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Autonomic efferents affect intake of imbalanced amino acid diets by rats

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Abstract

An anorectic response occurs following ingestion of imbalanced amino acid (IMB) diets. There are three phases o this response: 1, recognition of the IMB diet; 2, conditioned development of an aversion to the IMB diet; and 3, adaptation. Blockade of peripheral serotonin-3 (5-HT_3) receptors or vagotomy attenuates Phase 2 of the anorectic response. We investigated whether sympathetic efferents interact with the ventral gastric branch (VGB), by cutting it (X), or with the 5-HT_3 receptor in these responses. First, VGBX and sham-operated (SHAM) groups were injected with vehicle or phenoxybenzamine (α-blocker), or nadolol (β-blocker) before introducing the IMB diet. At 3 h suppression of the IMB diet ingestion was unchanged, showing no sympathetic efferent effect on Phase 1. Intake of the IMB diet increased 12–24 h later only in the SHAM+phenoxybenzamine group, so the VGB was necessary for α-blockade to enhance IMB diet intake during Phase 2 or possibly Phase 3. On days 2–5, intakes by the SHAM+phenoxybenzamine, VGBX+phenoxybenzamine and VGBX+nadolol groups were elevated. Therefore, α-blockade enhanced adaptation alone, but VGBX was necessary for β-receptor blockade to augment Phase 3 adaptation. Both sympathetic efferents and the VGB are involved in Phases 2–3. Second, rats received vehicle or nadolol or scopolamine (nonselective muscarinic blocker) or pirenzepine (muscarinic M-1 receptor blocker), w+/− tropisetron (5-HT_3 blocker). Pirenzepine attenuated the tropisetron effect between 6–9 h, but then pirenzepine and nadolol enhanced the tropisetron effect between 9–12 h. Scopolamine attenuated the tropisetron effect between 9–12 h. While neither experiment showed effects during the recognition phase, the autonomic and serotonergic systems interact in the learned and adaptive responses to IMB diets.

Keywords: Nadolol; Phenoxybenzamine; Pirenzepine; Scopolamine; Tropisetron

1. Introduction

Rats show reduced intake of an imbalanced amino acid (IMB) diet as early as 30 min after diet introduction (Gietzen et al., 1986; Gietzen, 2000; Koehnle et al., 2003). This is the recognition phase, in which the rat senses that it is ingesting an IMB diet, and is called Phase 1. Phase 1 is initiated in the anterior piriform cortex, with subsequent modulation in the hypothalamus (Bellinger et al., 1998; Bellinger et al., 1999; Blevins et al., 2000; Gietzen, 1993; Gietzen et al., 1998). Next, during Phase 2 the rat develops a conditioned aversion (CA) to the taste or other orosensory, or olfactory qualities of the IMB diet (Simson and Booth, 1973; Terry-Nathan et al., 1995). The initial learning for development of the CA appears to have taken place by 6 h and is fully established by the second day (Feurte et al., 2002). A role for 5-HT_3 receptors in the CA effect was demonstrated by the use of 5-HT_3 receptor antagonists, such as tropisetron, which attenuate, but do not completely reverse, the early Phase 2 anorectic effects of the IMB diet (Gietzen et al., 1987; Hammer et al., 1990a; Jiang and Gietzen, 1994). Hrupka et al. (1991) showed that the 5-HT_3

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antagonist acts, at least in part, in the periphery. In addition, rats with total subdiaphragmatic vagotomies increase their intake of the IMB diet during Phase 2, ~3–9 h, whether given tropisetron or vehicle injections (Pavelka et al., 1996; Washburn et al., 1994). However, the tropisetron effect is attenuated following vagotomy and blunted even more when a combination of vagal cuts and splanchnicectomy are used (Pavelka et al., 1996). This suggests that tropisetron could interact with both the vagal and sympathetic systems (reviewed in Bellinger et al., 1993; Sanger, 1992; Washburn et al., 1994).

The critical branches of the vagus supporting Phase 2 of the IMB diet effect are a combination cut (X) of the hepatic vagal branch and at least one of the gastric branches, either the ventral gastric (VGBX) or the dorsal gastric branch (Dixon et al., 2000; Pavelka et al., 1996). The topography of the salient vagal fibers points to innervation of the distal stomach and the proximal duodenum (Berthoud et al., 1991) and these segments may be involved in the development or maintenance of the aversion.

