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ICS 205-930 and Feeding Responses to Amino Acid Imbalance: A Peripheral Effect?

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HRUPKA, B. J., D. W. GIETZEN AND J. L. BEVERLY, III. *ICS 205-930 and feeding responses to amino acid imbalance: A peripheral effect?* PHARMACOL BIOCHEM BEHAV **40**(1) 83–87, 1991.—Serotonin$_3$ (5-HT$_3$) receptor antagonists (ICS 205-930 and MDL 72222) have been shown to block or ameliorate the anorectic response of the rat to amino acid imbalanced (IMB) diets. Two experiments were conducted to determine whether the effects of these antagonists are mediated through central or peripheral 5-HT$_3$ receptors. In Experiment One, ICS 205-930 (ICS) was injected centrally, into either the lateral ventricle (doses: 0.3 pmol to 10 nmol), or the cisterna magna (62 nmol). The intake of rats fed an isoleucine IMB diet was not affected by these injections. In Experiment Two, rats received an IP injection of either saline, ICS, or a quaternized derivative of ICS (Q-ICS) that should not cross the blood-brain barrier. Both ICS- and Q-ICS-injected rats ate significantly more ($p<0.05$) IMB diet than saline-injected rats. Intake of IMB diet was not different ($p>0.4$) between ICS and Q-ICS groups. From these results, it appears that ICS restores intake of IMB through a peripheral component.

Anorexia Serotonin Feeding behavior Rats Receptor Serotonin$_3$ Imbalanced diet Amino acid diet

IN studies using disproportionate amino acid diets, rats adapted to a low protein diet reduce their intake of an amino acid imbalanced (IMB) diet. Serotonin (5-HT) appears to be involved in the anorectic response of rats to IMB diets (11), as well as other depressions of food intake (3). We have previously reported that 5-HT activity (as measured by the 5-HIAA/5-HT ratio) was increased in some brain areas of rats that had ingested IMB diets (11). Enhancing 5-HT activity by injection of the agonist, quipazine, exacerbated the feeding depression to IMB, while depressing 5-HT activity by the 5HT$_{1A}$ agonist 8-hydroxy dipropylamino tetralin (8-OH-DPAT) attenuated the normal reduction in intake of IMB diet (11). However, blockade of 5-HT$_1$ and 5-HT$_2$ receptors with metergoline did not alter intake of IMB diets. Hammer et al. (14) reported that pharmacological blockade of 5-HT activity using the 5HT$_3$ antagonists ICS 205-930 (ICS) and MDL 72222 ameliorated the depression in intake of a severe isoleucine (Ile) IMB diet, and totally blocked the feeding depression to a mild Ile IMB diet. These investigators also observed that intake of IMB diets was not affected by blockade of dopamine or alpha-adrenergic receptors. From these experiments, blockade of the anorectic response to IMB appeared to be specific for the 5-HT$_3$ [or 5-HT$_4$ (6)] receptor.

Brain lesion studies (18,19) and microinjection studies (1,2) have shown that recognition and response to IMB diets in rats has a central component (22). It is not known whether these centrally mediated responses involve 5-HT, or whether the drugs and antagonists previously used to affect intake of IMB are acting on these central mechanisms. The present studies were designed to determine whether ICS blocks IMB-induced anorexia by acting centrally or peripherally. We used cisterna magna and lateral ventricle injections of ICS and peripheral injections of a quaternized form of ICS, which should not enter the brain (4,13), to examine this question. Our results indicate that ICS blocks the anorectic response, at least in part, by action on peripheral receptors.

METHOD

Male Sprague-Dawley rats (Bantin & Kingman, LaFayette, CA) were individually housed in stainless steel hanging wire cages in rooms maintained at 22°C on a 12:12-h light:dark cycle. Food and water were available ad lib.

