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Umami Taste Receptor Functions as an Amino Acid Sensor Via Gαs Subunit in N1E-115 Neuroblastoma Cells

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ABSTRACT

The sensing of the nutritional level of the body fluid is pivotal for maintaining homeostasis in animals. However, it is not yet understood how the cells detect nutritional levels. In the present study, we examined the function of umami taste receptor, which has a dimeric protein structure composed of Tas1r1 and Tas1r3, as amino acid sensor in the cells. We found that deprivation of amino acids induced neurite outgrowth in N1E-115 cells. The neurite outgrowth was inhibited by almost all of the amino acids tested. To investigate the involvement of the umami taste receptor, siRNA against each of Tas1r1 or Tas1r3 was administered, resulting in suppression of the inhibitory effects of amino acids on neurite outgrowth. In addition, inosine 5′-monophosphate, which potentiates the response to amino acids in the taste cells, enhanced the inhibitory effect of glutamine on neurite outgrowth. These results suggest that Tas1r1 + 3 functions as an amino acid sensor in N1E-115 cells. Because glutamine increased intracellular cAMP concentration, we investigated the involvement of the Gαs subunit of the heterotrimeric G protein in signal transduction. The treatments to inhibit the Gαs subunit significantly suppressed the increase of intracellular cAMP concentration induced by glutamine and the inhibitory effect of amino acids on neurite outgrowth. In addition, the reagents for increasing intracellular cAMP concentration inhibited neurite outgrowth induced by deprivation of amino acids. We concluded that Tas1r1 + 3 functions as an amino acid sensor and activates the intracellular signaling pathway through the Gαs subunit in N1E-115 cells. J. Cell. Biochem. 113: 1654–1662, 2012. © 2011 Wiley Periodicals, Inc.

KEY WORDS: AMINO ACIDS; G PROTEIN; Tas1r1, Tas1r3

Sensing the energy state of the body fluids is critical for maintaining the cellular homeostasis in animals. One of the mechanisms for detection of energy state is mediated by several hormones such as cholecystokinin, ghrelin, insulin, and leptin, which are secreted from peripheral organs including gut, stomach, pancreas, and adipose tissue, respectively [Dockray, 2009; Nikolopoulos et al., 2010; Sánchez-Lasheras et al., 2010]. Secretion of the hormones is dependent on the energy state and results in regulation of feeding behavior. Another mechanism of sensing energy state is by directly sensing energy substrates such as glucose and amino acids. Several cells such as glucose-responsive neurons in the area postrema have been shown to sense nutrient molecules [Funahashi and Adachi, 1993; Riediger et al., 2002]. However, the mechanism by which cells sense nutrient molecules remains to be elucidated.

The taste cells in the taste buds located on the tongue are examples of cells with sensitivity to nutrient molecules. Umami, the taste derived from amino acids, and sweet are especially important as taste sensations for energy substrates. Several molecules have been identified as umami taste receptors [Chaudhari and Roper, 2010]. The best understood receptor is Tas1r1 + 3, which is a heterodimeric receptor composed of Tas1r1 and Tas1r3 subunits coupled with the heterotrimeric G protein. Both of Tas1r1 and Tas1r3 are required for detection of amino acids because knockout mice of each molecule lack sensitivity to amino acids [Zhao et al., 2003].

Gustducin is a Gα subunit co-localized with Tas1r1 + 3 in almost all of the fungiform and palatal taste buds [Kim et al., 2003; Stone et al., 2007]. Transducin is one of the Gα subunits related to gustducin and is also expressed in taste buds. Glutamate decreases intracellular cAMP concentration in the taste cells [Abaffy et al., 2003; Trubey et al., 2006] probably via activation of phosphodiesterase (PDE), which hydrolyzes cAMP, by gustducin and/or transducin. The Gα pathway via gustducin and/or transducin plays an important role because the response to glutamate is greatly

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decreased in gustducin and/or transducin knock-out mice [He et al., 2004]. Gustducin and transducin are expressed in restrictive tissues such as the taste buds [McLaughlin et al., 1992], gastrointestinal tract [Wu et al., 2002], and retina [Lerea et al., 1986], indicating that the signal transduction from Tas1r1 + 3 via the Gα pathway may be also allowed in the restrictive cells. Otherwise, there is the possibility that the Gα subunits mediating umami signal transduction are not limited to gustducin or transducin because co-localization of gustducin with Tas1r1 + 3 is rare in circumvallate and folitate taste buds [Kusakabe et al., 1998; Sainz et al., 2007]. Thus, the Gα subunits other than gustducin or transducin may mediate signal transduction from Tas1r1 + 3.

In the present study, we found that a mouse neuroblastoma cell line, N1E-115 cells, formed neurites in amino acid-free culture media. The morphological changes were evaluated as the indicator to study the mechanism by which the cells detect amino acids. We demonstrate that the Gαs subunit, which is ubiquitously expressed and activates adenylate cyclase to produce cAMP, mediates Tas1r1 + 3 signal transduction in N1E-115 cells, and propose that Tas1r1 + 3 may function throughout the body as an amino acid sensor.

