Inhibitory effect of gurmarin on palatal taste responses to amino acids in the rat

SHUITSU HARADA AND YASUO KASAHARA
Department of Oral Physiology, Kagoshima University Dental School, 8-35-1 Sakuraagaoka, Kagoshima 890-8544, Japan

Harada, Shuitsu, and Yasuo Kasahara. Inhibitory effect of gurmarin on palatal taste responses to amino acids in the rat. Am J Physiol Regulatory Integrative Comp Physiol 278: R1513–R1517, 2000.—Gurmarin (10 μg/ml), a protein extracted from Gymnema sylvestre, depressed significantly (40–50%) the phasic taste responses to sugars (sucrose, fructose, lactose, and maltose) and saccharin sodium recorded from the greater superficial petrosal nerve (GSP) innervating palatal taste buds in the rat. However, no significant effect of gurmarin was observed for taste responses to NaCl, HCl, and quinine hydrochloride. Phasic responses to ω-amino acids that taste sweet to humans (His, Asn, Phe, Glu) were also depressed, but gurmarin treatment was without significant effect on taste responses to ω-Trp and ω-Ala, six L-amino acids (His, Asn, Phe, Glu, Trp, and Ala), and two basic amino acid HCl salts (Arg and Lys). With the exception of ω-Trp, these inhibitory effects of gurmarin on GSP taste responses were related to the rat’s preference for these substances.

Materials and Methods

Surgical procedures. The surgical procedure to dissect the GSP was similar to that described previously (6, 8). The trachea was cannulated with polyethylene tubing, and the head of the animal was fixed with a nontraumatic head holder made of Plexiglas that allowed exposure of the soft palate for stimulation. The area of the nasoincisor duct innervated by the GSP was covered by the head holder and sealed with petroleum jelly. An incision was made ventrally along the angle of the right mandible. The ventral wall of the right tympanic bulla was removed, and the tensor muscle was cut at the tendon attached to the malleus and removed carefully. The cochlea was left intact, and a portion of the temporal bone overlying the GSP was removed. The GSP was dissected free from the surrounding tissue and transected as it exited from the geniculate ganglion.

Electrophysiological recordings. The exposed GSP was placed on a 100 μm Ag-AgCl hook electrode, and an indifferent electrode was placed on the inner wall of the bulla. These electrodes were soaked in petroleum jelly mixed with an equal amount of liquid paraffin. The animal was grounded by an alligator clip attached to the surgical margin. Neural activity from the whole nerve was led to a high-impedance probe (B-101J, Nihon Kohden) and an AC amplifier (ABV-11, Nihon Kohden), monitored on an oscilloscope and an audio monitor, and recorded on a PCM data recorder (model RTA-1100M, Nihon Kohden) at a speed of 1 mm/s.

Taste stimulation. An outlet of polyethylene tubing (2.5 mm ID) was placed adjacent to the soft palate for application of taste stimuli and rinsing water at a flow rate of 2 ml/s. Distilled water (DW) constantly flowed over the palate. For stimulation, a 3-way electromagnetic valve controlled by a microcomputer (model PC9801RX, NEC) switched the flow from DW to a taste stimulus for 10 s. Stimulus solutions were made with reagent grade chemicals (Nacalai Tesque) in DW. The stimuli were 0.1 M NaCl, 0.1 M NH4Cl, 0.01 M saccharin sodium, 0.01 M HCl, 0.01 M quinine hydrochloride (QHCl), 0.5 M sugars [sucrose (Suc), lactose (Lac), maltose (Mal), fructose (Fru), glucose (Glc) and galactose (Gal)], 0.1 M L-basic amino acid hydrochloride salts (L-ArgHCl and L-LysHCl), and ω- and ω-neutral amino acids (His, Asn, Phe, Glu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Gln, and Ala) at 0.1 M and Trp at 0.05 M. Sugar and amino acid solutions were prepared weekly and stored at 5°C. All stimuli and rinsing water were presented to the tongue or palate at a room temperature. To examine effects on the taste responses, 10 µg/ml gurmarin was used according to Imoto et al. (9). Gurmarin was dissolved in Ringer solution at pH 7.4 and infused into the oral cavity for 10 min. Following the treatment, DW was used to rinse the palate for 10 min prior to testing the taste solution again.

