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PII: S0308-8146(96)00033-7

Food Chemistry, Vol. 56, No. 3, pp. 323–328, 1996

0308-8146/96 $15.00+.00

Species differences toward sweeteners

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(Received 1 February 1996; accepted 6 February 1996)

The understanding of the sense of taste in mammals has over the last few decades slowly changed from the misconception that all mammals are equal with regard to taste to a realization that there are profound differences between species. These differences probably pertain to all basic tastes, but have been especially documented with regard to the sweet taste. This study addresses two issues: the difference in taste fiber specificity between mammals and the related issue of species differences in ability to taste sweeteners. These issues are illustrated by single taste fiber recordings from hamster, pig, rhesus monkey and chimpanzee. The hamster, a rodent, is used as an animal model in taste research because of its especially well developed sweet taste sensitivity, but this study shows that many sweeteners do not taste sweet to the hamster. The same is true for the pig, an ungulate, and from this point of view quite unrelated to the human, but with similar internal anatomy, food preferences and diets, and therefore extensively used as an animal model. Even the rhesus monkey, an old world primate belonging to the same superfamily as human, Catarrhina, shows some differences in its sweet tasting ability and taste fibers specificity although much less so than the previously mentioned species. The only species in which studies of its sense of taste have not yet revealed any differences from the human sense of taste, is the chimpanzee, which by most accounts is our closest relative. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

For many years, physiological observations of the sense of taste obtained in one species were uncritically applied to another species, be it from a frog to a rat or a monkey without, as it seems, much consideration of species difference. Typical is Pfaffmann's description how he in the late 1930s embarked on the mission "to find objective evidence for the four basic taste sensitivities" by recording from single taste fibers in the cat (Pfaffmann, 1981). Needless to say, he found no relationship between fiber types in cats and human taste qualities (Pfaffmann, 1941).

In primates, Snell may have been the first (Snell, 1965) to stumble over phylogenetic differences when he studied the effects of gymnemic acids in the squirrel monkey. As is well known, humans lose their ability to taste sweet after gymnemic acids, as was described almost 150 years ago by Edgeworth (1847). Today we know that the underlying neural phenomenon is a suppression of the taste nerve response to sweeteners (cf. Zotterman, 1971). However, Snell found no suppression of the squirrel monkey taste nerve response to sucrose after gymnemic acids. In retrospect this shows that the effects of gymnemic acids in the squirrel monkey must have been different from that in humans. In spite of his negative findings and not surprisingly, when one considers the lack of understanding of comparative events then prevailing, he did not draw the conclusion that the cause must have been species differences. This is understandable. We had a similar problem to acknowledge species differences when we recorded for the first time the effects of miraculin in man, monkey and rat and found no effects in the rat (Diamant *et al.*, 1972).

In this context it should be mentioned that both Kitchell and Kare cautioned more than 30 years ago against conclusions in taste founded on comparative events and generalizations (cf. Kare & Ficken, 1963; Kitchell, 1963). They based their warning on data from ruminants and other farm animals. However, on the whole it is evident that phylogenetic considerations played a minor role in the study of taste for many years.

This view has over the last few decades changed to a realization that phylogenetic differences play a major role in taste. Species differences pertain to all basic tastes or taste qualities, but have been especially documented with regard to the sweet taste (e.g. Diamant *et al.*, 1972; Brouwer *et al.*, 1973; Hellekant, 1975; Glaser *et al.*, 1992). We and others (e.g. Glaser *et al.*, 1978) have in farm animals, laboratory species and primates presented comparative findings which stress, not only quantitative but also qualitative differences in the sense of taste (cf. Hellekant & van der Wel, 1989).

In this study we will summarize data from four species; two primates, *Pan troglodytes* (chimpanzee) and *Macaca mulatta* (rhesus monkey), one rodent, *Mesocricetus auratus* (syrian goldhamster) and one ungulate, *Sus scrota* (domestic pig) which illustrate comparative differences in sweet taste.

The choice of species was guided partly by phylogenetic considerations, as with the chimpanzee and the rhesus monkey, but also by the species' ability to taste sweetness and its liking of sweetness, as was the case with the hamster and the pig. The pig was also interesting, because of its similarity with humans in diets and internal anatomy, although the last can certainly be challenged when one considers the difference between the two digestive systems, especially the large intestines (cf. Sisson & Grossman, 1953).

