Behavioral responses to sweet compounds via T1R2-independent pathways in chickens

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ABSTRACT Elucidating the taste sensing systems in chickens will enhance our understanding of poultry nutrition and improve the feeding strategies used in poultry farming. It is known that chickens lack the sweet taste receptor subunit, taste receptor type 1 member 2 (***T1R2***), in their genome. Thus, the present study investigated T1R2-independent sweet-sensing pathways in chickens. RT-PCR analysis revealed that glucose transporters known to play an important role in T1R2-independent sweet sensing in mammals—namely sodium-glucose cotransporter 1 (***SGLT1***) and ATP-gated K^+ channel subunits—are expressed in the palate, the main taste organ in chickens. In behavioral tests, chickens slightly preferred glucose, galactose, sucrose, maltose, lactose, and stevioside, while high doses of sucrose and fructose were rejected. Chickens did not show any preference for noncaloric sweeteners or sugar alcohol, such as acesulfame K, aspartame, saccharin, sucralose, or sorbitol. The preference for galactose was inhibited by an inhibitor of SGLT1 in a dose-dependent manner. In addition, we found that glucagon-like peptide 1 (**GLP-1**) and mRNA of the GLP-1 receptor, which are involved specifically in sweet transmission in mice, are also present in the oral tissues of chickens. The present results imply that chickens can sense various sweet compounds via T1R2-independent pathways in oral tissues.

Key words: chicken, GLUT, K_{ATP} channel, SGLT1, sweet taste

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INTRODUCTION

The sense of taste guides animals to choose nutritious foods and avoid toxic substances, and it is deeply related to their feeding behavior. Thus, elucidating taste-sensing systems in chickens can enhance our understanding of poultry nutrition and improve feeding strategies in the poultry industry. It is widely accepted that tastes can be classified into 5 basic tastes: sweet, umami, bitter, sour, and salty. Sweetness is mainly mediated by a heterodimer of taste receptor type 1 members 2 and 3 (**T1R2/T1R3**) (Zhao et al., 2003). However, avian species including chickens lack the *T1R2* gene, which encodes the sweet taste receptor subunit, in their genome (Shi and Zhang, 2006). Consistent with this finding, several studies have suggested that chickens show no or low taste acuity to sugars (Gentle, 1972; Cheled-Shoval et al., 2017; Yoshida et al., 2018a).

On the other hand, it was reported that *T1R2*-knockout mice show residual responses to sugars, suggesting the existence of alternative, T1R2-independent oral sweet-sensing pathways (Zhao et al., 2003). Indeed, it was suggested that the glucose transporters (**GLUTs**), sodium-glucose cotransporter 1 (**SGLT1**), ATP-gated K^+ ($\mathbf{K_{ATP}}$) channels, and disaccharide-digesting enzymes are expressed in the taste buds and involved in oral sugar sensing in mice (Sukumaran et al., 2016; Yee et al., 2011). Other studies have demonstrated that T1R3 alone is activated by high concentrations of sucrose and maltose (Zhao et al., 2003), and that natural noncaloric sweeteners, including steviol glycosides such as stevioside, potentiate the activity of transient receptor potential melastatin 5 (**TRPM5**) (Philippaert et al., 2017), which is involved in sweet taste transduction (Zhang et al., 2007). However, it is not understood whether these T1R2-independent sweet-sensing pathways are involved in sweet taste sensing in chickens.

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Thus, the aim of the present study was to investigate the key molecules for T1R2-independent sweet-sensing pathways in chickens and the behavioral responses of chickens to various sugars and natural/artificial sweeteners.

MATERIALS AND METHODS

Chemicals

D-Glucose was purchased from Katayama Chemical Industries (Osaka, Japan). Sucrose was purchased from Nacalai Tesque (Kyoto, Japan). Sucralose was purchased from Tokyo Chemical Industry Co. (Tokyo). Stevioside was obtained from Ark Pharm (Arlington Heights, IL). The other chemicals were purchased from FUJIFILM Wako Pure Chemical (Osaka, Japan). They were dissolved in filtered tap water just before the intake experiments. Phloridzin was dissolved in 0.2% ethanol solution just before use.

Animals

The use of animals throughout the study was approved by the Committee for Laboratory Animal Care and Use at Kyushu University (approval no. A28-151-1) and was in compliance with 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). Rhode Island Red strain chicks were obtained from the National Livestock Breeding Center's Okazaki station (Okazaki, Japan), and the chicks and their offspring were used for this experiment. The sexes of the chicks were mixed. The chicks were maintained in a box brooder with a heating system (Showa Furanki, Saitama, Japan) at a temperature of around 30°C under 24 h lighting. The chicks were euthanized by intraperitoneal injection with an overdose of pentobarbital sodium solution.

Reverse Transcription Polymerase Chain Reaction

It was suggested that the taste buds of chickens are localized in the palate and the base of the oral cavity, and that only a few taste buds are present in the tongue tip (Kudo et al., 2008). Thus, we chose tongue tip as a representative non-taste tissue in the oral tissues. The palate, tongue tip, base of the oral cavity, gizzard, liver, pancreas, kidney, superficial pectoral muscle, and duodenum were collected from 3-day-old chicks (n = 7). Total RNA was isolated from these tissues with the use of the reagent ISOGEN II (Nippon Gene, Tokyo) according to the manufacturer's instructions. Then, the cDNA was synthesized using a PrimeScript RT reagent kit with gDNA Eraser (Takara Bio, Otsu, Japan) according to the manufacturer's protocol. The primers were designed with the aid of the nucleotide database of the National Center for Biotechnology Information and are listed in Table 1. The polymerase chain reaction (**PCR**) mixture had a total volume of 20 μL and consisted of 15.0 to 15.5 μL ultrapure water, 2.0 μL 10 × Ex Taq buffer (TaKaRa Bio), 1.6 μL dNTP mixture (2.5 mM) (TaKaRa Bio), 0.4 μL primer forward (10 μM), 0.4 μL primer reverse (10 μM), 0.5 to 1.0 μL cDNA, and 0.1 μL Ex Taq (5 Units/μL) (TaKaRa Bio). PCR reactions were conducted under the following conditions: 30 to 35 cycles of 98°C for 10 s, 55 to 62°C for 30 s, and 72°C for 1 min/1 kb (15−59 s). PCR products were electrophoresed on a 2.0% agarose gel.

Quantitative Reverse Transcription PCR

The palate and tongue tip were collected from 3-day-old chicks (n = 3). Total RNA was isolated from

Table 1. Primers used for the RT-PCR and quantitative RT-PCR.

