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Article

Screening and Identification of Food Extracts that Are Useful For Reducing Salt Intake - An Approach from a GABA-synthesizing Enzyme -

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Potential use of plant extracts for reducing salt intake was explored. Recent finding of GAD67 and GABA-gated chloride ion channel $GABA_A$ receptors being expressed in type III taste buds supported an idea that GABA could be a signal for salty taste mechanism. In this study, we have shown that food extracts effected on both GAD67 activity *in vitro* and taste sensations *in vivo*. Furthermore, those food extracts tended to enhance GAD67 activity showed direct correlation with salty taste enhancement. It is concluded that food extracts can be a useful salt enhancing materials.

Key words: GABA, GAD67, spices, teas, salt intake

1. Introduction

Comprehensive Survey of Living Conditions 2004 [1] reported that strokes were the main cause for nursing care and bedridden patients, as Japan becomes an aging society. Prevention of strokes would facilitate longevity by allowing people to maintain a healthy life. Fewer strokes could help reduce healthcare costs significantly. The main strategy for preventing strokes is to manage hypertension, a major risk factor for strokes [2-5].

It is widely accepted that reduced dietary salt intake can lower the possibilities of developing hypertension. On the other hand, there are difficulties in reducing the salt intake, since salt enhances the flavor of foods. It is desirable to seek alternative approaches that provide us with flavor in a low-salt diet without sacrificing the taste. In this context, understanding how the salty taste signal transduction mechanism in taste buds works is important.

Humans perceive five basic tastes, including umami, bitter, sweet, sour, and salty, and commonly recognized taste substances are monosodium glutamate (MSG), quinine sulfate, sucrose, citric acid, and sodium chloride (NaCl), respectively. The sense of taste is mediated by taste buds, approximately 5,000 located on the tongue. A single taste bud is constituted of approximately 50-100 cells, and these cells are

Abbreviations: GABA: γ-amino-butyrate, GAD67: an isoform of glutamate decarboxylase, GST: glutathione S transferase, PCA: perchloric acid

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morphologically divided into four groups, namely type I, II, III and IV [6, 7]. Type II cells are shown to contain receptors for umami, bitter and sweet substances and type III cells for sour and salt substances.

Recently, a number of the candidates for taste receptors have been clarified. Umami, bitter and sweet tastes are mediated by G protein-coupled receptors (GPCRs) located on type II cells [7, 8]. Sour tastes are transmitted by ion channel-linked receptors, such as PKD2L1 and PKD1L3, both of which belong to the transient receptor potential (TRP) family, expressed on type III cells [9]. Salty taste is similarly transmitted by the sodium ion channel since intracellular Na^+ influx induces depolarization to give the salty taste sensation. Currently, amiloride-sensitive epithelial sodium channels (ENaCs) that are located on taste cells, are regarded as one of the major contributors to Na^+ transduction. Details of ENaCs' molecular expression including the types of the taste cells are not fully understood. Several salty taste receptive mechanisms other than ENaCs are also proposed [10, 11].

NaCl, a chemical component for common table salt, is the prototypic salty tastant. Frequent use of table salt has been suspected as a cause of hypertension; however, NaCl intake itself is not directly associated with hypertension [5, 12]. The association is clear for Na^+ [5, 13]; hence, KCl is often used as a dietary substitute for NaCl. KCl exhibits saltiness together with bitter taste [14]. Since KCl has salty taste, Na^+ is not the only element that contributes to the salty sensation. Salty taste may be also attributed to chloride ion, Cl^-, because the substitution of Cl^- to iodine ion, I^-, gives completely different taste from NaCl [15]. It is important to note that KCl may not be a universal substitute for NaCl, since high blood K^+ level may cause heart failure and people with kidney and heart problems should avoid KCl intake. Hence, other suitable substitutes to NaCl are needed.