Diets

Rats were fed stock diet (Purina Rat Chow, Ralston Purina, St. Louis, MO) for 4–5 days after arrival to allow adaptation to our vivarium, and were then switched to a low-protein purified Ile basal (BAS) diet for 10 to 20 days before each experiment. The Ile BAS diet contained 11.7% amino acid mixture (wt./wt.) in which Ile was the growth-limiting amino acid. Starch and sucrose (2:1) were used as the carbohydrate source, and corn oil (5% wt./wt.) as the fat source. The necessary vitamins and minerals were included in the diet. To make the Ile IMB diet, 9.86% of the diet as the carbohydrate fraction in the BAS diet was replaced by an indispensable amino acid mixture that contained all the essential amino acids except Ile. These diets have been described in detail previously (14,16).

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Food Intake Procedures

Intake of BAS diet, corrected for spillage, was recorded for 3–5 days preceding each experiment. Each rat's average 24-h intake was called its "baseline food intake." On the day of the injections, a clean preweighed food cup filled with the appropriate diet was placed in each rat's cage at the time of lights-out. Food intake was recorded at 3, 6, 12, and 24 h after diet presentation and was determined by the difference in food cup weight between periods, corrected for spillage. A 25-watt red light bulb was left on at all times to help facilitate food intake measurements during the dark cycle.

EXPERIMENT ONE: CENTRAL INJECTIONS

Cisterna Magna (CM) Injections

A 2×2 factorial design was used to study rat's (N=32) intake of IMB or BAS diet after CM injections of either ICS (62 nmol) or saline (SAL). This experiment was conducted over 2 days, with 16 rats injected each day. Rats weighing an average of 223±2.0 g were blocked according to body weight and randomly assigned to treatment groups. One hour before lights out rats were lightly anesthetized with methoxyflurane (Metofane™, Pittman Moore, Washington Crossing, NJ) and placed in a stereotaxic apparatus. The neck was flexed so that a 28-gauge needle could be lowered between the base of the skull and the first cervical vertebra. Then 5 μl of ICS or SAL was slowly injected into the CM. Needle placement was verified by drawing a small amount of clear liquid before injection, and visualizing it in the clear tygon tubing that connected the needle to the syringe. Blood was observed in the tubing during the injections of four rats, and these animals were not included in the data analysis.

Lateral Ventricle (LV) Injections

Rats that had been prefed BAS diet for 6–8 days were surgically prepared with chronic unilateral guide cannulae positioned above the right LV. For surgery, animals were anesthetized with sodium pentobarbital (65 mg/kg, IP, Steris Laboratories, Phoenix, AZ) augmented with atropine (0.4 mg/kg, SC, Elkins-Sinn Inc., Cherry Hill, NJ). A twenty-gauge guide cannula was stereotaxically placed 1 mm above the LV at the coordinates: 0.8 mm posterior to bregma, 1.5 mm lateral to the midline and 1.5 mm ventral to the dura according to atlas of Paxinos and Watson (20). The cannula was fixed in position with cranioplastic cement anchored by stainless steel screws, and a 24-gauge stylette was inserted into the cannula. Daily stylette cleaning maintained cannula patency and adapted animals to handling procedures prior to the experimental injections. Injections were given through a 24-gauge needle attached to a 25 μl syringe by a 20 cm piece of tygon tubing (0.01″ i.d. × 0.03″ o.d.). The injection needle was inserted 1.5 mm past the end of the guide cannula, and a slight back pressure was applied to the syringe. The needle was then slowly withdrawn until movement of an air bubble in the tubing was observed, verifying placement in the LV. Five days after surgery, cannula placement was tested by injecting 100 ng angiotensin II (ANGII). Animals that did not drink after ANGII injections were eliminated from the trials. LV injections were administered to unanesthetized rats in volumes of 7 μl over a 30–60-second period. Experimental injections for each trial were given 5–8 days after ANGII injections, 10–50 minutes before lights out.