Target gene	Accession no.	Primer forward	Primer reverse	Product size (bp)
GLUT2	NM_207178.1	CCCCGCAGAAGGTGATAGAAGC	ACCATGCCTCCAACAGCAAAC	247
GLUT8	NM_204375.1	AAACTTCCAGGACAGCAGAATG	TAAAAGGCTGCAAAGTCACGC	516
GLUT9[a]	(X2) XM_420789.4 (X3) XM_004936166.1	ACGCAGGCCTCTTCTCATCGG	ATTTTTCCCCCAGCCACCCC	578
SGLT1	NM_001293240.1	ACCATTACAGGTGGCCTTGC	CTCCAGTGACAGCATCTCGG	244
Kir6.1	NM_001293287.1	AAGGATGCGAGTGAGCGAG	AAGCAGAGGTGAAAGACCTGAC	542
Kir6.2	XM_426402.4	CTGCCATGCTGAGTTGCACG	TGCACTGCCTGACCTTCTTGG	246
SUR2A	XM_003640404.2	CTGTGGCAAGTCCTCCCTTC	AAGTCTGATCAGCATTCCCG	977
SUR2B	XM_004937988.1	ATGGAATGGTGCTCAGGGAAGG	CGACAAGGAACCATGGAGTGGC	768
SUR1	(X2) XM_421005.4	GTTGCATCACGGGACCTTC	GGATCTCAACGTCTGTAAGGGG	711
	(X3) XM_004941409.1	AAGATGCCTTGGAGAGCATGTGG	GTGAGGAAGCATAAGAGCATAG	279
	(X4) XM_004941410.1	TCTCATTGCCTTGGTTCCCC	TTCATCTCTGTGCCCATACG	897
T1R3	XM_425740.3	TGTTACCACCGCAGTGAGAG	GGGAACTCTGTGAGCAGGAC	335
TRPM5	XM_003641321.2	TCATGTCCCAACGACTACGC	ACGCTCTTCTCTCTTTCAGCCC	516
AMY2A	NM_001001473.2	CAGAAGGCACCATGCAAGTC	GAGGAGAAACCTGAACTCCTCC	189
MGAM	XM_015273018.1	AACGGGAAAGGCGCCAAGAG	AATTCACCCCGGAAGCCGAC	338
SI	XM_015291762.1	AGCAGCAAGCAGGTCATGAGG	ACAGAAAGCTGTTAACACAAATCGC	778
β-actin	NM_205518.1	ACGCATTCTTCCTGATGGGG	GGGATGTCATCATGTCCTAAAGC	263
GLP-1R	NM_1135551.1	ATCCCTTAGCTGCCGTTTGG	CAGCCAATGCAGAGATACAG	156
GAPDH	NM_20435.1	ACTGTCAAGGCTGAGAACGG	ACCTGCATCTGCCCATTTGA	99

[a]There are two variants and the primer pair can amplify both variants.

these tissues with the use of the reagent ISOGEN II (Nippon Gene). Genomic DNA was removed with the use of DNase I (Nippon Gene). The PCR mixture had a total volume of 10 μL and consisted of 5.0 μL 2 × OneStep SYBR reverse transcription PCR (**RT-PCR**) Buffer 4 (Takara Bio), 0.4 μL PrimeScript 1 step Enzyme Mix 2 (Takara Bio), 0.4 μL forward primer (10 μM), 0.4 μL reverse primer (10 μM), 0.2 μL ROX Reference Dye II (50 ×) (Takara Bio), 1.0 μL total RNA (100 ng/μL), and 2.6 μL RNase Free dH_2O (Takara Bio). The PCR reactions were conducted under the following conditions: 42°C for 5 min, 95°C for 10 s, 40 cycles of (95°C for 5 s and 60°C for 34 s), followed by a melting curve analysis from 60°C to 95°C. The primers used are listed in Table 1. *GAPDH* was used as an internal control gene.

One-Bowl Drinking Test

The one-bowl drinking tests were performed as described previously with some modifications (Hirose et al., 2015). Briefly, male and female chicks that were 0 to 2 wk old at the start of the experiment were used. The tests were performed for 6 consecutive days. During the tests, chicks were supplied commercial layer feed (Powerlayer 17Y, JA Kitakyushu Kumiai Shiryo, Fukuoka, Japan) ad libitum, but the test solution or water (for the controls) was withheld for 23.5 h—that is, until 30 min before the tests—throughout the experimental period.

On D 0, the chicks were presented filtered tap water for 24 h. On D 1 and 2, the chicks were presented water (or control solutions) for 30 min. On D 3, the chicks were presented the test solutions for 30 min. On D 4 and 5, the chicks were randomly presented the test solutions or water (or control solutions) for 30 min. To compensate for evaporation from the bowl during the 30 min of exposure, control tap water was set in the box brooder, and the amount of evaporation was subtracted from the volume of solution drunk. To evaluate the preference for the test stimuli, the preference index was calculated as (test solution intake / (test solution intake + water (or control solution) intake) × 100). For statistical analyses, preference indexes were compared to the values obtained as follows: water (or control solution) intake / (test solution intake + water (or control solution) intake) × 100. A preference index >50 indicates preference, and an index <50 indicates aversion.

First, we investigated the preference for the monosaccharides: glucose, galactose, and fructose. Second, we investigated the involvement of SGLT1 on the preference for 0.5 M galactose in chickens using a competitive SGLT1 inhibitor, phloridzin (Garriga et al., 2006). We used 0.2% ethanol solution as the control solution, and we used 0.2% ethanol solution containing 0.5 M galactose with 0, 50, or 500 μM phloridzin or 0.2% ethanol solution containing 500 μM phloridzin alone as the test solutions. Then, we investigated the preference for the disaccharides (sucrose, maltose, and lactose), a sugar alcohol (sorbitol), a natural sweetener (stevioside), and the artificial sweeteners (acesulfame K, aspartame, saccharin, and sucralose).

Measurement of Glucagon-Like Peptide 1 Contents in Oral Tissues

A previous report suggested the involvement of glucagon-like peptide 1 (**GLP-1**) signaling in sweet taste transmission in mice (Takai et al., 2015). Thus, we investigated the GLP-1 content and mRNA expression of the GLP-1 receptor (***GLP-1R***) in the oral tissues of chickens. The palate and tongue tip were collected from 3 to 5-day-old chicks (n = 6). The amounts of GLP-1 contained in these tissues were measured using a Chicken GLP-1 ELISA Kit according to the manufacturer's instructions (Elabscience Biotechnology, Wuhan, China).

Statistical Analysis

Statistical analyses were performed by paired t test, using Microsoft Excel (2011; Redmond, WA) and one-way factorial ANOVA followed by post-hoc Tukey HSD test, using IBM SPSS Statistics (Version 27, Armonk, NY). A P-value <0.05 was used as the threshold to declare statistical significance.