In taste sensations, sometimes cross talks between two different tastes take place, which are called hidden tastes known in the field of cooking. One of the hidden tastes is a contrast effect in which a small amount of salt enhances both sweetness and umami taste [16, 17]. There is an inhibitory effect in which a small amount of salt can weaken bitter taste [18]. Although detailed molecular mechanisms of the hidden tastes are still under investigation, it is of interest to note that the phenomenon can be interpreted as cell-to-cell communication which occurs between type II and III cells. In order to examine the hidden tastes in molecular level, one has to consider how the taste signal be transmitted from the taste bud to the gustatory nerves. It has been shown that gustatory nerves are connected to the type III cells via synaptic connection but there is no such interaction with type II cells [7, 8]. It is not fully established how the signal from type II cells is transmitted to the gustatory nerves [19-21].

Nakamura *et al.* found that glutamate decarboxylase 67 (GAD67), one of the isoforms of GAD, is expressed in the type III taste cells [22]. GAD is an enzyme to catalyze the synthetic reaction of γ-aminobutyrate (GABA). In mammals, there are two distinct genes which encode GAD proteins, called isoforms, and those exhibit almost identical catalytic activity [23]. Nakamura *et al.* also found that GAD67 in the type III taste cells is enzymatically active and GABA is produced in the type III taste cells [24]. Since GABA is an amino acid that acts as a major inhibitory neurotransmitter in the central nervous system, its existence in the type III taste cells raises some questions: What is the role of GABA in the taste cells? Is GABA involved in taste? Since GABA is a neurotransmitter, it would be highly

likely to act as a part of taste signaling although it has yet to be ascertained. There are several kinds of evidence to support GABA being involved in taste: 1) By performing taste sensation tests with human subjects, GABA was shown to be a tasteless compound. Furthermore, it also gave an enhancement effect for sour and bitter when taking together with sour and bitter substances, respectively [25, 26]. 2) GABA is a ligand for GABA receptors in which $GABA_A$ and $GABA_C$ receptors are GABA-gated chloride ion channels. Since chloride ion participates as a salty substance, it is highly likely that GABA is involved in salt signal transduction.

Salty taste could be enhanced when a small amount of GABA is present [16]. It raises the possibilities that any substances that affect GABA levels and/or GABA production in the type III taste cells would alter the salty taste signaling. In the present study, we aim to identify food extracts that affect GAD67 activity. In order to find such food extracts, various food extracts from spices, herbs, teas, and common food materials are examined. The food extracts that influence GAD67 activity should be further examined for the effects on taste sensation by taste sensation tests with human subjects.

2. Materials and Methods

1. Spices, herbs and teas used for the screening

Fifteen types of spices, herbs and teas used in this study are listed in Table 1 They are classified into six categories of 1) leaves (oolong tea, lemongrass, celery, basil, oregano, peppermint, perilla, and parsley), 2) seeds (anise, cumin, and poppy seed), 3) aril (mace), 4) fruit (paprika), 5) root (ginger), and 6) peel (yuzu peel). These materials were gifts from Somatech Center of House Foods Corporation or purchased from commercially available sources.

2. Preparation of food extracts

Extraction of food was carried out by incubating the crushed food materials in 5 times the weight of water and powdered foods in 10 times the weight of water at 4-6 °C for overnight. The supernatant of the incubated samples was individually collected and they were used as food extracts. The pH and dry weight of the extracts are summarized in Table 1.

Table 1 Characteristics of Food Extracts

No.	Samples	Part of plant used	Food extract			Family
			pH	Dry weight (mg/ml)	GABA (μg/ml)	
1	anise	seed	5.3	32	0.29	Apiaceae
2	basil	leaf	6.1	33～48	0.14	Lamiaceae
3	celery	setm and leaf	7.5	66	1.70	Lamiaceae
4	cumin	seed	6.0	25～32	0.14	Apiaceae
5	ginger	root and rhizome	6.0	25～33	0.96	Zingiberaceae
6	lemongrass	leaf	5.6	27～28	0.21	Poaceae
7	mace	aril	4.0	13	0.10	Myristicaceae
8	oolong	leaf	5.8	30	0.30	Theaceae
9	oregano	leaf	6.5	30	0.16	Lamiaceae
10	paprika	fruit	4.6	22～28	0.30	Solanaceae
11	parsley	leaf	5.3	40	0.24	Apiaceae
12	peppermint	leaf	6.0	50	1.50	Lamiaceae
13	perilla	leaf	6.0	20～21	0.45	Lamiaceae
14	poppy seed	seed	7.0	13	0.03	Papaveraceae
15	yuzu peel	peel	3.7	71～76	0.21	Rutaceae