Space limitation allows only a summary of some of our findings on sweet taste. In all four species the conclusions are based on recordings from fine filaments from the chorda tympani nerve (CT). The CT conducts nerve impulses from the anterior two-thirds of the tongue. Behavioral data with the two-bottle preference technique (TBP) were utilized in our conclusions from monkey and hamster. In the chimpanzee we used a modification of the TBP method (Hellekant *et al.*, 1996). In monkey and hamster we also employed the conditioned taste aversion (CTA) method.

METHODS

Recordings of the right chorda tympani proper nerve (CT) were obtained under general anesthesia, which varied according to species used. The nerve was approached through an incision dorsal to the right mandibular angle. Single taste impulses were recorded from fine nerve filaments connected to an amplifier, loudspeaker, oscilloscope, recorder, a nerve impulse-amplitude analyzer and a computer. A computer program stored the intervals between pulses, together with information on the stimulus, time, animal and inputs from the keyboard, while it controlled the taste stimulation system. The stimulation system was recently described (Hellekant & Roberts, 1995). It can deliver taste stimuli at given intervals and over a preset time and under conditions of constant flow and temperature. Between stimulations, the tongue was rinsed with artificial saliva for up to 55 s.

The spontaneous nerve activity before each stimulation was deducted from the responses. We define here spontaneous activity as the impulse activity during rinsing of the tongue with artificial saliva during the 5 s preceding the stimulation. The impulse frequency before stimulation, maximum and average frequency during stimulation were both printed in tabular form and displayed on a terminal during the experiment (Hellekant & Roberts, 1995).

Single taste fibers were classified according to their response to salt, sour, sweet and bitter compounds. The fibers were designated sweet or sweet best fibers if sucrose elicited their largest responses (Frank, 1973). The sweet fibers were a part of a 2–3 times larger population of taste fibers characterized by their responses to some 30 different tastants.

We also used behavioral methods, two-bottle preference tests (TBP) in monkeys and hamsters, a one-bottle preference test in chimpanzees, and a conditioned taste aversion (CTA) test in monkeys and hamsters. The pig is the only species in which we did not apply a behavioral method. In the following we will briefly describe the behavioral methods.

It is generally thought that there is a linkage between a compound's sweetness and the amount consumed. This is the idea behind the TBP paradigm during which the animals were given a choice of two bottles, one with water and the other with the compound in question. In monkeys the intake was measured over 15 min or until one bottle was empty. In hamsters the bottles were left on the cages over night. In the chimpanzee we used a modification of this technique by offering one bottle at a time while we recorded the behavior of the chimpanzee according to a 6 point scale (Hellekant *et al.*, 1996).

In contrast, the CTA technique is not based on the liking for a compound but on the taste similarity between tastants. Here we used it to quantify the taste similarity between, on one side sucrose, and on the other acesulfame-K, alitame, aspartame, etc. This was done by linking the taste of sucrose to a negative experience, in this case the nausea created by LiCl injections following intake of sucrose, and then seeing to what extent the aversion created generalized to the other sweeteners. This was measured as a ratio between intake of these sweeteners by the conditioned animals and unconditioned animals. The intake was monitored either in ml consumed, as in monkeys, or as licks with a device that employs an infrared beam (King *et al.*, 1970) in hamsters.

The compounds included in this study are listed in Table 1. All compounds except quinine hydrochloride (QHCl), which for solubility reasons was dissolved in distilled water, were dissolved in artificial saliva during the electrophysiological experiments (Hellekant & Roberts, 1995). During the behavioral tests the compounds were dissolved in distilled water. The concentrations of the sweeteners were chosen so that they were about equisweet to humans and their concentrations were generally the same for all species. This was also the case with the non-sweet stimuli. The structure of SC-45647 has been given in a study of rats (Hellekant *et al.*, 1991) and that of super-aspartame in Hellekant *et al.* (1996).