RESULTS

Expression of GLUTs, SGLT1, K_{ATP} Channel Subunits, T1R3, and TRPM5

The present RT-PCR analysis demonstrated the mRNA expression profiles of GLUTs including *GLUT2, GLUT8*, and *GLUT9, SGLT1*, and K_{ATP} channel subunits, inwardly rectifying K^+ channels (***Kir6.1*** and ***Kir6.2***), and sulfonylurea receptors (***SUR2A***, ***SUR2B***, and ***SUR1*** variants), in addition to *T1R3* and *TRPM5*, in the palate, gizzard, liver, pancreas, kidney, and muscle of chickens (Figure 1). *GLUT2* expressions were confirmed in the gizzard, liver, pancreas, and kidney. Other gene RNA transcripts were expressed in all tissues examined in this study. We confirmed that no bands were observed in the negative control samples, which were generated without templates (H_2O) and reverse transcriptase (**RT-**) (data not shown).

Behavioral Responses to the Monosaccharides Glucose, Galactose, and Fructose

The present one-bowl drinking tests demonstrated that the intakes of 0.05, 0.1, 0.2, 0.3, and 1.0 M glucose solution did not differ from the intakes of water ($P > 0.05$; Figure 2A), but chickens showed a slight preference for 0.2 M glucose compared to water ($P <$

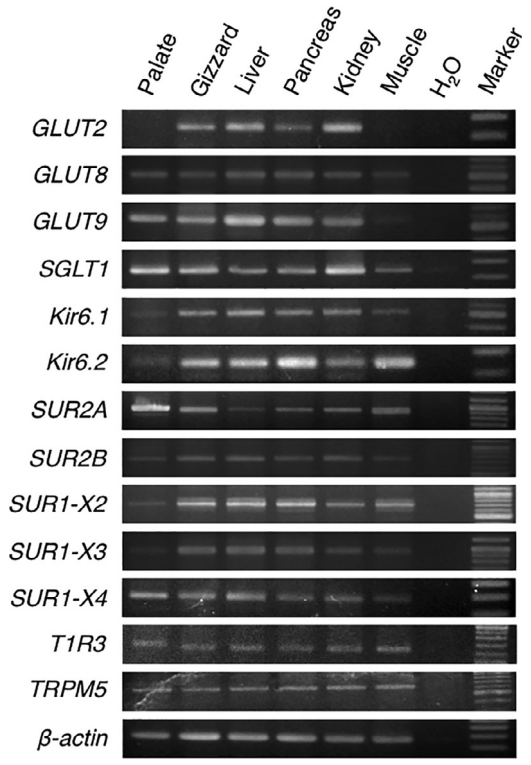

Figure 1. RNA transcripts of the glucose transporters *GLUT2, GLUT8, GLUT9*, and *SGLT1*; the K_{ATP} channel subunits *Kir6.1, Kir6.2, SUR2A, SUR2B*, and *SUR1* variants; and *T1R3, TRPM5*, and *β-actin* in the chicken palate, gizzard, liver, pancreas, kidney, and muscle. No bands were observed in the negative controls without templates (H_2O).

0.05; Figure 2D). We also found that the intakes of 0.05, 0.1, and 1.0 M galactose solution were not significantly different from the intakes of water ($P > 0.05$; Figure 2B), while the 0.5 M galactose solution intake was increased ($P < 0.05$; Figure 2B). We also found that chickens preferred 0.5 M galactose solution over water ($P < 0.05$; Figure 2E). On the other hand, the 1.0 M fructose solution intake was lower than the intake of water ($P < 0.001$; Figure 2C), while the 0.05, 0.1, and 0.5 M fructose solution intakes did not differ from that for water ($P > 0.05$; Figure 2C). We also found that chickens showed a significantly reduced preference for 1.0 M fructose compared to water ($P < 0.01$; Figure 2F).

Effects of an SGLT1 Inhibitor on Galactose Preference in Chickens

Although the intake of 0.5 M galactose solution was higher than that of the control solution ($P < 0.05$; Figure 3A), the intakes of 0.5 M galactose with 50 or 500 μM phloridzin solution were comparable to the intakes of the control solution ($P > 0.05$; Figure 3A). The intake of 500 μM phloridzin solution did not differ from that of the control solution ($P > 0.05$; Figure 3A). The preference indexes of this experiment showed that the preference of 0.5 M galactose solution was significantly inhibited by phloridzin in a dose-dependent manner (Figure 3B).

Behavioral Responses to the Disaccharides Sucrose, Maltose, and Lactose

We found that the 0.1 M sucrose solution intake was increased ($P < 0.05$; Figure 4A), and the 1.0 M sucrose solution intake was decreased, compared to intakes of water ($P < 0.05$; Figure 4A). On the other hand, intakes of the 0.05 and 0.3 M sucrose solutions did not differ compared to intakes of water ($P > 0.05$; Figure 4A). We found that chickens showed a slight preference for 0.1 M sucrose solution compared to water ($P < 0.05$; Figure 4D), but showed aversion to 1.0 M sucrose ($P < 0.05$; Figure 4D). We also found that the 0.1 M maltose solution intakes were increased compared to water intake ($P < 0.01$; Figure 4B), but the 0.05, 0.5, and 1.0 M maltose solution intakes were not different from those for water ($P > 0.05$; Figure 4B). We found that chickens preferred 0.1 M maltose solution compared to water ($P < 0.01$; Figure 4E). The 0.1 M lactose solution intake was also increased compared to water intake ($P < 0.05$; Figure 4C), while the intakes of the 0.05 and 0.5 M lactose solutions did not differ ($P > 0.05$; Figure 4C). Chickens preferred 0.1 M lactose solution over water ($P < 0.001$; Figure 4F).

Expression of Polysaccharide- and Disaccharide-Digesting Enzymes in the Oral Tissues of Chickens

Analysis of the mRNA expression profiles of polysaccharide- and disaccharide-digesting enzymes revealed that amylase (***AMY2A***), maltase-glucoamylase (***MGAM***), and sucrase-isomaltase (***SI***) were expressed in the palate, the base of the oral cavity, and the duodenum of chickens (Figure 5). *AMY2A* and *NGAM* were also expressed in the tongue tip and pancreas. We confirmed that no bands were observed in the RT-negative control tissues (data not shown).

Behavioral Responses to Sorbitol, Stevioside, Acesulfame K, Aspartame, Saccharin, and Sucralose

We found that the 0.05, 0.1, and 0.5 M sorbitol solution intakes did not differ compared to water intakes, and the chickens did not show responses to the 0.05, 0.1, or 0.5 M sorbitol solutions ($P > 0.05$; Figures 6A and 6C). On the other hand, although the 0.12, 3.73, 12.4, 124, and 373 mM stevioside solution intakes did not differ compared to water intakes ($P > 0.05$; Figure 6B), the chickens showed a slight preference for 12.4 mM stevioside solution compared to water ($P < 0.05$; Figure 6D).

