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Synergistic Responses of the Chorda Tympani to Mixtures of Umami and Sweet Substances in Rats

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Abstract

It has been known that umami substances such as monosodium L-glutamate (MSG) and 5′-inosine monophosphate (IMP) elicit a unique taste called 'umami' in humans. One of the characteristics of the umami taste is synergism: the synergistic enhancement of the magnitude of response produced by the addition of 5′-ribonucleotides to MSG. In addition to this well-documented synergism, we report here for the first time on another type of synergism between a glutamate receptor agonist, L-AP4, and sweet substances, by analyzing the chorda tympani responses in rats. The results are as follows: (i) when L-AP4 was mixed with one of the sweet substances, such as sucrose, glucose, fructose and maltose, large synergistic responses were observed. (ii) These synergistic responses, except to L-AP4 + sucrose, were not suppressed by sweet taste suppressants, gurmarin and pronase E. (iii) These synergistic responses were not suppressed by either metabotropic or ionotropic glutamate receptor antagonists. (iv) Fibers that responded well to the binary mixtures of L-AP4 and sweet substances also responded well to NaCl and HCl, but very weakly to sucrose. These findings are different from the characteristics of synergism between glutamate and IMP. The multiple transduction mechanisms for the umami taste in rat taste cells are discussed.

Key words: chorda tympani, rats, sweet, synergism, umami

Introduction

The umami taste has been recognized as a unique taste which does not belong to any of the four basic taste qualities, sweet, salty, sour or bitter. Many researchers have reported that a group of L-amino acids represented by monosodium L-glutamate (MSG) and the derivatives of 5′-ribonucleotides represented by inosine 5′-monophosphate (IMP) induce the umami taste (Kawamura and Kare, 1987).

Chaudhari *et al.* (Chaudhari *et al.*, 1996, 2000; Bigiani *et al.*, 1997; Chaudhari and Roper, 1998) found by using the reverse transcriptase (RT)-PCR method in rats that the metabotropic glutamate receptor, taste-mGluR4, a truncated form of brain-mGluR4, was expressed in only lingual tissues containing taste buds. They also showed that MSG and an agonist for mGluR4, L-amino-4-phosphonobutyrate (L-AP4), elicited a similar taste in a behavioral experiment in which the conditioned taste aversion technique was used, and suggested that the taste-mGluR4 was a chemosensory receptor responsible for the taste of glutamate. Despite findings, our recent neuropharmacological study (Sako and Yamamoto, 1999) showed that *S*-2-amino-2-methyl-4 phosphonobutylic acid (MAP4), an antagonist of mGluR4, did not suppress taste nerve responses to glutamate and L-AP4, but enhanced them in chorda tympani nerve recordings, and that gurmarin (Imoto *et al.*, 1991; Miyasaka and Imoto, 1995), an anti-sweet peptide, suppressed the synergistic response to the mixture of glutamate + IMP or the mixture of L-AP4 + IMP in rats. Recently, Nelson *et al.* (Nelson *et al.*, 2002) reported that T1R1 and T1R3, which are taste specific G-protein coupled receptors (CGCRs), combine to function as a broadly tuned L-amino acid receptor responding to the binary mixture of MSG + IMP.

In cytophysiological studies, the activation of sweet receptors is known to increase the cAMP level [for reviews see (Kinnamon and Margolskee, 1996; Lindeman, 2001)], but the mGluR4 binding of MSG or L-AP4 in the brain decreases the cAMP level (Flor *et al.*, 1995; Thomsen *et al.*, 1997). Yamamoto *et al.* (Yamamoto *et al.*, 1991) found, by using conditioned taste aversion techniques in their behavioral study, that the taste of umami substances was not unique, but was similar to the taste of sucrose for rats.

Synergism is known as one of the unique characteristics of the umami taste (Yamaguchi, 1991). Sato and his colleagues (Sato and Akaike, 1965) found that the synergistic enhancement of the magnitude of response occurred when

MSG was mixed with IMP in rats. However, this enhancement was observed in fibers that responded well to sucrose, but not to NaCl. Thus, the umami and sweet receptors are confused with each other. A more definitive explanation for the glutamate (umami) transduction mechanism is needed.