Materials and Methods

REAGENTS

Dulbecco's Modified Eagle Medium (DMEM) and glutamine-free DMEM were obtained from Invitrogen (CA). Amino acid-free DMEM were obtained from Cell Science & Technology Institute (Sendai, Japan). Nutrient-free DMEM was prepared according to the components of DMEM as follows: 1.8 mM $CaCl_2$, 0.248 μM $Fe(NO_3)_3$ $9H_2O$, 0.814 mM $MgSO_4$, 5.33 mM KCl, 110.34 mM NaCl, 0.906 mM NaH_2PO_4-H_2O, 0.0399 mM Phenol Red. The media containing an amino acid were prepared by the addition of each amino acid into amino acid-free DMEM. All of the amino acids and caffeine monohydrate were obtained from Wako Pure Chemical (Osaka, Japan). Dibutyryl adenosine-3′, 5′-cyclic-monophosphate (dbcAMP), inosine 5′-monophosphate disodium salt (IMP) and U-73122 were obtained from Sigma Aldrich (MO). NF449 was obtained from Merck (Darmstadt, Germany).

CELL CULTURE

N1E-115 cells were routinely maintained in DMEM containing 10% fetal bovine serum (FBS; Hyclone, UT), penicillin G sodium (1×10^5 U/L), and streptomycin sulfate (1×10^5 g/L) in 5% CO_2 at 37°C. The antibiotics were also added into the media used in each experiment.

COUNTING OF NEURITE BEARING CELLS

Numbers of neurite bearing cells are shown as a percentage of the 100 cells randomly selected in each well. The number of cells possessing neurites twice greater than the diameter of a cell body was assessed 3 h after culture in each medium. Each experiment was performed at least three times independently.

TRANSFECTION OF SMALL INTERFERING RNA

siRNAs against mouse Tas1r1 (Mm_Tas1r1_9228), Tas1r3 (Mm_Tas1r3_1168), and negative control siRNA (SIC-001) were obtained from Sigma Aldrich. N1E-115 cells were seeded on a 96-well culture plate to evaluate neurite formation (2×10^3 cells/well) or on a 35-mm dish for western blot analysis (3×10^4 cells/dish). The next day each siRNA was transfected into the cells using lipofectamine 2000 (Invitrogen) according to the manufacturer's protocol. After 48 h, neurite formation was tested or the cell lysates were collected for western blotting.

WESTERN BLOT ANALYSIS

The cells were solubilized in 100 μl of cell lysis buffer containing 20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM Na_2EDTA, 1 mM EGTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM Na_3VO_4, 1 μg/ml leupeptin, 1 mM phenylmethylsulfonyl fluoride, and 0.5% sodium deoxycholate. After 20 min on ice, the lysates were centrifuged at 10,000*g* for 5 min and the supernatants were collected. Protein concentration was determined using the protein assay kit II (Bio-Rad Laboratories, CA). Protein samples (50 μg) were boiled for 5 min in the SDS sample buffer containing 250 mM Tris-HCl (pH 6.8), 5% (w/v) SDS, 6% (v/v) β-mercaptoethanol, and 4% (v/v) glycerol, and then applied onto each lane of 10% SDS–PAGE gel, followed by transfer onto nitrocellulose membranes (Bio-Rad Laboratories) at 4°C in 25 mM Tris-HCl (pH 8.4), 192 mM glycine, 20% methanol, and 0.025% SDS. The membranes were blocked in Blockase (DS Pharma Biomedical, Osaka, Japan) dissolved with Tris-buffered saline containing 0.1% (v/v) Tween 20 (TBST) at room temperature for 1 h and then incubated with a rabbit polyclonal antibody to Tas1r1 (1:3000, OSR00182W, Osenses, SA, Australia), Tas1r3 (1:3000, ab74732, Abcam, Cambridge, UK) or mouse glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (1:3000, 6G7, Funakoshi, Tokyo, Japan) at 4°C overnight. After incubation, the membranes were washed three times in TBST, and incubated in donkey anti-rabbit (Promega, WI) or anti-mouse IgG-conjugated with HRP (1:3000, Promega) at room temperature for 1 h. After washing the membrane three times in Tris-buffered saline (TBS), the bands were identified using Lumi-Light Western bloting substrate (Roche Applied Science, IN), followed by detection with LAS3000 (Fuji Photo Film, Tokyo, Japan).

RT-PCR

Total RNAs were extracted from N1E-115 cells or the tongue from BALB/cAJcl (CLEA Japan, Tokyo, Japan) using TRIZOL Reagent (Invitrogen). Mice were maintained in accordance with the guidelines of the NIH (Guide for the Care and Use of Laboratory Animals, 1996) and all procedures were approved by the Animal Research Committee of the Obihiro University of Agriculture and Veterinary Medicine. One microliter of total RNA sample (250 ng/μl) was reverse-transcribed using an oligo dT primer and AMV reverse transcriptase with a Takara RNA LA PCR™ Kit (AMV) v.1.1 (Takara, Kyoto, Japan) in a 20 μl reaction volume according to the manufacturer's instructions. Two microliters of the cDNA reaction mixture were subjected to PCR using 0.5 units of ExTaq HS polymerase (Takara) in a final volume of 20 μl and PCR amplification was carried out in MJ Mini (Bio-Rad Laboratories).

The thermal profile for PCR was at 94°C for 10 min, followed by 40 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 30 s. Each primer was as follows: Gustducin (GenBank accession number: NM_001081143.1) (F: 5′-ggtggccagagatcagagag-3′, R: 5′-agaa-caatggaggtggttgc-3′), transducin (GenBank accession number: NM_008140.2) (F: 5′-cccgactacgatggacctaa-3′, R: 5′-cgcagtcttt-gaggttctcc-3′), GAPDH (GenBank accession number: NM_008084.2) (F: 5′-cccactaacatcaaatgggg-3′, R: 5′-ccttccacaatgccaaagtt-3′). Sequences of all PCR products were analyzed by an automatic DNA sequencer (ABI 3730xl, Applied Biosystems, CA).