We found that the chickens did not show any responses to 1, 10, or 100 mM acesulfame K, to 0.1, 1, or 50 mM aspartame, to 0.054, 2.73, 5.46, and 54.6 mM saccharin, or to 0.025, 0.25, 2.5, or 25 mM sucralose ($P > 0.05$; Figures 7A−7D). Chickens slightly rejected 10 mM aspartame ($P < 0.05$; Figure 7B).

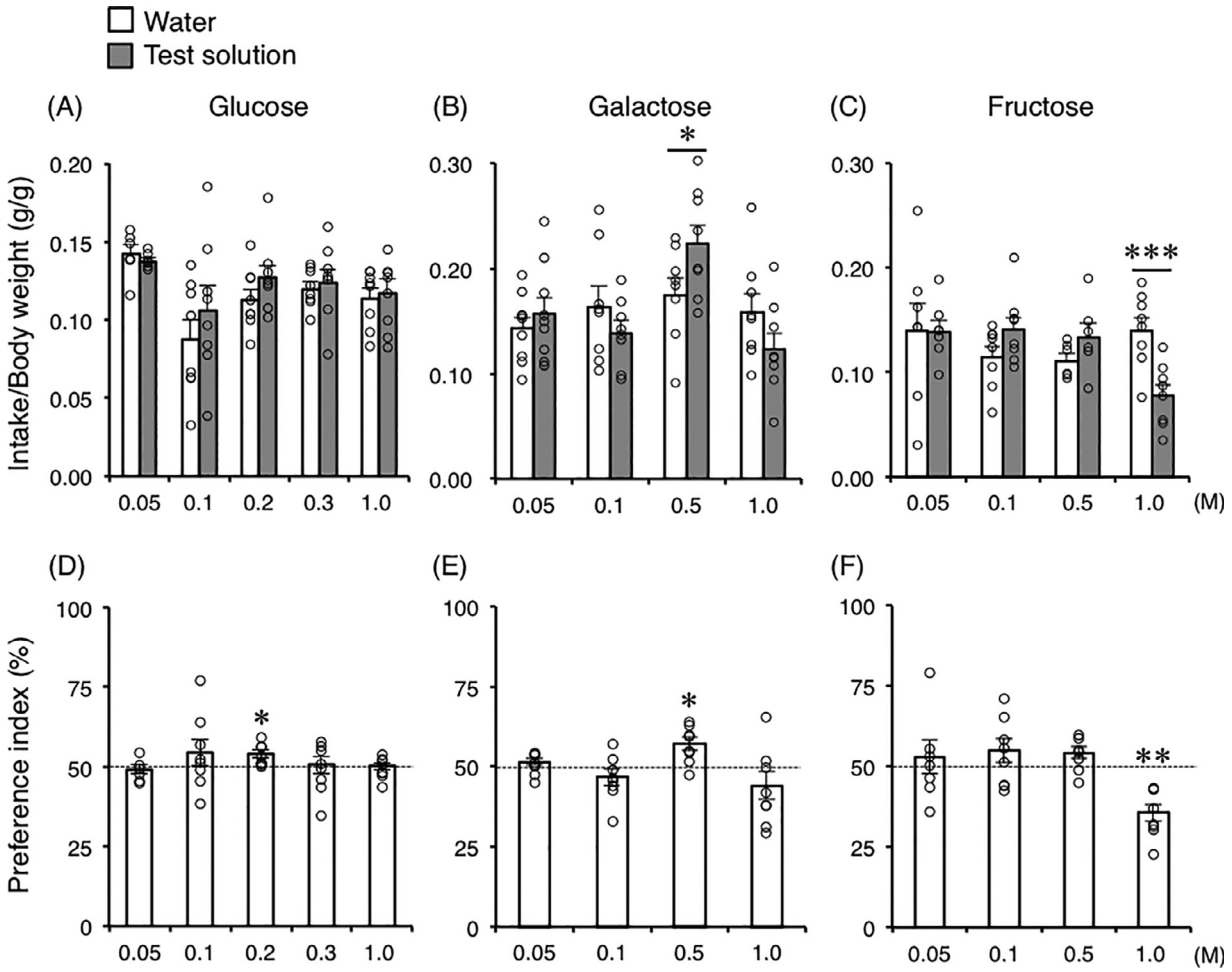

Figure 2. Behavioral responses to monosaccharides in chickens. (A–C) Water intake / body weight (BW) (white bar) and test solution intake / BW (gray bar) were compared. We used 0.05, 0.1, 0.2, 0.3, and 1.0 M glucose (A), 0.05, 0.1, 0.5, and 1.0 M galactose (B), and 0.05, 0.1, 0.5, and 1.0 M fructose (C) as the test solutions. (D–F) Preference indexes for the corresponding concentrations of glucose (D), galactose (E), and fructose (F) are shown. Values are the means ± SE (n = 6–9). $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ by paired *t* test, compared to water intakes.

GLP-1 Content and mRNA Expression of GLP-1 Receptor in the Oral Tissues of Chickens

We found that GLP-1 was abundantly present in the palate (more than 15 ng/mg tissue), compared to the tongue tip in chickens (Figure 8A). We also found that mRNA of *GLP-1R* was more highly expressed in the palate than in the tongue tip (Figure 8B).

DISCUSSION

In the present study, we focused on the T1R2-independent sweet taste-sensing systems in chickens, which lack the *T1R2* gene in their genome (Shi and Zhang, 2006). Previously, it was suggested that the key molecules for monosaccharide detection, such as GLUTs, SGLT1, and K_{ATP} channels, are expressed in the taste buds in mice (Yee et al., 2011), and that gustatory neural responses to glucose are inhibited by an SGLT1 inhibitor, phloridzin, in mice (Yasumatsu et al., 2020). Here, we found that mRNAs of GLUTs (*GLUT8* and *GLUT9*), *SGLT1*, and K_{ATP} channel subunits (*Kir6.1, Kir6.2, SUR2A, SUR2B*, and *SUR1* variants) are expressed in the palate of chickens as well as in other tissues (gizzard, liver, pancreas, kidney, and muscle). We also found that chickens have a slight preference for monosaccharides, such as glucose and galactose, and the preference for galactose is inhibited by an SGLT1 inhibitor, phloridzin. These results imply that chickens can sense monosaccharides through the glucose transporters and K_{ATP} channels.

