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Response properties of the pharyngeal branch of the glossopharyngeal nerve for umami taste in mice and rats

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Abstract

Many studies have reported the mechanism underlying umami taste. However, there are no investigations of responses to umami stimuli taste originating from chemoreceptors in the pharyngeal region. The pharyngeal branch of the glossopharyngeal nerve (GPN-ph) innervating the pharynx has unique responses to taste stimulation that differs from responses of the chorda tympani nerve and lingual branch of the glossopharyngeal nerve. Water evokes robust response, but NaCl solutions at physiological concentrations do not elicit responses. The present study was designed to examine umami taste (chemosensory) responses in the GPN-ph. Response characteristics to umami taste were compared between mice and rats. In mice, stimulation with compounds eliciting umami taste (0.1 M monosodium L-glutamate (MSG), 0.01 M inosine monophosphate (IMP) and the mixture of 0.1 M MSG + 0.01 M IMP) evoked higher responses than application of distilled water (DW). However, synergistic response of a mixture of 0.1 M MSG + 0.01 M IMP was not observed. In rats, there is no significant difference between the responses to umami taste (0.1 M MSG, 0.01 M IMP and the mixture of 0.1 M MSG + 0.01 M IMP) and DW. Monopotassium glutamate (MPG) was used in rats to examine the contribution of the sodium component of MSG on the response. Stimulation with 0.1 M MPG evoked a higher response when compared with responses to DW. The present results suggest that umami taste compounds are effective stimuli of the chemoreceptors in the pharynx of both mice and rats.

Keywords: Umami; Pharynx; Glossopharyngeal nerve; Glutamate; Rat; Mice

It has been established that umami taste is a distinct taste quality in addition to the traditional taste qualities of sweet, salty, sour and bitter. Umami taste is evoked by monosodium L-glutamate (MSG) and inosine monophosphate (IMP) [7]. Many researchers have investigated the receptor mechanism of umami taste. The metabotropic glutamate receptor, taste-mGluR4, truncated form of brain-mGliR4 was found in lingual tissues containing taste buds in rats [1,3–5]. Recently, it has been reported that the G-protein-coupled receptor complex T1R1+3 is a candidate of mammalian umami taste receptors [21]. Previously, it was suggested that at least two mechanisms are involved in the umami response based on behavioral [11,13] and electrophysiological [11–13] studies. When the lingual branch of the glossopharyngeal nerve (GPN-li) was sectioned bilaterally in mice, they were unable to discriminate between MSG and NaCl [11,12]. This result in mice suggests that chemoreceptors innervated by the GPN-li are the receptors responsible for discrimination between umami and NaCl taste. In addition, MSG best fibers were isolated in the GPN-li of mice [12]. Nerve recordings in mice and rats demonstrated that both the chorda tympani nerve (CT) and GPN-li respond to application of umami stimuli [11–13].

In rats behavioral studies have demonstrated that umami taste is not unique, and judged to be similar to the taste of sucrose. Rats cannot discriminate MSG from sucrose, when the epithelial sodium channel blocker amiloride is mixed with the MSG to eliminate the sodium component of the response [19].

Synergism is a further characteristic of the unique characteristic of umami taste [13,14,19]. Rats show preference for the mixture of MSG + IMP. In electrophysiological experiment in rats, CT responses to the mixtures of MSG + IMP are larger than each component of the mixture [19]. In addition, the synergistic enhancement was evoked in fibers that respond well to sucrose,

Abbreviations: MSG, monosodium L-glutamate; IMP, inosine monophosphate; MPG, monopotassium glutamate; DW, distilled water; GPN-ph, pharyngeal branch of the glossopharyngeal nerve; GPN-li, lingual branch of the glossopharyngeal nerve; CT, chorda tympani nerve; SLN, superior laryngeal branch of the vagus nerve

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but not to NaCl [13,14,19]. Thus, umami and sweet receptors are both involved in umami and sweet taste responses [13].

The receptors in the mucosa of the pharynx play an important role in controlling a number of reflexes, preventing aspiration of food and fluid to the upper air way [17]. Taste (chemosensory) responses of the glossopharyngeal nerve (GPN-ph), innervating the pharynx, are different from those obtained from the CT and GPN-li, but have some similarities to those recorded from the superior laryngeal branch (SLN) of vagus nerve; the GPN-ph does not respond to 0.15 M NaCl but respond to a lower and higher concentration of NaCl [6] and water evokes a vigorous response of the GPN-ph similar to that reported in studies of the SLN [2,6].

Umami taste is involved in diversity of foods such as fish, meat, milk, tomato and some vegetables [18]. This sensation is considered to have significant roles in food palatability and control of food intake [20]. It is necessary for perception of food palatability to swallow food bolus.

The present study was therefore designed to determine a physiological significance of umami taste in the pharynx. We examined the responses of the GPN-ph to various compounds eliciting umami taste in mice and rats.