In Phase 3, after day 2, rats begin to adapt to the IMB diet with altered feeding patterns, which are manifested by rats taking smaller meals and extending their feeding into the light phase (Erecius et al., 1996). These changes apparently prevent the rapid influx of large amounts of amino acids from the IMB diet into the gut and circulatory system (Sanger, 1992). Using this strategy the rats increase their intake of the diet slowly over the next few days. There also appears to be peripheral involvement in the adaptive phase of IMB diet consumption, because loss of the hepatic vagal branch enhances adaptation to the IMB diet (Bellinger et al., 1993; Bellinger et al., 1996).

The findings that tropisetron and/or vagal branch cuts attenuated, but did not eliminate the anorectic effects of the IMB diet suggests that pathways other than vagal may be involved in the rat's response to the IMB diet. Whether these additional pathways are central or peripheral is unknown. Peripherally the splanchnic nerves carry both efferent fibers and large numbers of afferent fibers (Aidar et al., 1952; Grundy, 1988) and they are known to influence feeding behavior (Stuckey et al., 1985). It is thus possible that sympathetic fibers could also be involved in the rat's early and/or late feeding responses to an IMB diet. In addition to possible afferent neuronal involvement, activation of vagal and/or sympathetic efferents could be involved in the rat's response to the IMB diet, possibly via recurrent stimulation of relevant portions of the gastrointestinal tract.

In the first experiment of the present study rats were given sympathetic α (phenoxybenzamine) or β (nadolol) receptor blocking agents to determine whether sympathetic efferents are involved in the rat's feeding response to IMB diets. Additionally, some of the rats were given a VGBX to determine whether the VGBX would interact with sympathetic efferent fiber blockade to increase Phase 2 and 3 intake of the IMB diet. In the second experiment, the 5-HT_3 receptor blocker tropisetron was used in place of cutting the VGB. The interaction of tropisetron with sympathetic and vagal efferents was investigated using nadolol to block sympathetic β-adrenergic receptors, scopolamine to block vagal muscarinic receptors non-selectively, or pirenzepine to block sympathetic-postganglionic cell body and myenteric ganglia muscarinic receptors of the M-1 subtype (Goyal, 1988).

2. Methods

Male Sprague–Dawley rats (150–300 g) were purchased from Harlan Industries, Houston, Texas or Bantin and Kingman, Lafayette, CA, and upon arrival they were housed individually in a temperature controlled room (22 ± 2 °C) under a reversed 12:12 h light:dark cycle with lights out at 1000 h in Experiment 1 and 1100 hours in Experiment 2. Rats were given a stock diet (Purina rat chow #5012, Ralston, St. Louis, MO) during habituation to the laboratory, followed by defined diets as described below and water, ad libitum. All procedures were done under the NIH guidelines for animal use and care and had previously been approved by the Institutional Animal Care and Use Committees.

2.1. Experiment 1

Eight days after arrival, rats were anesthetized prior to surgery with ketamine (90 mg/kg) and xylazine (9 mg/kg). At the time of surgery, rats were divided into groups that received either cuts of the VGB below the hepatic vagal branch, i.e., VGBX, or sham operations (SHAM). Surgical procedures were carried out as previously described (Bellinger and Williams, 1983; Lambert, 1965; Pavelka et al., 1996; Powley et al., 1987). The hepatic vagal branch was left intact because, as noted above, cutting it and the VGB decreases Phase 2 aversion and thus increases intake during Phase 2 (Bellinger et al., 1993; Bellinger et al., 1996). Additionally, cutting the hepatic vagal branch alone enhances adaptation to Phase 3. Therefore, if the hepatic vagal branch were transected in this study, with or without a VGBX, it might mask a sympathetic efferent effect during these two phases. Following surgery, all rats were presented with a complete liquid diet (Ensure, Ross Laboratories, Columbus, OH) for 2 days. On the next day, all rats were presented with a diet consisting of 17% casein in an agar gel (Difco Laboratories, Detroit, MI). After 5 days on the gel diet rats were switched to an isoleucine basal diet for 4 days. On day five the basal diet was removed at 0700 hours and rats were injected intraperitoneally (ip) with vehicle at 0930 hours and the basal diet was then returned at 1000 hours. This procedure was repeated on days 6 and 7, but on these days after return of the basal diet food intake, corrected for spillage, was recorded 3, 6, 12 and 24 h later.

