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Article

Relationship between GAD/GABA and Sweet, Umami and Bitter Tastes, Those Received in Type II Taste Buds

Kimiko Sasaki,[1,2,*] Kaori Hamano[1] and Hiroshi Ueno[1]

[1]Lab. Appl. Microbiol. & Biochem., Nara Women's University, Nara 630-8506, JAPAN
[2]Dept. Food, Mimasaka University, Tsuyama 708-8511, JAPAN

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Food extracts from spices and teas were examined for the taste altering ability on sweet, umami and bitter tastes by using the taste sensation test. The idea arose based upon the findings that 1) some food extracts from spices and teas act as salt taste enhancers and 2) the enhancing effect is directly proportional to glutamate decarboxylase 67 (GAD67) activity *in vitro*. Our results indicated that those extracts significantly activated or inhibited GAD67 enzyme activity were able to alter sweet, umami, and bitter tastes. However, there were no relationships between the taste potency of each of the examined extracts and the GAD67 relative activity ratio. While it is unlikely that the extracts have directly activated GAD67 activity and enhanced sweet, umami or bitter tastes, the results do not exclude the idea that GABA may participate in the taste cell-to-cell communications.

Key words: GAD, GABA, taste signal transduction

Introduction

Food supplies important nutrients and energy to sustain life for humans. Human eating behaviors vary depending on social circumstances and cultural environments. In Japan, several decades ago people used to have struggled to obtain enough food to satisfy their needs, but nowadays they can choose to eat from foods available in their daily lives. The purpose of eating gradually has shifted from satisfying huger to concerning nutritional aspects as well as enjoyment of eating, and taste becomes a critical factor in eating.

Five basic tastes, sweet, sour, bitter, salty, and umami, have been established (1).

*To whom correspondence should be addressed: E-mail: sasaki@mimasaka.ac.jp
Abbreviations: GABA: γ-amino butyric acid; GAD, glutamate decarboxylase; PCA, perchloric acid; PLP, pyridoxal 5'-phosphate

Taste substances first interact with corresponding receptor proteins and the signals are transmitted to the taste nerve system. Those receptor proteins locate on the taste buds, which are classified into 4 cell types, I, II, III, and IV (2). Type II cells express G-protein coupled receptors for sweet, bitter and umami tastes, and type III cells express ion-channel receptors for sour and salt tastes. Although taste signals transmitted from receptors to taste nerves, type II cells appears to have no synaptic connection with gustatory nerves (3). Electron microscopic observation showed only type III cells having synaptic connections (4). It has not been clear that how taste signals from type II cells transmitted to the nerves. Moreover, the role of the chemical mediators that may act on the taste signal transduction has not been clarified. Recently, Nakamura *et al*. have shown that glutamate decarboxylase (GAD) 67, one of the isoforms to synthesize γ-aminobutyric acid (GABA), is expressed in the type III taste cells (5)(Fig.1). They also found that GABA-gated chloride ion channel, also known as $GABA_A$ receptor, is expressed in the taste buds (5-7). Nakamura's study has expanded our understanding of the role of type III cells,

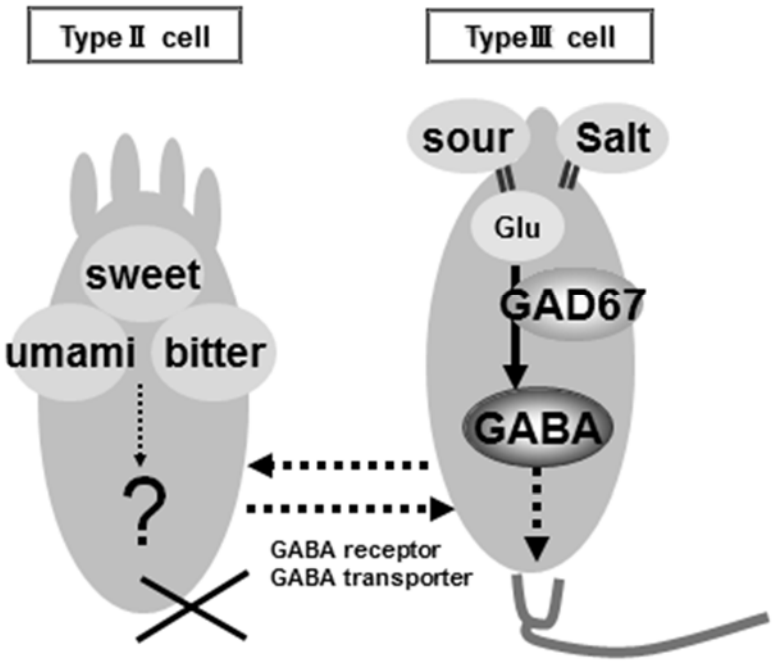

Fig. 1 Schematic view for taste transduction route between type II and type III cells
Sweet, umami and bitterness are received on type II cells. Salty and sourness are received on type III cells that have synaptic connections. GAD67 synthesizes GABA inside of type III cells.

where GABA may play an active role in the salt signal transduction.

