Reduction of the Suppressive Effects of Gurmarin on Sweet Taste Responses by Addition of β-Cyclodextrin

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Abstract

Cyclodextrins (CDs) have the remarkable ability to form inclusion complexes with a wide variety of guest molecules. In the present study, possible influences of CDs on gurmarin inhibition of the chorda tympani responses to sucrose were examined in C57BL mice. Responses to sucrose were suppressed to ~50% of control by treatment of the tongue with 30 μg/ml (~7.1 μM) gurmarin. Rinsing the tongue with 15 mM β-CD after gurmarin gave rapid recovery of the suppressed sucrose responses to ~85% of control, whereas 15 mM α- or γ-CD did not. When gurmarin was mixed with β-CD, the suppressive effects of gurmarin on sucrose responses were largely reduced. No such reduction was observed for mixtures with α- and γ-CD. Gurmarin includes tyrosine and tryptophan residues whose aromatic rings are directed outward and can probably form inclusion complexes with β-CD. Therefore, the observed reduction of the effects of gurmarin may be due to steric hindrances in inclusion complexes of gurmarin with β-CD that may interfere with gurmarin binding to sweet taste receptors.

Introduction

Gurmarin, a 35-amino-acid residue polypeptide (molecular weight: 4209) (Imoto et al., 1991; Arai et al., 1995), is known to be an inhibitor of responses to various sweet taste stimuli in rats (Imoto et al., 1991; Miyasaka and Imoto, 1995) and certain strains of mice (Ninomiya and Imoto, 1995; Ninomiya et al., 1997). The suppressive effect of gurmarin on chorda tympani responses to sucrose is long lasting—for more than several hours in rats (Imoto et al., 1991; Miyasaka and Imoto, 1995) and ~2 h in mice (Ninomiya and Imoto, 1995). This long-lasting effect gives rise to the question of whether or not the site of action of gurmarin is on the apical membrane of the taste cell. Evidence obtained from an electrophysiological study on the rat showing that rinsing the tongue with anti-gurmarin serum after gurmarin facilitates the recovery of the suppressed sucrose responses suggests that gurmarin may act on the receptor sites of taste cells (Miyasaka and Imoto, 1995). However, to establish this, stronger evidence is necessary.

Cyclodextrins (CDs), a family of cyclic oligosaccharides with α-1,4 bonds, can form inclusion complexes with a wide variety of guest molecules, ranging from organic or inorganic compounds of neutral or ionic nature to noble gases (Li and Purdy, 1992). Because of the relatively high hydrophobicity and size of the cavity formed inside the cyclic structure of CDs, chemical compounds with aromatic rings are suggested to be the best fitting guest molecules (Inoue and Miyata, 1981; Inoue et al., 1981). A previous chemical study using NMR (Arai et al., 1995) demonstrated that gurmarin includes tryptophan and tyrosine residues whose aromatic rings are directed outward in its three-dimensional chemical structure. This unique location of the aromatic rings of the amino acids may allow it to form inclusion complexes with CDs, and the complexes may modify the effect of gurmarin.

In the present study, therefore, to investigate the possible effects of CDs on the action of gurmarin, we examined if the extent of inhibition of responses of the mouse chorda tympani nerve to sucrose by gurmarin would be altered by rinsing the tongue with CDs and by mixing CDs with gurmarin in the gurmarin-sensitive C57BL/KsJ mouse strain (Ninomiya et al., 1997).

Materials and methods

Recording of taste responses of the mouse CT nerve

Subjects were adult mice of the C57BL/KsJ strain (8–25 weeks of age: male, $n = 13$; female, $n = 15$), ranging in weight from 20 to 31 g. Mice were anesthetized by an i.p. injection of sodium pentobarbital (40–50 mg/kg body wt) and maintained at a surgical level of anesthesia with

supplemental injections. The trachea was cannulated and the mouse was then fixed in the supine position with a head holder to allow dissection of the chorda tympani nerve. The hypoglossal nerve was transected bilaterally to prevent inadvertent tongue movements. The right chorda tympani nerve was exposed at its exit from the lingual nerve by removal of the internal pterygoid muscle. The chorda tympani nerve was then dissected free from surrounding tissues and cut near its entrance to the bulla. For whole nerve recording, the entire nerve was placed on a silver wire electrode. An indifferent electrode was positioned nearby in the wound. Neural responses resulting from chemical stimulation of the tongue were fed into an amplifier (Iyodenshikogaku K-1) and displayed on an oscilloscope screen (Nihon Kohden VC-10). Whole nerve responses were integrated and displayed on a recorder (Nihon Kohden WS-641G). The time constant of the integrator was 1.0 s.