We found that chickens have a slight preference for disaccharides, including sucrose, maltose, and lactose, and rejected high concentrations of sucrose. These results are consistent with reports showing that chickens have a preference for 5% sucrose (Gentle, 1972) and that chickens reject high concentrations of sucrose (Cheled-Shoval et al., 2017). Although monosaccharides such as glucose and galactose are the substrates for the transporters, a previous study suggested that polysaccharide- and disaccharide-digesting enzymes are expressed in the taste buds and are involved in disaccharide taste detection for digesting disaccharide to monosaccharide in mice (Sukumaran et al., 2016). Here, we found expressions of the polysaccharide- and disaccharide-digesting enzymes, including *AMY2A, MGAM*, and *SI,* in chicken

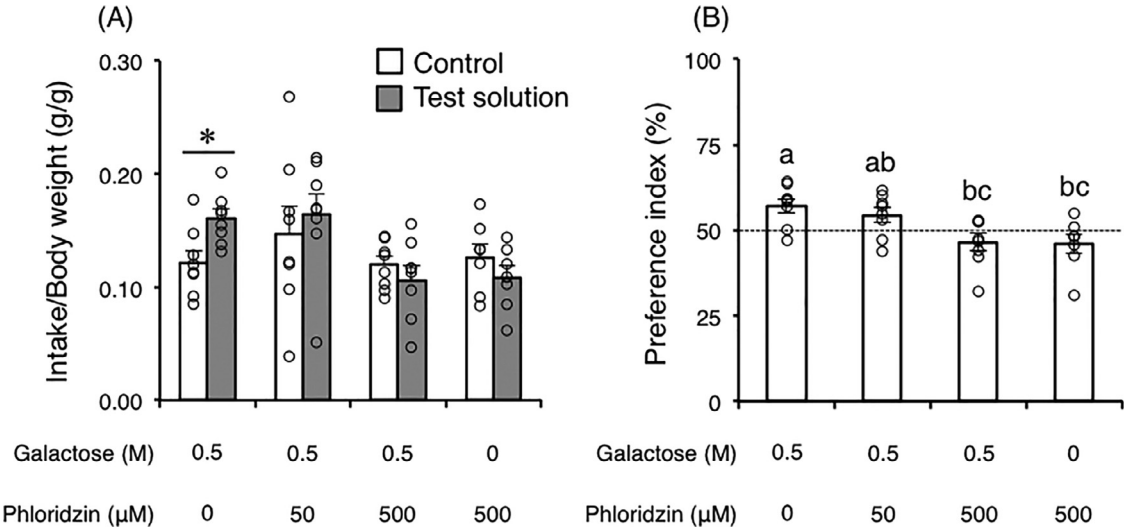

Figure 3. The effect of an SGLT1 inhibitor, phloridzin, on the preference for galactose in chickens. (A) Control solution intake / BW (white bar) and test solution intake / BW (gray bar) were compared. 0.2% ethanol solution was used as a control solution, and 0.2% ethanol solutions containing 0.5 M galactose alone, 0.5 M galactose with 50 μM phloridzin, 0.5 M galactose with 500 μM phloridzin, or 500 μM phloridzin alone as the test solutions. (B) Preference indexes for the test solutions are shown. Values are the means ± SE (n = 7−8). $^{*}P < 0.05$ by paired t test, compared to control solution intake. Bars without a common letter differ significantly; $P < 0.05$ by one-way factorial ANOVA followed by post-hoc Tukey HSD test.

oral tissues, implying that chickens can sense disaccharides using the disaccharide-digesting enzymes. Previously, it was also suggested that T1R3 alone is activated by high concentrations of sucrose and maltose (Zhao et al., 2003). Our previous report suggested that T1R3 is expressed in the taste cells of chickens (Yoshida et al., 2021a). And, we also confirmed the RNA transcript of *T1R3* in the palate as well as in other

