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Expressions of multiple umami taste receptors in oral and gastrointestinal tissues, and umami taste synergism in chickens

Yuta Yoshida [a], Yuko Kawabata [a, b], Fuminori Kawabata [c, *], Shotaro Nishimura [a, b], Shoji Tabata [a, b]

[a] Laboratory of Functional Anatomy, Department of Bioresource Sciences, Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu University, Fukuoka, Japan
[b] Faculty of Agriculture, Kyushu University, Fukuoka, Japan
[c] Institute for Advanced Study, Kyushu University, Fukuoka, Japan

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ABSTRACT

Umami taste is one of the five basic taste qualities, along with sweet, bitter, sour, and salty, and is elicited by some L-amino acids and their salts, including monopotassium L-glutamate (MPG). The unique characteristic of umami taste is that it is synergistically enhanced by 5′-ribonucleotides such as inosine 5′-monophosphate (IMP). Unlike the other four basic taste qualities, the presence of umami taste sense in avian species is not fully understood. In this study, we demonstrated the expression of multiple umami taste receptor candidates in oral and gastrointestinal tract tissues in chickens using RT-PCR analysis. We first showed the metabotropic glutamate receptors (mGluRs) expressed in these tissues. Furthermore, we examined the preference for umami taste in chickens, focusing on the synergistic effect of umami taste as determined by the two-feed choice test. We concluded that chickens preferred feed containing both added MPG and added IMP over feeds containing either added MPG or added IMP alone and over the control feed. These results suggest that the umami taste sense and synergism are conserved in chickens.

1. Introduction

In chickens, the taste sense is one of the most important senses for acquiring and choosing feeds, as are the smell and visual senses [1]. It is important to clarify the mechanism of taste sense in chickens in order to improve the efficiency of poultry feeding through the feeding of preferable feed and to elucidate the evolution of the taste sense from birds to mammals.

Umami taste is one of the five basic taste qualities, and is discriminated from the other four basic taste qualities (sweet, bitter, sour, and salty) in mammals [2–4]. L-glutamate and its salts, including monosodium L-glutamate and monopotassium L-glutamate (MPG), are used as seasonings and are known to be umami tastants for humans. Interestingly, the umami taste is synergistically potentiated by 5′-ribonucleotides, such as inosine 5′-monophosphate (IMP), which is contained in some foods such as dried bonito [5,6]. The umami taste receptor candidates in mammals are the G-protein-coupled receptors, the heterodimer of taste receptor type 1 (T1R1) and taste receptor type 3 (T1R3) and brain-expressed and taste-expressed type 1 and type 4 metabotropic glutamate receptors (brain-mGluR1, brain-mGluR4, taste-mGluR1, and taste-mGluR4, respectively) [7–10]. The umami taste synergism can be explained by stabilization of the conformation of the extracellular domain of T1R1 by its allosteric modulator, IMP [11].

Recently, many reports related to the taste sense in chickens have been published. Behrens et al. reported the activities of three chicken bitter taste receptors, the type 2 taste receptors (T2R1, T2R2, and T2R7), using a cell-based assay [12]. Hirose et al. found that the activities of T2R1 were compatible with behavioral sensitivities to bitterness in chickens [13]. Sawamura et al. revealed that a chicken fatty acid receptor candidate, GPR120 (G-protein-coupled receptor 120), cloned from chicken oral tissue, responded to fatty acids [14]. With respect to the sense of umami taste in chickens, one of the umami taste receptors, the T1R1/T1R3 heterodimer, was expressed in the oral and gastrointestinal tract tissues in chickens [15] and was activated by L-alanine and L-serine [16]. In another study, we revealed that primary culture cells from isolated taste buds from the chicken palate responded to a mixture of MPG and IMP [17]. Here, we speculated that, if the umami taste sense and its

* Corresponding author.
E-mail address: kawabata@agr.kyushu-u.ac.jp (F. Kawabata).

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unique characteristic, umami taste synergism, were conserved in chickens, feed intakes would be synergistically enhanced by umami taste enhancers such as IMP. In this study, we analyzed the expression of multiple umami taste receptor gene candidates by RT-PCR and tested the feed preference for umami tastants focusing on umami taste synergism, which is specific to this taste, using the two-feed choice test in chickens.

2. Materials and methods

2.1. Chemicals

MPG and IMP were purchased from Sigma–Aldrich (Tokyo). These compounds were stored at room temperature.

2.2. Animals

Rhode Island Red strain 3-day-old chicks were used for the RT-PCR, and 0-week-old chicks were used for the two-feed choice test. These studies were carried out according to the Guide for Animal Experiments issued by Kyushu University, the Law Concerning the Human Care and Control of Animals (Law No. 105; October 1, 1973), and the Japanese Government Notification on the Feeding and Safekeeping of Animals (Notification No. 6; March 27, 1980).