On day 8 rats were divided into six groups and injected ip at 0930 hours: VGBX+saline or propylene glycol (vehicle); VGBX+nadolol (Sigma, St Louis, MO) at a dose

of 5 mg/kg body weight (Yamashita et al., 1994; Yat and Cho, 1994), dissolved in distilled water and pH adjusted to 7.0 with HCl (Yamashita et al., 1994; Yat and Cho, 1994); VGBX+phenoxybenzamine (SmithKline Beecham, Philadelphia, PA) at a dose of 1 mg/kg body weight, dissolved in propylene glycol (Salles et al., 1994); SHAM+vehicle; SHAM+nadolol; and SHAM+phenoxybenzamine. Nadolol is a competitive non-selective β-adrenergic receptor blocker (Borchard et al., 1991) and phenoxybenzamine is an irreversible non-selective α-adrenergic receptor blocker (Borchard et al., 1991). At 1000 hours rats were presented with the isoleucine IMB diet and food intake recorded at the intervals described above. Over the next 4 days, 24 h intakes were recorded. Body weights were recorded daily throughout the study.

At the end of this period completeness of VGB transection was verified using techniques as previously described (Bellinger and Williams, 1983; Powley et al., 1987). Animals showing incomplete VGBX were eliminated from the data set.

2.2. Experiment 2

For the second experiment, tropisetron (Research Biochemicals Int., Natick, MA) was used, because it reliably increases IMB diet intake and likely works through pathways other than the VGBXs of Experiment 1. The tropisetron treatment was combined with treatments that affect autonomic efferents in parallel with the first experiment. Three trials were conducted using tropisetron, it was combined with either: nadolol, scopolamine, a nonselective muscarinic antagonist (Sigma, St Louis, MO) or pirenzepine, a selective muscarinic M_1 receptor antagonist (Sigma, St Louis, MO).

Following habituation to the laboratory, rats were given an isoleucine or threonine basal diet, depending on the limiting amino acid in the IMB diet to be used (either isoleucine or threonine) for 10 days. It should be noted that any indispensable amino acid can be used in the IMB diet model (see Harper et al. (1970) for a full review of the model); several different limiting amino acids have been used over the years, with similar results (see Gietzen et al., 1998; Hammer et al., 1990a for comparisons between isoleucine—and threonine—limited diets). Therefore, the imbalanced diets used in the present experiments are fully interchangeable.

Food intake was measured after 3, 6, 9, 12, and 24 h on basal diet days 9 and 10. On day 11 at 1000 hours, 1 h prior to lights out, rats received injections. Rats in the nadolol and scopolamine trials received the threonine IMB diet at lights out. In the pirenzepine trial the isoleucine basal diet was used during the basal diet pre-feeding period and the isoleucine IMB diet was given on Experimental days 1–3. Food intake of the IMB diet was measured after 3, 6, 9, 12, and 24 h on the first experimental day, followed by a 24 h food intake measurement on days 2 and 3. In the three trials all the groups had eight rats.

2.2.1. Trial 1, Tropisetron and Nadolol

The rats were randomly assigned by body weight to one of four groups: vehicle+vehicle; vehicle+tropisetron; nadolol+vehicle; nadolol+tropisetron. Tropisetron was given ip at a dose of 9 mg/kg body weight (Hammer et al., 1990a; Erecius et al., 1996) and nadolol was injected ip at a dose of 1 mg/kg body weight (Yamashita et al., 1994; Yat and Cho, 1994).

2.2.2. Trial 2, Tropisetron and Scopolamine

The rats were randomly assigned by body weight to one of four groups: vehicle+vehicle; vehicle+tropisetron; scopolamine+vehicle; scopolamine+tropisetron. Tropisetron was given as described in Trial 1 and scopolamine hydrochloride dissolved in saline was injected sc. The dose was selected by testing basal diet intake after published effective doses that range from 0.02 to 0.8 mg/kg (Drinkinburg et al., 1995). The highest dose that did not decrease intake of the basal diet was used, i.e., 0.5 mg/kg body weight.

2.2.3. Trial 3 Tropisetron and Pirenzepine

For the pirenzepine trial, a preliminary trial was conducted to find a dose that did not affect basal diet intake. This dose was 0.42 mg/kg (Safsten et al., 1994). The groups were: vehicle+vehicle; vehicle+tropisetron; pirenzepine+vehicle; pirenzepine+tropisetron. Tropisetron was given as in Trial 1 and pirenzepine dissolved in saline was injected ip.

2.2.4. Diets

After pretreatment as described above, rats were switched to a purified basal diet (with the appropriate limiting amino acid) for 10 days and then during the experimental day they received either isoleucine or threonine IMB diet. As noted above, these diets have been described previously (Bellinger et al., 1998; Gietzen et al., 1998; Hammer et al., 1990b) and are used routinely with similar anorectic responses and thus are completely interchangeable (Beverly et al., 1990; Hammer et al., 1990a).

