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Gurmarin Suppression of Licking Responses to Sweetener-Quinine Mixtures in C57BL Mice

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Abstract

Gurmarin (Gur) is a peptide that selectively suppresses responses of the chorda tympani nerve to sweet substances in rats and mice. In the present study, we examined the effect of Gur on behavioral responses to sweet substances in C57BL mice. To accomplish this, we developed a new short-term lick test and measured numbers of licks for 10 s for sweet substances mixed with quinine hydrochloride (QHCl) in water-deprived mice. Numbers of licks for sucrose mixed with 1 or 3 mM QHCl increased with increasing concentration of sucrose from 0.01 to 1.0 M. Oral infusion with 30 μg/ml Gur produced significant decreases in responses to concentration series for sucrose mixed with 3 mM QHCl, whereas no such effect by Gur was observed in responses to QHCl alone or QHCl-mixed HCl, NaCl or monosodium glutamate. The Gur suppression of QHCl-mixed sucrose responses, which otherwise lasted for 2–3 h, rapidly returned to ~80% of control levels after oral infusion with β-cyclodextrin. These results are comparable to neural data previously found in chorda tympani responses, and thereby provide further evidence for Gur as a sweet response inhibitor in C57BL mice. In the other aspect, our newly developed short-term test can also provide a tool for measurements of taste-guided behavioral responses to sweeteners.

Key word: β-cyclodextrin, gurmarin inhibition, licking behavior, mice, sweet taste

Introduction

Gurmarin (Gur), a polypeptide isolated from a plant *Gymnema sylvestre*, has been reported to suppress responses to sweet substances without affecting responses to salty, sour and bitter substances in rat (Imoto *et al.*, 1991; Miyasaka and Imoto, 1995; Harada and Kasahara, 2000) and mouse taste nerves (Ninomiya and Imoto, 1995; Ninomiya *et al.*, 1997; Ninomiya *et al.*, 1998), and gerbil taste cells (Uchida and Sato, 1997). Anti-sweet effects of Gur are normally long-lasting (>2–3 h), but they rapidly disappeared after rinsing the tongue with either anti-gurmarin serum in rats (Imoto *et al.*, 1991; Miyasaka and Imoto, 1995) or β-cyclodextrin (β-CD) in C57BL mice (Ninomiya and Imoto, 1995; Ninomiya *et al.*, 1998). These results suggest that Gur may act as a specific inhibitor for responses of peripheral taste system to sweet substances in rodents.

Previous electrophysiological studies (Ninomiya and Imoto, 1995; Ninomiya *et al.*, 1998) also demonstrated that the effect of Gur on responses to sweet substances in mice is strain- and nerve-specific. That is, sucrose responses of the chorda tympani innervating taste buds located in the anterior two-thirds of the tongue were suppressed to ~50% of control by Gur in C57BL mice, whereas no such suppression was observed in BALB mice. Furthermore, even in Gur-sensitive C57BL mice, responses of the glossopharyngeal nerve innervating taste buds located in the posterior one-third of the tongue were not affected by Gur (Ninomiya *et al.*, 1998). Therefore, in mice the effect of Gur on the peripheral receptor system might be considerably limited. This gives rise to the question of whether such a limited effect of Gur in mice would be strong enough to produce selective suppression on their behavioral responses to sweet substances. In a previous study (Katsukawa *et al.*, 1999), we examined behavioral preference for sucrose mixed with quinine in rats fed diets containing *G. sylvestre* leaves. We found a very weak suppression of preference for the sucrose–quinine mixture at a particular combination of

concentrations during the first 2 days of the trial (48 h) after the start of the diet, and such suppression disappeared rapidly on the second trial, which started several days after the start of the diet (Katsukawa *et al.*, 1999). So far, therefore, no convincing behavioral data on the effect of Gur have been reported in rodents.

