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Response of Pigs to Bitter-tasting Compounds

Sandra L. Nelson and John D. Sanregret

The Procter & Gamble Company, Health Care Research Center, Mason, OH 45040, USA

Correspondence to be sent to: Sandra L. Nelson, Box 721, PO Box 8006, Mason, Ohio 45040-8006, USA

Abstract

Two-bottle preference tests were done to determine whether pigs detect bitter-tasting compounds. Four standard bitter compounds and nine bitter-tasting pharmaceutical compounds were tested. Pigs detect and avoid taste compounds that humans perceive as bitter-tasting. A dose–response to varying concentrations of bitter tastants can be measured. **Chem. Senses 22: 129–132, 1997.**

Introduction

This study tested whether pigs would make good animal models for responsiveness to bitter tastants. Rodents have commonly been used as animal models for studies of taste and taste mechanisms. However, a larger animal capable of providing more taste tissue would be desirable for biochemical taste studies. It has also been postulated that pigs would make better models for human taste studies than rodents because their diet and physiology are more similar to humans.

Pigs are omnivores, like humans. Both their digestive system and their skin system are similar to humans, making them good animal models for studies on those organ systems. Few behavioral or biochemical studies on pigs have been reported in the literature. Pigs have been shown to prefer the sweet taste of sugars (Kare *et al.*, 1965; Kennedy and Baldwin, 1972). Other behavioral and electrophysiological measurements show that pigs perceive the basic tastes of sweet, sour, salty and bitter (Baldwin, 1976; Hellekant, 1976; Danilova *et al.*, 1995). Biochemical responses to sucrose were shown to be the same in pigs as responses of other mammals that have been studied (Naim *et al.*, 1991).

Materials and methods

Animals

Seventy-nine Hanford miniature pigs (*Sus scrofa*), bred from domestic agriculture pigs, were used for the taste tests. The pigs were males, 2–4 months old, weighing 8–12 kg.

Bitter-tasting compounds

Tastants were reagent grade chemicals from Sigma Chemical Company (St Louis, MO) or over-the-counter pharmaceutical compounds from The Procter & Gamble Company (Cincinnati, OH). All of the compounds were soluble in water at the concentrations tested. Each test solution was tasted by the authors before being used in a pig taste test to ensure that bitterness could be detected by humans.

Taste tests

The experimental protocol for the pigs was approved by the Institutional Animal Care and Use Committee (IACUC). Fifty-six pigs were used for only one taste test, 20 pigs were used for two taste tests and three pigs were used for four taste tests. If an animal participated in more than one test,

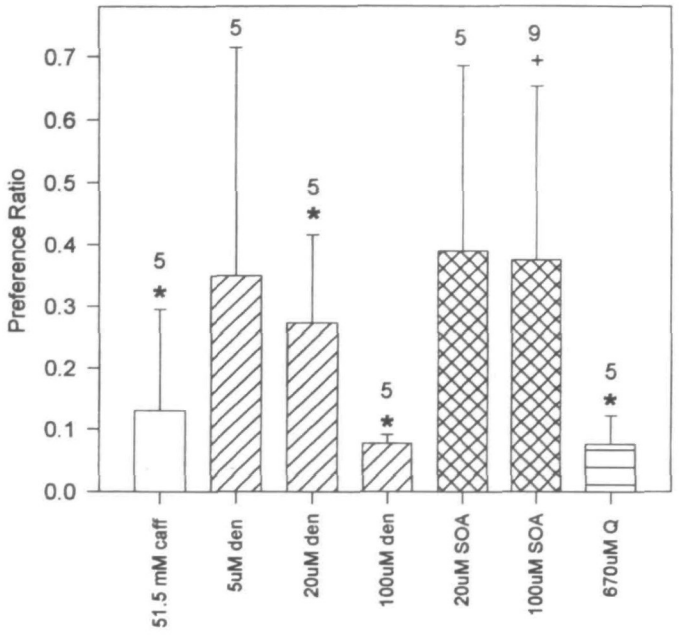

Figure 1 Preference ratio for standard bitter compounds. Mean with error bars for standard deviation (SD). The number of animals tested with each compound is shown above the error bar. Abbreviations: 51.5 mM caff = 51.5 mM caffeine (1%), den = denatonium benzoate, SOA = sucrose octaacetate, 670 μM Q = 670 μM quinine sulfate (0.025%). *marks preference ratios significantly less than 0.5 with $P < 0.05$. + marks a preference ratio significantly less than 0.5 with $P < 0.1$.