3. Preparation of GAD67

A recombinant rat brain GAD67 was prepared by growing *E. coli* strain *Rosetta-gamiB (DE3) pLysS* that was transformed by GST-GAD67 containing vector. Since amino acid sequence homology between the rat brain and human brain GAD67s is high enough, the commercial anti-GAD67 antibody from Sigma (St. Louis, MO, USA) which recognizes both species was used for the detection of rat brain GAD67 in the present study. Purification of the recombinant GAD67 was performing by glutathione affinity chromatography. The protein concentration was estimated by using a Bio-Rad protein assay dye reagent purchased from Bio-Rad Laboratories (Hercules, CA, USA) with bovine serum albumin as a standard. GAD enzyme activity was determined by incubating the enzyme solution with L-glutamate in the presence of PLP and pH 7.4 HEPES buffer at 37 °C. Amounts of GABA produced were determined by HPLC after derivatization with NBD-F and by using Agilent ZORBAX-C18 column (3.0 x 50 mm, 1.8 μm particle diameter). A typical purified GAD67 exhibited a specific activity of 0.4 μmol/min/mg.

Table 2 GAD67 Activity in the Presence of Food Extracts

No.	Samples	GAD67 activity %
1	anise	28.0
2	basil	-2.2
3	celery	15.2
4	cumin	15.8
5	ginger	-30.6
6	lemongrass	-66.7
7	mace	3.9
8	oolong	-63.6
9	oregano	-62.5
10	paprika	-16.7
11	parsley	-8.2
12	peppermint	-43.3
13	perilla	-30.9
14	poppy seed	-11.8
15	yuzu peel	36.0

4. Effects of food extracts on GAD67 activity

The amounts of GABA produced in GAD67 assay with and without the food extracts were evaluated. The effect of each of the food extracts was indexed as a ratio of relative activity (%) by comparing amounts of GABA produced in the presence and the absence of the food extracts. Positive and negative % numbers indicate that food extracts activate or inhibit GAD67 enzyme activity, respectively.

5. Quantitative analysis of GABA

GAD67 enzyme solution was mixed with 100 μL of assay mixture containing 0.5 M HEPES buffer (pH 7.0), 0.2 M sodium L-glutamate, and 2 mM PLP, where water and the food extracts were added to make up the total assay volume of 1 mL. The assay mixture was incubated at 37 °C for 1 h. At the end of the incubation period, 50 μL of 60% perchloric acid (PCA) was added to stop the reaction. As a blank, PCA solution was added at time zero. After terminating the reaction, the mixture was centrifuged and the supernatant was transferred to a vial and used for GABA analysis on an HPLC system as described previously [27, 28]. The obtained GABA peak area was compared with the standard GABA to estimate the GABA contents in the assay mixture. The effect of each of the food extracts on GAD activity was evaluated by comparing GABA contents in the reaction mixture in the presence and absence of the examined food extract.

6. Taste sensation tests

Forty one healthy women between the ages of 18 and 25 were chosen for the taste sensation test subjects. The tests were carried out in the cooking practice room at Osaka International College. Prior to the tests, we explained the purpose of the sensory taste testes to the subjects. Given the possible regulatory effect of the extracted food components from spices, herbs and teas on GAD67 activity, the effects of the prepared food extracts were evaluated on the intensity of the salty sensation. First, the subjects rinsed their mouth with water, then, standard 0.8% NaCl solution of 5 mL was put into their mouth and they were allow to taste for a while. After spitting out the solution, they rinsed their mouth again with water. Then, 0.5 - 1.0 mL of the sample solution containing the appropriately diluted food extract was tasted. They were allow to taste the solution for a while and could swallow it, if they wanted it. Finally, the subjects were given standard 0.8% NaCl solution again to test any changes in the salty taste. They evaluated the last solution for the saltiness on the basis of three step scale from strong, same, to weak. The scores of the taste sensation test were derived as follows. The number of subjects who answered weak was subtracted from those who answered strong. The subtracted number was divided by the total number of the subjects and expressed as salty taste enhancement points.

