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The role of G-protein-coupled receptor 120 in fatty acids sensing in chicken oral tissues

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ABSTRACT

Clarification of the mechanism of chickens' taste sense will provide meaningful information for creating and improving new feedstuff for chickens, because the character of taste receptors in oral tissues affects feeding behavior in animals. Although fatty acids are partly recognized via G-protein coupled receptor 120 (GPR120) for fat taste in mammalian oral tissues, the fat taste receptor of chickens has not been elucidated. Here we cloned chicken *GPR120* (*cGPR120*) from the chicken palate, which contains taste buds. By using Ca^{2+} imaging methods, we identified oleic acid and linoleic acid as cGPR120 agonists. Interestingly, in a behavioral study the chickens preferred corn oil-rich feed over mineral oil (control oil)-rich feed. Because corn oil contains high amounts of oleic acid and linoleic acid, this result was thought to be reasonable. Taken together, the present results suggest that cGPR120 is one of the functional fat taste receptors 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]. The recognition of chicken taste sense offers opportunities to create and improve the feedstuffs of chickens, and to contribute to the understanding of mammalian taste senses in comparisons with the functioning of mammalian taste receptors. In mammals, there are five basic tastes: sweet, bitter, umami, sour, and salty. However, in chickens, although there are four taste receptor genes (for bitter, umami, sour, and salty), the *T1R2* gene, which is for the sweet taste receptor, is absent [1].

We revealed that almost all of the taste buds of chicken exist in the palate and the floor of oral cavity rather than on the tongue [2], and we found that gustducin, which is a specific G protein in a subset of taste cells, is expressed in taste buds in chickens [3]. In another study our findings elucidated that the chicken strain which has a lower number of taste buds compared to other strains has lower sensitivity for bitter taste than the other strains [4]. We also observed that the primary culture cells of isolated taste buds from the chicken palate show sensitivity to bitter and umami tastants, and these tastants increased the intracellular calcium concentration of these primary culture cells [5].

It was reported that bitter taste receptors, i.e., T2Rs, and umami taste receptor, T1R1/T1R3, were cloned in chicken, and some agonists of these receptors were identified [6,7]. Although it is thought that these taste receptors expressed in chicken taste buds sense tastants, it is assumed that there are a number of unknown taste receptors in chickens. In the present study, we focused on fat-taste receptors in chickens. Although fat taste is not among the five basic tastes, it is an important taste to detect fatty acids, which are necessary nutrients for animals.

G-protein-coupled receptor 120 (GPR120) is one of the fatty acid receptors [8], and it is expressed in oral taste cells of mammals [9–11]. In mice, taste preference for linoleic acid and oleic acid is partly mediated by GPR120 [12]. In chickens, although it has been reported that gustation has important roles in the determination of oil preference [13], the detection mechanisms of fat taste have not

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been elucidated. The purpose of the present study was to clone the chicken fat-taste receptor GPR120 and analyze its functions.

2. Materials and methods

2.1. Chemicals

Oleic acid and linoleic acid were purchased from Sigma–Aldrich (St. Louis, MO, USA), dissolved in dimethyl sulfoxide (DMSO) and stored at −20 °C. The DMSO stock was diluted with a standard bath solution containing 140 mM NaCl, 5 mM KCl, 2 mM $MgCl_2$, 2 mM $CaCl_2$, 10 mM HEPES and 10 mM glucose at pH 7.4, adjusted with NaOH just before each experiment.

2.2. Animals

Rhode Island Red strain 0-wk-old chicks were used for this research. The study was 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. Chicken GPR120 construct

Total RNA was isolated from the palate of chicks, and first-strand cDNA was synthesized by reverse transcription. Then, deduced open reading frames (ORFs) of chicken *GPR120* (*cGPR120*) were amplified and sequenced. The polymerase chain reaction (PCR) primers were designed based on the NCBI nucleotide database of the *cGPR120* (XM_003641481.2). The primers were designed as exon-spanning. The PCR products of the ORFs were subcloned into the pcDNA3.1(+) mammalian expression vector by using the In-Fusion HD Cloning Kit (Takara Bio, Otsu, Japan). The entire sequence of *cGPR120* was confirmed.

2.4. Cell culture

Human embryonic kidney (HEK)-derived 293T (HEK293T) cells were maintained in Dulbecco's modified Eagle's medium (DMEM high glucose, Wako Pure Chemical Industries, Osaka, Japan) containing 10% fetal bovine serum (FBS, GE Healthcare, Buckinghamshire, UK), and Penicillin–Streptomycin Solution (×100) (Wako) at 37 °C in 5% CO_2.

2.5. Ca^{2+} imaging by confocal microscopy

For the Ca^{2+} imaging experiments, HEK293T cells were transfected with either empty vector pcDNA3.1(+) or cGPR120/pcDNA3.1 (+) by using Screen*Fect*™A (Wako) on coverslips coated by poly-D-lysine (0.1 mg/mL). After transfection, the cells were incubated for 48 h at 37 °C and 5% CO_2. Then, the cells were loaded with 1.25 μM Fluo 4-AM solution for 1 h at 37 °C and 5% CO_2 in the dark. Fluo 4-AM solution was prepared according to the manufacturer's manual (Dojindo Laboratories, Kumamoto, Japan).