Recently, we have found the occurrence of synergistic responses to mixtures of L-AP4 and sweet substances (Sako *et al.,* 2001). This phenomenon seems to be interesting, since no synergism has been reported previously for the mixture of MSG and sweet substances. In the present study, therefore, we conducted an electrophysiological study to further investigate the receptor synergistic mechanisms for the umami taste.

Materials and methods

General procedure

In total, 57 male Wistar rats (250–300 g) were used. The animals were deeply anesthetized by an i.p. injection of sodium pentobarbital (60 mg/kg). Each animal was tracheotomized and secured with a head holder. The left chorda tympani nerve was exposed, freed from its surrounding tissues, and cut at the point of its entry to the bulla. The whole bundle or single fiber of the nerve was dissected and lifted on a platinum recording-wire electrode (0.1 mm diameter). An indifferent electrode was attached to nearby tissues. The nerve activities were amplified, displayed on an oscilloscope, and monitored with an audio amplifier. In the whole nerve experiment, the amplified signal was passed through an integrator with a time constant of 0.3 s, and was displayed on a slip chart recorder. In the single nerve experiment, the amplified signal was recorded using a DAT recorder (RD-135T; TEAC Co., Tokyo, Japan) for future analyses.

Data analysis

In the whole nerve experiment, the magnitude of the nerve response was measured as the height of the integrated response from the baseline at 10 s after the onset of stimulation. Responses to the taste stimuli were expressed as the relative magnitudes of responses when the magnitude of response to 0.1 M NH_4Cl was taken as the standard. In the single nerve experiment, the number of impulses occurring before and after each stimulation were counted by WorkBench and the Discovery system (DataWave Technologies Co., Longmont, CO). The mean background activity per 5 s during rinsing of the tongue with distilled water was calculated. A fiber was considered to be responsive to a stimulus if the nerve impulse rate during the first 5 s of taste stimulation was larger than the mean ± SD of the background rate. The response magnitude of each fiber to a particular stimulus was the net number of impulses produced for the first 5 s after the stimulus onset, which was obtained by subtracting the mean background impulse discharge from the total number of impulses.

The synergistic effect was expressed as a potentiation ratio (= magnitude of response to mixture/sum of magnitudes of responses to individual components in the mixture). The suppressive effect by each suppressant was expressed as a suppression ratio (= magnitude of response after treatment with suppressant/magnitude of response before treatment with suppressant).

Stimuli and chemical substances

The taste solutions were (in M): 0.1 NH_4Cl (Wako Pure Chemical Industries, Osaka, Japan), 0.1 NaCl (Na; Wako), 0.01 HCl (H; Wako), 0.02 quinine hydrochloride (Q; Wako), 0.005 L-AP4 (Sigma-Aldrich Fine Chemicals, St Louis, MO), 0.1 monopotassium L-glutamate (MPG; Ajinomoto Co., Tokyo, Japan), 0.01 IMP (Ajinomoto), 0.1 sucrose (Suc; Wako), 0.1 glucose (Glu; Wako), 0.1 maltose (Mal; Wako), 0.1 fructose (Fru; Wako), 0.001 sodium saccharin (Sac; Sigma) and their binary mixtures. A higher concentration (0.5 M) of sucrose was used when necessary. MPG was used instead of MSG because the latter elicits a huge sodium response in rats (Yamamoto *et al.*, 1991).

As the sweet taste suppressants, 2% pronase E (Hiji, 1975), a proteolytic enzyme, was dissolved in a 5 mM phosphate buffer of pH 6.8, and 50 μM gurmarin, an antisweet peptide, was dissolved in a 5 mM acetate buffer, pH 4.5. As antagonists for mGluR4, NMDA and the AMPA/kinate glutamate receptors, 40 mM MAP4 (Tocris Cookson Inc., Ballwin, MO) (Jane *et al.*, 1994), 40 mM 3-((*R*)-2-carboxypiperazin-4-yl)-propyl-1-phosphanic acid (CPP; Tocris) (Davies *et al.,* 1986) and 3 mM 2,3-dihydroxy-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulphonamide disodium (NBQX; Tocris) (Gill *et al.*, 1992) were used, respectively. Each solution and the rinsing water were applied to the anterior part of the tongue at room temperature (25 ± 2°C). The tongue was rinsed for at least 45 s between successive stimulations.