2.2.5. Statistical analysis

For Experiment 1 the data (period food intake, daily food intake and change in body weight) were evaluated using two-way analysis (surgery and drug) of variance (ANOVA) with time as the repeated measure (Abstat version 1.94, Anderson-Bell, Arvada, CO). Data found to have a significant ANOVA were subsequently analyzed using Duncan's multiple-range test. For Experiment 2 the data (interval food intake, daily food intake and pre and post-basal feeding body weights) were analyzed using the type III sums of squares routine in the General Linear Model procedure (SAS Institute, Cary, NC) with error factors determined for tropisetron as drug 1, the second drug as

drug 2, and a third error factor for diet and, both diet and drug interactions in the feeding trials. Where body weights were analyzed, a simple one-way ANOVA was used. Where a significant ANOVA was seen, post-hoc differences among the group means were determined by the Fischer's protected Least Significant Difference means test. Using two tailed tests for the above comparisons, a P value of <0.05 was considered significant.

3. Results

3.1. Experiment 1

After eliminating rats with incomplete VGB transections or that died after surgery all groups had $n=11$, except the VGBX+phenoxybenzamine group which had $n=12$.

The body weights of the groups were similar at the time of surgery (group means ranged from 229 to 237 g) and at the end of basal diet period (group means ranged from 266–273 g).

The food intake of the VGBX+NADO or SHAM+NADO groups did not differ from each other or with their control groups during the 0–3, 3–6, or 6–12 h measurement periods, surgery × drug interaction [F(1,40)=0.07, ns], data not shown. The food intake (Fig. 1) of the VGBX+phenoxybenzamine and SHAM+phenoxybenzamine groups showed significant differences on day one, surgery × drug interaction [F(1,42)=5.23, $P<0.03$]. The of the VGBX+phenoxybenzamine and SHAM+phenoxybenzamine groups did not differ from each other or with their control groups during the 0–3 h or 3–6 h measurement period, however during the 9–12 h period the intake of the SHAM+phenoxybenzamine group was significantly

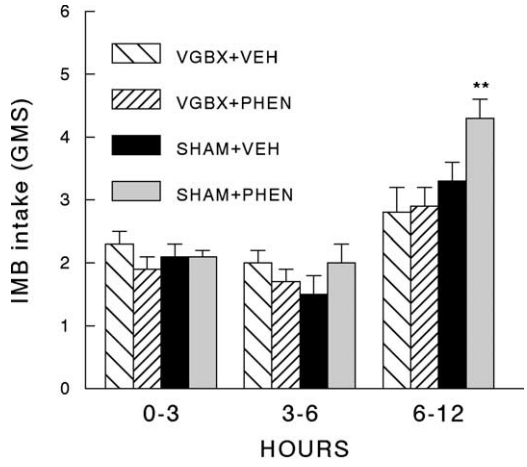

Fig. 1. Imbalance amine acid (IMB) diet intake, expressed as grams eaten, during intervals on Experimental day one. Groups are identified: VGBX=ventral vagal transected below the hepatic vagal branch; SHAM=sham operation; and ip injections of vehicle (VEH)=saline or propylene glycol; PHEN=Phenoxybenzamine. Statistical comparisons made with SHAM+VEH group; **=$P<0.01$. Means ± SEM.

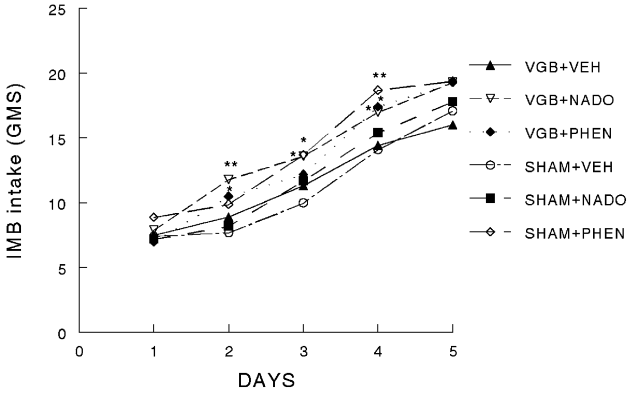

Fig. 2. Daily food intake after switching to the IMB diet. Abbreviations are the same as the legend for Fig. 1. Statistical comparisons made with SHAM+VEH group; *=$P<0.05$ and **=$P<0.01$. SEM range (0.36–1.92).

higher than the other groups. The groups ate little over the next 12 h.

Inspection of daily IMB diet intake (Fig. 2) indicates a significant drug effect occurred during the first 4 days of ingesting the IMB diet [F(2,62)=5.61, $P<0.01$]. There was also a significant day effect [F(4,248)=206.8, $P<0.001$], but non-significant surgery × day interaction, [F(4,248)=1.89, $P>0.11$] and drug × day interaction [F(8, 248)=1.67, $P>0.10$].