Chemical stimulation

The anterior half of the mouse's tongue was enclosed in a flow chamber. Solutions were delivered into the flow chamber by gravity flow and flowed over the tongue for a controlled period. Chemical stimuli used were 3.0 mM–1.0 M sucrose and 0.1 M NH_4Cl, which were dissolved in distilled water. During chemical stimulation of the tongue, test solution flowed for ~30 s at the same flow rate as the distilled water used for rinsing the tongue (0.5 ml/s). The tongue was rinsed during the interval of ~1 min between successive stimulations. The stability of each preparation was monitored by the periodic application of 0.1 M NH_4Cl. A recording was considered to be stable when the 0.1 M NH_4Cl response magnitudes at the beginning and end of each stimulation series deviated by no more than 15%. Only responses from stable recordings were used in the data analysis.

To examine gurmarin inhibition of responses, the tongue was treated with 1.0–100 μg/ml (~0.24–23.8 μM) gurmarin dissolved in 5 mM phosphate buffer (pH 6.8) for 10 min in the same manner as that described in our previous reports (Imoto et al., 1991; Ninomiya and Imoto, 1995; Ninomiya et al., 1997). To examine the possible effects of CDs on gurmarin inhibition of sucrose responses, we repeatedly recorded responses to sucrose and NH_4Cl for ~15 min after treatment with gurmarin, and then rinsed the mouse tongue for 10 min with the buffer, 15 mM α-, β- or γ-CD (we chose 15 mM because that is near the solubility limit of β-CD at room temperature). After rinsing, we again recorded responses to sucrose and NH_4Cl. We also examined the extent of inhibition of sucrose responses after treatment with mixtures of 15 mM CDs with various concentrations of gurmarin for 10 min. Adaptation of the tongue to the buffer without gurmarin by itself has no effect on the neural responses to sucrose.
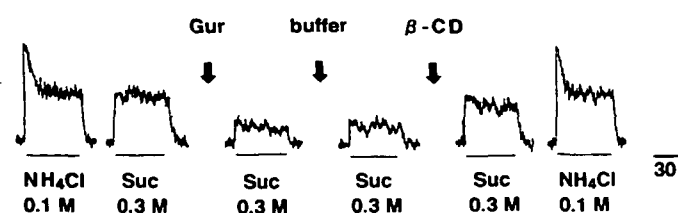

Figure 1   Sample recordings of integrated responses of the mouse chorda tympani nerve to 0.1 M NH_4Cl and 0.3 M sucrose before and after treatment of the tongue with 30 μg/ml gurmarin (Gur), 5 mM phosphate buffer (buffer) and 15 mM β-CD.

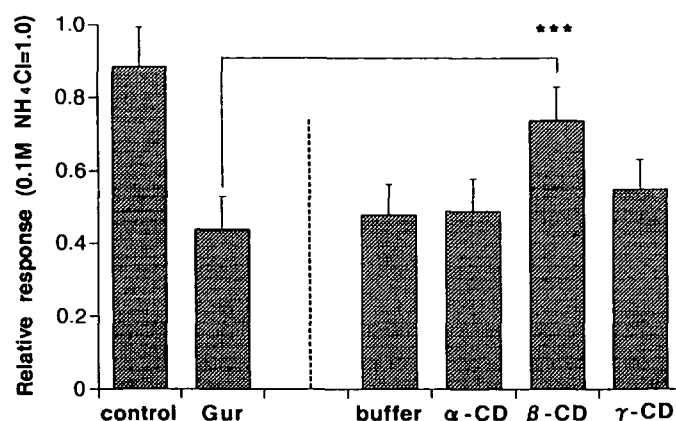**Figure 2**   Relative responses to 0.3 M sucrose before (control) and after lingual treatment with 30 μg/ml gurmarin (Gur), 5 mM phosphate buffer (buffer), and 15 mM α-, β- and γ-CD. Relative responses represented are mean ± SD. Data were obtained from 5–21 mice. The significant difference from sucrose responses after gurmarin was tested for those after α-, β- and γ-CD: t-test, ***P < 0.001.

Data analysis

In the analysis of whole nerve responses, the magnitude of the integrated response at 20 s after stimulus onset was measured. Relative response magnitudes for each stimulus were calculated by defining the response magnitude to 0.1 M NH_4Cl as unity (1.0), and these were used for statistical analysis.