PLASMID CONSTRUCTION AND TRANSIENT TRANSFECTION

The expression plasmid of 11 amino acids (QRMHLRQYELL) of the carboxy-terminal of the Gαs subunit was constructed according to the previous report with some modification [Gilchrist et al., 1999]. Briefly, the oligonucleotide encoding the 11 amino acids were synthesized by greiner bio-one (Tokyo, Japan) with the kozak sequence and a methionine for translation initiation in the 5′-end. The sequences were as follow: F; 5′-gccaccatgcagcgcatgcatctccgc-caatacgagctgctctaa-3′, R: 5′-ttagagcagctcgtattggcggagatgcatgc-gctgcatggtggc-3′ (the sequence encoding the 11 amino acids is

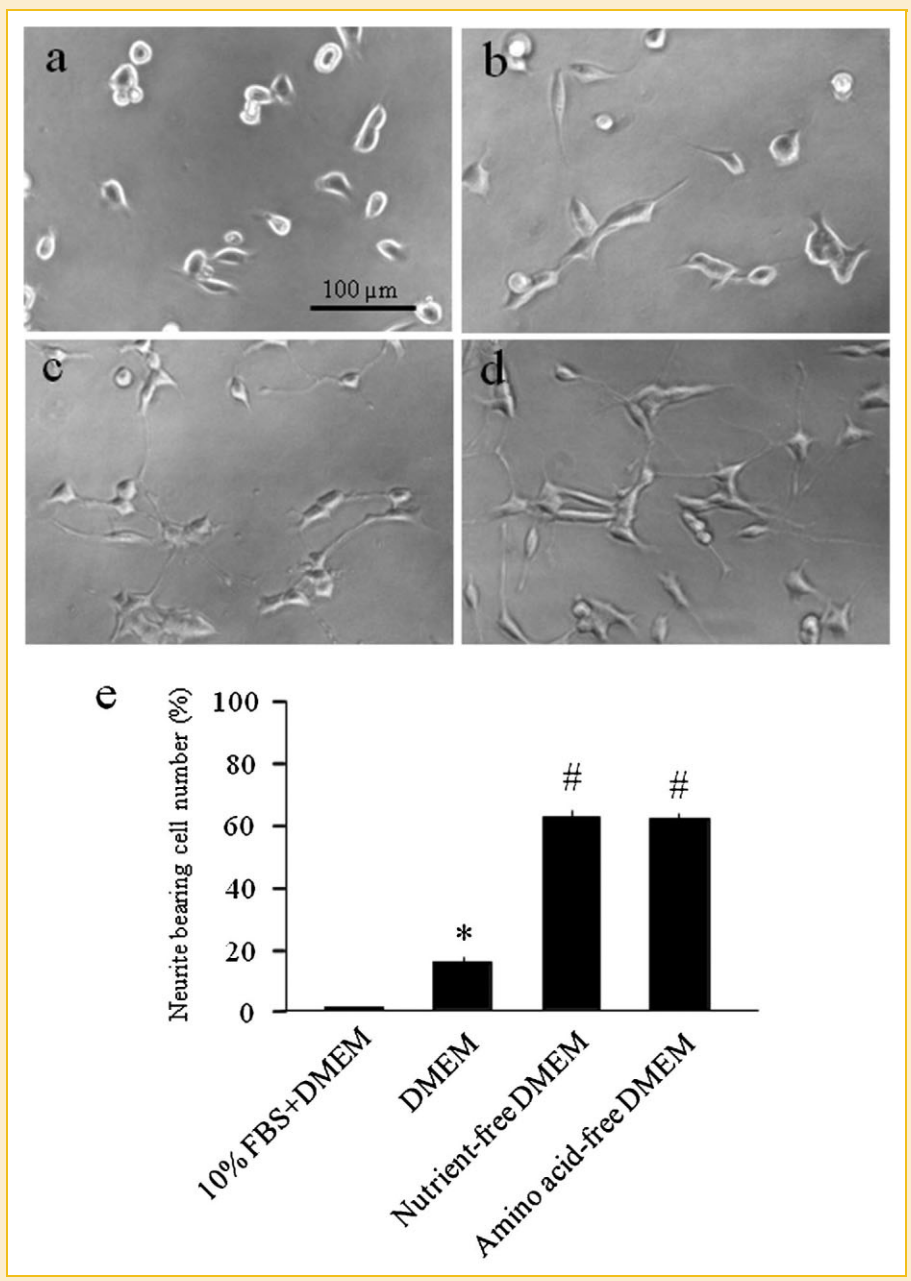

Fig. 1. Deprivation of amino acids induces neurite outgrowth in N1E-115 cells. a–d: Representative photomicrographs of N1E-115 cells grown on DMEM containing 10% FBS (10%FBS + DMEM) (a), FBS-free DMEM (DMEM) (b), nutrient-free DMEM (c), and amino acid-free DMEM (d). Scale bar: 100 μm. e: The number of cells possessing neurites greater than twice the length of a cell body was assessed 3 h after cultures in each DMEM condition. Values are means ± S.E.M. of three separate experiments. $^{*}P < 0.05$, compared with 10%FBS + DMEM, $\#P < 0.05$, compared with DMEM.

underlined). After heating at 94°C, the mixture of the nucleotides was annealed at room temperature. After treatment with T4 polynucleotide kinase (Takara), the DNA fragment was subcloned into a eukaryotic expression vector pRC/CMV (Invitorgen), resulting in generation of pRC/CMV-C-terminal. The correct insertion was analyzed by an automatic DNA sequencer (ABI 3730×l).

