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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