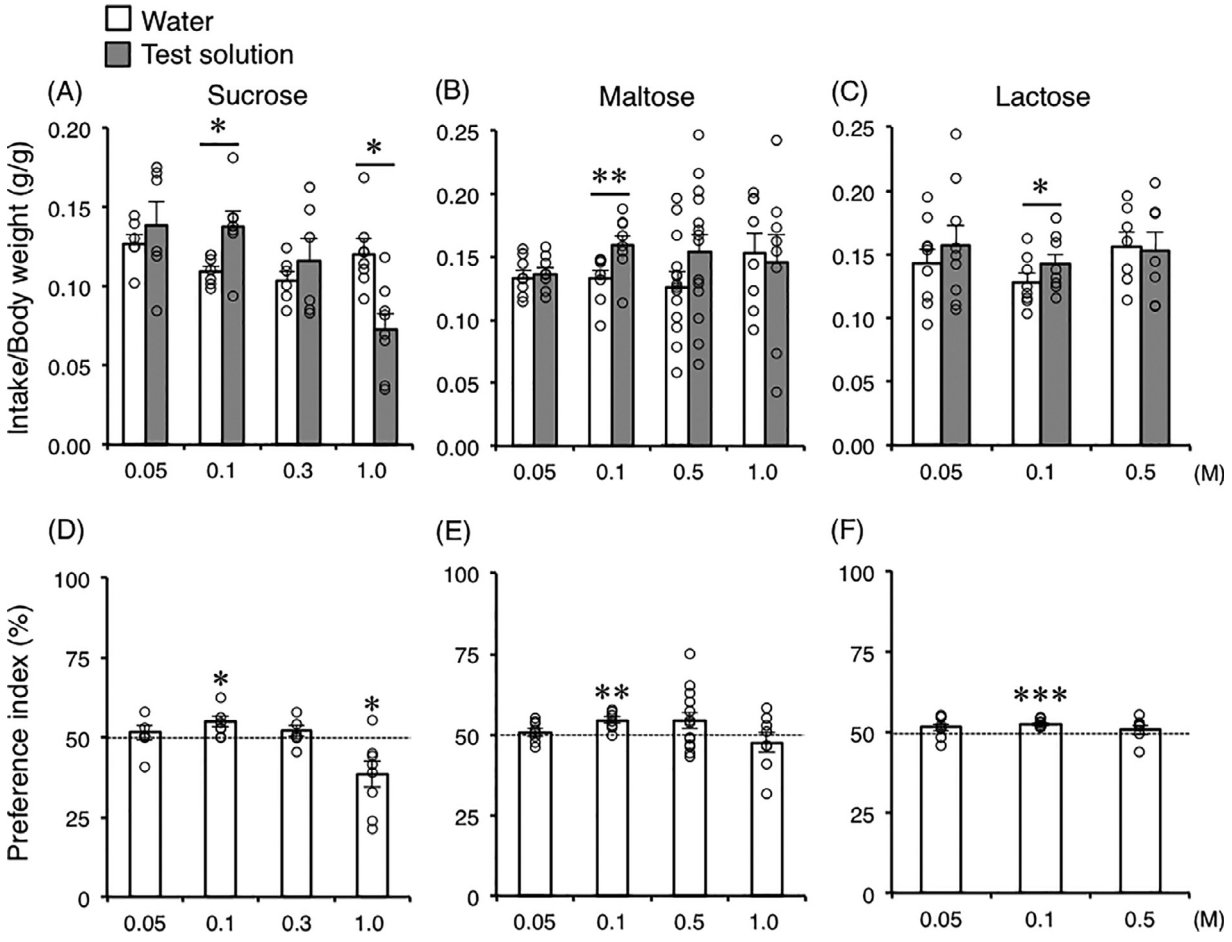

Figure 4. Behavioral responses to disaccharides in chickens. (A−C) Water intake / BW (white bar) and test solution intake / BW (gray bar) were compared. We used 0.05, 0.1, 0.3, and 1.0 M sucrose (A), 0.05, 0.1, 0.5, and 1.0 M maltose (B), and 0.05, 0.1, and 0.5 M lactose (C), as the test solutions. (D−F) Preference indexes for the corresponding concentrations of sucrose (D), maltose (E), and lactose (F) are shown. Values are the means ± SE (n = 6−14). $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.01$ by paired t test, compared to water intakes.

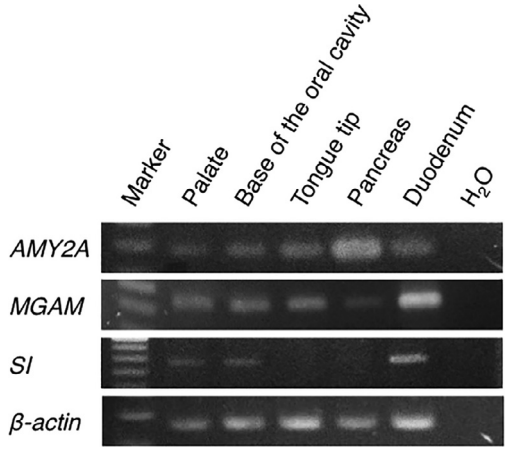

Figure 5. RNA transcripts of the polysaccharide-digesting enzyme *AMY2A*, disaccharide-digesting enzymes *MGAM* and *SI,* and *β-actin* in the chicken palate, base of the oral cavity, tongue tip, pancreas, and duodenum. No bands were observed in the negative controls without templates (H_2O).

tissues (gizzard, liver, pancreas, kidney, and muscle) in the present study. Therefore, the preference and aversion to sucrose and maltose observed in the present study may have been mediated by T1R3 expressed in the taste cells, in addition to the glucose transporters and K_{ATP} channels.

Here, we found that chickens do not show any preference to the sugar alcohol sorbitol or the artificial sweeteners acesulfame K, aspartame, saccharin, and sucralose although the preference ratio of 10 mM aspartame was slightly decreased. A previous report suggested that sorbitol can bind to the T1R2 subunit of the heterodimer of T1R2/T1R3, rather than the T1R3 subunit (Mahalapbutr et al., 2019). Another previous report suggested that *T1R2*-knockout mice show residual gustatory neural responses to sugars, but the responses to saccharin and acesulfame K are terminated (Zhao et al., 2003). The binding site of aspartame is also T1R2 in human sweet taste receptor (Maillet et al., 2015). Therefore, it is reasonable that chickens showed no responses to these sweeteners, because chickens lack the sweet taste receptor subunit T1R2. On the other hand, chickens slightly preferred stevioside solution. It was suggested that stevioside potentiates the activity of TRPM5 (Philippaert et al., 2017), which plays a major

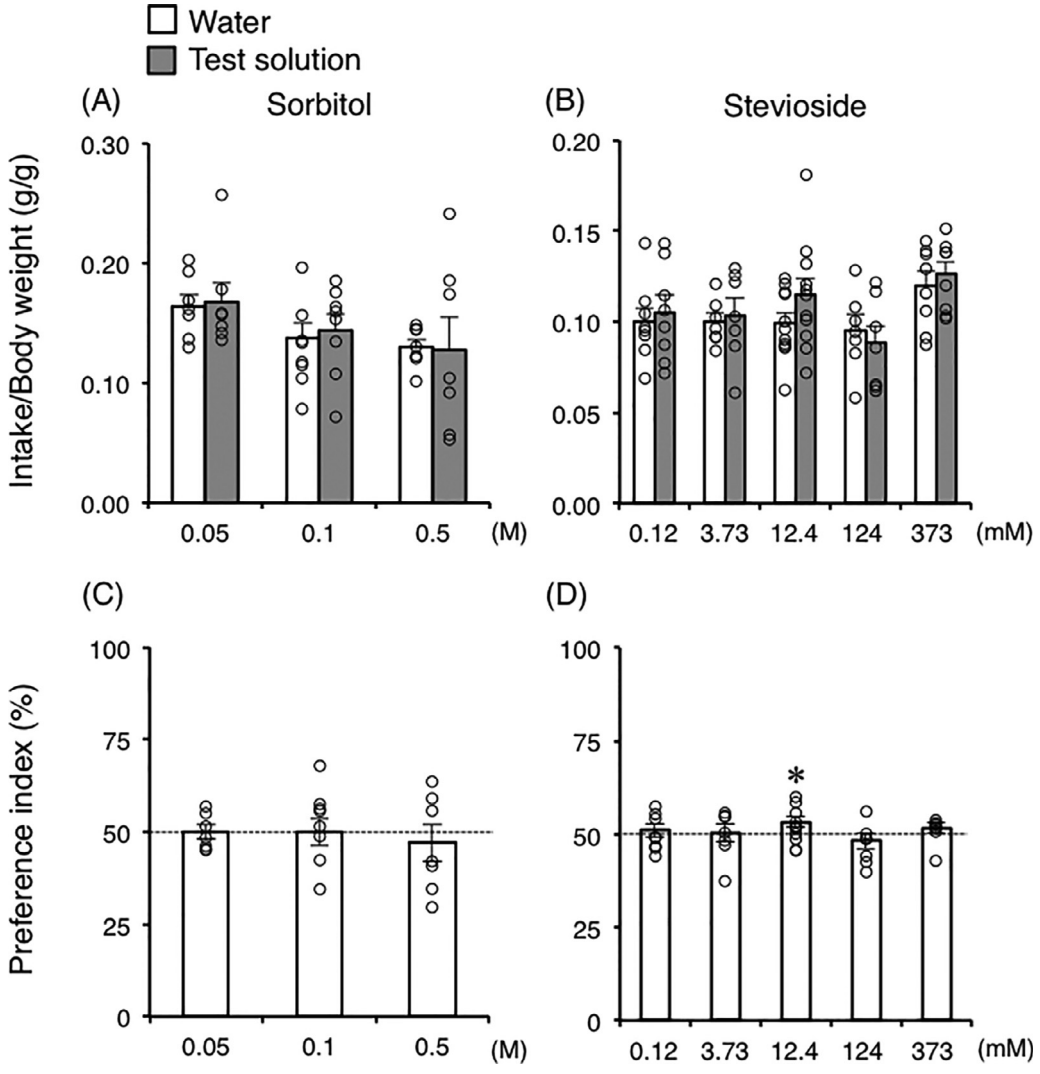

Figure 6. Behavioral responses to the sugar alcohol sorbitol and the natural sweetener stevioside in chickens. (A, B) Water intake / BW (white bar) and test solution intake / BW (gray bar) were compared. We used 0.05, 0.1, and 0.5 M sorbitol (A) and 0.12, 3.73, 12.4, 124, and 373 mM stevioside (B) as the test solutions. (C, D) Preference indexes for the corresponding concentrations of sorbitol (C) and stevioside (D) are shown. Values are the means ± SE (n = 7–11). $^{*}P < 0.05$ by paired *t* test, compared to water intake.