Several methods have been employed to examine behavioral responses to sweet substances in animals. Long-term (24–48 h) two-bottle preference tests may be unsuitable to examine anti-sweet effects of Gur because a significant effect of Gur on the CT nerves may disappear at ~5 h (Y. Ninomiya *et al.*, unpublished observation). A conditioned taste aversion paradigm may be available for a short-term test for the effect of Gur. However, this method involves extinction effects which may give some difficulties to evaluate the data. In rats, short-term lick tests with water deprivation have been used to assess behavioral responses to bitter substances (Contreras *et al.*, 1995). Water deprivation motivates licking to aversive taste solutions, and numbers of licks per 10–30 s were measured for dilute bitter compounds dissolved in a strong sucrose solution.

In the present study, to examine the effect of Gur, we developed a short-term test in which water-deprived mice were used to motivate licking to aversive taste of quinine hydrochloride (QHCl) and counted numbers of licks per 10 s for various sweet substances mixed with a low concentration of QHCl before and after Gur. The experiment was performed by using Gur-sensitive C57BL mice with purified Gur. Our results demonstrated that responses to sucrose–QHCl mixtures were inhibited by Gur and quickly recovered by rinsing the tongue with β-CD. These results further support the idea that Gur can act as a specific inhibitor for responses to sweet substances in C57BL mice.

Materials and methods

Subjects

Male and female C57BL/KsJ mice at 8–25 weeks of age (18–30 g body wt) were housed in plastic cages under a 12 h light/12 h dark cycle at 20–22°C and 50–55% relative humidity. Two or three mice were housed together in a cage and received food pellets (MF; Oriental Yeast, Tokyo, Japan) *ad libitum*. Tap water was also freely available except the training and testing sessions.

Taste stimuli

All chemicals were purchased from Wako Pure Chemical Industries (Osaka, Japan). Solutions used for the test stimuli were: 0.01, 0.03, 0.1, 0.3, 0.5, or 1.0 M sucrose, 0.3 M glucose, 0.3 M fructose, 0.3 M maltose, 0.1 M glycine, 0.1 M L-alanine, 0.3 M L-proline, 0.1 M D-phenylalanine, 0.03 M D-tryptophan, 0.1 M D-histidine, 0.02 M sodium saccharin, 10 mM HCl, 0.1 M NaCl and 0.1 M monosodium L-glutamate. These solutions were used alone or in binary mixtures with 3 mM QHCl. Mixtures of 0.01–1.0 M sucrose with 0.3, 1 or 10 mM QHCl were also used. All solutions were presented to mice at room temperatures.

Procedures

All training and testing sessions occurred during the light phase of the light/dark cycle. On the first day of training, the animal was placed in a test box and given free access to distilled water during a 1 h session from a polyethylene tube via a circular window (5 mm in diameter). The tip of a tube (1.5 mm in inner diameter) was located 2 mm outside the window. This arrangement prevented contact of the tip of the tube with the animal's lips. The number of licks per 10 s was measured by a lickometer with a laser beam sensor (Yutaka Electronics Company, Gifu, Japan) and recorded on a pen recorder. From day 2 to day 5, training session time was reduced from 1 h to 30 min. During this period, the animal was trained to drink distilled water on an interval schedule, consisting of 10 s periods of presentation of distilled water alternated with 20 s inter-trial intervals, resulting in 30–50 trials. On days 6–10, lick rates for each of the test stimuli and distilled water were measured during the first 10 s after the animal's first lick.

The first experiment was designed to establish a concentration–response relationship for 0.01–1.0 M sucrose so that we can choose an appropriate concentration of QHCl for mixing with sucrose and other sweet substances in subsequent experiments. Numbers of licks (per 10 s) were measured for 0.3, 1, 3 or 10 mM QHCl alone and in mixtures with concentration series of sucrose (0.01, 0.03, 0.1, 0.3, 0.5 or 1.0 M).