GAD67 expressed in the type III cells is an active enzyme and produces GABA (5). Since GABA is a known neurotransmitter in CNS, it is reasonable to assume that GABA plays some biological role(s) in the type III cells. In this extent, two types of GABA actions involving the taste signaling are proposed. One is an intracellular action of GABA in the sour taste signaling, and the other an extracellular action in sweet, bitter, and umami taste signaling. If any fluctuation of GABA level could occur within the type III cells, it should influence salty taste as well as other associated activities. In fact, Hisaki and Ueno have shown that extracts of spices and herbs significantly altered GAD67 enzymatic activity *in vitro* and this phenomenon coincided with the level of salty taste (6, 8). Hence, it is in value to examine the fluctuation of GABA level and its influence on sour, umami and sweet tastes.

Table 1 Characteristics of food extracts
A name and components of spices used in the extraction of food ingredients

Samples	Part of plant used
anise	seed
basil	leaf
celery	setm and leaf
cumin	seed
chamomile	flower
ginger	root and rhizme
lemongrass	leaf
mace	aril
oolong tea	leaf
oregano	leaf
paprika	fruit
parsley	leaf
peppermint	leaf
perilla	leaf
poppy seed	seed
rose rugosa	flower
yuzu peel	peel

The contrast effect has been known in the food cookery field. A small amount of added salt significantly enhances the sweetness and/or umami of foods. Inhibition effect is also known that a small added amount of salt weakens bitterness. It is still a puzzle that how salt which acts on type III cells could influence sweetness, bitterness and umami whose taste information is received on type II cells. Since any signals traveling from type II to type III, or vice versa, have not been clearly understood, the contrast and/or inhibition effects on foods could not be explained at molecular level. If GABA could be identified as a messenger acting between type II and type III cells, the mechanism of the contrast and/or inhibition effects could be explained. In order to demonstrate the GABA role as the messenger between the two cell types, we present preliminary results of 1) how food extracts affect sweet, bitter or umami tastes and 2) whether or not those effects are related to GAD67 activity.

Materials and Methods

Extraction and preparation of food ingredients

Spices, herbs and tea leaves listed in Table 1 were obtained from Somatech Center, House Foods Co., and commercially available sources. Most samples were provided as a dry form and were processed into a small pieces. Ultrapure water was added to the samples and placed in a refrigerator for overnight. Then, water soluble components were filtered using 110 mm diameter filter paper. Crushed food samples were typically mixed with 5 times weight volume of water and powdered samples were mixed with 10 times weight volume of water. The supernatants were collected and used as food extracts.

Preparation of GAD67

Recombinant rat brain GAD67 was expressed by growing *E. coli* strain transformed with Rosetta-gamiB (DE3) pLysS vector in which GST-GAD67 encoding cDNA was cloned in this laboratory. Purification of the recombinant GAD67 protein from GST-GAD67 fusion protein was carried out by glutathione affinity chromatography. GST-tag was removed with thrombin treatment to give an active GAD67**.**

Evaluating GABA production

GAD67 enzyme assay was carried out by quantitatively determining GABA production. GAD reaction was initiated by adding the appropriate amount of enzyme solution into final 1.0 mL assay solution to which 100 μL of the assay mixture containing 0.2 M L-glutamate and 2 mM pyridoxal 5'-phosphate (PLP) in 0.5 M HEPES buffer, pH 7.0 was added. The assay solution was incubated at 37 °C for 1 h. At the end of incubation period, 50 μL of 60% perchloric acid (PCA) was added to terminate the reaction. As a blank, PCA solution was added to the assay solution immediately after the enzyme addition. After the centrifugation, the supernatant was collected, where an appropriate amount of the aliquots was placed into the vial and injected to HPLC for GABA analysis as described previously (8)**.** **A**rea of the **GABA** peak was compared to that of the standard GABA and the amount of GABA produced per incubation time was estimated. Together with protein assay data obtained by Bradford method (BioRad), specific activity of GAD67 was calculated as expressed μmol/min/mg. Bovine serum albumin was used as a reference protein for the protein assays.

Evaluation of the effects of food extracts on GAD67 activity

Effects of food extracts on GAD67 activity was evaluated by carrying out the enzyme assay in the presence of an appropriate amount of food extracts. The amount of GABA produced was compared with that obtained without the addition of the

food extracts. Any increase or decrease in GAD67 activity was referred to relative activity ratio (%) where no change in activity was expressed as zero and any inhibition was expressed as a minus % number.