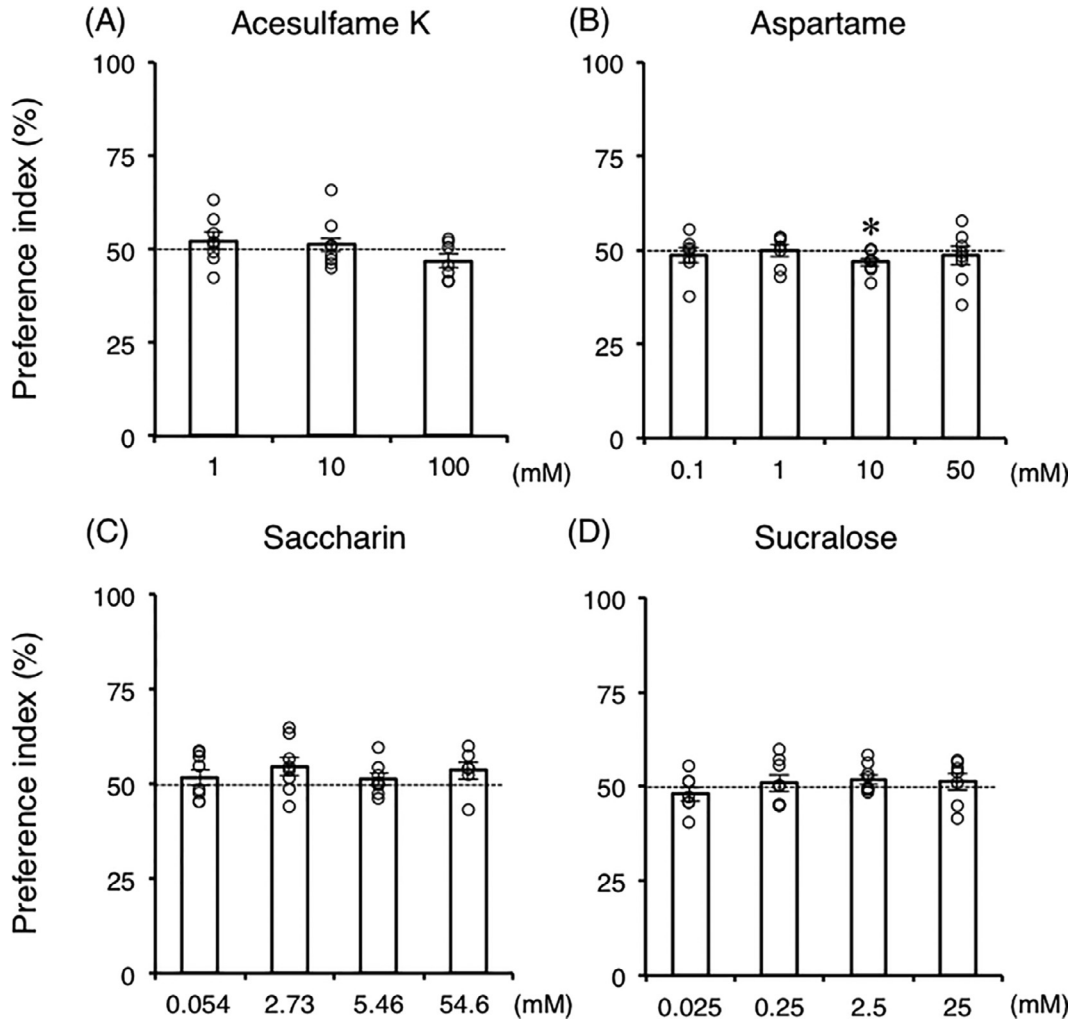

Figure 7. Behavioral responses to the artificial sweeteners acesulfame K, aspartame, saccharin, and sucralose in chickens. (A−D) Preference indexes for 1, 10, and 100 mM acesulfame K (A), 0.1, 1, 10, and 50 mM aspartame (B), 0.054, 2.73, 5.46, and 54.6 mM saccharin (C), and 0.025, 0.25, 2.5, and 25 mM sucralose (D) compared to water are shown. Values are the means ± SE (n = 6−8). $^{*}P < 0.05$ by paired t test, compared to water intake.

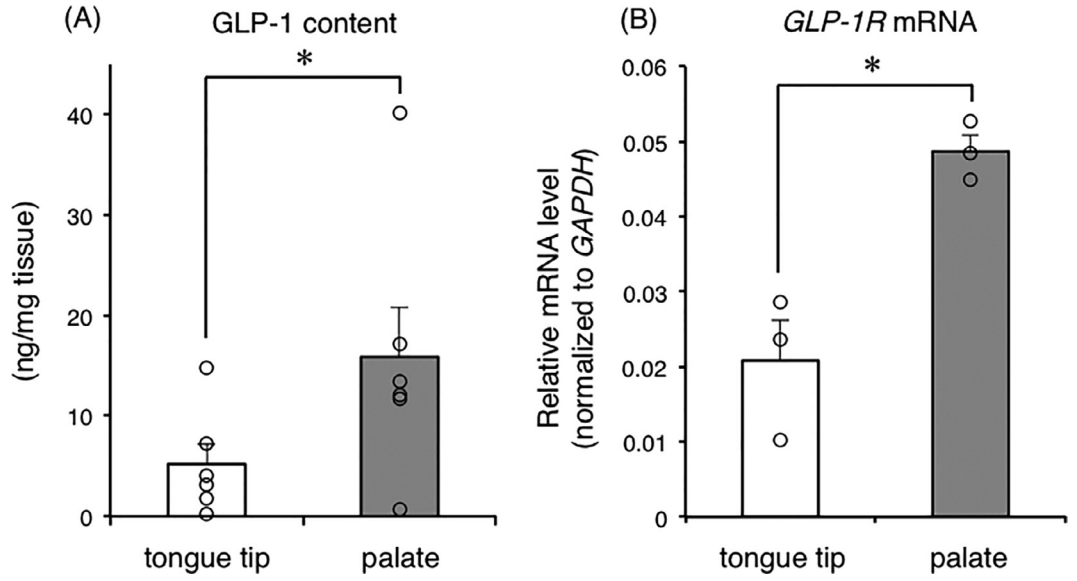

Figure 8. Measurement of GLP-1 content and mRNA of *GLP-1R* in chicken oral tissues. (A) GLP-1 contents (ng/mg tissue) in chicken palate and tongue tip are shown. Values are the means ± SE (n = 6). There are significant differences between two tissues ($P = 0.029$ by paired t test). $^{*}P < 0.05$. (B) The relative mRNA levels of *GLP-1R* in the chicken palate and tongue tip are shown. Values are the mean relative mRNA levels (normalized to *GAPDH*) ± SE (n = 3). There are significant differences between two tissues ($P = 0.023$ by paired t test). $^{*}P < 0.05$.

