. The degree of inhibition was partial when ICS-205,930 was paired with the two lower doses of naltrexone (58–61%), but was complete when ICS-205,930 was paired with the highest dose of naltrexone. These effects were all the more striking given the ability of ICS-205,930 to stimulate insulin hyperphagia when administered alone. In contrast, pairing the two lower doses of naltrexone with the lowest dose (0.25 mg/kg)

of ritanserin was most effective in inhibiting insulin hyperphagia after 2 h. The degree of inhibition of insulin hyperphagia was slightly more pronounced following ritanserin and insulin (55–76%) than following ritanserin alone (51%).

The ability of naltrexone and these serotonin receptor subtype antagonists to inhibit insulin hyperphagia was transient. Potent potentiations of insulin hyperphagia were observed after 4 (68–80%) and 6 (56–89%) h following naltrexone and ICS-205,930. These increases were less pronounced than the 190–272% increases in insulin hyperphagia induced by ICS-205,930 alone. Ritanserin paired with the high dose of naltrexone also increased insulin hyperphagia after 4 (56–65%) and 6 (68%) h; this effect was opposite to the 51% decrease in insulin hyperphagia induced by ritanserin alone. Finally, methysergide paired with the two higher doses of naltrexone significantly increased insulin hyperphagia by 56–92% despite the inability of either antagonist to exert effects themselves.

Previous studies demonstrated that pairing ICS-205,930 with opiate antagonists inhibited hyperphagia following food deprivation and 2DG (3,5). As suggested in the companion article (5), the interactions between ICS-205,930 and opiate antagonists upon these forms of hyperphagia are probably centrally mediated for three reasons. First, 5-HT_3 receptors are most densely localized in the CNS in the nucleus tractus solitarius (NTS), dorsal nucleus of the vagus, area postrema (AP), and substantia gelatinosa of the spinal trigeminal nucleus and the dorsal horn of the spinal cord (29,57,58). Second, one site of action by which general and specific opioid receptor antagonists produce central hypophagic effects (1,2,4,30) is the parabrachial region (11), which in turn receives dense innervation from the NTS and AP (50,56). Finally, the NTS, dorsal vagus, and AP are important sites for both the induction of glucoprivic feeding (45) and the maintenance of glucoprivic feeding following AP lesions [(6,12), but see (26)].

It would appear that the loci underlying the interactive inhibitory effects of naltrexone and either ICS-205,930 or ritanserin upon insulin hyperphagia observed in the present study are not in the CNS for the following reasons. First, whereas hyperphagia can be elicited following central administration of 2DG (37) central administration of insulin results in reductions of food intake and body weight (10,27,60). Second, dissociations between 2DG and insulin hyperphagia occur following opioid antagonists (see above), as well as following either subdiagphragmatic vagotomy or lesions placed in the ventromedial hypothalamus, zona incerta, medial forebrain bundle, or midbrain tegmentum (7,19,35,36,47,49,59). Third, given the importance of dorsal hindbrain circuits to the maintenance of 2DG hyperphagia it is important to note that 2DG hyperphagia, but not insulin hyperphagia, is reduced following transections anterior to the NTS [see review: (44)].

The peripheral location of what are presently called 5-HT_3 receptors was suggested by the classic work of Gaddum and Picarelli (18), who proposed the existence of two serotonin receptors (S and M). More recent data indicated that the 5-HT_3 receptor corresponded to the M-type receptor (43), and has been located in such peripheral tissue and nerves as the nodose ganglion, superior cervical ganglion, submucosal plexus, vagus nerve, and myenteric plexus [see reviews: (39,55)]. Receptor autoradiography has revealed peripheral 5-HT_2 binding sites in smooth muscle in addition to central 5-HT_2 binding sites in the frontal cortex, limbic system, basal ganglia, and dorsal raphe nucleus (32,39,40). There is other evidence of peripheral sites of action for serotonin antagonist effects upon ingestive behavior. The mediation of intake of an imbalanced diet by 5-HT_3 receptor antagonists (20) appears to be mediated by peripheral receptors since: a) both ICS-205,930 and its quarternary derivative, which is incapable of penetrating the blood-brain barrier, increase intake of an imbalanced diet; and b) injection of ICS-205,930 into either the lateral ventricles or cisterna magna were without effect (22).

In conclusion, insulin hyperphagia appears to be modulated differentially by serotonin receptor subtypes with 5-HT_3 antagonists potentiating and 5-HT_2 antagonists reducing the magnitude of insulin hyperphagia. Whereas opiate receptor antagonism marginally reduced insulin hyperphagia, pairing naltrexone with either ritanserin or ICS-205,930 resulted in marked, significant reductions in this ingestive response. Together with previous data evaluating deprivation-induced and 2DG-induced hyperphagia (3,5), it appears that serotonin and opioid receptors interact to modulate these ingestive responses.

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