Data analysis. All integrated taste responses recorded from a given preparation were calculated relative to the magnitude of the phasic response to the standard, 0.1 M NaCl. The standard solution was applied between every three to four test stimuli. The height of the peak of the initial phasic response and the height of the tonic portion of the integrated response at 10 s past stimulus onset were used as measures of the response magnitude to each stimulus. The proportion of response remaining was calculated as the response magnitude after gurmarin treatment divided by that before treatment. Differences in the effect of gurmarin treatment on taste responses to the different stimuli were analyzed by ANOVA with stimuli and pretreatment vs. posttreatment responses as the variables; a multiple comparison post test (Bonferroni/Dunn) was used for testing the statistical significance (P < 0.05) for the difference between each possible stimulus pair.

RESULTS

Treatment of the soft palate with 10 µg/ml gurmarin resulted in a significant reduction in the phasic response of the GSP to 0.5 M Suc (to 47% of control) and to 0.01 M saccharin sodium (to 51%), whereas phasic responses to 0.01 M HCl, 0.01 M QHCl, and 0.1 M NaCl were not significantly affected (Fig. 1 and 2). Tonic responses to 0.5 M Suc and 0.01 M saccharin sodium were not significantly affected by gurmarin treatment. In addition, the phasic and tonic responses to 0.1 M basic L-amino acid monohydrochlorides (ArgHCl and LysHCl) were not affected significantly by gurmarin treatment. The proportion of the phasic and tonic GSP responses remaining to both sucrose and saccharin sodium subsequent to gurmarin treatment was significantly less than that for both phasic and tonic responses to the other stimuli.

Phasic taste responses to 0.5 M Suc, Fru, Lac, and Mal were significantly inhibited by gurmarin treatment (Fig. 3), whereas the phasic taste responses to Gal and Glc were not significantly inhibited. Tonic responses to all sugars tested were not significantly reduced by gurmarin treatment (Fig. 3).

Gurmarin treatment greatly reduced the phasic taste responses to 0.1 M D-amino acids (His, Asn, Phe, and Gln). In contrast, the phasic taste responses to D-His and to D-Phe were reduced to only 52% and 68%, respectively. Tonic responses to D-His (to 35%) and D-Asn (32%) were also significantly inhibited by gurmarin treatment. However, there was little inhibition for both the phasic (reduced to 79% of the original) and tonic (reduced to 71%) response to 0.05 M D-Trp (Fig. 4 and 5). Inhibition of both the phasic and tonic GSP taste responses to 0.05 M Trp was significantly less than that for the other D-amino acids.

The phasic and tonic responses to the six L-amino acids tested were not significantly reduced by gurmarin treatment (Fig. 6).

DISCUSSION

Similar to its suppressing effect on rat CT taste responses (9), gurmarin markedly suppressed rat GSP taste responses to 0.5 M sucrose, fructose, lactose, and maltose despite having minimal effect on taste responses to NaCl, HCl, and QHCl. Also, gurmarin treatment inhibited GSP taste responses to 0.01 M saccharin sodium and 0.5 M sucrose by 40%, whereas gurmarin inhibition of CT taste responses in the rat to 0.003 M saccharin sodium was rather weak compared with that to 0.5 M sucrose (9). A possible reason for this difference in suppression for saccharin sodium between the two nerves may be the robust responsiveness of the CT to Na salts and the GSP to sweet substances (8, 16).
Fig. 2. Relative magnitudes (response to 0.1 M NaCl = 100) of phasic (A) and tonic (B) responses before (pre, open bars) and after treatment (post, solid bars) with gurmarin for 0.5 M sucrose, 0.01 M saccharin sodium, 0.01 M HCl, 0.01 M QHCl, 0.1 M L-Arg and L-Lys hydrochloride, and 0.1 M NaCl. Proportion of response remaining (%) for phasic (open bars) and tonic (solid bars) responses to each stimulus is shown in C. Bold square brackets indicate statistically significant difference (ANOVA, \( P \approx 0.0001 \); phasic, \( F = 13.29 \); tonic, \( F = 4.48 \); df = 13). Data are from 5 animals. Error bars indicate standard deviations.