3. Results

1. Screening of food extracts that affect GAD67 activity

Common spices, herbs and teas were chosen and extracts prepared were examined for their effects on GAD67 activity. Table 2 indicates that food extracts were able to alter GAD67 activity and the degree of inhibition/activation is varied widely. Lemongrass, oolong tea, and oregano extracts showed inhibition, while, yuzu peel, anise, cumin and celery extracts activated GAD67 extensively. Mace, poppy seed, and basil extracts hardly affected GAD67 activity. The results suggest that those common food extracts affect GAD67 activity *in vitro* and may also affect *in vivo*.

2. Effects of food extracts on the salty taste

The extracts from the chosen spices, herbs and teas exhibited various effects on GAD67 activity by *in vitro* assay system. All the food extracts listed in Table 1 were used for the taste sensation test. The results of the taste sensation test were summarized and shown in Table 3A. More than 50% of the subjects answered that peppermint, oregano and lemongrass extracts weakened salty taste. On the other hand, more than 50% of the subjects answered that celery, anise and cumin extracts strengthened salty taste.

3. Effects of GABA on sweet taste

The effect of GABA on salty taste sensation was evident in the taste test. As type III cells utilize GABA for the salty taste sensation, it is of interests to know if GABA might influence to another taste sensations, such as the sweet sensation located on type II cells. The effect on sweet taste was chosen since it has been known that sweet taste can be enhanced by adding a small amount of salt eliciting a contrast effect. Three-percent sucrose solution, which is a common concentration for sweet solutions in cooking, was used in the taste sensation test.

We have previously demonstrated that additional 0.0025% of GABA, about threshold level, did not enhance sweet taste for 3% sucrose with human subjects [26]. We have examined further whether or not higher concentration of GABA may enhance sweet taste using commercial soy

Table 3 Change in the Salt/Sweet Taste Sensitivity by Food Extracts

(A)

No.	Samples	Taste test (3% sucrose)				
		Subjects	Number of subjects			Enhancing effect of sweet taste points
		n	Strong	Same	Weak	
1	anise**	40	25	6	9	40.0
2	basil	65	22	16	27	-7.7
3	celery***	55	33	12	10	41.8
4	cumin	67	30	16	21	13.4
5	ginger	70	27	19	24	4.3
6	lemongrass**	64	33	17	14	29.7
7	mace***	56	39	9	8	55.4
8	oolong*	42	23	10	9	33.3
9	oregano***	72	16	7	49	-45.8
10	paprika	65	22	17	26	-6.2
11	parsley	57	20	16	21	-1.8
12	peppermint	42	15	9	18	-7.1
13	perilla	55	19	19	17	3.6
14	poppy seed***	42	28	9	5	54.8
15	yuzu peel	55	22	11	22	0.0

(B)

No.	Samples	Taste test (0.8% NaCl)				
		Subjects	Number of subjects			Enhancing effect of salty taste points
		n	Strong	Same	Weak	
1	anise	8	6	1	1	62.5
2	basil	52	19	17	16	5.8
3	celery*	15	12	1	2	66.7
4	cumin	30	16	6	8	26.7
5	ginger	64	27	19	18	14.1
6	lemongrass	30	10	4	16	-20.0
7	mace	30	13	11	6	23.3
8	oolong	31	6	11	14	-25.8
9	oregano**	33	5	8	20	-45.5
10	paprika	20	8	5	7	5.0
11	parsley	28	12	8	8	14.3
12	peppermint	10	2	1	7	-50.0
13	perilla	15	5	4	6	-6.7
14	poppy seed	32	11	12	9	6.3
15	yuzu peel	15	5	7	3	13.3

