Glutamate taste and appetite in laboratory mice: physiologic and genetic analyses

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ABSTRACT
This article provides an overview of our studies of variation in voluntary glutamate consumption in mice. In 2-bottle preference tests, mice from the C57BL/6ByJ (B6) strain consume more monosodium L-glutamate (MSG) than do mice from the 129P3/J (129) strain. We used these mice to study physiologic and genetic mechanisms that underlie the strain differences in glutamate intake. Our genetic analyses showed that differences between B6 mice and 129 mice in MSG consumption are unrelated to strain variation in consumption of sodium or sweeteners and therefore are attributed to mechanisms specific for glutamate. These strain differences could be due to variation in responses to either taste or postingestive effects of glutamate. To examine the role of taste responsiveness, we measured MSG-evoked activity in gustatory nerves and showed that it is similar in B6 and 129 mice. On the other hand, strain-specific postingestive effects of glutamate were evident from our finding that exposure to MSG increases its consumption in B6 mice and decreases its consumption in 129 mice. We therefore examined whether B6 mice and 129 mice differ in postingestive metabolism of glutamate. We showed that, after intragastric administration of MSG, the MSG is preferentially metabolized through gluconeogenesis in B6 mice, whereas thermogenesis is the predominant process for 129 mice. We hypothesize that a process related to gluconeogenesis of the ingested glutamate generates the rewarding stimulus, which probably occurs in the liver before glucose enters the general circulation, and that the glutamate-induced postingestive thermogenesis generates an aversive stimulus. Our animal model studies raise the question of whether humans also vary in glutamate metabolism in a manner that influences their glutamate preference, consumption, and postingestive processing.

INTRODUCTION
L-Glutamic acid is a nonessential amino acid commonly found in proteins and in free form in some foods. It is used as a food additive, most commonly in the form of monosodium L-glutamate (MSG). L-Glutamic acid, especially its salts (for brevity, in this article, these compounds are referred to as "glutamate"), is perceived by humans as having the umami taste quality (1, 2) and probably evokes an equivalent taste sensation in nonhuman animals (3–5). Because umami taste likely evolved to detect the presence of nutrients and to induce their consumption, variation in umami taste responsiveness is likely to affect ingestive behavior. When glutamate is consumed, it can have postingestive effects that may be mediated by several different mechanisms, such as its nutritive value (which includes energy generated from its catabolism), production of metabolites, release of hormones, stimulation of vagus activity, or changes in gastrointestinal secretion (6–19). Therefore, glutamate consumption may depend on both chemosensory perception of its taste and on its postingestive effects.

Humans differ in perception of glutamate taste (20), and recent studies suggest that this variation may have a genetic basis (21–23). There is also evidence that mice have genetic differences in taste responsiveness to stimuli including glutamate (24). However, it is not known whether this variation in taste responses to glutamate influences glutamate preference or intake. Because glutamate has many beneficial effects for health (13, 14, 25, 26), it is important to understand genetic and physiologic mechanisms that regulate its consumption.

This article summarizes our studies of variation in glutamate consumption and the physiologic and genetic mechanisms underlying it. These studies involve inbred strains of mice. Animals within each inbred strain are genetically identical. Differences between inbred strains are attributed to allelic variation of polymorphic genes. Our studies have focused on 2 inbred mouse strains that differ in glutamate consumption, C57BL/6ByJ (B6) and 129P3/J (129).

GLUTAMATE CONSUMPTION

Strain differences in MSG consumption
In 2-bottle preference tests with water and MSG solutions, mice from the B6 strain had higher MSG intakes and preferences than did mice from the 129 strain (Figure 1A). For preference

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scores, the strain differences were largest at lower MSG concentrations, which were near preference thresholds. These thresholds were lower in the B6 mice (0.3 mmol/L) than they were in the 129 mice (1 mmol/L). However, the strain differences in MSG solution intakes were largest at higher concentrations (300–600 mmol/L). Consumption of 300 mmol MSG/L by the B6 mice was remarkably high: some of the B6 mice consumed MSG solution during 24 h in amounts >50% of their body weight.