The SHAM+phenoxybenzamine group's consumption of the IMB diet remained elevated on days 2, 3, and 4 when compared to the SHAM+vehicle group (Fig. 2). The VGBX+phenoxybenzamine group's intake did not differ significantly from the VGBX+vehicle group on day 1, but that group's intake was elevated when compared to the VGBX+vehicle group on days 2 thru 4. Thus the phenoxybenzamine effect of enhancing adaptation to the IMB diet was delayed by 1 day in the VGBX+phenoxybenzamine group.

The daily intake of SHAM+nadolol group did not differ from the SHAM+vehicle over the course of the study (Fig. 2). The IMB diet intake of the VGBX+nadolol was significantly elevated by day 2 when compared either to the VGBX+vehicle, SHAM+vehicle, or SHAM+nadolol groups. The intake of the VGBX+nadolol group remained elevated ($P<0.05$) over the SHAM+vehicle group on days 3 and 4.

Body weight changes (Fig. 3), from the last day they were on the basal diet, differed with both significant surgery [F(1,62)=4.87, $P<0.03$] and drug [F(2,62)=5.75, $P<0.01$] effects, over the 5 days they were given the IMB diet and, in general, weight changes were reflective of the food intake data.

3.2. Experiment 2

3.2.1. Trial 1 Tropisetron and Nadolol

Prior to introduction of the basal diet the body weights of the groups were similar (group means ranged from 161

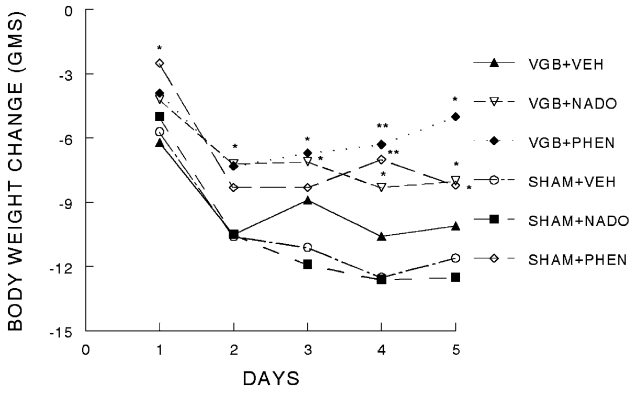

Fig. 3. Change in body weight, expressed in grams (GMS), after introduction of the IMB diet. The change in body weight is from the rat's weight at the end of the basal diet period. NADO=nadolol. Abbreviations are the same as the legend for Fig. 1. Statistical comparisons made with SHAM+VEH group; *=$P<0.05$ and **=$P<0.01$. Means ± SEM.

to 162 g) and on the injection day were again similar (group means ranged from 175 to 178 g).

On the first experimental day when intake was broken down into 3 h periods (Fig. 4) during the 9–12 h interval nadolol enhanced the tropisetron effect [overall $F(3,27)=7.10$, $P<0.001$]. The intake of the nadolol+tropisetron group tended to be higher, i.e., 34% ($P=0.09$), than the vehicle+tropisetron group (nadolol+tropisetron vs vehicle+vehicle, $P<0.001$; tropisetron alone vs vehicle+vehicle, $P<0.02$). The 12–24 h light-period intake is not shown here or in Trials 2 and 3 because as usual, rats consumed little IMB diet during the light phase. On the second and third day there were no differences in IMB diet intake among any of the groups (data not shown).

3.2.2. Trial 2 Tropisetron and Scopolamine

Prior to introduction of the basal diet the body weights of the groups were similar (group means for the body weights ranged from 165–166 g) and on the injection day were again similar (group means ranged from 166–169 g).

On the first experimental day when intake was broken down into periods (Fig. 5), during the 9–12 h period, scopolamine attenuated the tropisetron effect. Thus, while the vehicle+tropisetron group consumed significantly ($P<0.05$) more IMB diet than the vehicle+vehicle group as usual, the difference between the scopolamine+tropisetron group and the vehicle+vehicle group failed to reach significance for the 9–12 h period [overall ANOVA for period: $F(3,28)=2.52$, $P=0.08$, ns]. By the end of the second experimental day, the amount of IMB diet eaten by the vehicle+tropisetron group was significantly lower than all other groups (vehicle+vehicle, 9.9 ± 0.4 g; vehicle+tropisetron, 7.3 ± 0.3 g; scopolamine+vehicle, 9.2 ± 0.5 g; scopolamine+tropisetron, 9.3 ± 0.3 g) [$F(3,28)=5.85$, $P<0.01$]. This was due to a carryover tropisetron effect [$F(1,28)=7.09$, $P<0.02$] and a scopolamine+tropisetron interaction [$F(1,28)=8.59$, $P<0.01$].