(A) Standard solution for saltiness was 0.8% NaCl solution. (B) Standard solution for sweetness was 3% sucrose solution. *p>0.05, ** p>0.01, *** p>0.001 : By the comparison test (Strong : Weak) [17)]

sauce containing GABA. The salt content in the GABA soy sauce is a half of that in regular soy sauces [29]. Considering both GABA and salt contents in the GABA containing soy sauce, we have employed the same GABA level used in the soy sauce for our experiment. The amount of GABA in the soy sauce was determined as follows: According to the label of the GABA soy sauce, 1.3 g GABA is added per 100 g, and salt content is 7.8%. The amount of GABA per g of NaCl is converted to be 0.17 g. The contrast effect observed for sweetness with salt was demonstrated by adding 1.7% salt to the sweet material (Hisaki K, personal unpublished results). Based upon this concept, 0.01% GABA was estimated to give the contrast effect toward 3% sucrose solution. The estimated amount of GABA was equivalent to 0.05% salt.

When the test solution containing 0.01% GABA in 3% sucrose was examined, it did not show any enhancement of sweetness as shown in Fig. 1. For the comparison, our previous data using 0.0025% GABA, almost at the

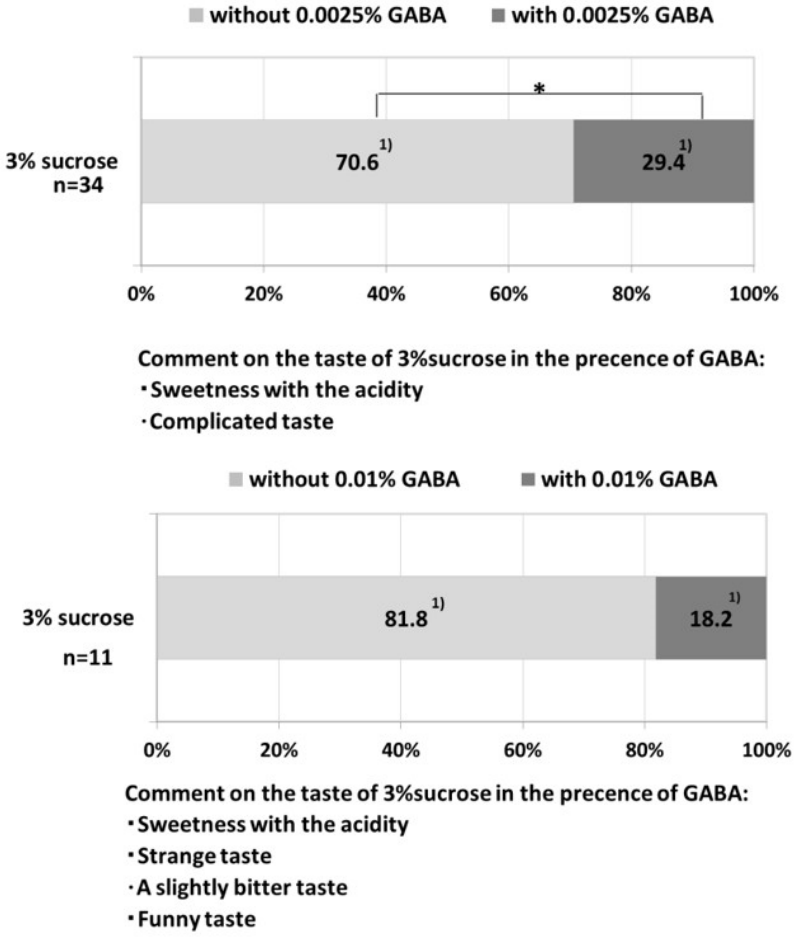

Fig. 1 Effect of GABA to the Hidden Taste on Sweetness
Three-percent sucrose solution was used in the taste sensation test. Two kinds of GABA concentrations were chosen for the test: 0.0025% GABA solution [26] and 0.01% GABA solution. *p<0.05: By the comparison test (without GABA : with GABA)[17]
1) Percentage of the subjects who evaluated that sweet taste was enhanced.

threshold value, was presented in Fig. 1 [26]. Significantly higher GABA showed no contrast effect, rather it gave some sour taste [25, 26]. We did not carry out any further experiment by raising GABA concentration because of the sour taste. Our results suggest that GABA itself may not replace the action of salty taste toward sweetness.