In the next series of experiments to examine the anti-sweet effects of Gur, licks for 0.01–1.0 M sucrose and other taste stimuli alone or in mixtures with 3 mM QHCl (decided from the first experiment) were measured before and after treatment with Gur. The purified Gur was dissolved in 5 mM phosphate buffer (PBS; pH 6.9) at a concentration of 30 μg/ml. Each mouse received an oral infusion for 1 min twice to a total of 20 μl of the Gur solution using a microsyringe. The concentration of Gur (30 μg/ml) was chosen because in the same C57BL/KsJ mice suppressive effect of Gur on sucrose responses in the CT nerve had been shown to be nearly maximal at this dose (Ninomiya *et al.*, 1997, 1998). Control animals received oral infusion with a similar volume of PBS in place of Gur. After 10 min following oral infusion with Gur, each animal received five consecutive 10 s lickings of distilled water, followed by measurement of the number of licks (per 10 s) for each of the test stimuli.

The time course of Gur-induced anti-sweet effects was examined by measuring numbers of licks for a mixture of 0.5 M sucrose and 3 mM QHCl before and at several time points following oral infusion with 30 μg/ml Gur, because previous electrophysiological studies showed suppressed CT nerve responses to sweeteners by Gur lasted for 2–3 h in C57BL mice (Ninomiya and Imoto, 1995). Control animals

received oral infusion with PBS in place of Gur. After 10 min following treatment with Gur, each animal received five consecutive 10 s lickings of distilled water, followed by measurement of number of licks (per 10 s) for the mixture. The numbers of licks were also measured at 35 min and at 1, 2 or 3 h after treatment with Gur.

To examine the effects of β-CD on Gur suppression of lick responses, the numbers of licks were measured for a mixture of 0.5 M sucrose and 3 mM QHCl before and after treatment with 30 μg/ml Gur and after two subsequent oral infusions with 20 μl of 15 mM β-CD (dissolved in PBS). The concentration of β-CD was chosen because our previous electrophysiological study showed that recovery of CT responses to sucrose after Gur was maximal at this concentration of β-CD (Ninomiya *et al.*, 1998). Control animals received oral infusion with PBS in place of either Gur or β-CD. After 10 min following treatment with either Gur or β-CD, each animal received five consecutive 10 s trials with distilled water. The numbers of licks for the mixtures were then measured.

Data analysis

The mean number of licks for 5 consecutive days was obtained for each of the test stimuli in each mouse. Data in each test stimuli were expressed as mean values ± standard error of the mean (SE) and were analyzed using a two-way repeated-measures analysis of variance (ANOVA). When the ANOVA indicated differences amongst the groups, pairwise comparisons of each value versus the control value (mean number of licks before Gur) were performed using a *t*-test. Data were also analyzed using a two-way factorial ANOVA and a Bonferroni-corrected *t*-test, where appropriate. All calculations were performed using the statistical software package StatView (Abacus Concepts, Inc., Berkeley, CA).

Results

Concentration–response functions for sucrose–QHCl mixtures

Numbers of licks (per 10 s) for distilled water were usually maximal to reach ~80. Licks for sucrose alone were also ~ 80 in a concentration range between 0.01 and 1.0 M. Thus, without mixing with QHCl, licks for sucrose were essentially the same as those for distilled water (data not shown). Figure 1 shows the concentration–response functions for mixtures of sucrose (0.01–1.0 M) and 0.3, 1, 3 or 10 mM QHCl. The two-way factorial ANOVA for the mixtures of QHCl and sucrose showed significant main effects for the concentration of QHCl [$F(3,140) = 984.4$, $P < 0.001$], for the concentration of sucrose [$F(5,140) = 180.5$, $P < 0.001$] and for their interaction [$F(15,140) = 39.37$, $P < 0.001$], indicating that licks for sucrose–QHCl mixtures varied dependent on the concentration of each component in the mixtures. Licks for sucrose mixed with lowest concentration