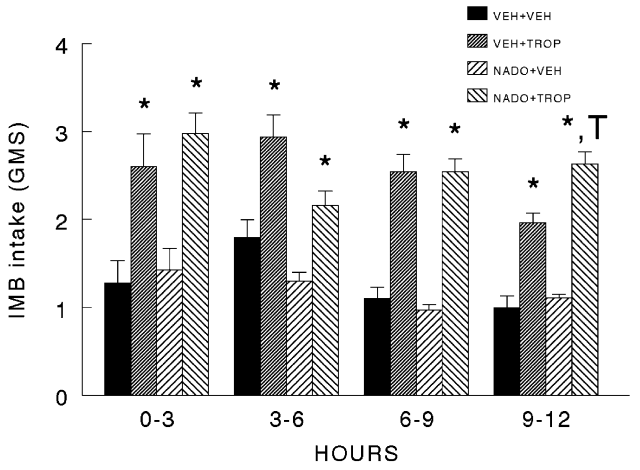

Fig. 4. IMB diet intake during intervals on experimental day one, expressed as grams eaten. TROP=tropisetron. Abbreviations are the same as the legend for Fig. 1. Statistical comparisons of TROP groups made with the VEH+VEH group; *=$P<0.05$, while the "*T*" indicates a $P<0.09$ trend towards a NADO effect, i.e., NADO+TROP compared to VEH+TROP. Means ± SEM.

3.2.3. Trial 3 Tropisetron and Pirenzepine

Prior to introduction of the basal diet the body weights of rats for the combined runs 1 and 2 were similar (group means ranged from 281–282 g) and on the injection day were again similar (group means ranged from 284–285 g).

On the first experimental day when intake was broken down into periods (Fig. 6), during the 6–9 h period pirenzepine inhibited the tropisetron effect [$F(3,28)=1.36$, $P<0.05$]. The opposite occurred during the 9–12 h period [$F(3,28)=15.15$, $P<0.001$]. While the vehicle+tropisetron group still had a significantly increased intake of the IMB diet, i.e., 150% increase over the vehicle+vehicle group's intake, the pirenzepine (pirenzepine+tropisetron group, $P<0.01$) further enhanced the tropisetron effect, i.e.,

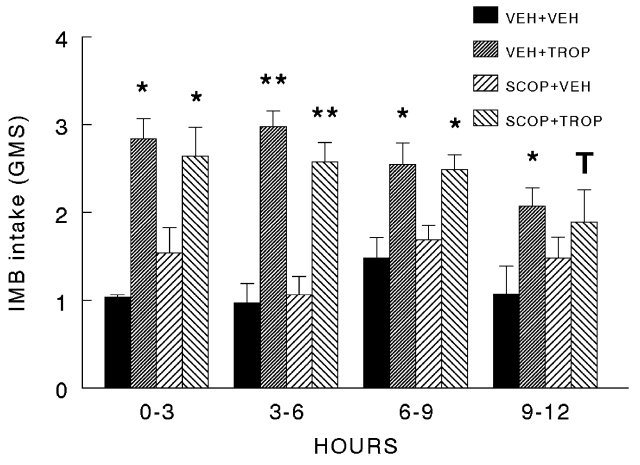

Fig. 5. IMB diet intake during intervals on experimental day one, expressed as grams eaten. SCOP=scopolamine. See legends of Figs. 1 and 4 for abbreviations. Statistical comparisons of TROP groups made with their respective VEH group; *=$P<0.05$ and **=$P<0.01$, while "*T*" indicates a trend, P=0.051 when TROP+SCOP group is compared with TROP+VEH group. Means ± SEM.

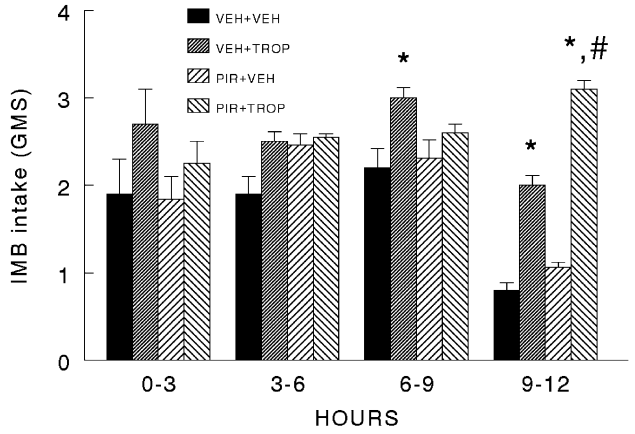

Fig. 6. IMB diet intake during intervals on Experimental day one, expressed as grams eaten. PIR = pirenzepine. See legends of Figs. 1 and 4 for abbreviations. Statistical comparisons of TROP groups are made with their respective VEH group; * = $P < 0.05$, while "#" indicates PIR interacted with TROP to enhance ($P < 0.01$) IMB diet intake when the PIR + TROP group was compared with TROP + VEH group. At 12–24 h the PIR + TROP group's intake was also higher ($P < 0.05$) than the TROP + VEH group (data not shown).

