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Intragastric infusion of denatonium conditions flavor aversions and delays gastric emptying in rodents

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Abstract

Because most naturally occurring toxins taste bitter to humans, any mechanism that reduces the rate at which bitter substances are ingested and digested should be adaptive. Based on the recent discovery of T2R bitter taste receptors in the gastrointestinal tract of rodents, we asked whether intragastric (IG) infusion of denatonium (a ligand for T2R receptors) would condition a flavor aversion and/or delay gastric emptying. Four experiments tested for post-oral responses to denatonium in rodents. First, Sprague–Dawley rats were trained to associate intake of a flavored solution (the CS+) with IG denatonium infusions, and intake of a different-flavored solution (the CS–) with IG water infusions during 30 min/day sessions. The rats acquired an aversion to the CS+ flavor when it was paired with IG infusions of 10 mM (but not 2.5 mM) denatonium. Intragastric infusions of 10 mM denatonium also delayed gastric emptying of food in the same rats. Second, we asked how long it took for rats to suppress their drinking while being infused IG with 10 mM denatonium. Rats drinking a palatable solution paired with IG infusions of 10 mM denatonium suppressed their licking within 6 min, as compared to rats infused IG with water. Third, we trained C57BL/6J (B6) mice 24 h/day to associate a CS+ flavor paired with IG infusions of 12 mM denatonium (diluted to 6 mM by orally consumed CS+). Like rats, the mice acquired a robust aversion to the CS+ flavor when it was paired with IG infusions of denatonium. A final experiment assessed the potential toxicity of denatonium. To this end, we gave B6 mice a 6 mM denatonium solution as their only source of water for 3 weeks. The mice grew normally and did not display any clinical signs of denatonium toxicosis. This study provides the first evidence that rodents respond to the presence of "bitter" substances in their gastrointestinal tract by generating both behavioral and physiological responses.
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1. Introduction

Many naturally occurring foods contain toxins [7,21,22]. The orosensory systems (taste, smell and somatosensation) constitute an important mechanism for detecting these dangerous foods because most toxins elicit aversive oral sensations (e.g., bitterness or astringency) in humans [2,6,7,14,35] and in other animals [16]. This oral protective mechanism is not foolproof, however. For example, the aversive oral sensations elicited by many bitter compounds can be masked (partially or completely) by the presence of sugars [27,31,51] or sodium [4,5]. Further, once animals adapt to the taste of some harmless bitter compounds [23,33,56,59], their tolerance to the taste of other toxic bitter compounds would increase [28,34,37]. There is evidence that leeches possess a "bitter taste" system in their gastrointestinal tract, which might function in situations where the oral mechanism failed [30]. We asked whether an analogous system exists in rodents.

Several observations provide support for the notion that mammals possess an extensive, but diffuse, network of chemosensory cells in their gut (i.e., stomach, small intestine, pancreatic duct and colon), which senses bitter substances. First, some of the chemosensory cells in the gut express the same

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proteins that mediate bitter taste in the oral cavity, including T2R bitter taste receptors [40,57,58] and three downstream signaling proteins, α-gustducin, PLC β2 and Trpm5 [3,12,24,25,50,52]. Second, two T2R ligands (denatonium and phenylthiocarbamide [38,43]) induce Ca^{2+} signaling and CCK release in enteroendocrine STC-1 cells [9]. Third, gastric infusion of denatonium elicits a strong excitatory response in the rat's vagus nerve [53], which relays input from chemosensory cells in the gut to the brainstem [45]. Fourth, bitter stimuli elicit a concentration-dependent decrease in stomach contractions when infused intragastrically in dogs [8,36].

Because rodents lack the vomiting reflex, they cannot eject poisons once they have been swallowed. For this reason, it has been suggested that rodents should have evolved multiple mechanisms for protecting them against toxic foods [10]. Here, we tested the hypothesis that rodents respond to the presence of "bitter" substances in their gastrointestinal tract by reducing the rate at which foods are ingested and digested. To evaluate this hypothesis, we conducted four experiments. First, we asked whether intragastric (IG) infusion of a bitter taste stimulus (denatonium) would generate negative feedback in rats, leading to a conditioned flavor aversion or delayed gastric emptying. Second, we determined how long it took for IG infusions of denatonium to inhibit licking for a palatable substance. Third, we ascertained whether IG infusions of denatonium would condition a flavor aversion in mice. Fourth, we explored the possibility that denatonium was conditioning an aversion in the mice through a toxicity mechanism.