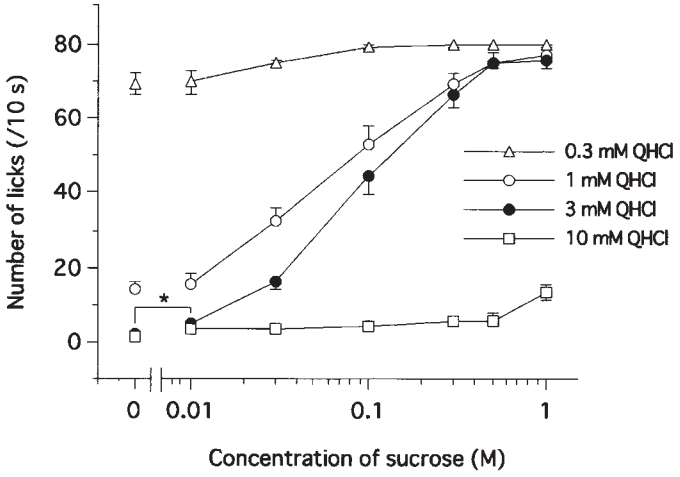

Figure 1 Concentration–response functions for sucrose (0.01–1.0 M) mixed with QHCl at 0.3 mM (open circles), 1 mM (closed circles), 3 mM (open triangles) or 10 mM (closed triangles). Numbers of licks for 0.3, 1, 3 or 10 mM QHCl alone are also indicated. Data were obtained from 5–7 mice, and expressed as mean values ± SE. Mean values between 3 mM QHCl alone and in mixture with 0.01 M sucrose are significantly different. *$P < 0.05$ (*t*-test).

of QHCl (0.3 mM) were found to be nearly maximal at any concentration of sucrose (70–80 licks). In contrast, 10 mM QHCl mixtures were strongly aversive, resulting in very low numbers of licks for the mixtures (<15), even at 1.0 M sucrose. Clear concentration-dependent changes in licks were observed when sucrose were mixed with 1 or 3 mM QHCl. Numbers of licks for sucrose (0.01, 0.03, 0.1, 0.3. 0.5, 1.0 M) mixed with 3 mM QHCl (means ± SE) were 5.3 ± 0.9, 16.4 ± 1.8, 45 ± 5, 66.2 ± 3.2, 74.8 ± 1.0 or 75.8 ± 2.5, respectively. There was a significant difference in licks for 0.01 M sucrose mixed with 3 mM QHCl and QHCl alone (*t*-test, $P < 0.05$), suggesting that the behavioral threshold for sucrose in this test can be 0.01 M or less in mice. Although a similar concentration–response curve was obtained for sucrose mixed with 1 mM QHCl, the difference in the number of licks for 0.01 and 1.0 M sucrose mixed with 1 mM QHCl was slightly smaller than that for sucrose mixed with 3 mM QHCl, and unlike the case for mixtures with 3 mM QHCl, there was no significant difference in licks for 0.01 M sucrose mixed with 1 mM QHCl and 1 mM QHCl alone. Thus, effects of Gur on lick responses to sucrose and some of other test stimuli were examined by using mixtures with 3 mM QHCl.

Selective suppression on licks for sucrose–QHCl mixtures

Figure 2 shows the mean numbers of licks for 0.01–1.0 M sucrose mixed with 3 mM QHCl when they were presented before (open circles) or after (closed circles) oral infusion with 30 μg/ml Gur. An ANOVA on the data for 0.1, 0.3 or 0.5 M sucrose, but not for 0.01, 0.03 or 1.0 M sucrose, revealed significant main or interactive effects (data not shown). After treatment with Gur, the mean numbers of

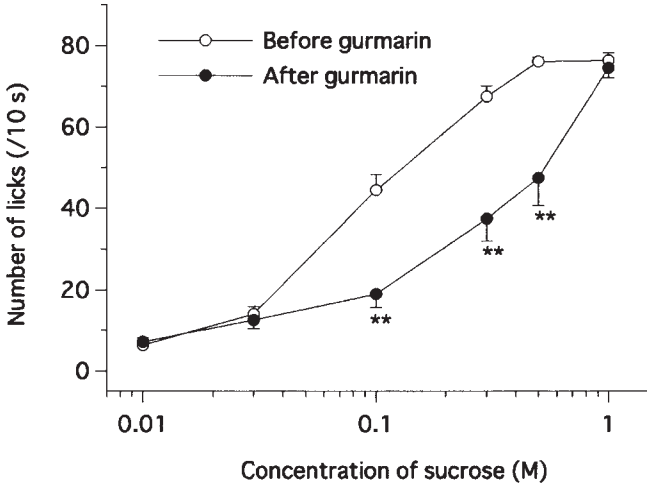

Figure 2 Mean numbers of licks for 0.01–1.0 M sucrose mixed with 3 mM QHCl before (open circles) and after (closed circles) oral infusion with 30 μg/ml gurmarin. Data were obtained from eight mice and expressed as mean values ± SE. $^{**}P < 0.01$ (*t*-test).