Experiment 1

A total of seven male Wistar rats were used. In this experiment, we examined the taste effectiveness of MPG and L-AP4, as well as the synergistic effects when each of these substances was mixed with one of the following sweet substances, Suc, Glu, Mal, Fru or Sac.

Experiment 2

A total of 14 male Wistar rats were used. In this experiment, the effects of 50 μM gurmarin or 2% pronase E on the chorda tympani responses to the mixtures of L-AP4 and one of the sweet substances were examined.

Experiment 3

A total of 24 male Wistar rats were used. The effects of 40 mM MAP4, 40 mM CPP or 3 mM NBQX on the chorda tympani responses to the mixtures of L-AP4 and one of the sweet substances were examined.

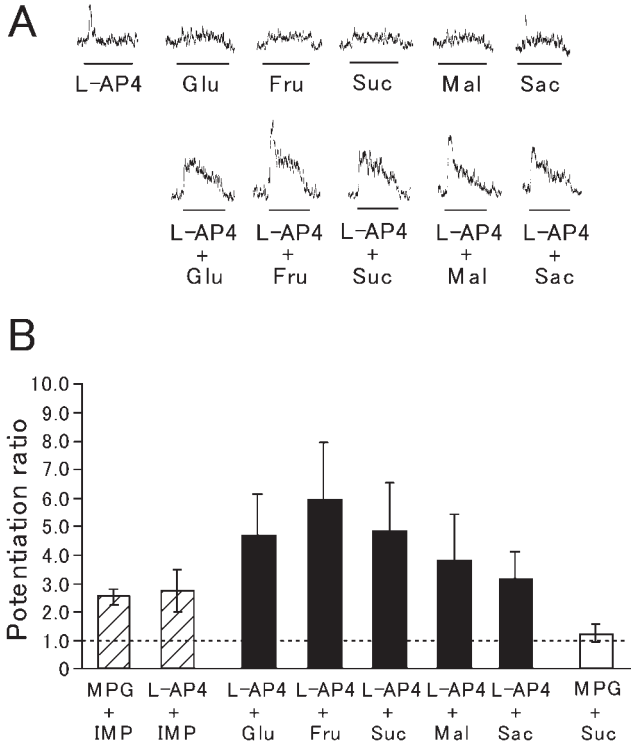

Figure 1 Sample integrated recordings **(A)** and potentiation ratios ± SE **(B)** for binary mixtures calculated from integrated response of the chorda tympani (n = 7).

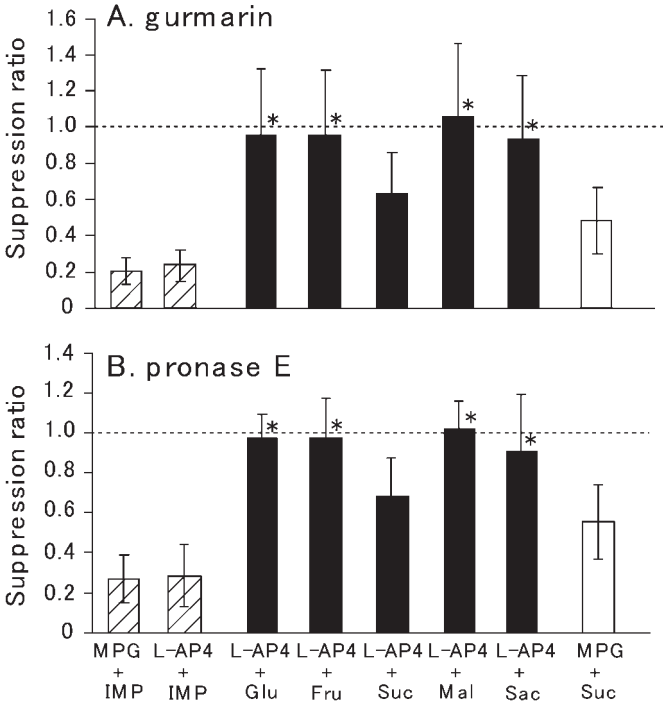

Figure 2 Suppression ratios ± SE (n = 7 for each) by the treatment with gurmarin **(A)** and pronase E **(B)**. Asterisks show significant differences between suppression ratio of the mixture of 0.1 M MPG + 0.01 M IMP and those of other binary mixtures (t-test; *$P < 0.05$).