Trial One. A 2×2 factorial design was used to study the effect of ICS (10 nmol) or SAL injected into the LV on intake of IMB or BAS diet. On the day of the experiment, 26 rats (n=6–7/group), weighing 280.4±3.2 g, were blocked according to body weight and randomly assigned to treatments. Injections and food intake procedures were performed as described above.

Trial Two. Thirty-three rats, weighing 248±2.0 g, were blocked according to body weight, and randomly assigned to one of 5 treatments (n=6–7 rats/group), receiving LV injections of either 0, 0.31, 3.1, 31, or 310 pmol ICS, followed by feeding of IMB diet. Three weeks after these injections, control (previously injected with SAL) rats were injected with 310 pmol of ICS to test the effect of the largest dose of ICS on BAS intake. Intake of BAS was recorded for one day.

EXPERIMENT TWO: PERIPHERAL INJECTIONS

A quaternized derivative of ICS (Q-ICS), which should not cross the blood-brain barrier (4,13), was injected IP to determine its effect on intake of IMB. We questioned whether Q-ICS would increase intake of IMB as do IP injections of ICS. Forty-two rats weighing 237±3.1 g were divided into 6 groups (n=7 rats/group). The two control groups received SAL injections followed by BAS or IMB feeding. ICS and Q-ICS were injected at doses of 7.8 and 28 μmol/kg b.wt. followed by IMB feeding. These molar amounts correspond to 2.5 and 9 mg/kg b.wt. of ICS which have been shown to increase intake of IMB (14).

Drugs and Materials

ICS 205-930 [3-tropanyl-indole-3-carboxylate (ICS)] was a gift from Sandoz Research Institute (East Hanover, NJ). Quaternized ICS 205-930 (3-tropanyl-indole-3-carboxylate methiodide) was purchased from Research Biochemicals Incorporated (Natick, MA). Angiotensin II was obtained from Sigma (St. Louis, MO). Drugs were dissolved in 0.9% NaCl (SAL) for all experiments. In Experiment 4, the 28.1 μmol dose of Q-ICS did not dissolve completely and was injected as a suspension. Stainless steel tubing was purchased from Small Parts, Inc. (Miami, FL). Analytical grade reagents were from Fisher Scientific (Santa Clara, CA).

Statistics

Food intake data were analyzed by General Linear Model (GLM) procedures (SAS, 1988) programmed for either a 2×2 factorial design with blocking, or a randomized block design. For the CM study, day of injection was included as a variable. Baseline food intake was included as a covariate in all models.

RESULTS

CM Injections

During the time intervals at which food intake was measured, rats fed IMB diet ate significantly less ($p<0.001$) than rats fed BAS. No main effect of drug or drug × diet interaction was observed during any time period (all $p>0.2$). Cumulative 24-h food intakes (means±SEM) were: BAS/SAL=14.8±0.5; BAS/ICS=14.7±0.9; IMB/SAL=3.8±0.5; IMB/ICS=4.2±0.7. ICS administered into the CM did not increase intake of IMB in this experiment.

LV Injections, Trial One

From 0–3 and 3–6 hours after lights-out, rats fed the IMB diet ate significantly less ($p<0.001$; $p<0.05$, respectively) than rats fed the BAS diet. (Fig. 1). ICS injections did not affect food intake during these periods ($p>0.3$). BAS-fed/ICS-injected rats