licks for sucrose mixed with 3 mM QHCl significantly decreased from 44.5 ± 3.8 to 19.0 ± 3.3 at 0.1 M sucrose (*t*-test, $P < 0.01$), from 67.6 ± 2.5 to 37.5 ± 5.5 at 0.3 M sucrose ($P < 0.01$) and from 76.1 ± 1.1 to 47.5 ± 6.8 at 0.5 M sucrose ($P < 0.01$), respectively. The number of licks for 0.01, 0.03 or 1.0 M sucrose mixed with 3 mM QHCl was not affected by treatment with Gur ($P > 0.05$).

Effects of Gur on licks for 3 mM QHCl alone or 0.01 M HCl, 0.1 M NaCl or 0.1 M MSG mixed with 3 mM QHCl are shown in Figure 3. ANOVAs on these data revealed no significant main or interactive effects (data not shown). The mean numbers of licks for 3 mM QHCl alone or 0.01 M HCl, 0.1 M NaCl or 0.1 M MSG mixed with 3 mM QHCl were similar before (open columns) and after (closed columns) Gur. Therefore, data obtained from behavioral study also suggest that Gur selectively suppresses responses to sweet substances, although they were mixed with QHCl.

Effects of Gur on licks for various sweeteners mixed with QHCl

Figure 4 shows mean numbers of licks for 11 sweet substances when they were presented alone (open circles) or in combination with 3 mM QHCl before (open columns) and after (closed columns) oral infusion with 30 μg/ml Gur. When they were mixed with 3 mM QHCl, numbers of licks for sucrose, fructose, glucose, maltose, L-alanine, L-proline and glycine significantly decreased (*t*-test, $P < 0.05$–0.001). Licks for another four sweeteners also slightly decreased by mixing with 3 mM QHCl, although differences were not statistically significant ($P > 0.05$). ANOVAs on the data obtained from 11 sweet substances before and after Gur revealed significant main or interactive effects (data not shown). After oral infusion with Gur, numbers of licks for

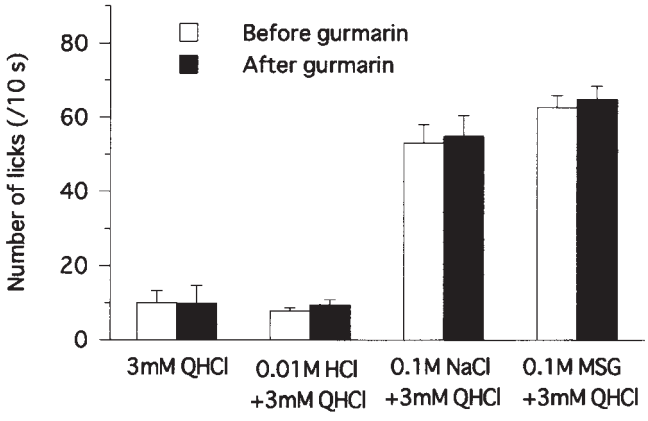

Figure 3 Mean numbers of licks for 3 mM QHCl alone or 0.01 M HCl, 0.1 M NaCl or 0.1 M monosodium L-glutamate (MSG) mixed with 3 mM QHCl before (open columns) and after (closed columns) oral infusion with 30 μg/ml gurmarin. Data were obtained from 5–8 mice and expressed as mean values ± SE.

all 11 sweet substances mixed with QHCl significantly decreased (*t*-test, $P < 0.05$–0.001).