Experiment 4

A total of 12 male Wistar rats were used. The single fiber responses to the conventional four basic taste stimuli and the mixtures of L-AP4 + Suc and MPG + IMP were recorded.

Results

Experiment 1

Figure 1 shows sample records of the integrated chorda tympani responses (A) and the representative integrated responses (B) to the mixture stimuli. The mixtures of L-AP4 and any of the sweet substances, including Glu, Fru, Suc, Mal and Sac, showed synergistic responses with potentiation ratios ranging from 3.1 to 5.9.

These values tended to be larger than those shown by the mixture of 5 mM L-AP4 and 0.01 M IMP or the mixture of 0.1 M MPG and 0.01 M IMP. However, the mixture of 0.1 M MPG and 0.1 M Suc showed essentially no synergism, the value being 1.2 ± 0.3 (mean ± SEM, n = 7).

Experiment 2

Figure 2 shows a graphical presentation of the suppression ratios by gurmarin (A) or pronase E (B). After treatment with one of the sweet suppressants, the responses to the mixture of 0.1 M MPG + 0.01 M IMP and 5 mM L-AP4 + 0.01 M IMP were suppressed to 20–29% of the pre-treatment level. However, the mixtures of L-AP4 and all of sweet substances, with the exception of Suc, were not suppressed by either grumarin or pronase E. There were significant differences in the suppression ratios between the mixture of 0.1 M MPG + 0.01 M IMP and those of 5 mM L-AP4 with one of the sweet substances except for Suc. No statistically significant difference was detected in the suppression ratios between the mixture of 0.1 M MPG + 0.01 M IMP and that of 0.1 M MPG + 0.1 M Suc.

Experiment 3

Figure 3 shows the integrated responses to the mixtures of L-AP4 and the sweet substances before and after treatment of the tongue with one of the glutamate antagonists for 10 min. No statistically significant differences were detected for the mixtures of L-AP4 and the sweet substances between before and after treatment with either glutamate antagonist (t-test; $P > 0.05$).

Experiment 4

Figure 4 shows a graphical presentation of the number of impulses per 5 s obtained from a single fiber in the chorda tympani nerve. This fiber responded best to H, followed by Na and Q. Suc elicited the least discharges among the conventional four basic taste stimuli. Although essentially

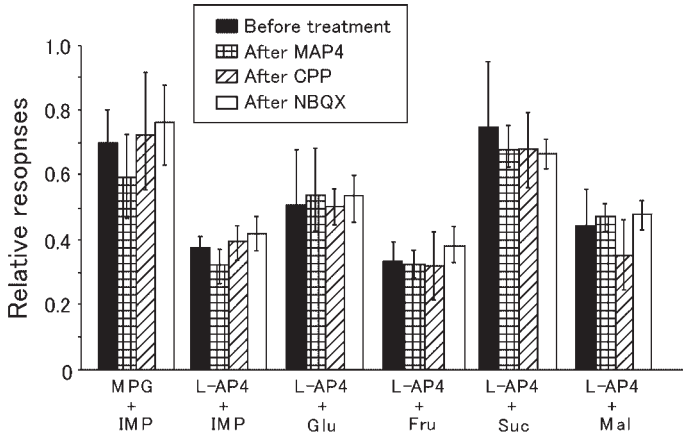

Figure 3 Relative magnitudes ± SE of responses of the chorda tympani before and after treatment with one of glutamate receptor antagonists (n = 7 for each).

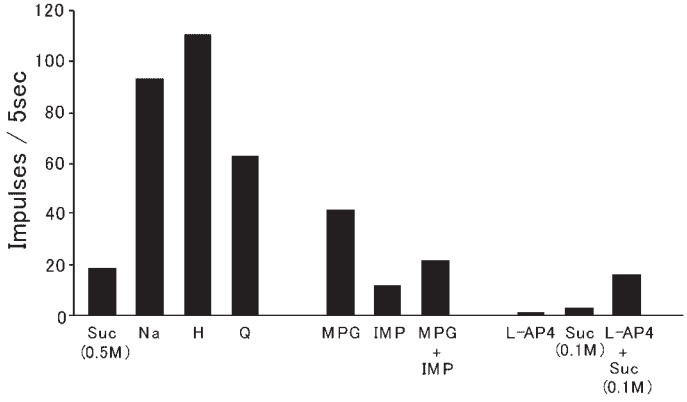

Figure 4 Graphical presentation of the number of impulses per 5 s obtained from a single fiber in the chorda tympani.