Taste sensory test

Seventeen extracts obtained from the spices, herbs and teas were examined for their effects on human taste sensation (9). Basic tastes for sweet, umami and bitter were 3% sucrose, 0.08% MSG and 0.05% caffeine, respectively (Table 2) (10-12). Reagents used were commercial grade purchased from NacalaiTesque, Ltd. Each of the extract solutions was diluted with ultrapure water to the appropriate concentrations and placed in a paper cup. Typically, about 20 ml was supplied for the

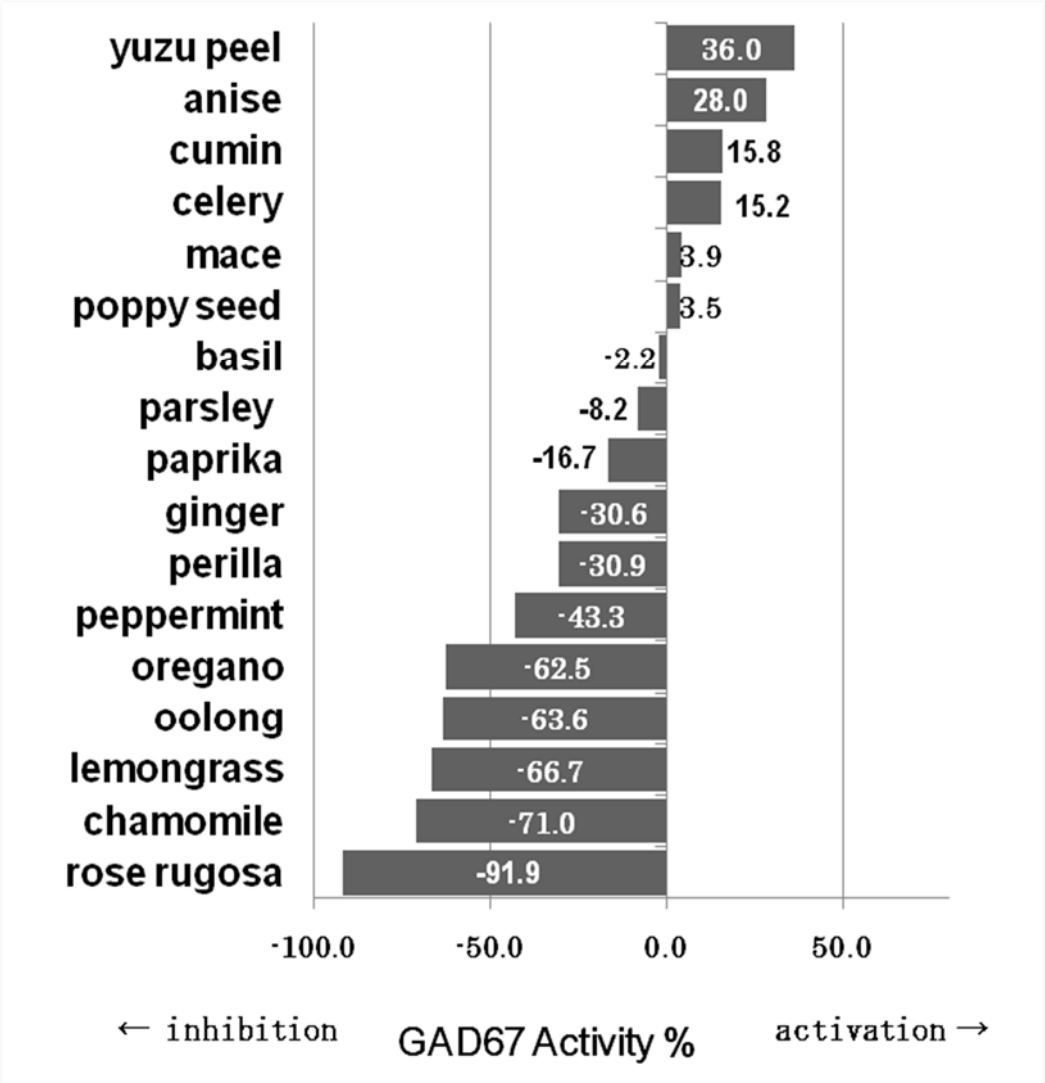

Fig.2 Effects of food extracts on GAD67 Activity
GAD67 activity is plotted in the presence and absence of food extract. Increased or decreased activity is indicated as positive or negative number in %, respectively. Water was used in place of food extracts as control that gave 100%.