N1E-115 cells were seeded on a 96-well culture plate (2×10^3 cells/well) to evaluate neurite formation or on a 35-mm dish (3×10^4 cells/dish) for the cAMP assay. Next day the expression plasmid was transfected into the cells using lipofectamine 2000 according to the manufacturer's protocol. After 48 h, each test was performed.

cAMP ASSAY

The cAMP concentration was determined using a Cyclic AMP EIA Kit (Cayman Chemical Company, MI). In the experiments presented in Figure 4b and Figure 5a, 30,000 cells were seeded in DMEM containing 10% FBS on a 35-mm dish 1 day before the test. In Figure 4b, the cells were grown in amino acid-free DMEM for 60 min and then the samples were collected 30 min after addition of 4 mM glutamine. In Figure 5a and Figure 6a, the samples were collected 30 min after the culture in amino acid-free DMEM with or without 4 mM of glutamine and/or 10 μM NF449. The collected samples were subjected to enzyme-linked immunosorbent assay after acetylation according to the manufacturer's protocol. The absorbance was measured at a wavelength of 415 nm.

STATISTICAL ANALYSIS

The data presented in Figure 4b and the lower graphs of Figure 5b were analyzed using Student's *t*-test. The other data were analyzed using a one-way analysis of variance, followed by a Tukey–Kramer test. A P-value < 0.05 was considered statistically significant.

Results

N1E-115 cells exhibit neurite outgrowth in response to serum deprivation [Ishii et al., 2001]. Serum is one of the nutrient-rich supplements for cultured cells. We tested the possibility that N1E-115 cells may change morphology depending on nutritional conditions. N1E-115 cells grown under nutrient-free conditions where the medium contains inorganic salts without FBS and other nutrients such as glucose and amino acids (described in Materials and Methods section), formed more neurites within 3 h compared with the cells grown in serum-free DMEM (Fig. 1). To determine which nutrients affected morphological changes, N1E-115 cells were grown under conditions lacking specific nutrients. We found that deprivation of amino acids causes neurite outgrowth (Fig. 1). The neurite outgrowth reached the peak 3 h after deprivation of amino acids (Supplementary Fig. 1). The neurites were stained with an antibody to microtubule associated protein 2, which is a marker protein of neurites (Supplementary Fig. 2).

To investigate which amino acids inhibit neurite outgrowth, N1E-115 cells were grown in media containing each amino acid in DMEM. In the cultures where the concentration of each amino acid is matched to the concentration in DMEM (L-arginine; 0.398 mM, L-cystine; 0.201 mM, L-glutamine; 4 mM, Glycine; 0.4 mM, L-histidine; 0.2 mM, L-isoleucine; 0.802 mM, L-leucine; 0.802 mM, L-lysine; 0.798 mM, L-methionine; 0.201 mM, L-phenylalanine; 0.4 mM, L-serine; 0.4 mM, L-threonine; 0.798 mM, L-tryptophan; 0.0784 mM, L-tyrosine; 0.398 mM, L-valine; 0.803 mM), glutamine alone inhibited neurite outgrowth induced by deprivation of amino acids (Fig. 2a). To confirm whether metabolism of glutamine is required for inhibition of neurite outgrowth, we evaluated the effect of glutamic acid and ammonia, which are the first metabolites of