These differences between the B6 and 129 strains allowed us to use these mice for physiologic and genetic analyses of mechanisms underlying glutamate consumption. Results of these analyses are described below.

Specificity of strain differences in MSG consumption

MSG contains sodium, which contributes a salty component to MSG taste and has its own postingestive effects when consumed. The salty (sodium chloride–like) taste quality component of MSG was shown in conditioned taste aversion experiments with mice (3, 4), rats (27), and hamsters (28) and in psychophysical experiments with humans (1, 2, 29, 30). Sodium consumed with MSG may affect sodium metabolism and osmotic equilibrium of the body. Therefore, strain differences in MSG consumption potentially could be influenced by differential responses to the Na+ anion of MSG. However, this is unlikely in this case because the B6 mice have lower sodium chloride intakes and preferences than do the 129 mice (1 mmol/L). Consumption of 300 mmol MSG/L by the B6 mice was remarkably high: some of the B6 mice consumed MSG solution during 24 h in amounts >50% of their body weight.

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Genetics of MSG consumption

To identify some of the genetic influences on MSG consumption, we out-crossed B6 mice and 129 mice to produce the first filial generation of hybrid mice, which were intercrossed to produce the second filial generation of hybrid mice (F2). The F2 mice (n = 455) were tested in 4-d, 2-bottle preference tests, with 1 and 300 mmol MSG/L and with 3 sweeteners: sucrose, saccharin, and D-phenylalanine (49). We showed that elevated MSG intake and preference in B6 mice is inherited as a recessive trait in the F2 generation.

We next analyzed correlations in preference scores of the solutions tested. Interestingly, preferences for 1 and 300 mmol MSG/L did not correlate in the F2 hybrid mice (Table 1), which suggested that ingestive responses to these solutions depend on different mechanisms and are determined by different genes. It is possible that weak and strong MSG solutions have different sensory properties. However, the difference in responses to these 2 solutions is also likely to depend on the postingestive effects of 300 mmol MSG/L (but not 1 mmol MSG/L), as discussed below in Postingestive Effects of Glutamate.

Preferences for sucrose, saccharin, and D-phenylalanine correlated strongly with each other in F2 mice (Table 1). However, no significant correlations were shown between MSG and...
sweetener preferences (Table 1), even though B6 mice have higher consumption of both MSG and sweeteners compared with 129 mice (Figure 1, A and C). Independence of the strain differences in taste responses to glutamate and sweeteners is also evident from studies of allelic variation of the \textit{Tas1r3} (taste receptor, type 1, member 3) gene (formerly the \textit{Sac}, saccharin preference, locus). The \textit{Tas1r3} gene encodes the T1R3 protein (50–54). When T1R3 is coexpressed in a heterologous system with T1R2, it functions as a broad-spectrum sweet taste receptor (43–45). In contrast, a heterodimer of T1R1 and T1R3 proteins functions as an umami taste receptor in humans and is more broadly tuned in rodents to respond to \textit{l}-amino acids (44, 45, 55). Thus, the T1R3 protein is involved in transduction of both sweet and umami tastes, and a disruption of the \textit{Tas1r3} gene diminishes taste responses to both sweet and umami taste stimuli (55, 56). Hence, natural allelic variation of the \textit{Tas1r3} gene could also affect umami taste responses. However, experiments with the F2 hybrids between the B6 and 129 inbred mouse strains (57) and with 129.B6-\textit{Tas1r3} congenic mice (58) have shown that although the \textit{Tas1r3} allelic variants influence taste responses to sweeteners, they do not affect behavioral or neural taste responses to umami stimuli, including MSG. Thus, although T1R3 is involved in the reception of umami taste, the B6/129-\textit{Tas1r3} sequence variants that affect its sensitivity to sweeteners do not affect taste responses to umami compounds, including MSG. These data demonstrate that the strain variation in MSG consumption depends on different genes from the strain variation in sweetener consumption.