Recovery of licks for sucrose–QHCl mixtures suppressed by Gur and its facilitation by treatment with β-CD

Mean numbers of licks for a mixture of 0.5 M sucrose and 3 mM QHCl before and at various time points after Gur are shown in Figure 5. An ANOVA on these data revealed significant main effects for the treatment condition (either Gur or PBS) [$F(1,77) = 16.44$, $P < 0.01$], for lick responses [$F(5,77) = 6.58$, $P < 0.001$] and for their interaction [$F(5,77) = 5.32$, $P < 0.001$]. The mean number of licks before Gur (control = 100%) significantly decreased to 55% (*t*-test, $P < 0.01$) of control after Gur. The suppressed licks for 0.5 M sucrose mixed with QHCl, then slowly returned to 58% of control at 25 min ($P < 0.01$), 73% at 1 h ($P < 0.05$), 81 % at 2 h ($P < 0.05$) and 96% at 3 h, respectively. This suggests that the suppression in licks by Gur normally lasted for 2–3 h.

Figure 5 also shows mean numbers of licks for a mixture of 0.5 M sucrose and 3 mM QHCl before and after β-CD. An ANOVA on these data revealed significant main effects for treatment condition (either Gur/β-CD or PBS/PBS) [$F(1,47) = 17.50$, $P < 0.001$], for lick responses [$F(2,47) = 19.33$, $P < 0.001$] and for their interaction [$F(2,47) = 15.90$, $P < 0.001$]. The mean number of licks for 0.5 M sucrose mixed with 3 mM QHCl before Gur (control = 100 %) significantly decreased to 57% (*t*-test, $P < 0.01$) of control after Gur. The value rapidly recovered after treatment with β-CD, to 81% of control level, although the level was still significantly different from control (*t*-test, $P < 0.05$). There was also a significant difference in mean values between at 25 min after Gur and after β-CD ($P < 0.01$). These results suggest that rinsing the tongue with β-CD reduced the effect of Gur and facilitated the recovery of licks for sweet

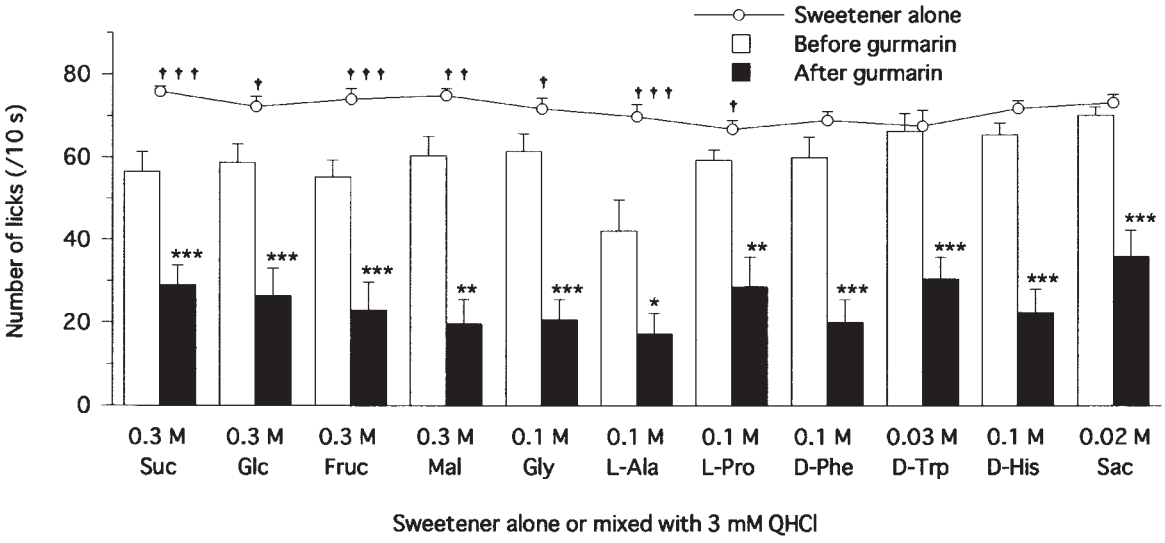

Figure 4 Mean numbers of licks for 11 sweeteners alone (open circles) or mixed with 3 mM QHCl before (open columns) and after (closed columns) oral infusion with 30 μg/ml gurmarin. Data were obtained from 8–13 mice and expressed as mean values ± SE. Suc, sucrose; Glc, glucose; Fruc, fructose; Mal, maltose; Gly, glycine; L-Ala, L-alanine; L-Pro, L-proline; D-Phe, D-phenylalanine; D-Trp, D-tryptophan; D-His, D-histidine; Sac, sodium saccharin. Mean values between sweetener alone and in combination with 3 mM QHCl are significantly different, †$P < 0.05$; ††$P < 0.01$; †††$P < 0.001$ (*t*-test). Mean values between before and after gurmarin are significantly different, *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ (*t*-test).