285% ($P < 0.001$) increase over the vehicle + vehicle group's intake. During this time period pirenzepine by itself (pirenzepine + vehicle group) did not significantly increase the group's intake of the IMB diet. On the second experimental day, pirenzepine had no significant effect and the vehicle + tropisetron group alone only tended ($P < 0.09$) to be higher than the double vehicle group.

4. Discussion

IMB diet intake by the vehicle treated groups and the groups treated with the irreversible adrenergic α-receptor antagonist, phenoxybenzamine, the competitive adrenergic β-receptor antagonist, nadolol, the selective ganglionic M-1 cholinergic receptor blocker, pirenzepine, or the general muscarinic blocker, scopolamine, were similarly low 3 h after eating the IMB diet. The only drug treated groups showing increased IMB diet intake in the first 3 h were those given the 5-HT_3 antagonist, tropisetron. These data indicate that neither the sympathetic efferent neurotransmitters that affect α or β receptors, nor parasympathetic muscarinic receptors are involved in Phase 1 when rats recognize the amino acid deficiency caused by an IMB diet. These data support previous results showing that recognition (Phase 1) occurs centrally (Bellinger et al., 1998; Bellinger et al., 1999; Beverly et al., 1990; Blevins et al., 2000; Gietzen et al., 1986; Gietzen et al., 1998; Gietzen, 2000; Leung and Rogers, 1971; Meliza et al., 1981). Additionally, the data also confirm an earlier report (Pavelka et al., 1996) that cutting the VGB alone (VGBX + vehicle group) does not interfere with the rat's ability to recognize that it is consuming an IMB diet.

Previously it has been demonstrated that CA (early Phase 2) to the IMB diet is manifested by the sixth hour of ingesting the diet (Erecius et al., 1996; Feurte et al., 2002). It was also shown that tropisetron can block the development of the CA (Terry-Nathan et al., 1995). An IMB diet alters meal patterns during Phase 2 (Feurte et al., 2002) and these changes are ameliorated by either tropisetron or a total subdiaphragmatic vagotomy (Erecius et al., 1996). Neither of the sympathetic blockers in the present study, i.e., the α-blocker phenoxybenzamine or the β-blocker nadolol, affected the early (first 6 h) development of Phase 2; similarly, the selective muscarinic M-1 blocker pirenzepine and the non-selective blocker scopolamine did not affect IMB intake in this period. This suggests that the serotonergic system, but not the parasympathetic or sympathetic systems, is an important mediator of early Phase 2.

In the present study, phenoxybenzamine ameliorated the anorexia during late Phase 2 at 6–9 and 9–12 h, suggesting involvement of the α−adrenergic receptor in the presence of the vagus nerve and intact 5 HT function.

Scopolamine blocked the tropisetron enhancement of IMB diet intake during the 9–12 h interval suggesting that vagal efferents are involved in the reinforcement of late Phase 2. Pirenzepine, which attenuates activation of both sympathetic and myenteric ganglia, enhanced the tropisetron effect during the 9–12 and 12–24 h intervals. This suggests sympathetic efferents are also involved in late Phase 2. As noted, the 12 and 24 h intake of IMB diet was elevated in the SHAM + phenoxybenzamine group, but not the VGBX + phenoxybenzamine group. Therefore, an intact VGB was necessary for α-adrenergic blockade to enhance adaptation late in Phase 2. The phenoxybenzamine-induced enhanced adaptation was first observed 12 h after introduction of the IMB diet and this 20–35% increase in intake of the IMB diet lasted for 4 days. Therefore, α-adrenergic receptors apparently are involved in the ability of the IMB diet to induce long-term food intake suppression, and thus appear to be associated with Phase 2 and Phase 3, the adaptation phase. These data are consistent with the pirenzepine data. Pirenzepine, which would decrease sympathetic activity via ganglionic blockade, augmented the tropisetron induced increased IMB diet consumption during the 9–24 h interval. However, pirenzepine also acts at muscarinic receptors and its activities were similar to that of scopolamine, which suggest interactions among these receptors.