The independent genetic determination of MSG and sweetener consumption must be a consequence of distinct physiologic mechanisms underlying strain differences in ingestive responses to these stimuli. This is consistent with available experimental data. Differences between B6 mice and 129 mice in sweetener consumption depend to a large degree on the T1R3-related peripheral taste mechanisms. Compared with 129 mice, B6 mice have higher peripheral neural responses to several sweeteners (59–61), and allelic variation of the \textit{Tas1r3} gene underlies the strain differences in both behavioral and neural taste responses to sweeteners (57, 58, 61). On the other hand, data presented below indicate that differences between B6 mice and 129 mice in consumption of concentrated MSG solutions depend on strain-specific postingestive effects of glutamate rather than on variation in umami taste responsiveness. Together, these results demonstrate that the elevated MSG and sweetener intakes by B6 mice and the decreased consumption of these compounds by 129 mice are the results of the incidental association of these traits during inbreeding of these 2 strains.

Possible mechanisms underlying strain differences in MSG consumption

Several possible mechanisms may be responsible for differences in MSG consumption between B6 mice and 129 mice. Moreover, strain variation in MSG preference at lower concentrations may depend on different mechanisms than does strain variation in intake of concentrated MSG. As discussed in the Introduction, when glutamate is consumed, it evokes taste sensations and may also produce postingestive effects. Therefore, strain variation in MSG consumption can be due to either differential umami taste responsiveness or differences in post-ingestive handling of glutamate or possibly to both. We have examined both of these possibilities in experiments that are described below in Taste Responses to Glutamate and Post-ingestive Effects of Glutamate.

TASTE RESPONSES TO GLUTAMATE

To examine whether differences between B6 mice and 129 mice in MSG consumption depend on peripheral taste responsiveness, we measured integrated responses of the whole chorda tympani and glossopharyngeal nerves to lingual application of MSG and several other taste stimuli in these mice (62). We showed that the B6 mice and the 129 mice have similar responses to MSG in both nerves (Figure 2A).

As discussed above in Specificity of Strain Differences in MSG Consumption, MSG contains sodium, which evokes a salty taste sensation and activates receptors distinct from the umami taste receptors (63). If there are strain differences in taste responses to Na⁺, they could also affect responses to MSG. We assessed the role of the Na⁺ component by measuring responses to a series of sodium chloride concentrations and showed that the B6 mice and the 129 mice had similar responses to sodium chloride in both nerves (Figure 2B). This is consistent with results of previous studies that used the B6 and 129 strains (33, 64–66) and implies that gustatory neural responses of B6 mice and 129 mice to MSG are not affected by differential responses to sodium present in this compound.

These data demonstrate that although B6 mice and 129 mice differ in MSG consumption in long-term 2-bottle tests (49), they have similar gustatory neural responses to MSG. In addition, there are no differences between these 2 strains in qualitative taste perception of MSG (3). Therefore, the strain differences in MSG consumption most likely depend on nongustatory mechanisms. Data presented below in Post-ingestive Effects of Glutamate suggest that strain-specific postingestive effects of glutamate can affect its intake.

<table>
<thead>
<tr>
<th>Solutions</th>
<th>MSG (300 mmol/L)</th>
<th>Sucrose (120 mmol/L)</th>
<th>Saccharin (20 mmol/L)</th>
<th>D-Phenylalanine (30 mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSG (1 mmol/L)</td>
<td>0.07</td>
<td>0.11</td>
<td>0.10</td>
<td>0.03</td>
</tr>
<tr>
<td>MSG (300 mmol/L)</td>
<td>0.00</td>
<td>0.01</td>
<td></td>
<td>−0.04</td>
</tr>
<tr>
<td>Sucrose (120 mmol/L)</td>
<td></td>
<td></td>
<td>0.72(^2)</td>
<td>0.50(^2)</td>
</tr>
<tr>
<td>Saccharin (20 mmol/L)</td>
<td></td>
<td></td>
<td></td>
<td>0.56(^2)</td>
</tr>
</tbody>
</table>

\(^1\) Reproduced with permission from reference 49. F2, second filial generation of hybrid mice.