Fig. 3. Relative magnitudes (response to 0.1 M NaCl = 100) of phasic (A) and tonic (B) responses before (pre, open bars) and after treatment (post, solid bars) with gurmarin for six sugars at 0.5 M: sucrose (Suc), fructose (Fru), lactose (Lac), maltose (Mal), galactose (Gal), and glucose (Glc). Proportion of response remaining (%) for phasic (open bars) and tonic (solid bars) responses to each stimulus is shown in C. Bold square brackets indicate statistically significant difference (ANOVA, \( P < 0.0001 \); phasic, \( F = 29.52 \); tonic, \( F = 5.42 \); df = 11). Data are from 5 animals. Error bars indicate standard deviations.

Fig. 4. Integrated GSP responses to five D-amino acids at 0.1 M, as well as 0.05 M D-Trp, 0.5 M sucrose, and 0.1 M NaCl before (A) and after (B) gurmarin treatment. Stimuli were applied for 10 s. Recordings are from the same animal.
On the other hand, the CT taste responses to 0.02 M saccharin sodium were markedly suppressed by 100 µg/ml gurmarin treatment, similar to that observed to 0.5 M sucrose in the C57BL/KsJ mice (19), because of the larger responsiveness to sweet substances of the mice CT (11).

Behavioral experiments in the hamster using the generalization of learned taste aversions revealed that saccharin sodium generalized to sucrose well in the rat and hamster (21). Also, preference tests indicated that ddy mice preferred 0.01 M saccharin sodium and 0.2 M sucrose similarly (13). In the present experiments, GSP taste responses to saccharin sodium and sucrose were similarly reduced by gurmarin treatment, although the transduction mechanisms for saccharin depends on inositol triphosphate pathway and is different from that for sucrose (1, 15, 26). Thus the encoded taste information for saccharin and sucrose appears to be quite similar in peripheral nerves of rodents.

Phasic taste responses of the rat GSP to 0.1 M L-Lys and L-Arg mono-hydrochloride salts were not significantly suppressed by gurmarin treatment. Neurophysiological experiments in C3H mice showed that the basic amino acid hydrochlorides cross-adapted the response to NaCl (7), and single fiber analysis in the rat CT indicated that the taste response to L-Arg·HCl was similar to that to NaCl (23). Also, preference magnitude for 0.1 M basic amino acids was much lower than those for L-Gly, L-Pro, L-Ala, and L-Thr (10, 22). Furthermore,
the results from behavioral experiments in mice employing a conditioned taste aversion paradigm indicated that the taste of a basic amino acid hydrochloride generalized to other basic amino acid hydrochlorides and to QHCl solutions (5). These results all suggest that basic amino acids may produce little sweet sensation in rat and mice, and that gurmarin has little effect on GSP taste responses to basic amino acids.

Gurmarin treatment significantly reduced the phasic and tonic taste responses to 0.1 M D-amino acids (His, Asn, Phe, and Glu); however, the inhibitory effect of gurmarin treatment on both the phasic (reduced to 79%) and tonic (reduced to 71%) responses to 0.05 M D-Trp was smaller than that for the other 0.1 M D-amino acids tested. Preference tests in mice showed that D-Trp is one of the most preferable amino acids (13), and in humans it produces a strong sweet sensation (25). Saccharin sodium (0.02 M) enhanced CT responses to D-Phe, D-Trp, and D-His among eight D-amino acids in C57BL mice, and responses to D-Trp and D-His were enhanced by saccharin sodium in BALB mice (20). Although these reports indicate that the taste of D-Trp may be similar to sugars or to other sweet compounds, the taste response to D-Trp was not specifically inhibited by gurmarin. Therefore, the transduction mechanism for D-Trp may contain different components responsible for the sweet (or preferable) component than that for other D-amino acids. In contrast to D-amino acids, the phasic and tonic GSP responses to six L-amino acids (0.1 M) were not significantly reduced by gurmarin treatment. With the exception of D-Trp, the selective inhibitory effects of gurmarin on taste responses to D-amino acids and carbohydrates that taste sweet to humans suggest that both rats and humans perceive these substances similarly.

We thank Dr. Toshiaki Imoto for providing gurmarin, and we thank Dr. John Caprio for valuable comments on the manuscript. This work was supported in part by Grant-in-Aid 10470358 for Scientific Research from the Ministry of Education of Japan.

Address for reprint requests and other correspondence: S. Harada, Dept. of Oral Physiology, Kagoshima Univ. Dental School, 8-35-1 Sakuragaoka, Kagoshima 890-8544, Japan (E-mail: harada@phy.hal.kagoshima-u.ac.jp).

Received 6 May 1999; accepted in final form 7 January 2000.

REFERENCES


