Published in final edited form as:

Sens Neur. 2001 January 1; 3(3): 205–212. doi:10.1163/156856501750387256.

Genetics of intake of umami-tasting solutions by mice

ALEXANDER A. BACHMANOV and GARY K. BEAUCHAMP*

Monell Chemical Senses Center, 3500 Market St., Philadelphia, PA 19104-3308, USA

Abstract

Inbred strains of mice provide a powerful tool for genetic dissection of quantitative behavioral traits. We have investigated intake of the umami-tasting substances monosodium glutamate (MSG) and inosine 5'-monophosphate (IMP) in inbred mice. Studies with two inbred strains, C57BL/6ByJ and I29P3/J have revealed strain differences in voluntary consumption of 300 mM MSG which depend, at least partially, on postingestive effects of solution consumption, as well as on strain differences in preferences for much lower MSG concentrations, which depend on perception. The strain difference in MSG acceptance was in the opposite direction to the strain difference in NaCl acceptance and was unrelated to sweetener preference in the F₂ generation. Thus, the strain differences in MSG acceptance are not related to the strain differences in salty or sweet taste responsiveness and most likely represent specific umami taste responsiveness. High acceptance of MSG solutions by the C57BL/6ByJ mice was inherited as a recessive trait in the F₂ hybrid generation. Further genetic linkage analyses using the F₂ hybrids are being conducted to map chromosomal locations of genes determining the strain difference in MSG acceptance. At the same time, a wider range of inbred strains is being phenotyped in a search for new model systems for studying umami substance acceptance.

Keywords

Taste; sweet; glutamate; inbred mice

When added to certain foods in relatively small quantities, monosodium glutamate (MSG) increases their palatability (Yamaguchi, 1998). The sensory basis for this effect is that MSG stimulates the sense of taste (Dove, 1948; Kawamura and Kare, 1987). Recent studies have shown that the taste quality elicited by MSG and related substances such as inositol monophosphate (IMP), herein labeled 'umami', is most likely unique in that it is not some combination of sweet, sour, salty and bitter, the presumed other primary taste qualities.

The mechanism(s) underlying detection and recognition of umami substances are now beginning to be understood (Chaudhari *et al.*, 2000) but the basis for their powerful ability to enhance palatability and increase intake remain unknown. Physiological and behavioral studies using animal models and genetic dissection of umami taste responses can help unravel these mechanisms. Umami substances are detected and preferred by several species of animals, with most work concentrated on rats and mice (a, b, e.g. Ninomiya and Funakoshi, 1989a, b; Yamamoto *et al.*, 1988, 1991). Mice are particularly attractive models since much is known about their genetics. Moreover, many inbred strains of mice exist that provide tools for genetic analysis.

We recently reported differences in MSG acceptance between two inbred mouse strains, C57BL/6ByJ and I29P3/J (Bachmanov *et al.*, 2000). Here, we summarize the results of these

*To whom correspondence should be addressed. beauchamp@monell.org.

studies and discuss future approaches to understanding genetic aspects of the acceptance of umami substances.

METHODS AND RESULTS

Two strains of mice, C57BL/6ByJ (B6) and 129P3/J (129) were used in these experiments. Within each strain, all mice are genetically identical whereas between strains there are substantial genotypic and phenotypic differences. In particular, these strains consume umami-tasting substances to very different degrees as described below.

The test mice were housed in individual cages in a temperature-controlled room. For genetic analyses, the B6 and 129 mice were outcrossed to produce the first filial generation of hybrids (F_1), and these were intercrossed to produce the second hybrid generation (F_2 , see Bachmanov *et al.*, 2000 for more detail). The primary behavioral measure was intake of fluids (umami substances and water) as measured using two-bottle preference tests (each lasting 48 h or 96 h). Methods are as previously described (Bachmanov *et al.*, 1996).

The umami substances used consisted of the monosodium salt of L-glutamic acid (MSG), and the disodium salt of inosine 5'-monophosphate (IMP). We also tested sucrose, D-phenylalanine and sodium saccharin (Sigma Chemical Company, St. Louis, MO) in some of the same animals to determine the relationship between intake of sweet substances and intake of umami substances. In the 2-bottle tests, the mice were presented with one bottle containing a taste solution and the other containing deionized water.