Table 2 Reagents used in sensory test

taste	taste substance	concentration (%)	pH
sweet	sucrose	3.00	7.4
umami	monosodium glutamate (MSG)	0.08	6.6
bitter	caffeine	0.05	7.2

tests. Detailed test procedure is described as follows:

(1) Sensory test subjects were selected from a group of students ranged in age from 19 to 21 at Mimasaka University where the tests were performed. They agreed with the purpose of this study. They were volunteers and did not receive any special training prior to the tests. The study was approved by the Mimasaka University Ethics Review Committee.

(2) The sensory test was proceeded according to the guideline "Fact of Food Sensory Test to Measure the Taste" (13).

(3) Subjects were allowed to repeat the tests if they were not fully confidence of the results.

(4) Tests were limited to three or four times in order to avoid fatigue of the subjects.

(5) The test site was at the cooking laboratory on campus, where room temperature was kept at 23-24 °C. All tests were performed between 9:00 and 12:00 a.m.

(6) Test results were statistically processed according to the two-sample preference test (14).

(7) The subjects proceeded the sensory test protocol as follows:

1. Rinsed mouth with ultrapure water
2. Take the first tasting solution into mouth trying to spread over the entire surface of the tongue, and spit out or swallow it.
3. Rinse mouth with ultrapure water, then, take the second tasting solution into mouth.
4. Complete the sensory evaluation sheet in three step scale, “strong”, “same” and “weak” on any changes.

Results

Search of spices that affect the activity of GAD67

We have examined if spice, herb and tea extracts influenced the activity of GAD67 by incubating with those ingredients and measured any changes in GABA production with and without the extracts (Fig. 2). Yuzu peel, anise, cumin, and celery raised GABA production whereas rose rugosa, chamomile, lemongrass, oolong, and oregano showed strong inhibition. Among the examined, a small number of the extracts activated GAD67 activity. The level of GABA production varied among the extracts. GABA contamination in the extracts was considered; however, it is

Table. 3 Change in the sweet taste sensitivity by food extracts （n＝30）
The subjects were evaluated three levels "strong", "same" and "weak" the strength of bitter taste after tasting the food ingredients.

enhancing effect of sweet taste

Relationship between activity of GAD67 and enhancing effect of sweet by Food Extracts.

Enhancing effect of taste by the food extract is subtracted a ratio of panelists who evaluated that taste was weak from the rate of them who evaluated that taste was strong.

Enhancing effect of sweet taste points = strong - weak /n (number of subjects)

samples	strong	same	weak	enhancing effects of sweet taste points	significant difference
	n	n	n	strong - weak	strong vs weak
anise	20	2	8	0.40	*
basil	8	4	18	-0.33	
celery	19	6	5	0.47	**
cumin	12	5	13	-0.03	-
chamomile	8	3	19	0.37	-
ginger	8	5	17	0.30	-
lemongrass	17	7	6	0.37	*
mace	19	5	6	0.43	*
oolong tea	18	5	7	0.37	*
oregano	10	4	16	-0.20	-
paprika	17	5	8	0.30	-
parsley	10	7	13	-0.10	-
peppermint	11	4	15	-0.13	-
perilla	13	7	10	-0.10	-
poppy seed	21	3	6	0.50	**
rose rugosa	8	4	18	-0.33	-
yuzu peel	12	6	12	0.00	-

*: $p<0.05$ **: $p<0.01$

unlikely occurred since endogenous GABA was subtracted from GAD assay results by the control experiments. Hence, it is likely that there is a direct interaction between GAD67 protein and food ingredient(s). Our results support the idea that spice or tea components have an impact on GAD67 activity *in vitro*.

Sensory test

We conducted a sensory test in which 30 female college students answered the strength of sweet, umami and bitter tastes, whereas the 17 extracts were tested due to

their affects on GAD67 activity.

(1) Sweet taste

Sweet taste tests were carried out with 3% sucrose solution in the presence and absence of the extracts. This concentration of sucrose was chosen according to the reference (10). The threshold concentration for sucrose was shown to be 0.5%. The amounts of the extract were determined based upon their effects on GAD67 activity. Results are summarized in Table 3. Those who tasted the extracts of all 17 extracts answered that anise, poppy seed, celery, mace, lemongrass and oolong tea enhanced sweet taste. On the other hand,

Table. 4 Change in the umami taste sensitivity by food extracts （n＝29）

The subjects were evaluated three levels "strong", "same" and "weak" the strength of bitter taste after tasting the food ingredients.

enhancing effect of umami taste

Relationship between activity of GAD67 and enhancing effect of umami by Food Extracts .