RESULTS

Figure 1 summarizes average response profiles of single sweet fibers of pig, hamster, monkey and chimpanzee. The number of sweet fibers were in chimpanzee 20, monkey 14, pig 16 and hamster 12. The top three bars in each graph show their responses to NaCl, citric acid and

Table 1. List of solutions and their concentrations used in experiments

Stimuli	Pig	Hamster	Monkey	Chimpanzee
NaCl	0.1 M	70 mM	70 mM	70 mM
Citric acid	20 mM	20 mM	40 mM	40 mM
Quinine HCl	5 mM	10 mM	5 mM	5 mM
Sucrose	0.3 M	0.2 M	0.3 M	0.3 M
Fructose	0.3 M	—	0.3 M	0.3 M
Glucose	0.5 M	—	0.3 M	0.5 M
Acesulfame-K	5 mM	4 mM	3.5 mM	3.5 mM
D-Tryptopan	20 mM	20 mM	30 mM	15 mM
Saccharin	1.6 mM	1.6 mM	1.6 mM	1.6 mM
SC-45647	0.08 mM	0.04 mM	0.04 mM	0.04 mM
Suosan	1 mM	1 mM	1 mM	2mM
Stevioside	0.9 mM	—	0.87 mM	0.87 mM
Cyclamate	22 mM	10 mM	10 mM	10 mM
Alitame	0.3 mM	0.15 mM	0.1 mM	0.3 mM
Aspartame	5.1 mM	2.7 mM	5.1 mM	5.1 mM
Super-APM	0.11 mM	0.06 mM	0.11 mM	0.11 mM

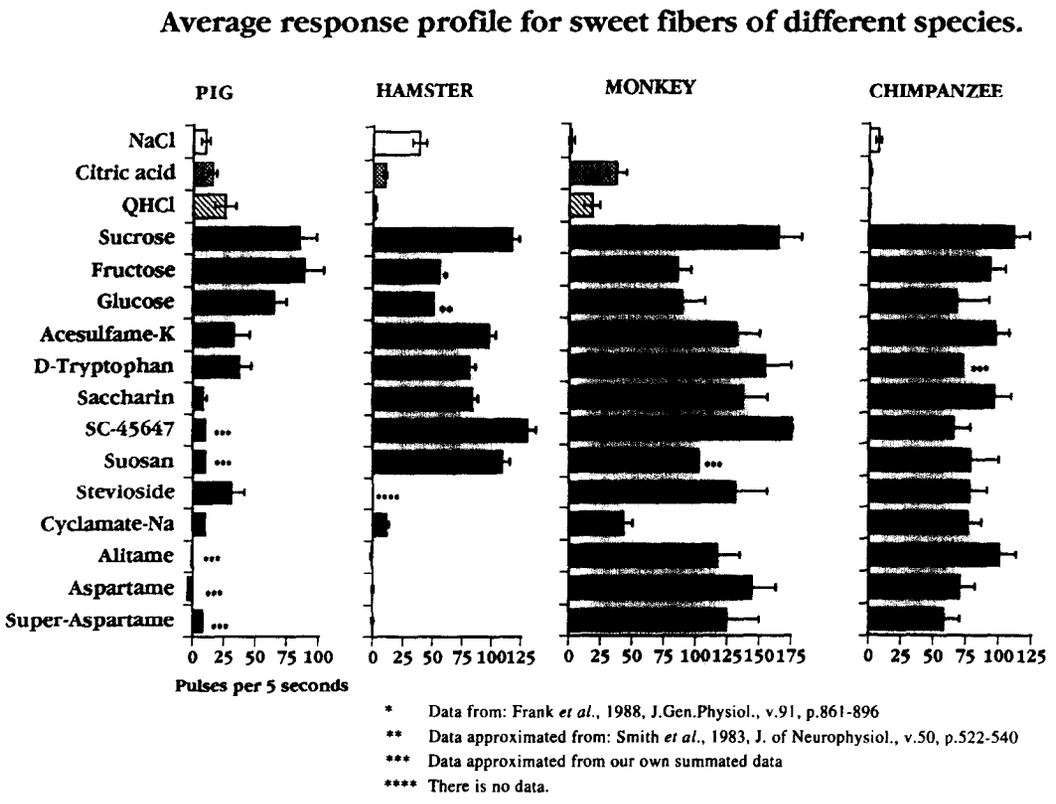

Fig. 1. Average responses of sweet fibers in pig, hamster, rhesus monkey and chimpanzee to NaCl, citric acid, quinine hydrochloride, sucrose and 12 compounds perceived by humans to be sweet.

quinine hydrochloride (QHCl) which, as expected since the recordings were obtained from sweet fibers, elicited a small or no response at all. The fact that there was virtually no response to these non-sweet compounds in the sweet fibers of the chimpanzee corroborates our earlier conclusions that chimpanzee sweet fibers respond more specifically to sweet compounds than the sweet fibers of any other species (Hellekant & Ninomiya, 1991).