Experiments were carried out using 12 adult C57BL/6NCrj male mice weighing 20–30 g and eight adult male Wistar rats weighing 250–300 g. These animals were deeply anesthetized with urethane (1.0 g kg^{-1}, ip) and placed supine. Rectal temperature was monitored and maintained at 37–38 °C with a thermostatically controlled heating pad. A longitudinal midline incision was made in the ventral surface of the neck. The trachea was isolated and cut transversely. A forked polyethylene tube was inserted into the trachea for artificial ventilation.

In all experiments, the right GPN was dissected from the surrounding connective tissue. The GPN-li and GPN-ph nerves were isolated and the right GPN-ph nerve was transected just distal to junction of the GPN-li and GPN-ph. The peripheral cut end of the GPN-ph was desheathed and put on a pair of platinum recording-wire electrodes (0.1 mm diameter) to record whole nerve responses to taste stimuli [8,9]. To apply taste solutions to the pharynx, the thyroid cartilage was cut and the edges held apart by using silk threads. In addition, the epiglottis was removed to expose the inside of the pharynx. During the recordings, the animals were paralyzed by pancuronium bromide (1 mg kg^{-1} h^{-1}, iv).

This study was approved by the Animal Use and Care Committee of Niigata University.

Taste stimuli were (in M): 0.1 MSG (Ajinomoto Co., Tokyo, Japan), 0.01 IMP (Ajinomoto Co.), the mixture of 0.1 MSG + 0.01 IMP, 0.15 NaCl (physiological saline; Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan), 0.1 NaCl (Wako pure Chemical Industries, Osaka, Japan), 0.1 KCl and distilled water (DW). Umami substances, NaCl and KCl were dissolved in DW. To control for the sodium component of MSG to the umami response, 0.1 monopotassium glutamate (MPG; Ajinomoto Co.) was used [19].

The selection of these solutions, concentrations and the mixtures used in this study were based on a number of previous studies examining umami taste. The taste solutions, at room temperature (25 °C approximately), were applied with a 1 ml syringe. About 0.2 ml of solution was flowed over the region of the pharynx for 1 s and remained for about 15 s. The order of stimulus application was random. At the end of the stimulation period, the taste solution was removed by aspiration. The pharyngeal region was rinsed with a saline (0.15 NaCl), because water itself stimulates the pharyngeal region innervated by the GPN-ph.

Neural responses induced by taste stimulation of the pharynx were amplified and integrated with time constant of 0.3 s. Neural responses were measured as the area of the integrated response above baseline for 10 s after the onset of stimulation. Responses to the taste stimuli were expressed as the relative magnitudes of response. These values for each taste stimulus were calculated with respect to the magnitude of a response to 0.15 NaCl which was taken as unity (1.0) and used for statistical analysis. Statistical analysis was performed using ANOVA followed by Dunnett's test. Results are presented as mean ± S.E.M. Differences were considered as significant at $p < 0.05$.

Fig. 1a is a typical whole nerve and integrated recordings from a mouse GPN-ph in response to the test solutions (DW, 0.1 MSG, 0.01 IMP and the mixture of 0.1 MSG + 0.01 IMP). The nerve activity of the GPN-ph to stimulation of the pharynx with DW is characterized by a high initial response followed by a lower level of activity. In contrast, stimulation with 0.1 MSG resulted in a high amplitude initial response with a much higher level of adapted response. Stimulation with 0.01 IMP induced a higher amplitude response immediately after stimulus onset, when compared to stimulation with 0.1 MSG, and this initial response lasted much longer than that of 0.1 MSG. The response to stimulation with 0.1 MSG + 0.01 IMP mixture was similar to the pattern of the integrated response evoked by stimulation with 0.01 IMP.

Fig. 1b is a comparison of the relative magnitudes of the responses of the GPN-ph in mice ($n = 12$) to the four test solutions (DW, 0.1 MSG, 0.01 IMP, the mixture of 0.1 MSG + 0.01 IMP). The value of the relative magnitude for DW was 1.92 ± 0.06. The values of 0.1 MSG, 0.01 IMP and the mixture of 0.1 MSG + 0.01 IMP were 2.67 ± 0.19, 3.13 ± 0.27 and 3.22 ± 0.34, respectively. The relative magnitudes of 0.1 MSG, 0.01 IMP and the mixture of 0.1 MSG + 0.01 IMP were significantly higher than that of DW ($^{**}p < 0.01$ for each). The synergy effect was estimated by using a potentiation ratio (magnitude of response to mixture/sum of magnitudes of responses to individual components in the mixture). The potentiation ratio evoked by synergism is more than 1.0. In this experiment, the stimulation with the mixture of 0.1 MSG + 0.01 IMP showed essentially no synergism, because the potentiation ratio was less than 1.0.

Fig. 2a shows typical recordings from a GPN-ph in a rat to the taste stimuli (DW, 0.1 NaCl, 0.1 MSG, 0.1 MPG, 0.01 IMP and the mixture of 0.1 MSG + 0.01 IMP). When DW was applied to the pharynx, high frequency discharges lasted for the whole stimulation period. 0.1 NaCl stimulation evoked a smaller response when compared to the response to DW. Stimulation with 0.1 MSG, 0.01 IMP and the mixture of 0.1 MSG + 0.01 IMP