\(^2\) Significant correlations; significance level was calculated with Bonferroni’s correction for 10 correlation coefficients, which estimates critical \(P\) level as 0.05/10 = 0.005.
POSTINGESTIVE EFFECTS OF GLUTAMATE

Strain-specific effects of exposure to MSG

Mice given concentrated MSG solutions, in particular 300 mmol MSG/L, consume substantial amounts of glutamate (Figure 1A). It is likely that consumption of this amount of glutamate has post-ingestive effects. Such effects, if present, could alter initial levels of consumption by increasing (if the effects are rewarding) or decreasing (if the effects are aversive) intakes. To examine the possibility that MSG has post-ingestive effects that influence its consumption, we measured intake of 300 mmol MSG/L during the 4-d 2-bottle tests (49). We showed that MSG consumption changed over time in a strain-specific manner (Figure 3). Compared with the first 2 d of the test, during the last 2 d, MSG intakes and preferences increased in the B6 mice and decreased in the 129 mice (all changes were significant with the exception of non-significant increase in preference scores of the B6 mice). As a result, the strain differences in MSG consumption were larger at the end of the test than they were in the beginning of the test. These observations suggest that the post-ingestive effects of MSG are rewarding to B6 mice and aversive to 129 mice and that the strain differences in MSG consumption depend on its post-ingestive effects (at least for concentrated solutions).

To investigate the nature of the strain-specific post-ingestive effects of glutamate, we conducted experiments, which are described below in Glutamate Metabolism. In these experiments, we examined whether B6 mice and 129 mice differ in post-ingestive metabolism of glutamate.

Glutamate metabolism

To examine whether strain-specific post-ingestive effects of MSG in B6 mice and 129 mice involve differences in glutamate metabolism, we administered MSG to mice from these strains intragastrically (by gavage) and measured perturbations in the main pathways of glutamate metabolism (67).

Metabolic pathways of ingested glutamate are complex. Glutamate is extensively metabolized in the small intestine into carbon dioxide, lactate, glutathione, glutamine, alanine, and several other amino acids (6, 10). If dietary glutamate is not completely metabolized in the intestine, then it (as well as some of its intestinal metabolites) is released into the hepatic portal circulation, and most of it is further metabolized in the liver. Because glutamate is a gluconeogenic amino acid, it (and its intestinal metabolites, alanine and lactate) can be converted to glucose in the liver. This glucose is either released into the general circulation (and stimulates insulin secretion) or stored in the liver in the form of glycogen. Alternatively, the carbon skeleton of glutamate can be oxidized through the tricarboxylic acid cycle to generate energy, and its nitrogen can be converted to urea, which is excreted in urine (68).

Our experiment was designed to characterize the main pathways of metabolism of ingested glutamate: its intestinal metabolism (by measuring plasma concentrations of glutamine, alanine, glutathione, and lactate), deamination (by measuring plasma and urine concentrations of urea and ammonia), gluconeogenesis (by measuring plasma glucose and insulin and liver glycogen), and thermogenesis (by measuring body temperature).

In this experiment, we intended to approximate conditions that mice experience during the 24-h 2-bottle tests. We calculated
a dose of glutamate with voluntary intakes of 300 mmol MSG/L (the most consumed solution; Figure 1) in the 2-bottle tests. Mice were intragastrically administered (by gavage) 1000 mg MSG/kg body wt, which is a dose equivalent to the average amount of MSG voluntarily consumed in 1 h by a mouse from the 129 strain (49). Therefore, the strain differences in postigestive metabolism of glutamate shown in this experiment are likely to also influence postigestive processing of these amino acids consumed during the long-term 2-bottle tests.

After administration of MSG, plasma glucose significantly increased above the baseline concentration in the B6 mice but not the 129 mice (Figure 4A). Correspondingly, plasma glucose and insulin (Figure 4B) concentrations were higher in the B6 mice than they were in the 129 mice \( [P < 0.05, \text{effect of strain; analysis of variance (ANOVA)}] \). The opposite pattern of changes was present for plasma alanine concentration: it was significantly increased above baseline in the 129 mice but not the B6 mice (Figure 4C; \( P < 0.05, \text{effect of strain, ANOVA} \)). Body temperature measured with subcutaneously implanted temperature transponders did not increase above the baseline level in the B6 mice, but in the 129 mice significant increases above baseline and significantly higher levels than in the B6 mice were recorded (Figure 4D; \( P < 0.0001, \text{effect of strain, ANOVA} \)). The B6 mice and the 129 mice did not differ significantly in postigestive changes in plasma glutamine, lactate, glutathione, urea, or ammonia concentrations; in liver contents of glycogen; or in total nitrogen (ammonia plus urea) excretion in urine (67).