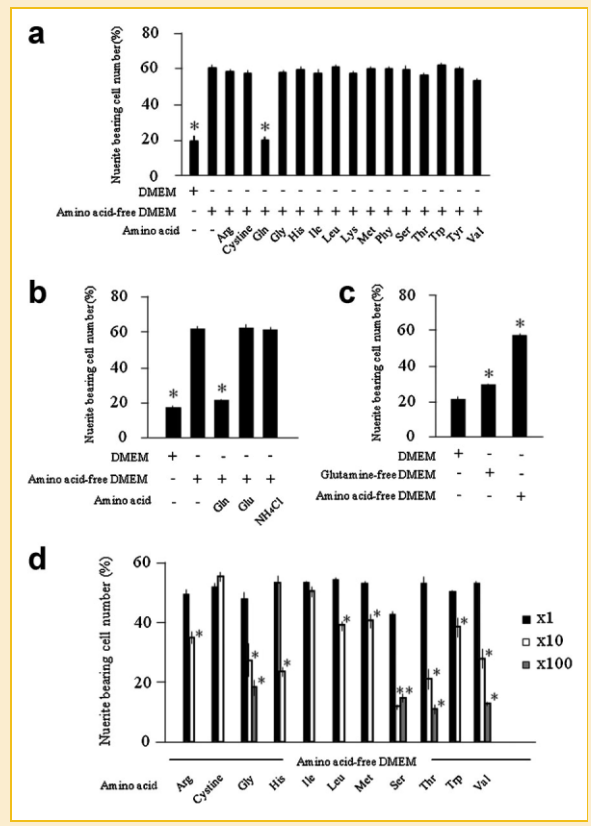

Fig. 2. Each amino acid inhibits neurite outgrowth induced deprivation of amino acids. The number of cells possessing neurites greater than twice the length of a cell body was assessed 3 h after culture in each medium. Values are the means ± S.E.M. of three separate experiments. a: The comparison of the number of cells bearing neurites in DMEM or amino acid-free DMEM added with or without each amino acid at the following concentration (in mM): Arginine (Arg); 0.398, Cystine; 0.201, Glutamine Gln); 4, Glycine (Gly); 0.4, Histidine (His); 0.2, Isoleucine (Ile); 0.802, Leucine (Leu); 0.802, Lysine (Lys); 0.798, Methionine (Met); 0.201, Phenylalanine (Phy); 0.4, Serine (Ser); 0.4, Threonine (Thr); 0.798, Tryptophan (Trp); 0.0784, Tyrosine (Tyr); 0.398, Valine (Val); 0.803. The concentration is consistent with that in DMEM. $^*P < 0.05$, compared with amino acid-free DMEM. b: The comparison of the number of cells bearing neurites in DMEM or amino acid-free DMEM added with or without 4 mM of glutamine, glutamic acid or NH_4Cl. $^*P < 0.05$, compared with amino acid-free DMEM. c: The comparison of the number of cells bearing neurites in DMEM, glutamine-free DMEM, or amino acid-free DMEM. $^*P < 0.05$, compared with DMEM. d: The comparison of the number of cells bearing neurites in amino acid-free DMEM added with each amino acid at 1-, 10-, or 100-fold concentration compared with those described in a. $^*P < 0.05$, compared with onefold concentration in each amino acid.

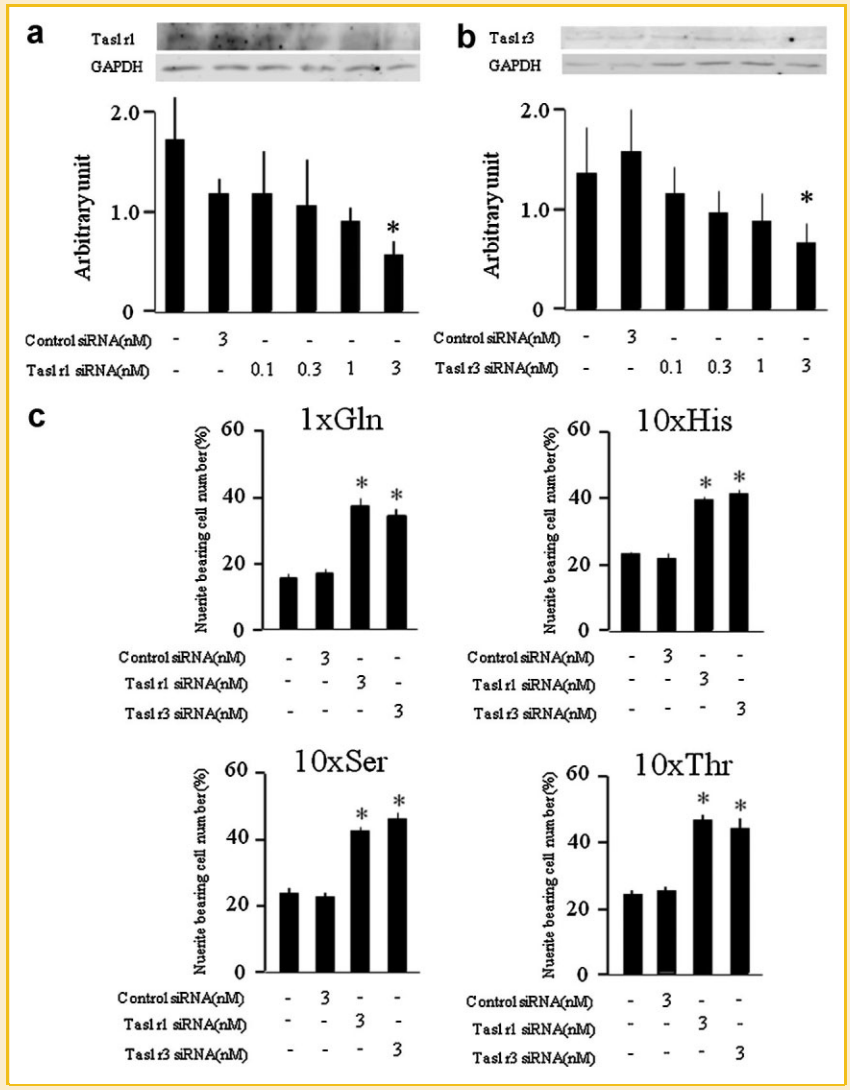

Fig. 3. Tas1r1 and Tas1r3 mediate amino acid-induced signal transduction. a and b: Western blot analysis of Tas1r1, Tas1r3, and GAPDH in N1E-115 cells transfected with negative control siRNA or each siRNA against Tas1r1 or Tas1r3 at the indicated concentration. The images show the representative result of each analysis (upper; Tas1r1 or Tas1r3, lower; GAPDH). The data were analyzed by ImageJ software (NIH). Expression levels of Tas1r1 or Tas1r3 normalized by GAPDH are presented as means ± S.D. from four independent experiments. c: The effects of the siRNA against Tas1r1 or Tas1r3 on inhibition of neurite outgrowth by 4 mM glutamine (1×Gln), 2 mM histidine (10×His), 4 mM serine (10×Ser), or 7.98 mM threonine (10×Thr). $^{*}P < 0.05$, compared with negative control siRNA.

