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TOLERANCE OF BITTER COMPOUNDS BY AN HERBIVORE, *Cavia Porcellus*

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Abstract—Many plant defensive chemicals are bitter to humans. Because of this taste characteristic, and because bitter compounds are often toxic, such substances, and the plants that contain them, are regarded as generally unpalatable to wildlife. These assumptions may be unwarranted. To test the hypothesis that herbivores are indifferent to 'bitter' tastants, we investigated the responsiveness of guinea pigs (*Cavia porcellus*) to denatonium benzoate, denatonium saccharide, limonene, L-phenylalanine, naringin, quebracho, quinine, Ro-Pel (a commercial animal repellent containing denatonium saccharide) and sucrose octaacetate. Only quinine and sucrose octaacetate slightly but significantly reduced feeding ($P < 0.05$). Our findings are inconsistent with the notion that herbivores generally avoid what humans describe as bitter tastes.

Key Words—Avoidance, bitter, *Cavia porcellus*, denatonium benzoate, denatonium saccharide, guinea pig, limonene, L-phenylalanine, naringin, quebracho, quinine, sucrose octaacetate.

INTRODUCTION

Many plants taste bitter to humans. Because of this taste characteristic, and because bitter compounds are often toxic, such substances, and the plants that

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contain them, are regarded as generally unpalatable to wildlife (Garcia and Hankins, 1975). Because of their plant diet, however, a priori rejection of what humans call bitter substances should be maladaptive for herbivores. Consistent with the view that herbivores tolerate so-called bitter tastants in food, Jacobs (1978) demonstrated that guinea pigs were essentially indifferent to quinine hydrochloride and sucrose octaacetate presented in two-bottle drinking tests. Jacobs et al., (1978) reasoned that the categorical exclusion of bitter forages by herbivores might result in the underexploitation of otherwise valuable and nutritious plants.

The present experiment was designed to elaborate Jacobs' earlier work. Although guinea pigs failed to avoid quinine and sucrose octaacetate, it is possible that the animals would respond to other putative bitter tastants. Accordingly, we evaluated the responsiveness of guinea pigs (*Cavia porcellus*) to nine bitter tastants chosen from seven structural classes (Belitz and Weizer, 1985; Maga, 1990): peptides (denatonium benzoate, denatonium saccharide); flavonoids (naringin); carbohydrates (sucrose octaacetate); alkaloids (quinine); terpenes (limonene); tannins (quebracho); and amino acids (L-phenylalanine). Except for sucrose octaacetate, denatonium benzoate, and denatonium saccharide, all of these tastants occur naturally in plants. A commercially available animal repellent (Ro-Pel), which contains denatonium saccharide as the active ingredient at a concentration of 0.065%, also was tested.

METHODS AND MATERIALS

Subjects. Eighteen experimentally naive adult guinea pigs were individually caged (50 × 50 × 30 cm) at 23°C, under a 12:12 hr light–dark cycle (light onset at 0700 hr). Water was freely available, and, prior to testing, Purina RP Guinea Pig Chow (Purina Mills, St. Louis, Missouri) was available ad libitum in cups with attached trays that caught spillage.

Chemicals. Naringin (NAR), CAS #10236-47-2), sucrose octaacetate (SOA, CAS #126-14-7), quinine monohydrochloride dihydrate (QUI, CAS #6119-47-7), limonene (LIM, CAS #5989-27-5), and L-phenylalanine (L–P, CAS #63-91-2) were obtained from Aldrich Chemical Company (Milwaukee, Wisconsin). Quebracho (QUE) was purchased from the Van Dyke Supply Company (Woonsocket, South Dakota). Denatonium benzoate (DB, CAS #3734-33-6), denatonium saccharide (DS, CAS #90823-03-84) and Ro-Pel[5] (ROP, commercial repellent) were donated by Atomergic Chemicals (Farmingdale, New York).

[5] Use of trade names in this manuscript is for the purpose of identification and does not indicate endorsement of commercial products by the U.S. Department of Agriculture.

For simplicity, we refer to these chemicals as bitter tastants throughout the remainder of the manuscript.

Stimulus Preparation. We chose to prepare stimuli in foods rather than solutions because bitter tastants are usually encountered in the former. Each chemical was dissolved in diethyl ether. The ether solutions were mixed with guinea pig pellets (250 ml of ether/250 g of feed) to produce 1.0% (mass/mass) tastant concentrations. This concentration was chosen because bitter tastants are typically avoided by omnivores at such high concentrations (i.e., ≥ 1.0%; Glendinning et al., 1990; Glendinning, 1992; Mason et al., 1985). Control pellets were prepared by mixing feed with ether alone. After mixing, the pellet samples were placed under a fume hood for 72 hr to ensure complete evaporation of the ether.

Procedure. Three weeks prior to the experiment, all animals were adapted to an 18 hr food-deprivation schedule that continued throughout the study. This deprivation period was implemented because a pilot study had indicated that deprivation was necessary to assure consistent, measurable consumption during the test period.

The adaptation period was followed immediately by four days of pretreatment. On each pretreatment day, 20 g of control pellets were presented at 0900 hr. At 1200 hr, the cups were removed from the cages, spillage was returned to the cups, and the weight of the cup contents was determined. Between 1200 and 1500 hr, animals were given free access to untreated pellets. Consumption during the measurement period was used to assign animals to nine pairs (i.e., those animals with the highest and lowest consumption were assigned to the first pair, those with the second highest and second lowest consumption were assigned to the second pair, and so forth.)