Indexes of fluid acceptance were calculated using average daily (24 h) fluid intakes for each mouse for each solution concentration. We determined (a) raw intakes per mouse, (b) intakes per 30 g of BW (the approximate weight of an adult mouse), and (c) preference scores [the ratio of the averaged solution intake to averaged total fluid (solution + water) intake, in percent].

In the first experiment 0.1–1000 mmol/l MSG solutions were tested in ascending order in 5½-month old male B6 ($n = 12$) and 129 ($n = 12$) mice. Each MSG concentration was tested with water for 2 days. The data are shown in Fig. 1. The two strains drank similar amounts of 0.1–100 mmol/l MSG, but the B6 mice drank more 300–1000 mmol/l MSG than did the 129 mice (Fig. 1, top). Consumption of 300 mmol/l MSG by the B6 mice was remarkably high: for some B6 mice daily consumption of MSG exceeded ½ of their body weight. The B6 mice also preferred a wider range of MSG concentrations and had higher MSG preference scores than did the 129 mice, although both strains preferred several concentrations (Fig. 1, bottom).

This experiment demonstrated concentration-specific strain differences in MSG acceptance. At lower MSG concentrations B6 mice had higher preference scores even though the two strains did not differ significantly in intake. However, at higher MSG concentrations (especially 300 and 600 mmol/l) the B6 mice consumed much more MSG than did the 129 mice. This pattern of differences was confirmed in several additional experiments that used only 1 and 300 mmol/l MSG and in tests with animals of different age and gender (Bachmanov *et al.*, 2000, experiments 2–5).

Next an additional umami substance, IMP, was tested with the same 2 inbred strains. IMP solutions (0.01–100 mmol/l) were tested in ascending order in a new group of male B6 ($n = 12$) and 129 ($n = 12$) mice. The results were similar to those with MSG. Compared with the 129 mice, the B6 mice had higher IMP intakes at 0.03–0.1 and 1–30 mmol/l concentrations. Also, B6 mice had significantly higher IMP preference scores (at 0.03–0.1 and 1–10 mmol/l concentrations). Thus, the B6 mice preferred a wider range of IMP concentrations and had higher IMP preference scores than did the 129 mice (Fig. 2).

The next step was to investigate acceptance of MSG and sweeteners by B6 × 129 F₂ hybrids to obtain information on possible genetic mechanisms underlying the pattern of strain differences described above. The F₂ animals ($n = 455$) were 4–7-months old when the tests began. For each animal each taste solution was tested with water for 4 days. The solutions were tested in the following sequence: 30 mmol/l D-phenylalanine, 20 mmol/l saccharin, 120 mmol/l sucrose, 1 mmol/l MSG and 300 mmol/l MSG. One or two days with only water available separated tests of each solution.

MSG solution intakes and preferences in the F₂ mice were lower than in the B6 mice, and did not differ significantly from the 129 mice. The F₂ values for 300 mmol/l MSG were also significantly lower than the midparental values (see Bachmanov *et al.*, 2000 for details). This demonstrates that high MSG acceptance by the B6 mice is inherited as a recessive trait. Acceptance of three sweetener solutions by the F₂ mice was significantly different from both parental values and did not differ from corresponding midparental values, except for sucrose preference that did not differ significantly from the B6 value and exceeded the midparental level. This demonstrates that for sweetener acceptance the mode of inheritance was additive with partial dominance of B6 alleles.

Correlations among preferences for solutions of MSG and sweeteners for all F₂ mice (males and females together) are shown in Table 1. Preferences for sucrose, saccharin and D-phenylalanine correlated strongly with each other. However, no significant correlations were found between preferences for two MSG solutions and between MSG and sweetener preferences indicating that differences in umami acceptance and sweet acceptance are controlled by different genetic mechanisms.

The data presented here demonstrate that mice from the B6 strain have a greater avidity for MSG and IMP solutions than do mice from the 129 strain. The strain difference in ingestive responses to MSG was confirmed in several experiments, and it appeared robust across different genders, various ages and previous experiences with taste solutions. This high MSG avidity is inherited as a recessive trait.