glutamine. Neither glutamic acid nor ammonia inhibited neurite outgrowth induced by deprivation of amino acids (Fig. 2b). These results suggest that glutamine itself inhibited neurite outgrowth induced by deprivation of amino acids. To test whether lacking glutamine is sufficient for inducing neurite outgrowth, N1E-115 cells were grown in glutamine-free DMEM, resulting in the slight increase of neurites but less than those in amino acid-free DMEM (Fig. 2c). L-glutamine has the highest concentration of the amino acids contained in DMEM. We tested whether the other amino acids may also have inhibitory effects on neurite formation at higher concentrations. The data in Figure 2d show the effect of each amino acid on neurite outgrowth at 10× or 100× concentration compared with that in DMEM. The tests were performed at concentrations at which each amino acid is soluble and non-toxic. All of the amino acids tested except cystine inhibited neurite outgrowth induced by deprivation of amino acids (Fig. 2d). These results suggest that N1E-115 cells sense amino acids in the media to inhibit neurite outgrowth.

Next, we studied how the cells sense amino acids. To investigate whether Tas1r1 + 3 functions as an amino acid sensor, we used the siRNA against Tas1r1 or Tas1r3, which significantly decreased expression of Tas1r1 or Tas1r3 at 3 nM, respectively (Fig. 3a,b). Treatment with each siRNA at 3 nM suppressed the inhibitory effects of 4 mM L-glutamine, 2 mM L-histidine, 4 mM L-serine, and 7.98 mM L-threonine (Fig. 3c). In addition, we examined whether IMP affects the inhibitory effect of amino acids on neurite formation because IMP enhances cellular responses induced by amino acids via Tas1r1 + 3 [Nelson et al., 2002]. IMP elicited inhibitory effects of 40 μM L-glutamine though glutamine alone did not inhibit neurite outgrowth at the tested concentration (Supplementary Fig. 3). These

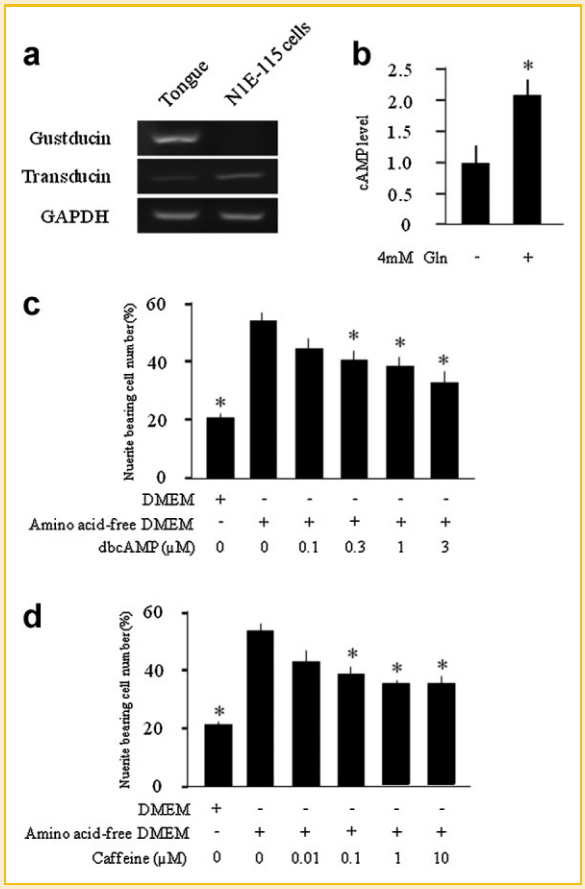

Fig. 4. cAMP inhibits neurite outgrowth induced by deprivation of amino acids. a: RT-PCR analysis of gustducin (upper), transducin (middle), and GAPDH (lower) mRNA in the tongue (left) and N1E-115 cells (right). b: Measurement of intracellular cAMP concentration 30 min after addition of 4 mM glutamine in amino acid–free DMEM. c and d: The inhibition of neurite outgrowth by dbcAMP (c) or caffeine (d). The cells were cultured in DMEM, or amino acid–free DMEM with or without each reagent at the indicated concentration for 3 h.

results suggest that Tas1r1 + 3 functions as an amino acid sensor in N1E-115 cells.

To investigate whether gustducin and/or transducin, which are the Gα subunits to mediate the signal transduction from Tas1r1 + 3 in the taste cells, are involved in the signal transduction in N1E-115 cells, we investigated mRNA expression of gustducin and transducin. The mRNA expression of transducin, but not gustducin, was detected by RT-PCR in N1E115 cells (Fig. 4a). If transducin mediates amino acid-induced signal transduction in N1E-115 cells, amino acids should decrease intracellular cAMP concentration because transducin activates PDEs. However, 4 mM glutamine increased intracellular cAMP concentration after 30 min (Fig. 4b). In addition, each of dbcAMP, a membrane permeant analog of cAMP, or caffeine monohydrate, an inhibitor of PDEs, inhibited neurite outgrowth induced by deprivation of amino acids (Fig. 4c,d). These results indicate that any molecules except transducin mediate the signal transduction by Tas1r1 + 3 in N1E-115 cells. We investigated the involvement of the Gαs subunit, a ubiquitous Gα subunit activating adenylate cyclase to increase intracellular cAMP concentration. NF449, a specific inhibitor of the Gαs subunit, significantly suppressed the increase of intracellular cAMP concentration induced by glutamine (Fig. 5a) and the inhibitory effect of neurite outgrowth by several amino acids (Fig. 5b,c). Because 11 amino acids of the carboxy-terminal of Gαs subunit inhibit Gαs subunit [Rasenick et al., 1994], we also investigated the effect of transfection with pRC/CMV-C-terminal on cellular responses to amino acids. Transfection with pRC/CMV-C-terminal significantly suppressed the increase of intracellular cAMP concentration induced by glutamine (Fig. 6c) and the inhibitory effect of neurite outgrowth by several amino acids (Fig. 6b). These results suggest that the Gαs subunit mediates amino acid-induced signal transduction in N1E-115 cells.