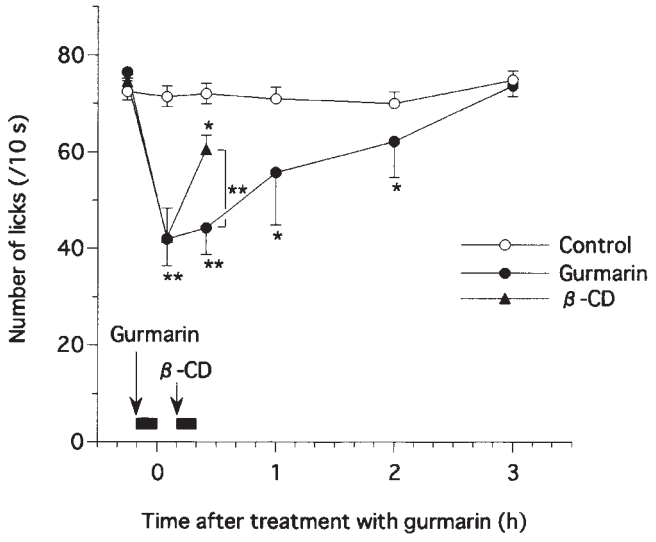

Figure 5 Mean numbers of licks for a mixture of 0.5 M sucrose and 3 mM QHCl before and at various times following oral infusion with either PBS (open circles) or 30 μg/ml gurmarin (Gur, closed circles). Mean numbers of licks for the mixture before and after treatment with gurmarin, and after subsequent oral infusion with 15 mM β-cyclodextrin (β-CD) are also indicated (closed triangles). Data were obtained from eight mice and expressed as mean values ± SE. *$P < 0.05$ (t-test) ; **$P < 0.01$.

substances suppressed by Gur as previously shown by electrophysiological studies (Ninomiya *et al.*, 1998).

Discussion

In the present study, we newly developed a short-term (10 s) lick test for measurements of behavioral responses to sweeteners in water-deprived C57BL mice. Short-exposure tests could minimize possible side effects by post-ingestive feedback, thereby allowing taste afferent inputs to determine licking responses (Contreras *et al.*, 1995). By using this test, we were able to repeat several trials in a single test session of ~30 min in each animal. Then we obtained a concentration–response function for 0.01–1.0 M sucrose when they were mixed with 3 mM (or 1 mM) QHCl, but not with 0.3 or 10 mM in water-deprived mice. Mixtures of sweet and bitter compounds have previously been employed in behavioral experiments. For example, Contreras *et al.* (Contreras *et al.*, 1995) used some bitter compounds mixed with 0.8 M sucrose to motivate water-non-deprived rats to lick bitter stimuli and obtained reliable concentration–response function for bitter stimuli. With regards to responses to sweet stimuli, studies in rats (Berridge and Grill, 1983, 1984; Hsiao and Fan, 1993) have shown that sucrose solutions when mixed with quinine produced both ingestive and aversive responses which reflected the stimulus intensity of each component of the mixture. The concentration–response curve for sucrose–QHCl mixtures obtained from behavioral responses in this study corresponds to that for sucrose alone obtained from the CT nerve responses (Ninomiya and Imoto, 1995). As shown in Figure 1, the mean number of licks for a mixture of 0.01 M sucrose + 3 mM QHCl was significantly higher than that for 3 mM QHCl alone, suggesting that the behavioral threshold for sucrose is 0.01 M or less. This threshold is about the same as that (0.01 M) for sucrose responses of the CT nerve (Ninomiya and Imoto, 1995), or that from a long-term (48 h) two-bottle preference test in the same C57BL/KsJ

mice without water deprivation. Thus, a short-term lick test by using mixtures with QHCl developed in this study may provide a new tool for measurements of taste-guided behavioral responses to sucrose in mice.