Enhancing effect of taste by the food extract is subtracted a ratio of panelists who evaluated that taste was weak from the rate of them who evaluated that taste was strong. Enhancing effect of umami taste points = strong– weak /n (number of subjects)

samples	strong	same	weak	enhancing effects of umami taste	significant difference
	n	n	n	strong - weak	strong vs weak
anise	17	9	2	0.54	* * *
basil	9	13	6	0.11	-
celery	16	7	5	0.39	*
cumin	19	7	2	0.61	* * *
chamomile	13	10	5	0.26	-
ginger	9	10	9	-0.04	-
lemongrass	18	9	1	0.61	* * *
mace	16	7	5	0.39	*
oolong tea	11	9	8	0.11	-
oregano	18	7	3	0.54	* *
paprika	12	7	9	0.11	-
parsley	11	15	2	0.32	*
peppermint	12	8	8	0.14	-
perilla	8	10	10	-0.71	-
poppy seed	15	7	6	0.32	-
rose rugosa	10	14	4	0.19	-
yuzu peel	12	8	8	0.07	-

*: $p<0.05$ **: $p<0.01$ ***: $p<0.001$

over 60% of the test subjects responded that basil, chamomile, ginger and rosa rugosa weakened sweet taste. It is evident that spices, herbs and teas could alter sweet taste.

(2) Umami taste

L-Monosodium glutamate solution at concentration of 0.08% was used as an umami compound. This concentration was chosen as it was above the threshold (0.03%) (10, 11). Subjects were asked to taste the umami solution in the absence and presence of an appropriate amount of each of the extracts. The subjects marked “strong”

Table. 5 Change in the bitter taste sensitivity by food extracts （n＝29）
The subjects were evaluated three levels "strong", "same" and "weak" the strength of bitter taste after tasting the food ingredients.
enhancing effect of bitter taste
Relationship between activity of GAD67 and enhancing effect of bitter by Food Extracts .
Enhancing effect of taste by the food extract is subtracted a ratio of panelists who evaluated that taste was weak from the rate of them who evaluated that taste was strong.
Enhancing effect of bitter taste points = strong - weak /n (number of subjects)

samples	strong	same	weak	enhancing effects of bitter taste	significant difference
	n	n	n	strong - weak	strong vs weak
anise	14	11	4	0.35	*
basil	12	8	9	0.1	-
celery	14	9	6	0.28	-
cumin	8	17	4	0.14	-
chamomile	17	7	4	0.45	* *
ginger	11	10	7	0.14	-
lemongrass	9	12	8	0.03	-
mace	10	12	7	0.1	-
oolong tea	14	7	8	0.21	-
oregano	13	8	8	17	-
paprika	18	4	7	0.38	*
parsley	16	7	6	0.35	-
peppermint	9	12	8	0.03	-
perilla	11	11	7	0.14	-
poppy seed	12	11	6	0.21	-
rose rugosa	10	16	2	0.29	*
yuzu peel	12	5	12	0.00	-

<0.05 **: p<0.01

and “weak” when they sensed umami taste being enhanced and diminished, respectively. Table 4 summarized the results. Anise, cumin, lemongrass, oregano, celery, mace and parsley significantly enhanced umami taste. Perilla and ginger significantly inhibited umami taste. Similar to sweet taste, the components of spices and teas could have affected umami taste.

(3) Bitter taste

Caffeine concentration of 0.05% was chosen for the bitter taste tests. This concentration of caffeine was typical for regular coffee and tea (12). Chamomile, anise, paprika and celery enhanced the bitter taste (Table 5). Interestingly, there were no extracts that significantly reduced the bitter taste. In addition, the dynamic range for the effects on bitter taste was relatively narrow as compared with those for sweet and umami tastes. Similar to sweet and umami tastes, the examined extracts showed various effects on bitter taste.

Discussion

Modern biological and nutritional sciences have shown that food not only provides energy, but also its components play significant physiological roles on our health (15). Recently, Hisaki *et al.* reported that food extracts could be used as a salty taste enhancer (6). In their study, for example, a small amount of anise exhibited salty taste enhancement. They suggested that the presence of food extracts could reduce salt intake and the reduction of salt intake could lead to the prevention of hypertension. Although detailed mechanism has not been clarified, the study suggests that the observed salt enhancement is probably due to the effects on salt signal transduction pathway locating on the type III taste buds. In this study, we have focused on the effects of spice, herb, and tea extracts on sweet, umami, and bitter tastes.

Spices, herbs, and teas may have some unexplored activities besides on the taste sensations, possibly altering sweetness, umami and bitterness. It has not been rigorously investigated on how spices, herbs and teas affect the tastes except few cases (16, 17). Recently, a salty taste signal transduction study has reported that GAD67 is involved via producing GABA in type III cells, and some spice extracts exhibit salt enhancing effects. This finding has encouraged us to study the roles of spices in relation with tastes on human subjects.