Results

Effects of rinsing the tongue with CDs on the recovery of sucrose responses after gurmarin

Figure 1 shows integrated responses of the chorda tympani nerve of a mouse to 0.3 M sucrose and 0.1 M NH_4Cl before and after treatment of the tongue with 30 μg/ml (~7.1 μM) gurmarin, 5 mM phosphate buffer and 15 mM β-CD. The magnitude of the response to sucrose was suppressed by gurmarin, was unchanged after rinsing the tongue with the buffer but had recovered considerably after rinsing with β-CD. Reduction of the sucrose response by gurmarin and its recovery by rinsing with β-CD were repeatable. Figure 2
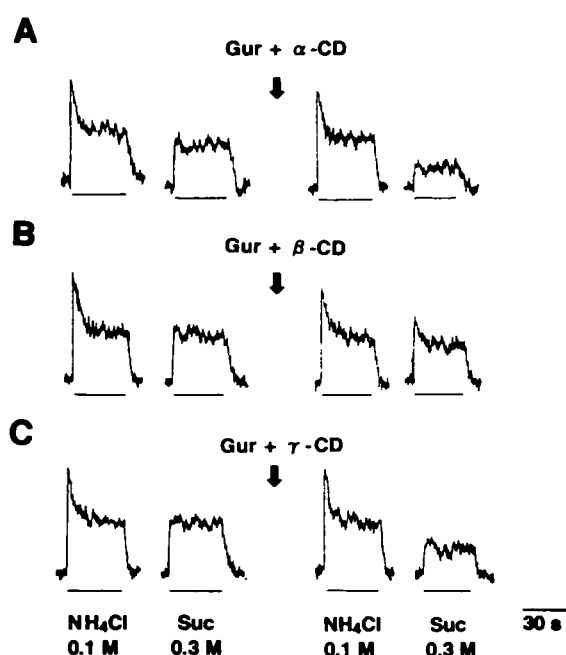
A

Gur + α-CD

B

Gur + β-CD

C

Gur + γ-CD

| NH₄Cl | Suc | NH₄Cl | Suc | 30 s |
| 0.1 M | 0.3 M | 0.1 M | 0.3 M | |

Figure 3 Sample recordings of integrated responses of the mouse chorda tympani nerve to 0.1 M NH₄Cl and 0.3 M sucrose before and after lingual treatment with mixtures of 30 μg/ml gurmarin with 15 mM α- (**A**: Gur + α-CD), β- (**B**: Gur + β-CD) or γ-CD (**C**: Gur + γ-CD).

shows the mean relative magnitudes of responses to 0.3 M sucrose before and after 30 μg/ml gurmarin, and after rinsing the tongue with 5 mM phosphate buffer, or 15 mM α-, β- or γ-CD. The responses to sucrose which was suppressed by gurmarin (49.7% of control) significantly increased after rinsing with β-CD to 83.6% of control (t-test, $P < 0.001$), whereas no significant change in response to sucrose was found after rinsing with the buffer, α- or γ-CD (t-test, $P > 0.05$). A minor tendency of recovery of the sucrose response after γ-CD (62.1% of control) was observed.

Effects of gurmarin mixed with CDs on sucrose responses

Figure 3 shows sample records of the mouse chorda tympani responses to 0.3 M sucrose and 0.1 M NH₄Cl before and after the lingual treatment with mixtures of 30 μg/ml (~7.1 μM) gurmarin with 15 mM concentrations of each of the CDs. The mixture of gurmarin with α-CD suppressed sucrose responses to about one-half of the control level, whereas a much smaller reduction of sucrose responses was observed after the mixture of gurmarin with β-CD. The suppressive effect of the mixture of gurmarin with γ-CD on sucrose responses was intermediate.