When the ventral vagal trunk was transected below the hepatic vagal branch, the enhanced adaptation to the IMB diet induced by phenoxybenzamine did not occur until the second day of ingesting the diet. This would indicate that either the VGB or the accessory celiac branch is necessary for the early effects of phenoxybenzamine. An earlier study (Pavelka et al., 1996) indicated that the accessory celiac is not involved in the rats' response to IMB diet. Taken as a whole these data suggest that, in the phenoxybenzamine trial, the important tract that was cut was the VGB.

Nadolol competitively blocks peripheral, but not central, β-adrenergic receptors. If β-adrenergic receptors were directly involved in the Phase 3 adaptation to an IMB diet then the SHAM+nadolol groups should have had an elevated consumption of the IMB diet. This did not occur and the intake of the SHAM+nadolol group was very similar to the SHAM+vehicle control throughout both experiments. The data do show that, when the VGB was transected and the animals were given the β blocker, their intake was elevated some 50% over the SHAM+vehicle group on the second day of ingesting the IMB diet. In the second experiment nadolol enhanced the effects of tropisetron at the 9–12 h period of day one. Therefore, interactions between the sympathetic efferents, the VGB and the serotonin system may occur in late Phase 2 and early Phase 3. The mechanism of action by which nadolol in the VGBX and tropisetron-treated groups increases intake is uncertain, but together with phenoxybenzamine data it appears that these effects may be generalized across receptor types of sympathetic efferents.

It is not established in the intact animal whether it is sensory or motor information carried in the VGB that is important in the parasympathetic interactions in Phase 2 and 3. Peripheral afferents may affect central sites that could in turn increase autonomic nervous system outflow to the periphery, where activation of various receptors in the gastrointestinal tract could reduce the consumption of the IMB diet during late Phase 2 and Phase 3. IMB diet intake might be reduced by the adrenergic receptor activation changing motility in the gut (Ganong, 2003), which could indirectly decrease intake. Moreover, tropisetron, which has gastrokinetic properties, can activate vagal efferents (reviewed in [Bellinger et al., 1993]), while also attenuating the activity of afferents by acting on 5-HT_3 receptors on both spinal and vagal afferent neurons (Bellinger et al., 1993). As noted above, it is thought that the amino acid deficiency is detected centrally (Beverly et al., 1990; Gietzen et al., 1998; Gietzen, 2000; Leung and Rogers, 1971), yet, sensory afferents in the VGB could reinforce that the animal is consuming an IMB diet, and that it should continue to eat less of the IMB diet. If this is the case, one would need afferents from both hepatic and ventral gastric branches of the vagus (Pavelka et al., 1996).

Additionally, the amino acid deficiency could activate both sympathetic and VGB efferent neurons, as suggested by the results with pirenzepine and scopolamine. A third possibility might be that the amino acid imbalance simultaneously activates competing VGB and sympathetic efferent neuronal activity to the stomach and pylorus. Giving phenoxybenzamine or nadolol should block the sympathetically-driven decreases in gastrointestinal motility and reduce the tone of the various intestinal sphincters. This would allow an unopposed parasympathetic effect and an increase in gastric emptying and upper intestinal activity. The anatomy of this effect would be consistent with the pattern of effective cuts seen in Dixon et al. (2000), which pointed to the distal stomach and proximal duodenum as effective sites. Since no single treatment fully restores IMB diet intake to basal diet levels, several systems appear to be involved in these responses. The present results point clearly to such interactions.

In conclusion, taken together these data support the hypothesis that during Phase 1 the IMB diet is recognized centrally and that a subsequent vago-vagal loop is activated, which reinforces the CA of Phase 2. Such activation would be consistent with previously shown circuits (Gietzen et al., 1998; Wang et al., 1996b) in the brain involving projections from the anterior piriform cortex with the dorsomedial hypothalamic nucleus (Bellinger et al., 1998; Bellinger et al., 1999), lateral hypothalamic areas (Blevins et al., 2000), and the amygdala (Wang et al., 1996a). Activation of these brain areas is known to initiate vago-vagal and sympathetic loops in many models (Rogers et al., 1995). Such a loop could reinforce the gastrointestinal input to the central feeding circuits as well as the aversive components of the responses, maintaining the learned aversion to the IMB diet, the primary feature of Phase 2. Thus, the available data support that both afferent and efferent limbs of the autonomic system are important in the maintenance of the CA to IMB diets throughout late Phase 2. Finally, both parasympathetic and sympathetic pathways appear to be involved in Phase 3.

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