The second general observation is that there were substantial species differences in the ability of these sweeteners to stimulate sweet fibers. In the following we will describe the findings in each species separately and begin with the chimpanzee because it is more closely related to humans than any other species.

Chimpanzee

The chimpanzee's sweet fibers show two major features; all the sweeteners elicited a response; these responses were approximately of the same size. As can be seen from Fig. 1 this was not the case in the other species. Thus, although in the monkey all sweeteners gave a response, it varied considerably. In hamster and pig not all sweeteners gave a response.

The fact that the nerve responses were of about the same magnitude indicates that the sweeteners gave a taste sensation of about the same intensity. This, since the concentrations of the sweeteners used were based on their sweetness to the human tongue, suggests a close similarity between the sense of taste in chimpanzee and human.

These conclusions become more interesting when one considers that the sweeteners and their concentrations were guided by psychophysical data of their sweetness (DuBois *et al.*, 1991). Besides the fact, shown in Fig. 1 that all sweeteners evoked a response in sweet fibers, these compounds also elicited a positive hedonic response in preference tests (Hellekant *et al.*, 1996). This supports the conclusion that all the compounds tasted sweet to the chimpanzee.

Rhesus monkey

As indicated above, the monkey sweet fibers show some deviations from the picture in the chimpanzee. Thus although all sweet compounds elicited a response, the uniformity of these responses, expressed as number of impulses, was less than in the chimpanzee.

Stimulation with Na-cyclamate evoked an especially small response. This suggests that cyclamate is not very sweet to the monkey. Behavioral tests support this conclusion. Although cyclamate was significantly preferred over water in TBP tests, its intake ranked among the last of the sweeteners. Further, sucrose did not generalize to Na-cyclamate in CTA tests (unpublished own observations).

Hamster

Two features, varying sweet fiber responses and inability to evoke a sweet fiber response by some sweeteners, were evident in the hamster. Thus, as can be seen in Fig. 1, while sucrose, fructose, glucose, acesulfame-K, D-tryptophan, saccharin, SC-45647 and suosan gave a response, cyclamate, alitame, aspartame and super-aspartame elicited no response at all. Data are missing for stevioside.

The results of behavioral experiments in hamster were corroborative. Thus, in TBP tests, sucrose, fructose, glucose, acesulfame-K, D-tryptophan, saccharin, SC-45647 and suosan were significantly preferred over water, while the animals did not discriminate between water and Na-cyclamate, alitame, aspartame or super-aspartame. CTA tests confirmed this. The hamster generalized from sucrose to acesulfame-K, D-tryptophan, saccharin, SC-45647, and suosan, but not to Na-cyclamate, alitame, aspartame or super-aspartame.

Pig

It should be mentioned that the results with SC-45647, suosan, alitame, aspartame and super-aspartame are based on recordings from the whole CT nerve. As can be seen in Fig. 1 these sweeteners elicited little or no CT response. The obvious conclusion is that they basically don't taste sweet to the pig, because if there is no response in the whole nerve, there will no response in individual fibers.

Thus, the pattern from the pig extends the one from the hamster; in addition to the lack of sweetness by alitame, aspartame and super-aspartame, the small nerve response to saccharin, SC-45647 and suosan indicated that these sweeteners do not taste sweet to the pig. Recordings of the NG taste nerve support these conclusions.

With regard to saccharin, behavioral tests support this conclusion. Aldinger *et al.* (1959) and Wahlstrom *et al.* (1974) found saccharin less desirable or of no value in practical feeding. Further, considering the smaller response to glucose in comparison with that of sucrose, it is interesting that diets with sucrose were much more preferred than diets with glucose (Aumaitre, 1980).