For humans MSG and IMP have a similar taste. Although we assume that the response to IMP is due to the interaction of IMP with taste receptors, it has been suggested that IMP itself is tasteless, but it enhances the taste of glutamate naturally contained in saliva (Yamaguchi, 1991, 1998).

The B6 mice had higher 1 mmol/l MSG preferences than did the 129 mice, and they consumed much more 300 mmol/l MSG than did the 129 mice. However since preferences for 1 and 300 mmol/l MSG did not correlate in the F₂ hybrid mice (Table 1), acceptance of these solutions appears to depend on different mechanisms determined by different genes. The different mechanisms may involve different post ingestive effects of low versus high osmotic solutions.

For two reasons it is evident that postingestive mechanisms play some role in MSG acceptance and consumption. As discussed in more detail in Bachmanov *et al.* (2000), B6 and 129 animals gained weight differently when exposed to this high MSG concentration and they showed changes in intake over the 4 days of testing suggesting differential reinforcing effects of MSG consumption. Thus for the high MSG concentration it is unlikely that strain differences only in taste perception are responsible.

The strain differences in MSG acceptance do not depend on sweet or salty taste. The evidence for this is that the modes of inheritance were different for MSG (recessive B6 alleles) and sweeteners (additive or partial dominance of B6 alleles) and for MSG (B6 preference greater than 129) and salt (129 preference greater than B6: Bachmanov *et al.*, 1996, 1998b; Beauchamp and Fisher, 1993; Gannon and Contreras, 1993; Lush, 1991). Also, among the F₂ hybrid mice,

preferences for the sweeteners correlated positively with each other, but not with preferences for MSG (Table 1). Apparently high acceptance of MSG and sweeteners fortuitously coincides in the two inbred strains.

DISCUSSION AND PROSPECTUS

Using the B6 $\times$ 129 F₂ mice, we are currently conducting a genome screen to identify chromosomal regions containing genes that determine the strain difference in MSG acceptance. Based on chromosomal locations, it should then be possible to identify the genes themselves (Collins, 1992).

Although the two inbred strains tested here had very different ingestive responses to both low and high MSG concentrations, it is possible that even more extreme differences might be found if more strains were tested. Consequently, we (Bachmanov and Beauchamp, unpublished) have initiated a study comparing ingestive responses to MSG and IMP in 28 inbred mouse strains. Preliminary evidence suggests the existence of extensive strain diversity in MSG consumption and preference in 2-bottle tests. There appears to be substantially less strain variation for IMP. If this tendency is maintained in the completed study it will provide further evidence that strain difference in MSG consumption and preference, particularly at high concentrations, are due in large measure to post-ingestive effects that may involve osmotic effects. And it also should provide further opportunities to investigate mechanisms underlying differential umami (both MSG and IMP) solution acceptance. Thus, we expect that this research program will eventually result in greater understanding of the molecular genetic basis of umami taste responsiveness.

Acknowledgments

This work was supported by NIH DC 00882.

References

- Bachmanov AA, Tordoff MG, Beauchamp GK. Ethanol consumption and taste preferences in C57BL/6ByJ and 129/J mice. *Alcoholism: Clin Exp Res* 1996;20:201–206.
- Bachmanov AA, Schlager G, Tordoff MG, Beauchamp GK. Consumption of electrolytes and quinine by mouse strains with different blood pressures. *Physiol Behav* 1998a;64:323–330. [PubMed: 9748100]
- Bachmanov AA, Tordoff MG, Beauchamp GK. Voluntary sodium chloride consumption by mice: differences among five inbred strains. *Behav Gen* 1998b;28:117–124.
- Bachmanov AA, Tordoff MG, Beauchamp GK. Intake of umami-tasting solutions by mice: a genetic analysis. *J Nutr* 2000;130:935s–941s. [PubMed: 10736356]
- Beauchamp GK, Fisher AS. Strain differences in consumption of saline solutions by mice. *Physiol Behav* 1993;54:179–184. [PubMed: 8327600]
- Beauchamp GK, Bachmanov AA, Stein L. Development and genetics of glutamate taste preference. *Ann N Y Acad Sci* 1998;412–416. [PubMed: 9929634]
- Collins FS. Positional cloning: let's not call it reverse anymore. *Nat Gen* 1992;1:3–6.
- Chaudhari N, Landlin AM, Roper SD. A metabotropic glutamate receptor variant functions as a taste receptor. *Nature Neurosc* 2000;3:113–119.
- Dove, WF., editor. Quartermaster Food and Container Institute for the Armed forces, and Associates. Food and Container Institute; Chicago, IL: 1948. Flavor and Acceptability of Monosodium Glutamate: proceedings of the Symposium.
- Gannon K, Contreras RJ. Sodium intake linked to amiloride-sensitive gustatory transduction in C57BL/6J and 129/J mice. *Physiol Behav* 1993;57:231–239. [PubMed: 7716197]
- Halpern, BP. Psychophysics of taste. In: Beauchamp, GK.; Bartoshuk, L., editors. Tasting and Smelling. Academic Press; San Diego, CA: 1997. p. 77–123.