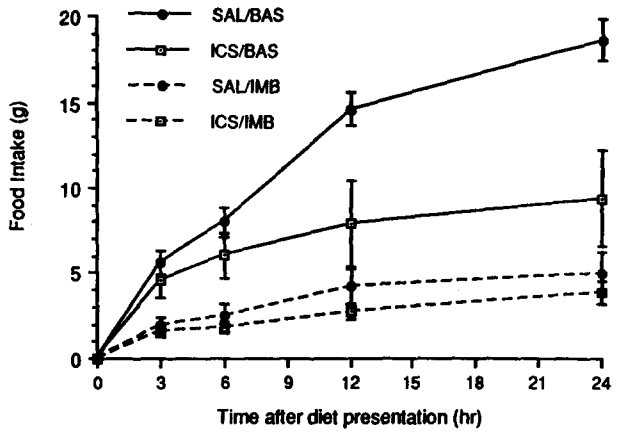

FIG. 1. Cumulative food intake in grams for rats given ICS 205-930 or saline into the lateral ventricle within one hour prior to introduction of BAS or IMB diets. Points represent mean ± SEM of 6–7 rats/group. Main effect of diet was significant during all time intervals at which food intake was measured (all $p<0.5$). From 6–12 hour after diet presentation, a significant drug ($p<0.01$) and diet × drug interaction ($p<0.05$) was observed. A significant diet × drug interaction ($p<0.05$) was also observed during the light cycle (12–24 h).

ate significantly less than BAS-fed/SAL-injected rats during the second half of the dark cycle and during the light cycle, while rats fed IMB ate nearly the same small amount regardless of drug. This resulted in a significant diet × drug interaction ($p<0.05$).

LV Injections, Trial Two

Intake of IMB was not affected by ICS injection at any of the doses used in this trial during any time period ($p>0.05$) (Table 1). All groups ate between 5 and 6.5 g of IMB diet during the 24 hours. When control rats were later injected with 310 pmol ICS and fed BAS, their 24-hour food intake was significantly less than baseline food intake. Intakes were 9.3 ± 1.75 g (mean ± SEM) for BAS and 16.3 ± 0.4 g for baseline ($p<0.01$).

Experiment Two, Peripheral Injections

From 0–6 h, rats receiving ICS and Q-ICS injections ate similar amounts of IMB ($p>0.10$, Fig. 2). Rats injected with either 28.1 μmol/kg ICS or Q-ICS ate significantly more IMB ($p<0.01$) from 6–24 h than rats injected with 7.8 μmol/kg of these drugs, demonstrating a dose-dependent response to these 5-HT_3 antagonists. There were no significant differences ($p>0.10$) between ICS and Q-ICS groups in IMB intake.

DISCUSSION

There is considerable evidence that central serotonergic systems are involved in the control of feeding, and that increases in serotonergic activity have generally been associated with a decrease in food intake (3). We have also shown that the rat's normal depression of IMB intake was attenuated pharmacologically by depressing 5-HT activity (11), and by 5-HT_3 receptor antagonist administration (14). In addition to pharmacological studies, brain lesioning studies have shown that both the prepyriform cortex (PPC) and the amygdala are necessary for the rat's normal depression to IMB diets, and that lesioning these areas will also attenuate the rat's depression to IMB [reviewed in (17, 21, 22)]. While Gietzen et al. (10) showed increases in 5-HIAA/5-HT ratios in several brain areas including the PPC, there is no direct evidence that central serotonergic systems are necessary for the depression of IMB intake. Fletcher and Burton (7) demonstrated that peripherally administered serotonin can also affect food intake. The present studies were conducted to investigate whether the effect of ICS in remediating the anorectic response to IMB is acting centrally or peripherally.

In the CM study, rats fed IMB diet reduced their food intake as expected, but CM injections of ICS 205-930 did not alter intake of either IMB or BAS diets. ICS injected into the CM would most likely affect receptors in the brain stem areas and the spinal cord. Waeber et al. (26) observed binding of ^{3}H ICS 205-930 to the area postrema (AP), vagus nerve, the nucleus of the solitary tract and the nucleus of the spinal trigeminal nerve. Because of the blood-brain barrier's higher permeability at circumventricular organs such as the AP, peripheral injections of Q-ICS are more likely to reach this area than other brain areas with a less permeable blood-brain barrier. The inability of CM injections to influence IMB intake argues against a central action of peripherally administered Q-ICS on receptors localized to the AP.