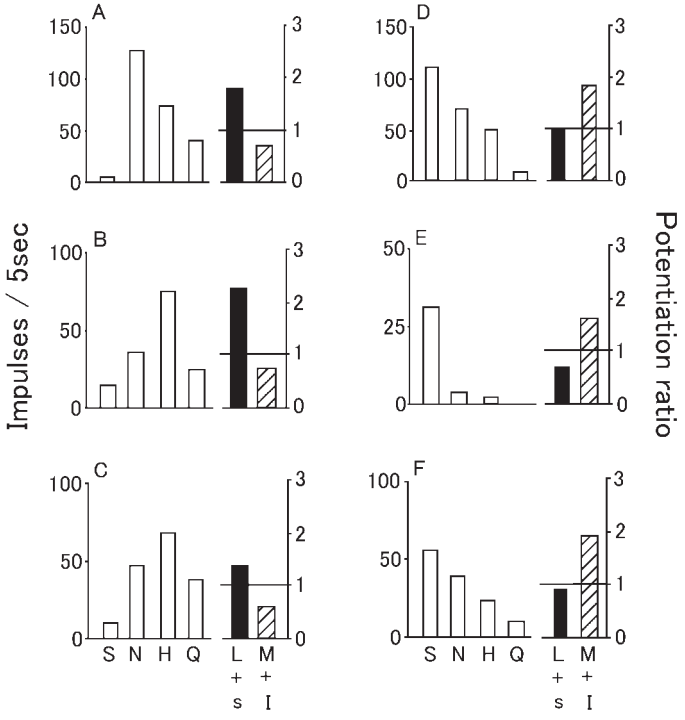

Figure 5 Taste responses obtained from six single fibers in the chorda tympani. **(A–C)** Fibers which showed synergistic responses to the mixture of 5 mM L-AP4 + 0.1 M sucrose (L + s) did not show synergism to the mixture of 0.1 M MPG + 0.01 M IMP (M + I). These fibers responded well to Na, H and Q. **(D–F)** On the other hand, fibers which showed synergistic responses to the mixture of 0.1 M MPG + 0.01 M IMP did not show synergism to the mixture of 5 mM L-AP4 + 0.1 M sucrose. These fibers respond to 0.5 M sucrose (S) , but not to Na, H and Q.

no responses were elicited by 5 mM L-AP4 or 0.1 M Suc, the mixtures of these substances elicited responses. Small numbers of impulses were elicited by 0.1 M MPG, 0.01 M IMP, and the mixture of these stimuli, but no potentiated response was detected for this mixture. Figure 5 shows the responses profiles of six representative fibers to the conventional four basic taste stimuli, the mixture of L-AP4 + Suc and the mixture of MPG + IMP analyzed with 30 chorda tympani fibers. Shown in Figure 5A–C are fibers that had good responses to Na, H and Q, but very weak responses to Suc. These fibers showed synergistic responses to the mixture of L-AP4 + Suc, but no such potentiated responses were detected for the mixtures of MSG + IMP. However, the fibers shown in Figure 5D–F responded well to Suc, then Na, H, and very weakly to Q. These fibers showed synergism to the mixture of MPG + IMP, but no such potentiated responses were detected for the mixture of L-AP4 + Suc.

Discussion

One of the characteristics of the umami taste is the occurrence of synergism. When the two groups of umami substances are mixed, the umami responses of the mixture become stronger than the sum of umami responses of the individual components in the mixture (Sato and Akaike, 1965; Kawamura and Kare, 1987; Yamamoto *et al.,* 1991).