DISCUSSION

Our study combines single fiber and behavioral techniques to assess the sweetness of a number of compounds sweet to humans. The results show that only five of these 13 sweeteners tasted sweet to all four species. As a matter of fact, only the carbohydrates elicited responses in the same types of fibers in all species. The difference was particular striking for high potency sweeteners, such as aspartame, alitame and super-aspartame. The latter two are, depending on how they are compared, at least 1000 times sweeter than sucrose.

We will first briefly discuss the single taste fiber technique before we discuss the major conclusion that this study attempts to make.

The idea employed here is to identify and isolate with an array of sweet and non-sweet compounds sweet taste fibers from other taste fibers. The sweetness of the compound is reflected in the impulse activity evoked. It is implicit that the sweetness of a compound can only be expressed in comparison with a standard, e.g. sucrose.

One major advantage of the technique is that a large number of compounds can be tested at the same time. The drawback is that the conclusions must be based on observations in several taste fibers which necessitates recording from more than one animal.

The taste fiber response can also be used to provide information about the temporal pattern of the compound as was used in an earlier study (Hellekant *et al.*, 1991). A 'slow' sweetener such as thaumatin elicits a slowly increasing nerve activity, while the 'fast' saccharin elicits a transient nerve response. Finally, the duration of the evoked nerve activity supplies information on how long the taste will linger on the tongue.

The major conclusion from this study is that there are important species differences toward sweeteners. This is not only important from theoretical points of view but also from practical points of view. It emphasizes the importance in choosing the right animal model for testing and development of tastants or food additives.

There is no difference in this regard whether the animal species in question is chosen as the target for a new compound, or serves as the animal model for development of a new tastant. In the following we will briefly apply this conclusion on the compounds used here.

The lack of a response to alitame, aspartame and super-aspartame in hamster and pig cannot be attributed to a low taste potency of these sweeteners as perhaps can be surmised for cyclamate. They are all high potency sweeteners, recently discovered and now being used because of their good sweet taste qualities to humans. Aspartame is extensively used in diet products, and alitame was recently approved for some use in human consumption (Hendrick, 1991). Super-aspartame, a combination of suosan and aspartame (Nofre *et al.*, 1987, 1988) has high sweet intensity and a pleasant taste.

In spite of this alitame, aspartame and super-aspartame elicited neither a nerve response nor a hedonic response in hamster and pig. This indicates that they lack taste to these non-primates and corroborates other studies which show that at least aspartame is generally not sweet to non-primates (e.g. Hård af Segerstad & Hellekant, 1989*a*, 1989*b*)

It may be of interest that the limitation of aspartame does not only apply to its taste in non-primates; aspartame lacks sweetness to Prosimii (half monkey) and Platyrrhina (new-world monkey) primates (Hellekant *et al.*, 1981; Glaser *et al.*, 1992). Apes and old-world primates seem to be the only group in which every compound sweet to humans, tastes sweet. At least, we have not observed one single sweetener that tastes sweet to humans but not to this group of primates. There are other differences, for which space does not allow elaboration (cf. Hellekant & van der Wel, 1989). As a rule of thumb, the number of human sweeteners that lack sweetness to an animal species increases with the species' increasing phylogenetic distance from humans.

Aspartame and the other sweeteners in Fig. 1 are a few examples of a growing number of compounds which are tasteless or taste different to animals. For example, there is no doubt that denatonium benzoate, an additive used to discourage human and animal consumption, is considerably less bitter to rodents; tannins, which have an aversive taste to humans, are readily consumed by many lemuriforme primates; acids are certainly less sour to a number of non-human primates than to humans, etc. The list is growing and potentially so extensive that no compound should and could be used as food additive or sweetener without solid scientific data.

In this context the chimpanzee is of course the ideal model for the study of human taste. However, as shown in Fig. 1, the more easily available old-world monkey, *M. mulatta*, serves the same purpose well. In general it seems that species within the catarrhina infra order can be used. We have recorded from other species within this group, such as baboon (*Papio anubis*), cynomolgus monkey (*Macaca fascicularis*), *Cercopithecus aethiops* and gibbon (*Hylobates lar*) and found that all compounds that are sweet to humans seem to elicit the same sensation in these species.

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