Concentration–response relationships for sucrose before and after 30 μg/ml gurmarin and its mixtures with 15 mM concentrations of each of the CDs are shown in Figure 4. The results of the analysis of variance indicated that the magnitudes of responses to sucrose at six different concentrations were different among treatments [$F(4,37) =$
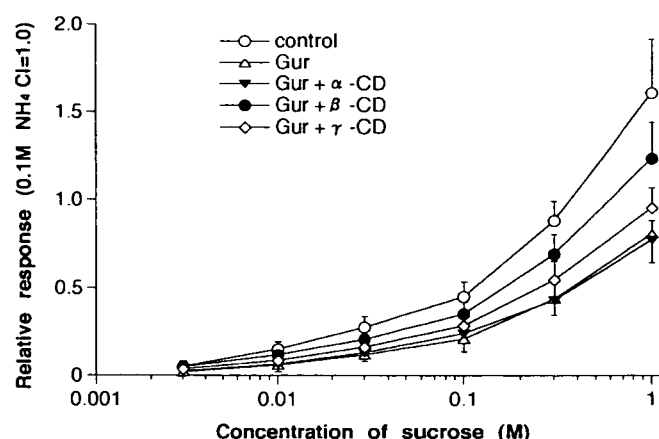
Figure 4 Concentration–response relationships of the mouse chorda tympani nerve for sucrose before (open circles) and after 30 μg/ml gurmarin alone (open triangles), and mixtures of gurmarin and 15 mM α- (filled triangles), β- (filled circles) and γ-CD (open squares). Relative responses represented are mean ± SD. Data were obtained from 4–21 mice.
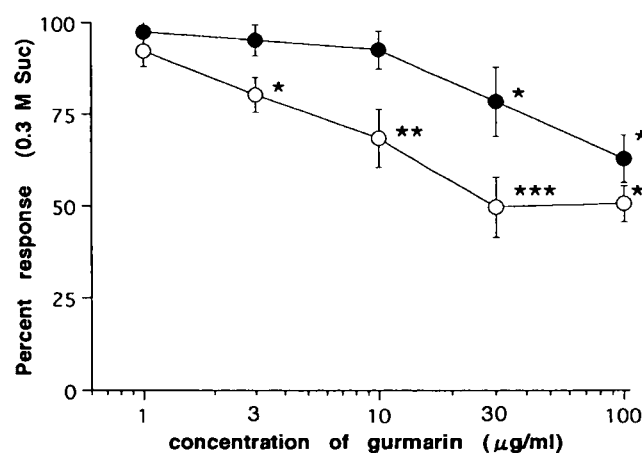
Figure 5 Normalized response (percent response; control = 100%, before treatment) of the mouse chorda tympani nerve to 0.3 M sucrose after treatment with gurmarin at different concentrations (open circles) and its mixtures with 15 mM β-CD (filled circles). Percent responses represented are mean ± SD. Data were obtained from 5–21 mice. The significant difference from control was tested with data of relative magnitudes of responses when the magnitude of responses to 0.1 M NH₄Cl was taken as unity (1.0): t-test, $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

30.531, $P < 0.001$], and that those after gurmarin alone and its mixtures with each of three CDs were significantly smaller than those of control (Fisher's post-hoc test, $P < 0.01–0.001$). The magnitudes of sucrose responses after the mixture of gurmarin with β-CD were significantly larger than those after gurmarin alone and its mixtures with α- or γ-CD (Fisher's post-hoc test, $P < 0.05–0.01$). The mixture of gurmarin with γ-CD suppressed sucrose responses to levels intermediate between those with α- and β-CD. These results suggest that the order of CD in strength for reducing gurmarin action is β > γ > α.

The concentration dependency of gurmarin and its mixture with 15 mM β-CD on responses to 0.3 M sucrose is shown in Figure 5. Significant suppression of sucrose responses by gurmarin alone was observed at 3 μg/ml (~0.7 μM) or more (t-test, $P < 0.05$) and reached a plateau (responses of control = 49.7 ± 8.2%) at 30 μg/ml (~7.1 μM). The suppressive effect of gurmarin at 3 and 10 μg/ml (~0.7–2.4 μM), however, was eliminated by mixing with 15 mM β-CD (t-test, $P > 0.05$), although 30 and 100 μg/ml gurmarin still significantly suppressed sucrose responses to 78.4 and 62.8% of control even when mixed with 15 mM β-CD. The magnitudes of sucrose responses suppressed by the mixtures were significantly larger than those suppressed by gurmarin alone at each concentration (t-test, $P < 0.05–0.001$).