Discussion

In the present study, we investigated the mechanism for sensing amino acids in N1E-115 cells. The cells formed neurites under amino acid-free conditions (Fig. 1). We evaluated the number of cells bearing neurites as an indicator of the response to amino acids. Neurite formation was inhibited by almost all of the amino acids tested at concentrations between 0.1 and 10 mM (Fig. 2). The effective concentration of the amino acids was consistent with the sensitive concentration of Tas1r1 + 3 [Nelson et al., 2002]. The inhibitory effect of the amino acids was attenuated by treatment with siRNA against Tas1r1 or Tas1r3 (Fig. 3) and potentiated by IMP in a dose-dependent manner (Supplementary Fig. 3). These results suggest that Tas1r1 + 3 functions as an amino acid sensor in N1E-115 cells.

Almost all of the amino acid tested inhibited neurite outgrowth induced by deprivation of amino acids (Fig. 2). The sweet taste receptor, which is composed of Tas1r2 and Tas1r3, are also sensitive to a variety of sweeteners. Tas1r3 is a common subunit between umami and sweet taste receptors. The discrete binding sites for each sweetener were identified in Tas1r2 and Tas1r3 (Cui et al., 2006). Similarly, the different binding sites may make it possible for Tas1r1 + 3 to accommodate the broad range of amino acids (Yarmolinsky et al., 2009).

In the taste cells, gustducin and/or transducin mediate the signal transduction from Tas1r1 + 3 [He et al., 2004]. In our analysis using RT-PCR, mRNA expression of transducin, but not gustducin, was detected in N1E-115 cells (Fig. 4a). Transducin may mediate the signal transduction from Tas1r1 + 3 in N1E-115 cells. If so, addition with glutamine should decrease intracellular cAMP concentration because transducin activates PDEs to hydrolyze cAMP. Addition with glutamine, however, increased intracellular cAMP concentration (Fig. 4b). Thus, even if transducin mediates the signal transduction, the pathway may be minor. In contrast, our data indicate that Gαs mediates the signal transduction in N1E-115 cells. Expression of gustducin and/or transducin in restrictive tissues such as the taste cells [McLaughlin et al., 1992], gastrointestinal epithelium [Wu et al., 2002], and retina [Lerea et al., 1986] indicates that Tas1r1 + 3 may function only in the cells expressing gustducin and/or transducin. Our data, however, suggest that Tas1r1 + 3

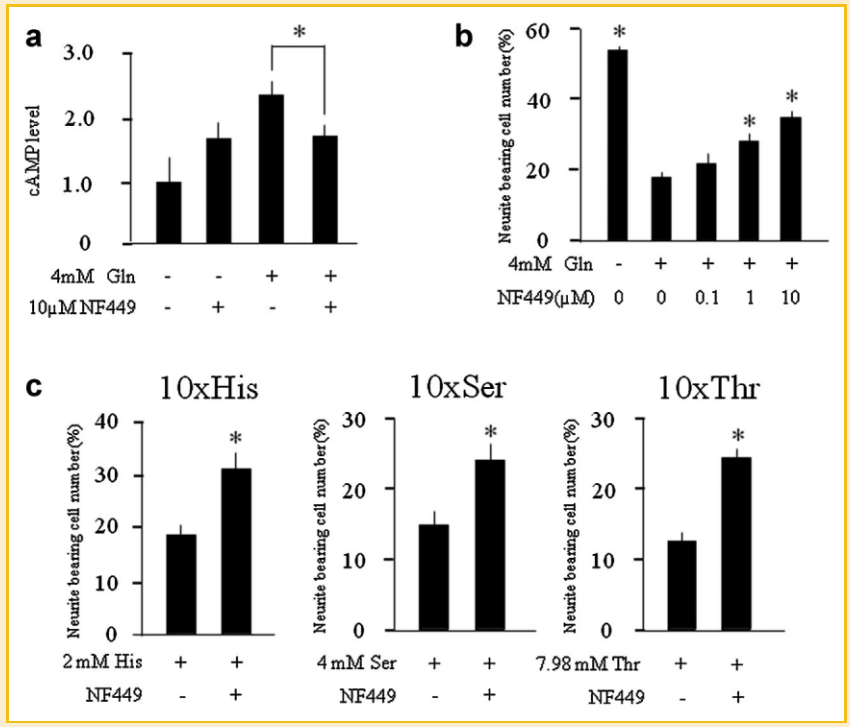

Fig. 5. NF449 inhibits amino acid-induced cellular responses. a: The suppression of glutamine-induced increase of intracellular cAMP concentration by NF449. The cells were cultured for 30 min in amino acid-free DMEM with or without 4 mM glutamine and/or 10 μM NF449. b and c: The suppression of inhibitory effect on neurite outgrowth by NF449. The cells were cultured in amino acid-free DMEM with or without glutamine and NF449 at the indicated concentration for 3 h (b) or in amino acid-free DMEM added with 2 mM histidine (10×His), 4 mM serine (10×Ser), or 7.98 mM threonine (10×Thr) with or without 10 μM NF449 (c). $^{*}P < 0.05$, compared with each amino acid alone.

functions ubiquitously as an amino acid sensor because Gαs is expressed extensively in the body.