2.3. RT-PCR analysis

Total RNA was isolated from the brain, oral tissues (palate beak, tongue tip, and floor beak), and gastrointestinal tissues (ingluvies, stomach, gizzard, duodenum, jejunum, ileum, and colon), using ISOGEN II (Nippon Gene Co., Ltd., Tokyo) according to the manufacturer's instructions. The first-strand cDNA was synthesized by reverse transcription, with the application of 1.0 μg total RNA with or without reverse transcriptase using the PrimeScript RT reagent kit with gDNA Eraser (TaKaRa Bio Inc., Otsu, Japan) following the manufacturer's protocol. Primers were designed with the aid of the nucleotide database of The National Center for Biotechnology Information and are shown in Table 1. Although the primer set of brain-mGluR1 has the ability to amplify two splice variants of brain-mGluR1 (Accession no. XM_004935585.1 and XM_419652.3), we thought it reasonable to elucidate the whole expression patterns of brain-mGluR1 without discriminating among these variants in this study. The PCR mixture had a total volume of 10 μl and consisted of 5.85 μl distilled water, 1.0 μl 10 × Ex Taq buffer (TaKaRa Bio Inc.), 0.8 μl dNTP mixture (2.5 mM each) (TaKaRa Bio Inc.), 0.4 μl primer forward (10 μM), 0.4 μl primer reverse (10 μM), 1.5 μl cDNA (100 ng/μl), and 0.05 μl Ex Taq (5 Units/μl) (TaKaRa Bio Inc.). PCR reactions were conducted under the following conditions: 38–40 cycles of 98 °C for 10 s, 60 °C for 30 s, and 72 °C for 1 min/1 kb (19–48 s). Five μl PCR products were electrophoresed on a 2.0% agarose gel.

2.4. Two-feed choice test

This experiment was conducted using a box brooder (Showa Furanki, Saitama, Japan) to keep the temperature at 30 °C. The box brooder was partitioned using woven metal wire so that the chicks could see other chicks through the wire. The chicks could freely drink tap water during the entire experimental period. As the experimental feed, PL1 (Oriental Yeast Co., Tokyo) was used. The feed is a normal and suitable feed for fulfilling the nutritional requirements of chicks. First, the umami feed (PL1 with the addition of either MPG or IMP or both) was presented to the chicks for 24 h, and then the umami feed and the control feed (PL1) were presented simultaneously to the chicks for 7 h. This training period was conducted to help them become accustomed to the experimental condition. After that, the chicks fasted for 17 h. The umami feed and the control feed were then presented simultaneously to the chicks again for 7 h, and the feed intake for this 7 h period was measured. This test was carried out for each concentration of umami substances, using 8–12 chicks, and for each of 5 conditions: 1) 0.05% MPG, 0.005% IMP, and 99.945% PL1; 2) 0.5% MPG, 0.05% IMP, and 99.45% PL1; 3) 5% MPG, 0.5% IMP, and 94.5% PL1; 4) 0.55% MPG and 99.45% PL1; and 5) 0.55% IMP and 99.45% PL1.

2.5. Statistical analysis

The data are expressed as the means ± SEM. Statistical analyses were done by paired *t*-test. Differences with *p*-values < 0.05 were considered significant.

3. Results

3.1. RT-PCR analysis

RT-PCR analysis showed that the expression of multiple umami taste receptor candidates, namely taste-mGluR1, brain-mGluR1, taste-mGluR4, brain-mGluR4, and T1R1/T1R3, was observed in the brain, palate beak, floor beak, ingluvies, stomach, gizzard, duodenum, jejunum, ileum, and colon in chickens, but not in the tongue tip, where almost no taste buds are distributed (Fig. 1A) [18]. We confirmed that no band was observed in the negative control reactions without reverse transcriptase (Fig. 1B).

3.2. The two-feed choice test

In the two-feed choice test, the intakes of the feed with the addition of both 0.5% MPG and 0.05% IMP were significantly larger than those of the control feed (Fig. 2). On the other hand, the intakes of the feed with the addition of either 0.55% MPG or 0.55% IMP showed no significant differences with those of the control feed (Fig. 2). The addition of both MPG and IMP at higher concentrations (5% MPG and 0.5% IMP) and lower concentrations (0.05% MPG and 0.005% IMP) had no significant effect on the feed intakes

Table 1
Primers used for the RT-PCR.

Target gene	Accession no.	Primer forward	Primer reverse	Product size (bp)
Taste-mGluR1	XM_004935586.1	CATCATAGCCAAGCCCGAGA	TCTGATGAAGTCCTGGGTGC	219
Brain-mGluR1	XM_004935585.1 XM_419652.3	GGAGACACGTCTCTTCCTGC	TAGGTCATGTCTGGGGGTGT	456
Taste-mGluR4	XM_004935045.1	CTCTCCCGTCTCCAACCAAA	GATCATGCTCTACACAGTCATCG	309
Brain-mGluR4	XM_003642745.2	GACAACAACCGCCGAAACAT	CACCATCCACTGGGTCCATC	271
T1R1	XM_425734.4	CAGCTACGAAGCCTCTCTGG	GTAGGAGCTGCCAGGGATAG	794
T1R3	XM_425740.3	TGTTACGACCGCAGTGAGAG	GGGAACTCTGTGAGCAGGAC	335
β-actin	NM_205518.1	ACACGGTATTGTCACCAACT	TAACACCATCACCAGAGTCC	263