In the LV injection experiments, ICS was not only ineffective at increasing intake of IMB, but it also reduced intake of rats fed BAS diets. In both trials using LV injections of ICS, 24-h BAS intakes were reduced. This is in contrast with peripheral and CM injections which did not reduce intake of BAS. In trial one, in which interval food intake measurements were made, rats fed BAS and injected with ICS ate similar amounts of food as control BAS-fed/SAL-injected rats during the first 3 h, but significantly reduced their food intake after 6 h. It is peculiar that BAS-fed/ICS-injected rats did not noticeably reduce their food intake until the period 6–12 h after lights out. If the doses were too high or the drug was causing malaise, the animals should have decreased their food intake much sooner and then recovered by eating more later. Our middle doses of ICS in

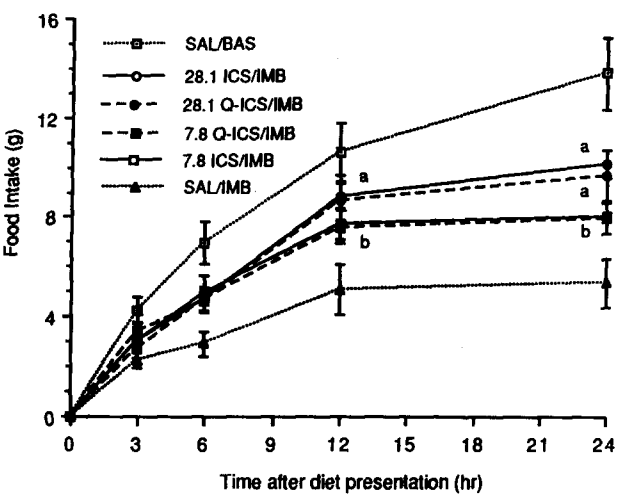

FIG. 2. Cumulative food intake in grams for rats given intraperitoneal injections of ICS 205-930, quarternized ICS 205-930, or saline within one hour prior to lights out, and introduction of basal or imbalanced diets. Points represent means ± SEM of 7 rats/group. Drug (ICS vs. Q-ICS) and dose effects were analyzed by orthogonal contrasts. No drug difference (ICS vs. Q-ICS) was observed at any period; however, dose effect was significant ($p<0.05$) by 6–12 and 12–14 h after diet presentation.

TABLE 1

INTAKE OF IMBALANCED DIET BY RATS RECEIVING INJECTIONS OF ICS 205-930 INTO THE RIGHT LATERAL VENTRICLE

	Dose (pmol)				
Period (h)	SAL	0.31	3.1	31	310
0–3	1.4 ± 0.2	1.3 ± 0.4	1.6 ± 0.2	1.4 ± 0.3	1.8 ± 0.3
3–6	0.4 ± 0.1	0.7 ± 0.3	0.3 ± 0.1	0.7 ± 0.3	0.9 ± 0.1
6–12	2.1 ± 0.6	2.0 ± 0.5	2.1 ± 0.7	1.5 ± 0.6	2.9 ± 0.4
12–24	1.2 ± 0.1	1.1 ± 0.4	0.9 ± 0.3	0.9 ± 0.3	0.8 ± 0.3
24 h Cum	5.1 ± 0.6	5.1 ± 1.1	4.9 ± 0.8	4.6 ± 0.9	6.3 ± 0.6

Values represent mean ± SEM for food intake in grams over the stated period for 6–7 rats/group. All rats received 7 μl injections of SAL or ICS at the doses listed (3.1 pmol = 1.0 ng), into the right LV within 1 h of lights out. IMB diet was presented to all rats at the time of lights out.

trial two were similar to those used by Costall et al. (5) who observed a significant attenuation of aversive behavior in mice injected with 1 to 10 ng of ICS (3.1 to 31 pmol) into specific nuclei. Also, we have previously observed no decrease in BAS intake in rats given peripheral injections of ICS up to 12 mg/kg (14). These doses of ICS should have yielded higher overall brain concentrations than the LV doses. IMB-fed/ICS-injected rats in trial one ate approximately the same amount of IMB as SAL-injected rats. Rats fed IMB diets will normally recognize the IMB and avoid the diet within 30 min to 3 h after ingestion of the first meal (9). The mechanism that causes the reduction in IMB intake is probably separate from the mechanism ICS acts upon to reduce intake of BAS, since ICS did not decrease intake in BAS-fed rats until 6 h after lights out. Differential effects on BAS intake after injections of ICS into the CM from those seen with LV injections are intriguing, and so far unexplained.