We have measured the potency of taste for each of the extracts in the sensory test. The potency of taste was derived by subtracting the number of subjects who responded that the extract "weakened" the taste from the number of subjects who reported that the same extract "enhanced”. An index of the enhancing effect of taste by the food extract was thus calculated based

upon the taste potency that was divided by the total number of subjects (Tables 3 - 5). The results indicate that almost all the examined extracts did alter the sweet, umami and bitter tastes. Some enhanced while some others reduced sensation of one or more of the three tastes. At the moment, it is not clear which chemical components in the extracts are responsible for the alteration of the tastes, and what the molecular basis or mechanism for such actions.

In addition to the roles of spice and tea extracts on sweet, umami and bitter tastes as described above, the relationship between the effect on GAD67 activity (Fig. 2) and each of the examined taste sensations were

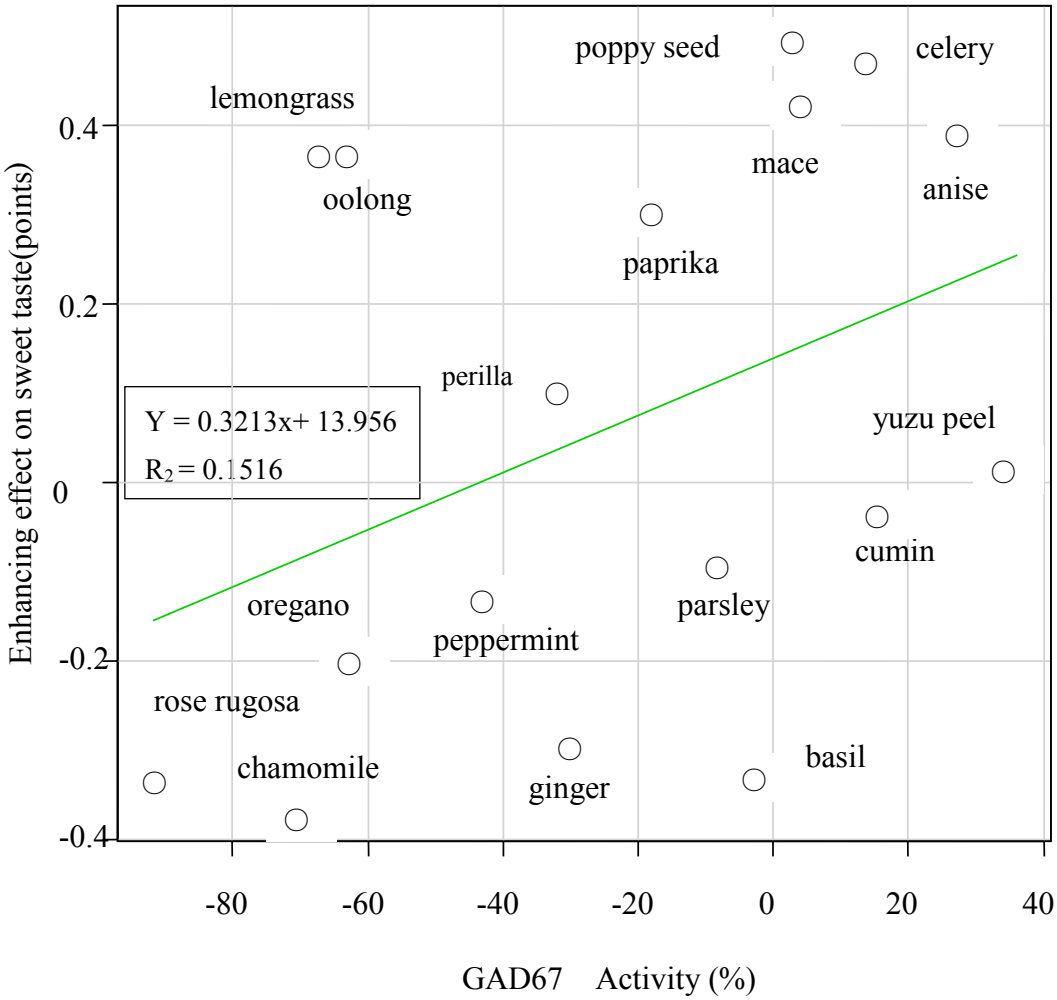

Fig. 3 Relationship for the effect of 17 food extracts on sweet taste sensation and that on GAD67 activity
X axis indicates effects on GAD67 activity and Y axis indicates effect on the sweet taste. Correlation between the effects is shown as a line (R2 = 0.1516).

analyzed (Fig. 3 - 5):