The time course of change was different for different measures. Peak alanine concentration in the 129 mice occurred 30 min after MSG administration, peak glucose concentration in the B6 mice occurred 60 min after MSG administration, and body temperature increase in the 129 mice occurred only at >90 min after MSG administration. These temporal differences point to different organs responsible for differential processing of glutamate. The relatively early time of the peak plasma alanine concentration suggests that it is produced from glutamate in the intestine. The later peak for glucose suggests that it is produced from absorbed glutamate (and its metabolites) in the liver through the gluconeogenic pathway and then released into the general circulation rather than stored as glycogen. The delayed body temperature rise suggests that the thermogenesis mainly occurred in the liver and/or other tissues but not in the intestine.

These observations indicate that the metabolic fate of oral glutamate differs in B6 mice and in 129 mice. B6 mice tend to use glutamate as a gluconeogenic precursor in the liver, whereas 129 mice convert glutamate to alanine in the intestine; then, alanine and possibly glutamate are further used for thermogenesis (Figure 5).

Can these strain differences in glutamate metabolism explain the differential consumption of glutamate by B6 mice and 129 mice? Our data suggest that rewarding postigestive effects of glutamate in B6 mice are associated with elevated blood glucose and insulin. Previous studies have shown that glucose can act as a metabolic signal in the intestine to promote reward associated with consumption of sugars. Glucose and insulin are known to be important in the control of food intake, and insulin has been shown to be a key factor in the regulation of food intake and body weight. Glucose may also be involved in the regulation of body weight and energy expenditure through its effects on the hypothalamus, which is a major brain region involved in the control of energy balance. Glucose and insulin are known to be important in the control of food intake, and insulin has been shown to be a key factor in the regulation of food intake and body weight. Glucose may also be involved in the regulation of body weight and energy expenditure through its effects on the hypothalamus, which is a major brain region involved in the control of energy balance.
a rewarding unconditioned stimulus. Elevated blood glucose was shown to suppress intake in some (69–71) but not all (72) studies. At the same time, systemic intravenous glucose administration is not effective for conditioning flavor preferences (72, 73). On the contrary, intragastric and hepatic portal infusions of glucose and some other nutrients can condition flavor preferences (72, 74–76), which suggests that the rewarding signal of ingested glutamate in B6 mice may be initiated by glucose generated during gluconeogenesis in the liver before it enters the general circulation. In contrast, aversive postigestive effects of glutamate in the 129 mice are associated with thermogenesis. It was shown that changes in body temperature positively correlate with satiety (77–79). It is, therefore, likely that thermogenesis induced by glutamate consumption in the 129 mice suppresses ingestive behavior and thus limits glutamate intake in this strain.

These data are consistent with a hypothesis that the metabolic fate of glutamate plays an important regulatory role in control of its intake. We speculate that glucose produced during the gluconeogenesis of the ingested glutamate generates the rewarding stimulus in the liver before it enters the general circulation and that glutamate-induced postigestive thermogenesis generates the aversive stimulus. However, additional studies are needed to examine this hypothesis.

CONCLUSIONS

Our studies have identified genetically determined differences in glutamate consumption in mice and suggested that the metabolic fate of glutamate may play an important regulatory role in the control of its intake. In addition, these studies provide the first characterization of genetic variation in amino acid metabolism. Given the many similarities between mice and humans in metabolism and behavior, it is a small step to infer that genetic variation in glutamate metabolism may also exist in humans and may influence their glutamate preference, consumption and postigestive processing. (Other articles in this supplement to the Journal include references 14–17, 21–23, and 80–101.)

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FIGURE 5. Differences in glutamate metabolism between B6 mice and 129 mice. Arrows indicate predominant metabolic pathways in each strain. Glu, glutamate; Ala, alanine; B6, C57BL/6ByJ mouse strain; 129, 129P3/J mouse strain. Modified with permission from reference 67.

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