It is clear that LV injections of ICS can affect food intake, as shown by the decreased intake of BAS diet. If ICS is working centrally, however, it may not be reaching the site(s) necessary to increase intake of IMB diet. For example, Hartman (15) was unable to increase intake of IMB diets by injecting the dietary limiting amino acid into the LV, while Beverly et al. (1) showed that injection of the dietary limiting amino acid directly into the PPC could result in an increase intake of IMB diet.

When ICS and Q-ICS where injected peripherally, both increased intake of the severe Ile IMB diet in a dose-dependent manner, but there were no significant differences in intake between ICS- and Q-ICS-injected rats. If ICS was working centrally to increase IMB intake, rats receiving Q-ICS should have eaten similar small amounts as SAL-injected rats. If Q-ICS does not cross the blood-brain barrier, these results support the conclusion that ICS acts on peripheral $5HT_3$ receptors to increase intake of IMB diets. Quaternized drugs should not cross the blood-brain barrier (4,13). Nonquaternized ICS has been demonstrated to cross the blood-brain barrier rapidly, and become evenly distributed throughout the brain with no detectable metabolites (Investigators Brochure, Sandoz). However, given the large number of $5\text{-}HT_3$ receptors in the gut, and the apparent ability of 5-HT to affect food intake through peripheral mechanisms [e.g., (7)], it is not unlikely that the antagonists may be working on peripheral receptors to increase intake of IMB.

The plasma half-life for ICS 205-930 in rats and dogs is about 3.5 h (Investigators Brochure, Sandoz). Most studies using ICS were of relatively short duration, and used lower doses of the drug. For example, Tyers et al. (25) used nmol doses of ICS, but their trials only lasted 15 min. Reduction of intake with IMB does not occur until one to two h after ingestion, and the restoration of feeding occurred after 3 h with IP injections of ICS (12,14). Since the action of the antagonist, whatever the mechanism, must be present over a prolonged period (following successive eating bouts), a higher dose may be required than is normally considered to be specific for the $5\text{-}HT_3$ receptor in the short term. High doses of ICS have been reported to be specific for antagonizing the $5\text{-}HT_4$ receptor as well (6), so that receptor should also be considered.

An important component of the IMB paradigm involves the development of conditioned taste aversion (CTA) to the IMB diet (21). In addition to increasing intake of IMB with ICS (14), we have shown that ICS (9 mg/kg IP) blocked the CTA to saccharin paired with lithium chloride (8). If ICS is working similarly in the IMB and LiCl models. it may do so by its antiemetic effects (24) or by interfering with some other gastrointestinal signal. In this case, two possible sites of action would be 1) the $5HT_3$ receptors on vagal afferents that innervate the 5HT-containing enterochromaffin cells of the intestine, or 2) the nucleus of the solitary tract (NTS) and/or AP, which receive input from vagal afferents, and also contain $5HT_3$ receptors. However, as noted above, IMB intake was unaffected by central injections of ICS which should have reached the NTS-AP complex. Further studies of vagal involvement are under way.

The ability of $5HT_3$ antagonists to reduce anxiety in some animals models (25) may also be important in the IMB diet paradigm. Triet (23) has suggested that blockade of the development of CTA is a reasonable test for anxiolytic drugs, but the role of anxiety in the rat's responses to IMB is not clear. Whatever the mechanism, the present data lead us to the conclusion that the effect of ICS 205-930 in increasing the rat's intake of IMB must have a significant peripheral component.

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