Recent molecular investigations have led to the identification of several taste receptor candidates. Ugawa *et al.* (Ugawa *et al.*, 1998) demonstrated that MDEG1 (mammalian degenerin-1) was a candidate receptor for the sour taste. Since the first study by Hoon *et al.* (Hoon *et al.*, 1999), in which they cloned and characterized the new taste receptors T1R1 and T1R2 from rat taste cells, the possible identifications of taste receptor candidates with GPCRs have been reported (Kitagawa *et al.*, 2001; Max *et al.,* 2001; Montmayeur *et al.,* 2001; Sainz *et al.,* 2001). Nelson *et al.* (Nelson *et al.*, 2001) suggested that these receptors (T1Rs) were candidates for sweet taste receptors. However, identification of a family of candidates for bitter receptors (T2R/TRB) that are members of the GPCRs superfamily has been reported (Adler *et al.*, 2000; Chandrashekar *et al.,* 2000; Matsunami *et al.,* 2000).

For the umami taste, taste-mGluR4, T1R1 and T1R3 are the likeliest receptors. Chaudhari *et al.* (Chaudhari *et al.*, 1996, 2000) employed molecular approaches using reverse transcriptase and *in situ* hybridization to find that a specific metabtropic glutamate receptor (taste-mGluR4), a subtype of mGluR4, was expressed exclusively in taste buds from the foliate and circumvallate papillae in the rat. Toyono *et al.* (Toyono *et al.*, 2002) also demonstrated that both brain-mGluR4 (brain-expressed mGluR) and taste-mGluR4 were expressed in the taste tissues by using immunoblot analysis. They also showed that the antibody against taste-mGluR4 exhibited intense labeling of the taste pores and taste hairs in all the taste buds of the gustatory papillae which they examined. However, Nelson *et al.* (Nelson *et al.*, 2002) also found that T1R1 and T1R3, which are taste-specific GPCRs, combine to function as a broadly tuned L-amino acid receptor responding to the binary mixture of MSG + IMP. Despite these findings, the mechanisms of synergism by the umami substances have not been clarified.

In the present study, we showed the possibility that two different synergistic mechanisms associated with different taste modalities exist in rats. One is the MPG + IMP type, which is suppressed by antisweet substances. This type of synergism is observed in fibers which respond well to sucrose. The other is the L-AP4 + sweet substance type synergism, which is not suppressed by antisweet substances or by any antagonists for the glutamate receptors. This type of synergism is observed in fibers which respond to NaCl, HCl and quinine.

The synergism of umami substances has been reported for many species, such as rats (Sato and Akaike, 1965; Yoshii, 1987; Yamamoto *et al.*, 1991; Sako and Yamamoto, 1999; Sako *et al.*, 2000), mice (Ninomiya and Funakoshi, 1987), dogs (Kumazawa and Kurihara, 1990), bovines (Torii and Cagan, 1980) and humans (Yamaguchi, 1991). Yoshii (Yoshii, 1987) showed that the purine-based 5′-phosphate ribonucleotide leads to an increase in the affinity of amino acids for the respective receptor sites, and has a synergistic effect on the response to amino acids. This finding suggests that there are specific binding sites for these nucleotides on the taste receptor membrane. However, Torii and Cagan (Torii and Cagan, 1980) showed that the number of exposed sites increased, but the affinity was not changed.

In the present study, we would like to hypothesize the existence of at least two types of synergistic mechanisms concerning umami substances. One involves taste-mGluR4 and sweet-taste receptors co-localized in single taste cells, which are responsible for the potentiation between glutamate (or L-AP4) and IMP. MSG and IMP may combine with sweet taste receptors at different sites, and synergism may occur within these receptors. This assumption explains (i) why the synergistic enhancement is exclusively seen in the subset of taste fibers highly responsive to sweet substances (Figure 5) (Sato and Akaike, 1965); (ii) why synergistic taste responses to mixtures of glutamate + IMP are greatly suppressed by gurmarin (Yamamoto *et al.*, 1991; Sako and Yamamoto, 1999); and (iii) why the mixture is very highly preferred (Yamamoto *et al.*, 1991), and the taste is similar to the taste of sucrose as shown by the conditioned taste aversion paradigm (Yamamoto *et al.*, 1991). The other type of synergism, which we found in the present study, is for the potentiation between L-AP4 and sweet substances. These substances may combine with unidentified receptors which may be colocalized with Na^+, H^+ and bitter-taste receptors.

In conclusion, two types of synergy with different mechanisms exist for umami and sweet substances in the taste cells of rats. One is the MSG (MPG) + IMP type, and the other is the L-AP4 + sweetener type.

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