Sweet Taste

We examined whether or not the effects of those food extracts on sweet taste have some of correlation with GAD67 activity. When the sweet taste potency vs relative activity for GAD67 are plotted (Fig. 3), there seems to be no direct correlation between them. The results indicate that any activation or inhibition of GAD67 activity may not influence sweet taste. This may deny the possibility that any GABA produced in the type III cells would influence the sweet taste signal transduction. It has been reported that the degree of the sweetness was unaffected by the GABA presence in the food samples (18, 19). This

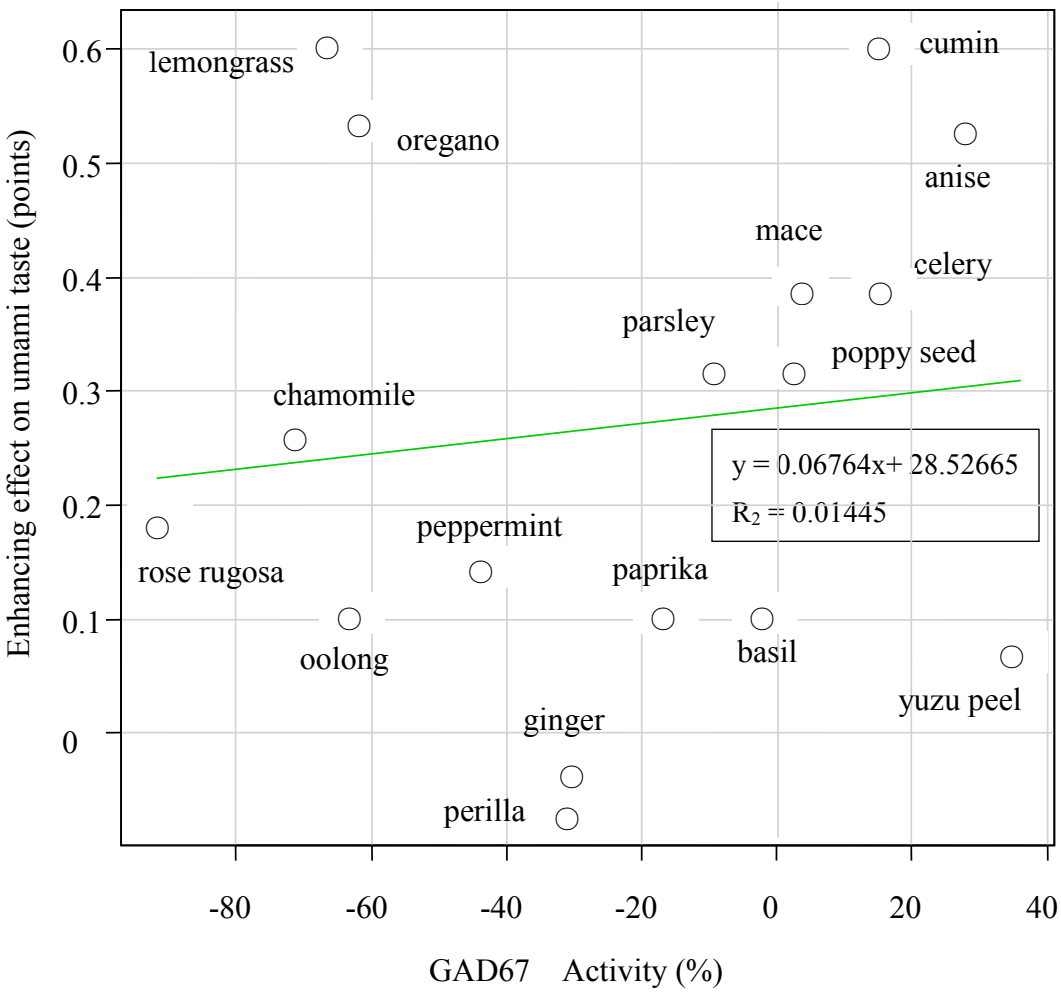

Fig. 4 Relationship for the effect of 17 food extracts on umami taste sensation and that on GAD67 activity
X axis indicates effects on GAD67 activity and Y axis indicates effect on the umami taste. Correlation between the effects is shown as a line (R2=0.01445).

suggests that GAD67 and GABA possibly locating in the type III cells have no communication with the sweet taste transduction that is received by the type II cells.