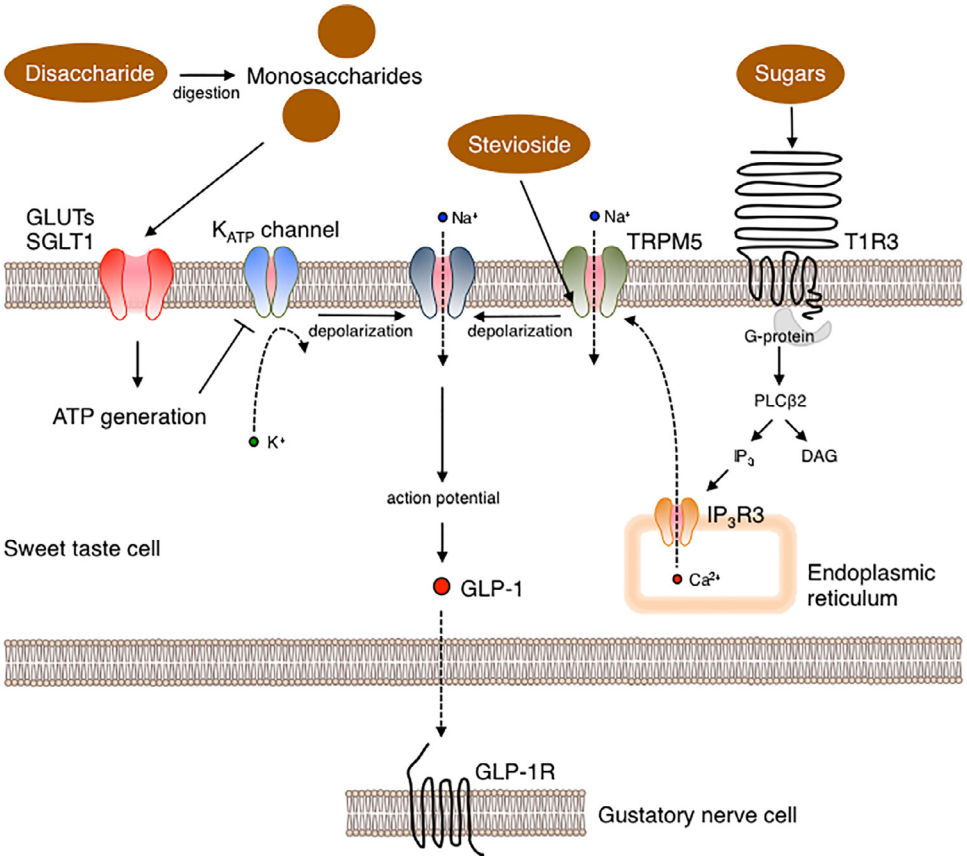

Figure 9. Summary of the possible mechanisms of T1R2-independent sweet-sensing pathways in chickens. Monosaccharides are the substrates for the GLUTs and SGLT1. ATP, generated by transported monosaccharides, leads to the closure of K_{ATP} channels and causes membrane depolarization. Disaccharides can be digested by disaccharide-digesting enzymes to monosaccharides, which can be transported by GLUTs and SGLT1. T1R3 is activated by high concentrations of sucrose and maltose, and the activation of T1R3 induces a cytosolic Ca^{2+} increase. TRPM5 is activated by cytosolic Ca^{2+} and induces membrane depolarization. TRPM5 activity is potentiated by stevioside. Membrane depolarization induced by these T1R2-independent pathways triggers a neurotransmitter, GLP-1, release to GLP-1R expressed in afferent taste nerves. Abbreviations: DAG, diacylglycerol; IP_3, inositol triphosphate; IP_3R3, inositol triphosphate receptor type 3; PLCβ2, phospholipase Cβ2.

role in sweet taste transduction (Zhang et al., 2007). We previously reported that *TRPM5* is expressed in the palate (Yoshida et al., 2018b). Furthermore, we also confirmed the RNA transcript of *TRPM5* in the palate as well as in other tissues (gizzard, liver, pancreas, kidney, and muscle) in the present study. Thus, these results imply that chickens can respond to stevioside through the potentiation of TRPM5 activity.

A previous study suggested that *GLP-1R* knockout mice show reduced gustatory neural and behavioral responses to sweet stimuli without any effect on the other taste stimuli (Takai et al., 2015). The same study also suggested that GLP-1 and GLP-1R are expressed in the taste cells and gustatory neurons, respectively, and that GLP-1 is secreted from taste cells in response to sweet stimuli, suggesting that GLP-1 plays a role in the neurotransmission of sweet stimuli (Takai et al., 2015). The present study suggests that GLP-1 and the mRNA of *GLP-1R* are abundantly present in the palate, where taste buds are mainly localized (Kudo et al., 2008), compared to the tongue tip. The results imply that sweet-specific neurotransmission by GLP-1 can be conserved in the chicken taste organs. Although gustatory neurotransmission has not been investigated in chickens, our previous report showed the localization of synaptosome-associated protein 25 and neural cell adhesion molecules, which are involved in neurotransmission in mammalian taste systems, in chicken taste cells (Yoshida et al., 2021b). Further analyses will be needed to fully elucidate the mechanisms of sweet taste transmission in chickens.

In conclusion, the present study demonstrated that chickens have a slight preference for monosaccharides, disaccharides, and stevioside, but not for artificial sweeteners. We also found that glucose transporters, K_{ATP} channels, polysaccharide- and disaccharide-digesting enzymes, GLP-1, and *GLP-1R* are present in chicken oral tissue, and that phloridzin inhibits the preference for galactose in chickens. The possible mechanisms of chicken sweet-sensing systems are summarized in Figure 9. Taken together, the results of the present study provided evidence that chickens can sense sweet taste via T1R2-independent sweet-sensing pathways.

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DISCLOSURES

The authors declare no conflict of interest.

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