- Jungas RL, Halperin ML, Brosnan JT. Quantitative analysis of amino acid oxidation and related gluconogenesis in humans. *Physiol Rev* 1992;72:419–448. [PubMed: 1557428]
- Kawamura, Y.; Kare, MR. Umami: a Basic Taste. Marcel Dekker; New York: 1987.
- Lush, IE. The genetics of bitterness, sweetness, and saltiness in strains of mice, in: Genetics of Perception and Communication. Wysocki, CJ.; Kare, MR., editors. Marcel Dekker; New York: 1991. p. 227–241.
- Ninomiya Y, Funakoshi M. Behavioural discrimination between glutamate and the four basic taste substances in mice. *Comp Biochem Physio* 1989a;92:365–370.
- Ninomiya Y, Funakoshi M. Peripheral neural basis for behavioural discrimination between glutamate and the four basic taste substances in mice. *Comp Biochem Physiol* 1989b;92:371–376.
- Ninomiya Y, Nakashima K, Fukuda A, Nishino H, Sugimura T, Hino A, Danilova V, Hellekant G. Responses to umami substances in taste bud cells innervated by the chorda tympani and glossopharyngeal nerves. *J Nutr* 2000;130:9505–9535.
- Yamaguchi S. Basic properties of umami and effects on humans. *Physiol Behav* 1991;49:833–841. [PubMed: 1679557]
- Yamaguchi S. Basic properties of umami and its effects on food flavor. *Food Rev Int* 1998;14:139–176.
- Yamamoto T, Matsuo R, Kiyomitsu Y, Kitamura R. Taste effects of umami substances in hamsters as studied by electrophysiological and conditioned taste aversion techniques. *Brain Res* 1988;451:147–162. [PubMed: 2855211]
- Yamamoto T, Matsuo R, Fujimoto Y, Fukunaga I, Miyasaka A, Imoto T. Electrophysiological and behavioral studies on the taste of umami substances in the rat. *Physiol Behav* 1991;49:919–925. [PubMed: 1653433]

Figure 1.

Average daily MSG intake (top) and preference (bottom) by male B6 and 129 mice in two-bottle 48 h preference tests (Experiment 1). Vertical bars represent SEM. Reprinted from Bachmanov *et al.* (2000). *Significant difference between B6 and 129 mice ($P < 0.05$, planned comparisons).

Figure 2.

Average daily IMP intake (top) and preference (bottom) by male B6 and 129 mice in two-bottle 48 h preference tests. Vertical bars represent SEM. Reprinted from Bachmanov *et al.* (2000). *Significant difference between B6 and 129 mice ($P < 0.05$, planned comparisons).

Table 1

Correlations among MSG and sweetener solution preferences in F2 mice (n = 450–454)

Solutions	MSG 300 mmol/l	Sucrose 120 mmol/l	Saccharin 20 mmol/l	D-phenylalanine 30 mmol/l
MSG, 1 mmol/l	+0.07	+0.11	+0.10	+0.03
MSG, 300 mmol/l		0.00	+0.01	−0.04
Sucrose, 120 mmol/l			+0.72 [*]	+0.50 [*]
Saccharin, 20 mmol/l				+0.56 [*]

^{*} Significant correlations; the significance level was determined using Bonferroni correction for 10 correlation coefficients estimating critical *P* level as $0.05/10 = 0.005$.