The cover slips were washed with the standard bath solution, and Fluo-4 fluorescence was measured in the standard bath solution by using a confocal laser scanning microscope (Nikon A1R, Nikon Co., Tokyo). The coverslips were mounted in a chamber connected to a gravity flow system to deliver various stimuli. Chemical stimulation was applied by running a bath solution containing various chemical reagents. Cell viability was confirmed by responses to 5 μM ionomycin (Dojindo).

2.6. Dose response assay of Ca^{2+} imaging

For the dose–response assay of the intracellular Ca^{2+} concentration, we seeded cells in a 96-well clear bottom plate (Corning, Lenexa, KS, USA) coated with poly-D-lysine (0.1 mg/mL) after transfection. Forty-eight hours after transfection, we loaded 100 μL of 5 μM Fura 2-AM solution per well. Fura 2-AM solution was prepared according to the manufacturer's manual for the Calcium Kit II–Fura 2 (Dojindo). After incubation for 1 h in the dark at 37 °C, calcium imaging was done using an Infinite® 200 PRO microplate reader (Tecan Group, Männedorf, Switzerland). The assay was done at about 20 °C, and 30 μL of 100–3000 μM oleic acid or linoleic acid solution diluted by the standard bath solution was applied. Cell activity was analyzed by the differences between R_{max} (the max value of the ratio of fluorescence intensity at 340 nm and 380 nm) after stimulus and R_0 (average value of the ratio) before stimulus.

2.7. The two-feed choice test

Rhode Island Red strain chicks were raised in a box brooder (Showa Furanki, Saitama, Japan) under a 14:10 h light/dark cycle

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atggtcggggctgggtacacacaaggggagaacaagacttacttcccattcttctcagac   60
  M  V  G  A  G  Y  T  Q  G  E  N  K  T  Y  F  P  F  F  S  D

ttcaggggtggcaacgtgacggccctgcgtgtcggcgagtcgactgcactgggctctgtc  120
  F  R  G  G  N  V  T  A  L  R  V  G  E  S  T  A  L  G  S  V

ttcctgctggccttggtgggcaacatctggggcatctggctgctggtgtggaggcagcaa  180
  F  L  L  A  L  V  G  N  I  W  G  I  W  L  L  V  W  R  Q  Q

cggctgtgtgctgccaactaccttgtcctcaacctcttctgcgctgacctgctcttcatc  240
  R  L  C  A  A  N  Y  L  V  L  N  L  F  C  A  D  L  L  F  I

accgccatcccattcatcgccatcgtgcgctggaccgagacctgggtgctgggcgacgtc  300
  T  A  I  P  F  I  A  I  V  R  W  T  E  T  W  V  L  G  D  V

atctgccacatgctcttctatgtgatgaccctcagtggcactgttgtcattgtctctctc  360
  I  C  H  M  L  F  Y  V  M  T  L  S  G  T  V  V  I  V  S  L

tcggccgtcagcctggagagggtcatcagcatcgctcggctgcaccacactgccttccgc  420
  S  A  V  S  L  E  R  V  I  S  I  A  R  L  H  H  T  A  F  R

cgccgcaagctgctggctgctgcccttctcatctggggcttcgctgctatagtcaccctg  480
  R  R  K  L  L  A  A  A  L  L  I  W  G  F  A  A  I  V  T  L

ccactctgctgcttcttcactgtggtgcagctgcccagtgtcactggtgaggagattcac  540
  P  L  C  C  F  F  T  V  V  Q  L  P  S  V  T  G  E  E  I  H

atttgtactttggattggcccagccatgcaggagaaatagtctgggatgtgacctatgct  600
  I  C  T  L  D  W  P  S  H  A  G  E  I  V  W  D  V  T  Y  A

gttgctgtctttttaataccaggattaatcactgtcatcagttattccaaaatcttacag  660
  V  A  V  F  L  I  P  G  L  I  T  V  I  S  Y  S  K  I  L  Q

attacaaaagcatcaagaagaagtttaaatgctggattagcctactcagaaaatcatcag  720
  I  T  K  A  S  R  R  S  L  N  A  G  L  A  Y  S  E  N  H  Q

attcgtgtttcccagcaagactataaactattccgagccctcattgtgttgatgatttcc  780
  I  R  V  S  Q  Q  D  Y  K  L  F  R  A  L  I  V  L  M  I  S

ttcttcatcatgtggagcccaattatcataatttatttttttaattttaatccggaactac  840
  F  F  I  M  W  S  P  I  I  I  I  I  F  L  I  L  I  R  N  Y

aaacaagatttaaatattttgccatcagttttcttctggatcatgctattcacatttgcc  900
  K  Q  D  L  N  I  L  P  S  V  F  F  W  I  M  L  F  T  F  A

aactctgctgtcaacccagttttatataatgttgcccatttcagacgtaaatgtcaggaa  960
  N  S  A  V  N  P  V  L  Y  N  V  A  H  F  R  R  K  C  Q  E

attctcctctgttgtacagggaaccctgtaaggaccagggttggttcagaaacgtctgca 1020
  I  L  L  C  C  T  G  N  P  V  R  T  R  V  G  S  E  T  S  A

agaagtaaacgtgaacaacctaagttgtctgtcatcaccagataa  1065
  R  S  K  R  E  Q  P  K  L  S  V  I  T  R  *
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Fig. 1. Nucleotide and protein sequences of chicken GPR120. The cDNA sequence of cloned *cGPR120* matches that in the NCBI database (XM_003641481.2) except for three bases (gray box). We identified one amino acid of unknown region (gray box). Other protein sequence matches the NCBI protein database of cGPR120 (XP_003641529.2).