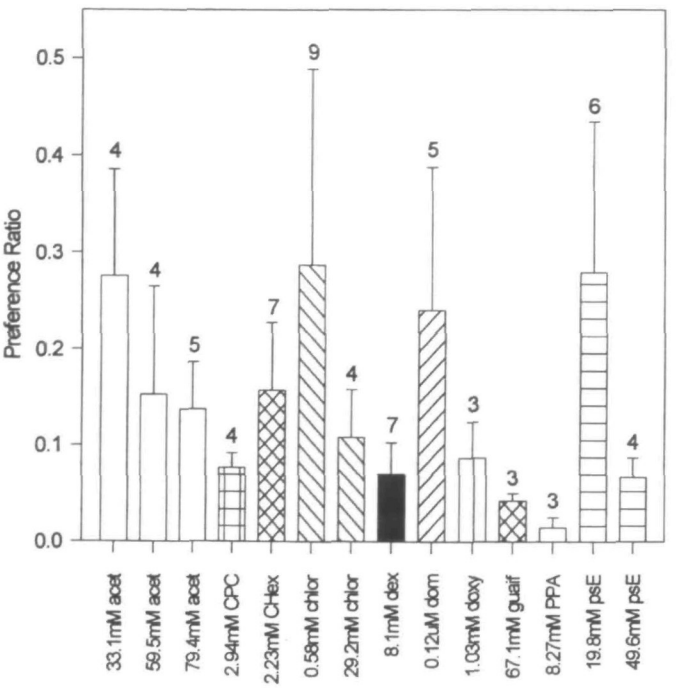

Figure 2 Preference ratio for pharmaceutical compounds. Mean with error bars for SD. Number of pigs tested with each compound is shown above each error bar. Preference ratio for each compound tested was significantly ($P < 0.05$) less than 0.5. Abbreviations: 33.1 mM acet = 33.1 mM acetaminophen (0.5%); 59.5 mM acet = 59.5 mM acetaminophen (0.9%); 79.4 mM acet = 79.4 mM acetaminophen (1.2%); 2.94 mM CPC = 2.94 mM cetylpyridinium chloride (0.1%); 2.23 mM CHex = 2.23 mM chlorhexidine; 0.58 mM chlor = 0.58 mM chlorpheniramine maleate (0.02%); 29.2 mM chlor = 29.2 mM chlorpheniramine maleate (1%); 8.1 mM dex = 8.1 mM dextromethorphan hydrobromide (0.3%); 0.12 μM dom = 0.12 μM domiphen bromide (0.005%); 1.03 mM doxy = 1.03 mM doxylamine succinate (0.04%); 67.1 mM guaif = 67.1 mM guaifenesin (1.33%); 8.27 mM PPA = 8.27 mM phenylpropanolamine (0.125%); 19.8 mM psE = 19.8 mM pseudoephedrine hydrochloride (0.4%); 49.6 mM psE = 49.6 mM pseudoephedrine hydrochloride (1%).

the testing periods were separated by at least 1 week. Animals were individually housed and deprived of water for an 8–16 h period. Animals were then given free access to two water bottles over the next 24 h period. One bottle contained water and the second bottle contained an equal volume of water with a tastant dissolved in it. The relative position of the bottles was randomized. The amount of liquid remaining in each bottle was measured at the end of the 24 h period.

The preference ratio was calculated as follows. Preference ratio = [(volume test liquid consumed)/(volume test liquid consumed) + (volume water consumed)].

Statistics

Significance was determined using Student's *t*-test. It was assumed that the preference ratio for water (in both bottles) would be 0.5. The probability that consumption of the test compound was less than consumption of water was determined using a one-tailed paired *t*-test.

Results

Results for standard bitter-tasting compounds are shown in Figure 1. Concentrations of denatonium benzoate and sucrose octaacetate (SOA) were chosen based on levels reported to be bitter for various species in the scientific literature. Concentrations of caffeine (1%) and quinine sulfate (0.025%) were those used as standards for human sensory studies at our institution and are normally determined on a w/v basis rather than a molar basis. Higher concentrations of caffeine, denatonium benzoate and quinine were significantly aversive to the pigs. SOA was not strongly aversive at the concentrations tested. Great variability between animals in the preference ratio for SOA were observed.