Umami Taste

We examined if the umami taste was affected by GAD67 activity and/or GABA. The effects of the extracts on GAD67 relative activity ratio were plotted against those on umami taste potency. As a result, there was no correlation between the GAD67 relative activity ratio and the umami taste potency (Fig. 4). It is of interests to note that the degree of umami taste was not affected when GABA alone was added (18, 19). This suggests that GAD67 activity and

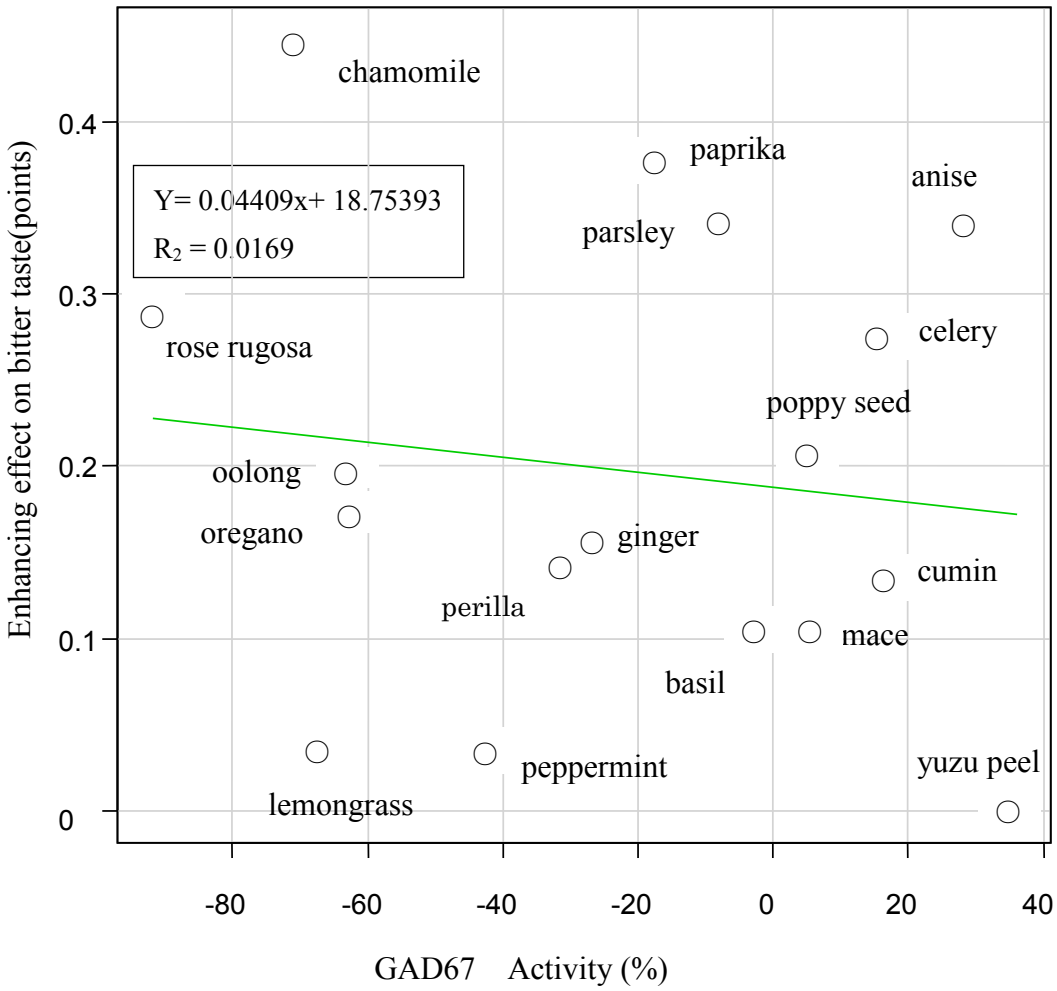

Fig. 5 Relationship for the effect of 17 food extracts on bitter taste sensation and that on GAD67 activity
X axis indicates effects on GAD67 activity and Y axis indicates effect on the bitter taste. Correlation between the effects is shown as a line (R2=0.0169).

GABA are unlikely to participate in the umami taste transduction pathway, which possibly is located in the type II cells.

Bitter Taste

Bitter taste is sensed by the type II cells since they express receptors for bitter taste. We have examined if effects of food extracts on bitter taste would be affected by GAD67 activity and/or GABA. For this purpose, effects of each of the food extracts on GAD67 relative activity ratio was plotted against those on bitter taste potency (Fig. 5). As a result, there seems to be no relationship between the GAD67 relative activity ratio and bitter taste potency (Fig. 5) for the 17 spice food extracts used for the sensory test. In addition, the degree of bitter taste was not affected even when GABA was added (18, 19). This suggests that bitter taste transduction pathway may not be influenced by both GAD67 activity and GABA.

From the present study, it is suggested that GAD67 and GABA may not take part in sweet, umami or bitter taste signal transduction, where GAD 67 is projected in the type III cells and GABA is received by the type II cells. Our present experiments have focused on the effects of the extracts from spices, herbs and teas upon the defined taste sensation. Since there was a limitation in the taste sensory testing method, we were not able to clearly identify the involvement of GAD67 and GABA on the taste signal transduction; however, our present data does not deny any possibility that GABA may be involved in "contrast effect" known in cooking field.

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