It has been shown in both humans and rodents that responses to sucrose–QHCl mixture are lower than the sum of responses to each component of mixture presented alone. This so-called mixture suppression between sucrose and QHCl in humans is proposed to involve both central and peripheral mechanisms (Lawless, 1979; Kroeze and Bartoshuk, 1985). Electrophysiological recordings in hamster single parabrachial neurons have revealed mutual suppression of responses between sucrose and QHCl (Vogt and Smith, 1993). In contrast, in hamster CT nerves, suppressive effects of QHCl on sucrose responses, but not the reverse, occurred in sucrose-best fibers (Formaker and Frank, 1996; Formaker *et al.*, 1997), suggesting that mixture suppression may occur at the intracellular level of sucrose responsive taste cells. In the present study using mice, however, peripheral mixture suppression may be negligible because the short-term lick responses to sucrose + QHCl mixtures are comparable with the CT responses to sucrose alone and 48-h preference for sucrose as mentioned above.

The present study revealed that Gur selectively suppressed lick responses to sweeteners, but not to QHCl, HCl, NaCl or monosodium L-glutamate. This confirms that Gur is an selective inhibitor for responses to sweet substances in rodents that have been shown in previous electrophysiological studies in rats (Imoto *et al.*, 1991; Miyasaka and Imoto, 1995) and mice (Ninomiya and Imoto, 1995; Ninomiya *et al.*, 1997, 1998, 1999). However, inconsistent with the CT nerve responses (Ninomiya *et al.*, 1997), Gur did not suppress short-term licks for mixtures of lower or higher concentration (0.03 or 1.0 M) of sucrose and 3 mM QHCl. One reason for such a difference between the CT nerve and behavioral responses may be attributable to information from the other peripheral taste nerves. It is reported that Gur suppresses responses to sweeteners to ~50% of control in the CT nerve and the greater superficial petrosal nerves (Ninomiya and Imoto, 1995; Harada and Kasahara, 2000) but produces no effect on the glossopharyngeal nerve responses (Ninomiya *et al.*, 1997). The threshold for sucrose in the Gur-insensitive glossopharyngeal nerve is about one log unit higher (0.1–0.3 M) than that of the chorda tympani (0.01–0.03 M). Sucrose responses of the glossopharyngeal nerve sharply increase from 0.3 to 1.0 M (Ninomiya *et al.*, 1997), and thereby produce a much larger relative Gur-insensitive component at 1.0 M in a total of sucrose responses from the peripheral taste nerves. The Gur-insensitive component at 1.0 M may be great enough to produce responses similar to that obtained before Gur. Also, dilution of orally infused Gur (30 μg/ml) with saliva may be involved in the difference especially in responses to the lower concentration (0.03 M). The diluted Gur may make the effect more obscure in such low lick rates (< 15 licks per 10 s) to 0.03 M sucrose + 3 mM QHCl already before Gur.

The present behavioral study revealed that anti-sweet effects of Gur lasted for 2–3 h, but that most of them were rapidly and largely weakened by subsequent treatment with β-CD. The long-lasting effects of Gur and its quick recovery by β-CD observed in the present behavioral test correspond quite well with data obtained from peripheral CT nerve responses (Ninomiya *et al.*, 1998).

In summary, the present study employed a newly developed short-term lick test to examine behavioral responses to a variety of sweeteners in mice. Data obtained from this test correspond quite well with peripheral CT responses to various sweeteners, and their inhibition by Gur and anti-Gur effect of β-CD. This consistency between behavioral and neural data further supports the idea that Gur can act as a specific inhibitor for responses to sweet substances in C57BL mice. In the other aspect, the present data also suggest that the newly developed short-term test can provide a tool for measurements of taste-guided behavioral responses to sweeteners without major disturbance by post-ingestive feedback.

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