A four-day treatment period followed pretreatment. Treatment trials were similar to pretreatment tests, except that pairs of guinea pigs were given pellets adulterated with each of the bitter tastants during the 3-hr measurement period.

A three-day rest period, during which animals were given untreated pellets, immediately followed treatment. This rest period was followed by another four-days of pretreatment and four-days of treatment, as previously described. The cycle of pretreatment, treatment, and rest periods was repeated until each of the animals had been presented with all tastants in a counterbalanced design (i.e., each of the tastants was presented during each of the nine treatment periods).

Analysis. A two-way repeated measures analysis of variance (ANOVA) was used to assess consumption. The factors in this analysis were tastant (nine levels) and periods (pretreatment, treatment). Tukey tests (Winer, 1971, p. 201) were used to isolate significant differences among means subsequent to the omnibus procedure ($P < 0.05$).

RESULTS

There was a significant interaction between tastants and periods ($F = 4.6$; 8,136; $P < 0.0001$). Post-hoc tests revealed small but significant reductions in consumption of QUI and SOA pellets relative to pretreatment consumption (Figure 1). Otherwise there were no significant effects ($P > 0.25$).

DISCUSSION

Guinea pigs were generally indifferent to the bitter tastants evaluated in the present experiment. Only QUI and SOA moderately reduced feeding relative to pretreatment levels, and these reductions were small (23.9%, and 14.5% of pretreatment, respectively). Although animals were moderately food-deprived, these findings, together with the high bitter tastant concentrations tested, are consistent with the notion that herbivores either ignore or are insensitive to natural compounds that humans reject as bitter.

Indifference to bitterness probably does not reflect an overall inability to detect and avoid (or prefer) chemical stimuli in feed. Guinea pigs avoid citric acid, avidly consume at least some carbohydrate sweeteners, and show preferences for sodium chloride and sodium saccharin (Beauchamp and Mason, 1991; Jacobs, 1978).

Rather than insensitivity, indifference could reflect tolerance (i.e., guinea

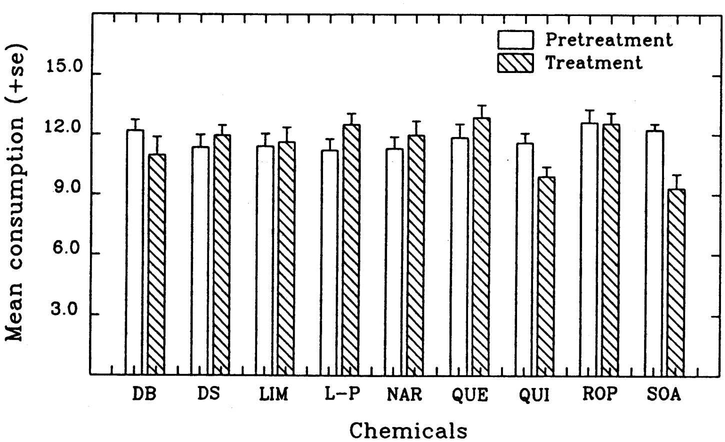

FIG. 1. Mean consumption by guinea pigs during pretreatment and treatment periods. Abbreviations: DB = denatonium benzoate; DS = denatonium saccharide; LIM = limonene; L-P = L-phenylalanine; NAR = naringin; QUE = quebracho; QUI = quinine; ROP = Ro-Pel; SOA = sucrose octaacetate.

pigs detect the tastants, but ignore them; Jacobs and Labows, 1979). Alternatively, it could be that guinea pigs perceive bitter tastants in terms of another taste quality. In either case, when SOA and QUI are paired with gastrointestinal distress, guinea pigs reliably acquire avoidance responses (Jacobs and Labows, 1979). Further, guinea pigs can learn to feed on those parts of bittersweet nightshade (*Solanum dulcamara*) that contain the lowest levels of toxicant (Jacobs and Labows, 1979). Other herbivores also exhibit this capability. For example, goats can learn to discriminate and avoid high tannin concentrations when foraging on blackbrush (*Coleogyne ramosissima*) (Provenza et al., 1990). These aversions are rapidly acquired within the first feeding bout (Provenza, et al., 1993).

More broadly, although herbivores generally tolerate bitter tastants, there is evidence that interspecific differences may exist. For example, guinea pigs tolerate QUE, but meadow voles (*Microtus pennsylvanicus*) avoid it. Cattle and guinea pigs respond similarly to SOA, yet guinea pigs are much more tolerant of QUI (Jacobs and Labows, 1979). Some rodents avoid DS (Davis et al., 1986; Langley et al., 1987), while others demonstrate a preference for it (Davis et al., 1987). Although the reasons for these differences remain obscure, it is plausible that they reflect differences among species in their evolutionary history or ecology (Freeland and Janzen, 1974; Lindroth, 1988).

Management Implications. Bitter tastants are generally regarded as unpalatable to wildlife, despite growing evidence to the contrary. In the present experiments, guinea pigs were indifferent to concentrations of denatonium benzoate and denatonium saccharide that were nearly three orders of magnitude higher than those that humans perceive as intensely bitter (Mason, personal observation). These denatonium compounds are used as the principle active ingredient in some commercial repellents. Although species differences exist among herbivores in avoidance of bitter tastants, the present results are inconsistent with the notion that bitter chemicals can serve as broadly effective repellents, particularly against herbivores.

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