Discussion

Reduction of gurmarin inhibition of sucrose responses by β-CD

Our previous studies in C57BL/6 and C57BL/KsJ mice (Ninomiya and Imoto, 1995; Ninomiya et al., 1997) demonstrated that gurmarin suppressed the chorda tympani responses to various sweeteners to ~50% of control without affecting responses to other basic taste stimuli, such as NaCl, HCl and quinine HCl. Such specific inhibition by gurmarin of sweet taste responses was not evident in the chorda tympani responses of BALB strain mice (Ninomiya and Imoto, 1995) or in the glossopharyngeal responses in C57BL mice (Ninomiya et al., 1997). In this study, we examined gurmarin inhibition of sweet taste responses in the gurmarin-sensitive C57BL/KsJ strain by recording their gurmarin-sensitive chorda tympani responses. We confirmed that the maximum magnitude of inhibition of sucrose responses by 30 and 100 μg/ml gurmarin was ~50% of control. In this study, we first found that rinsing the tongue with 15 mM β-CD for 10 min after the lingual application of gurmarin produced a rapid recovery of the suppression of sucrose responses to a level of ~85% of control, which was not significantly different from the control (t-test, $P > 0.05$). A previous study using rats (Miyasaka and Imoto, 1995) found a similar rapid recovery from the suppression of sucrose responses after gurmarin by rinsing the tongue with anti-gurmarin serum. Therefore, it is probable that β-CD and anti-gurmarin serum could stereospecifically bind gurmarin and remove it from the taste cell membrane. In the present study, mixing β-CD with gurmarin also reduced the suppressive effect of gurmarin on sweet taste responses. No significant suppression of sucrose responses was observed after mixtures of 3 and 10 μg/ml gurmarin with 15 mM β-CD (Figure 5). This effect of β-CD on the action of gurmarin further indicates the existence of a strong interaction between the two chemical compounds.

Possible mechanisms of reduction of gurmarin inhibition by β-CD

The effects of CDs on gurmarin inhibition of sucrose responses were considerably different among the three CDs tested, being absent for α-CD and very weak for γ-CD. It is known that α-, β- and γ-CD contain six, seven and eight μ-1,4-linked D-glucopyranose units respectively, and have different sizes of hydrophobic cavity leading to specific binding of guest molecules (diameters of the cavity are 0.57 nm for α-CD, 0.78 nm for β-CD and 0.95 nm for γ-CD) (Li and Purdy, 1992). The cavity size in some cases determines the strength of coupling with guest molecules. For example, β-CD is shown to be the best fitting CD for amino acids with phenyl rings, such as phenylalanine and tyrosine, where the phenyl ring of the guest is deep and tightly included in the cavity of β-CD (Inoue and Miyata, 1981; Inoue et al., 1981). The inclusion of the phenyl ring into the cavity of α-CD is shallow and loose, whereas that of γ-CD is deep and loose (Inoue and Miyata, 1981; Inoue et al., 1981). Correspondingly, our recent chemical study (Imoto and Ninomiya, 1997) showed that, unlike that of α- and γ-CD, addition of β-CD to gurmarin produced significant changes in the UV absorption spectra which were characteristic of those found in the inclusion complex formation of tyrosine. This strongly suggests that the inhibitory effect of β-CD on gurmarin suppression of sucrose responses found in this study is due to formation of inclusion complexes between β-CD and tyrosine residues of gurmarin.

Our chemical study (Imoto and Ninomiya, 1997) further showed that the binding constant (K_d) between β-CD and gurmarin was 3.9 mM. At 15 mM β-CD, ~80% of gurmarin would form inclusion complexes with β-CD. This implies that ~20% of gurmarin at each concentration is free. Therefore, in this study when 30 and 100 μg/ml gurmarin were mixed with 15 mM β-CD, 6 and 20 μg/ml gurmarin would have been available. As shown in Figure 5, the sucrose responses suppressed by mixtures of 15 mM β-CD with 30 and 100 μg/ml gurmarin are comparable to those of the presumptive concentrations of free gurmarin. Therefore, our neural data fit very well with the chemical data. From these results, we hypothesize that inactivation of gurmarin by β-CD is due to steric hindrances formed by inclusion complexes between its tyrosine residues and β-CD, which interfere with binding to the sucrose receptor sites on the taste cell membrane.

A previous chemical study using NMR (Arai et al., 1995) demonstrated that there exists a unique domain in the three-dimensional structure of gurmarin in which five aromatic amino acid residues—one histidine, two tryptophans and two tyrosines—are all directed outward and form a hydrophobic cluster. If inactivation of gurmarin by β-CD could occur through formation of inclusion complexes at the tyrosine residues, that domain is a possible candidate for the site which binds to the gurmarin-sensitive sweet taste

receptor. Weaker effects of γ-CD, which could form inclusion complexes with tryptophan residues, on the action of gurmarin may also imply participation of the domain as a possible site for interaction with the taste receptor. However, since little is known about receptor mechanisms for sweet taste, further extensive studies including those using gurmarin and β-CD as experimental tools are needed to examine the possibility.

Acknowledgements

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