2. Do IG infusions of denatonium elicit behavioral or physiological responses in rats? (Experiment 1)

Prior studies reported that denatonium and other T2R ligands can both inhibit feeding [1,27] and delay gastric emptying [13] in rats. However, because the T2R ligands were ingested, it is impossible to determine the relative contribution of oral vs. post-oral stimulation to the responses. In this experiment, we asked whether IG stimulation by denatonium alone is sufficient to condition a feeding aversion and delay gastric emptying in rats.

2.1. Methods

2.1.1. Animals

We used 15 male Sprague–Dawley rats born in our laboratory from stock purchased from Charles River Laboratories (Wilmington, MA). The rats were 100 days old at the start of testing. We housed each rat individually in a standard wire-mesh cage in a vivarium maintained at 21 °C with a 12:12 h light/dark cycle (lights on 0800 h). We provided powdered chow (No. 5001, PMI Nutrition International, Brentwood, MO) and tap water *ad libitum*. We subjected the same 15 rats to Experiments 1A-D.

2.1.2. Surgery

We anesthetized the rats with ketamine (63 mg/kg) and xylazine (9.4 mg/kg) and then fitted them with an IG catheter based on a technique adapted from Davis and Campbell [11]. In brief, a silastic tube (i.d.: 1.02 mm; o.d.: 2.16 mm) was inserted into the fundic region of the stomach. We routed the silastic tube under the skin to the back of the neck, and then connected it to a Luer-lock assembly, which was fixed to the skull with dental cement and stainless-steel screws.

2.1.3. Apparatus

We trained and tested the rats in plastic infusion cages. For the IG infusions, we ran plastic tubing from a syringe pump to the input port of a swivel on a counter-balanced lever. We connected the swivel's output port to the rat's IG catheter via additional plastic tubing, which was protected by a stainless-steel spring.

We offered the conditioning stimuli (i.e., two different fruit-flavored solutions; see below) in two stainless-steel drinking spouts. The spouts could be accessed through slots in the front wall of the plastic infusion cage, centered and 32 mm apart. We attached each drinking spout to a separate fluid reservoir, each of which was mounted on a motorized holder. These holders positioned the spouts in front of the slots (so that they could be accessed by the rats) at the start of the 30-min test session, and retracted them at the end of the session. Trays below the sipper tubes collected spillage. We monitored licking with an electronic lickometer (Med Electronics, St. Albans, VT) and a microcomputer, which controlled the syringe pumps.

During each training or testing session, the computer software accumulated licks and turned the infusion pumps on or off, as required, every 3 s. The infusion pump delivered the test solution directly into the stomach at a rate of 1.3 ml/min; the oral intake/infusion ratio was maintained at ~1:1 by computer software. With this system, the animal controlled the infusion volume by its licking behavior, and the concentration of infused denatonium was diluted by the orally consumed fluid; e.g., an infusion of 2.5 mM denatonium was diluted to 1.25 mM denatonium in the stomach. We recorded CS intakes to the nearest 0.1 g and IG infusions to the nearest 0.5 ml.

2.1.4. Test solutions

Each conditioning stimulus (CS) contained a 0.05% Kool-Aid flavor (General Foods, White Plains, NY) and 0.2% sodium saccharin (Sigma-Aldrich, St. Louis, MO) dissolved in water. We used three different flavor pairs: cherry and grape in Experiment 1A; orange and lemon–lime in Experiment 1B; and raspberry and arctic green apple in Experiment 1C. The unconditioned stimulus (US) contained one of three concentrations of denatonium benzoate (Sigma-Aldrich) dissolved in water: 2.5 mM in Experiment 1A; 10 mM in Experiment 1B; and 1.25 mM in Experiment 1C. In this and all subsequent experiments, the CS and US solutions were presented at room temperature.

Because 1.25 mM is the lowest concentration of denatonium that rats avoid reliably in oral taste tests [49], we used concentrations that were equal to or greater than 1.25 mM. To this end, we infused the rats IG (in Experiment 1A–B) with 2.5 and 10 mM denatonium, which would be diluted in the stomach to 1.25 and 5 mM denatonium, respectively, by the ingested CS

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