In contrast to N1E-115 cells, amino acids such as glutamate decrease intracellular cAMP concentration in the taste cells [Abaffy et al., 2003; Trubey et al., 2006]. Though the decrease is presumably caused by activation of PDEs via gustducin and/or transducin, Gαi, which inhibits adenylate cyclase to decrease cAMP concentration, may mediate the signal transduction because expression of Gαi2 and Gαi3 are found in the taste buds [Kusakabe et al., 2000]. Thus, Tas1r1 + 3 may diversely transduce the signal via several Gα subunits.

In the taste cells, the signal transduction via Tas1r1 + 3 is also mediated by the complex of Gβγ subunits [Zhang et al., 2003], which results in activation of phospholipase C (PLC) β to transduce the umami signal [Huang et al., 1999]. If the signaling from Tas1r1 + 3 activates PLCβ2 in N1E-115 cells, inhibition of PLCβ should result in enhancement of neurite outgrowth in N1E-115 cells. However, U-73122, an inhibitor of PLCβ, inhibited neurite outgrowth induced by deprivation of amino acids in N1E-115 cells (Supplementary Fig. 4), suggesting that PLCβ positively regulates neurite outgrowth and is not activated by the signal from Tas1r1 + 3. This result indicates that the complex of Gβγ subunits mediating the signal transduction from Tas1r1 + 3 may be different among the cells.

Expression of functional taste receptors has been reported in non-taste cells. For example, bitter taste receptors regulate enteroendocrine in the gastrointestinal tract [Jeon et al., 2011] and sweet taste receptors in the hypothalamus function as glucose sensors [Ren et al., 2009]. A recent study reported that Tas1r1 + 3 mediates amino acid-induced signal transduction to regulate secretion of insulin in a pancreatic β-cell line, MIN6 cells [Oya et al., 2011]. These findings indicate that taste receptors function as chemical sensors in non-taste cells. In those cells, it appears that gustducin and/or transducin mediate the signal transduction [Ren et al., 2009; Jeon et al., 2011; Oya et al., 2011]. Our data, however, suggest that the signaling pathway from Tas1r1 + 3 is diverse in the G proteins involved. Variation of Gα and Gβγ may enable Tas1r1 + 3 to function as a chemical sensor in a variety of cells.

Deprivation of amino acids increases expression of some types of the amino acid transporters such as cationic amino acid transporter (CAT)-1 and CAT-2 in several cells [Closs et al., 2006]. In neuronal cells, the increase in amino acid transporters are involved in formation of neuronal processes through meeting the increase of metabolic demands [Burkhalter et al., 2007]. In N1E-115 cells, deprivation of amino acids increased mRNA expression of CAT-1 but not CAT-2 (Supplementary Fig. 5). The increased was detected 9 h after deprivation of amino acids. Neurite formation reaches the peak at 3 h in N1E-115 cells (Supplementary Fig. 1). Therefore, amino acid transporters may not be involved in neurite formation induced by deprivation of amino acids in N1E-115 cells.

In conclusion, Tas1r1 + 3 functions as the amino acid sensor, which transduces signals via Gαs to increase intracellular cAMP concentration in N1E-115 cells. We propose that Tas1r1 + 3 may function ubiquitously as an amino acid sensor supported by a diversity of G proteins.

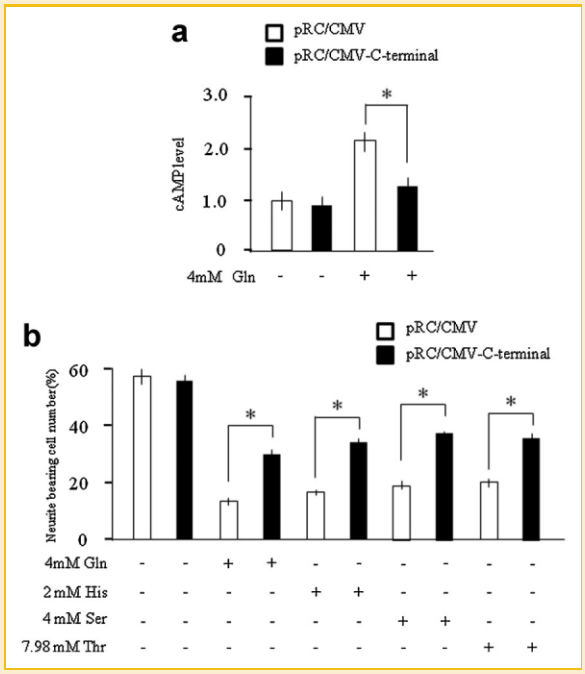

Fig. 6. Transfection with pRC/CMV-C-terminal inhibits amino acid-induced cellular responses. a: The suppression of glutamine-induced increase of intracellular cAMP concentration by transfection with pRC/CMV-C-terminal. The cells transfected with pRC/CMV or pRC/CMV-C-terminal were cultured for 30 min in amino acid-free DMEM with or without 4 mM glutamine. b: The suppression of inhibitory effect on neurite outgrowth by transfection with pRC/CMV-C-terminal. The cells were cultured in amino acid-free DMEM with or without 4 mM glutamine, 2 mM histidine (10×His), 4 mM serine (10×Ser), or 7.98 mM threonine (10×Thr). $^{*}P < 0.05$.

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