Preference ratios for bitter-tasting pharmaceutical compounds are shown in Figure 2. Concentrations tested were based on levels in marketed product which are normally measured on a w/v basis. All compounds at the

concentrations tested were significantly ($P < 0.05$) more aversive than water to the pigs.

Discussion

Caffeine at 51.5 mM was rejected by the pigs. Concentrations of 2 mM caffeine are detected as bitter by humans (Yokomukai *et al.*, 1993; Schiffman *et al.*, 1994). Mice respond to 1 mM caffeine (Tanimura *et al.*, 1994) and guinea pigs do not respond to caffeine at all (Nolte *et al.*, 1994).

A dose response relationship was observed for the preference ratio of denatonium benzoate by pigs. All animals tested rejected 100 μM denatonium benzoate. Humans seem to be especially sensitive to this compound, with an average detection threshold of 10 nM (Schiffman *et al.*, 1994). Mice reject denatonium benzoate at 100 μM (Whitney and Harder, 1994) and guinea pigs do not detect the compound (Nolte *et al.*, 1994). Based on these data, pigs are not as sensitive to denatonium benzoate as humans. Pigs have the same denatonium sensitivity as mice and are more sensitive to denatonium benzoate than guinea pigs.

Perception of SOA by pigs was extremely variable. With 20 μM, the preference ratio ranged from 0.13 to 0.82. With 100 μM SOA, four animals had a preference ratio <0.2 and four had a preference ratio ≥0.5. Due to the small number of pigs tested, no firm conclusions can be made. In mice, the ability to detect SOA is genetically determined; different inbred mouse strains detect and avoid SOA at different concentrations (Harder *et al.*, 1984; Whitney *et al.*, 1991). There is evidence the SOA sensitivity differences of mice may be due to differences in a taste cell receptor. Most humans can detect SOA.The detection threshold of SOA for humans is 4 μM (Schiffman, *et al.*, 1994); 6 μM is described as moderately bitter and 10 μM is described as strongly bitter (Yokomukai *et al.*, 1993). A possible explanation for pig response to SOA is the existence of two subpopulations that are differentially sensitive to SOA, as is the case for mice. Since Hanford miniature pigs are outbred and not genetically identical, identical taste sensitivity and identical taste receptors would not be expected. An alternative explanation is that the SOA concentrations tested are near the detection threshold for pigs. The results may simply be due to side or position preference of the animals. Further studies with more pigs are needed to clarify this point.

All pigs tested found 670 μM quinine sulfate to be extremely aversive. Humans have a detection limit of ~3 μM for quinine (Schiffman, *et al.*, 1994), label 8 μM quinine as moderately bitter and label 15 μM quinine as strongly bitter (Yokomukai *et al.*, 1993). By comparison, mice have a preference ratio ≤0.2 at 10–500 μM quinine, depending on their genetic make-up (Whitney and Harder, 1994). Guinea pigs have a small aversive response to 670 μM quinine (Nolte *et al.*, 1994).

Interestingly, humans describe 0.58 mM chlorpheniramine and 0.12 μM domiphen bromide as slightly bitter. These compounds were less aversive to the pigs, based on preference ratio, than other pharmaceutical compounds tested. Similarly, the pharmaceutical compounds found to be more aversive to pigs are perceived as more bitter by many humans (unpublished data).

For all compounds tested, animal to animal variability was generally higher at lower concentrations, which may have been near the taste threshold.

A dose response is observed with most of the tastants tested at more than one concentration. Denatonium benzoate, acetaminophen, chlorpheniramine maleate, and pseudoephedrine hydrochloride demonstrated a dose response. Only SOA showed no dose response at the concentrations tested.

These results demonstrate that pigs do perceive and respond aversively to compounds that humans find bitter-tasting. Although pigs respond to different concentrations than humans do for some of the compounds, their discernment of bitter-tasting compounds seems to be similar to human perception, based on comparisons with literature data. Due to their behavioral similarity to humans and large size, pigs (and pig taste tissue) should provide a good model for biochemical studies of bitter taste mechanisms.

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