4. Relationship between GAD67 activity and taste alteration with food extracts

We examined the effects of food extracts on the salty taste alteration. The salty taste enhancement in points were compared with the effects on GAD67 activity (%) (Fig. 2A).

The results represent a strong positive correlation between GAD67 relative activity and the enhancement of salty taste (R^2=0.6707), and suggest that a promoted GAD67 enzyme activity by the presence of food extracts enhances salty taste. It appears likely that the salty taste is enhanced as a result of GABA action that could be synthesized inside the type III cells.

5. Effects of food extracts on GAD67 activity and sweet taste

As described in the previous section, the food extracts, that activated GAD67, enhanced salty taste strongly. This suggests a possibility that food extracts acts on GAD67 located in type III taste cells; hence, GABA production level might be elevated. It is likely that GABA may act as a taste effector or messenger. Based on this assumption, we examined the possible effect of GABA on the intensity of the sweet taste. For the evaluation method used in sweet taste, 3% sucrose solution was used for the taste sensation test. The 15 types of food extracts examined for the salty taste tests were used. The methodology of the taste sensation test is the same as described for the salty taste tests.

As shown in Table 3B, more than 50% of the subjects replied that mace, poppy seed, anise, celery, oolong tea and lemongrass enhanced the sweet taste, where mace, poppy seed and celery showed significant differences of $p<0.001$, anise and lemongrass showed $p<0.01$, and oolong tea showed $p<0.05$. On the other hand, it was only oregano in which more than 60% of the subjects expressed the sweet taste was weakened, $p<0.01$. The obtained relative sweet taste enhancement point was compared with GAD67 relative activity (%). The results of the sweet taste enhancement point were shown in Table 3B. When the effect of food extracts on GAD67 activity was plotted in X axis and that on sweet taste enhancement was in Y axis (Fig. 2B), almost no correlation between the effects of the GAD67 activity and the degree of sweet taste enhancing effect of food extracts was observed (R^2=0.0846) with a greater variability of the data compared to the salty taste enhancement shown in Fig. 2A. These results suggest that GABA synthesized in taste bud cells would be unlikely to influence the sweet taste. The results do not conflict with the finding that addition of free GABA in sucrose solution did not enhance the sweetness as described in the previous section.

4. Discussion

A number of questions have been raised since the discovery of GAD67 protein expression in type III taste cells [22, 24]. One major question was about the role of GABA in taste signaling mechanisms: Whether or not it is involved in the salty taste signal transduction, and if it is involved, how GABA acts as a messenger to other cell types. We have attempted to answer to the questions by using the taste sensation tests. Since experiments utilizing animals cannot indicate clearly the perception of tastes, it is necessary to

include human as a subject.

Our preliminary data indicate that GAD67 and possibly its product GABA are active in type III taste buds [30]. When GABA alone was subjected to the smell and taste tests, it was found that the majority of subjects expressed GABA exhibiting sour and bitter tastes but very little smell [25]. Additional studies were carried out in order to examine the effects of GABA with the pre-existing basic 5 taste substances. The concentrations for 5 taste substances are 3% sucrose, 0.5% citric acid, 0.6% sodium chloride (NaCl), 0.00125% quinine sulfate, 0.06% monosodium glutamate (MSG), and 0.0025% GABA (GABA). The presence of GABA had no effect on sweet taste (p<0.01), bitter taste (p<0.001), and sour taste. On the contrary, the presence of GABA significantly enhanced umami and salty tastes. In particular, the addition of GABA enhanced salty taste without any changes in taste quality [25, 26].

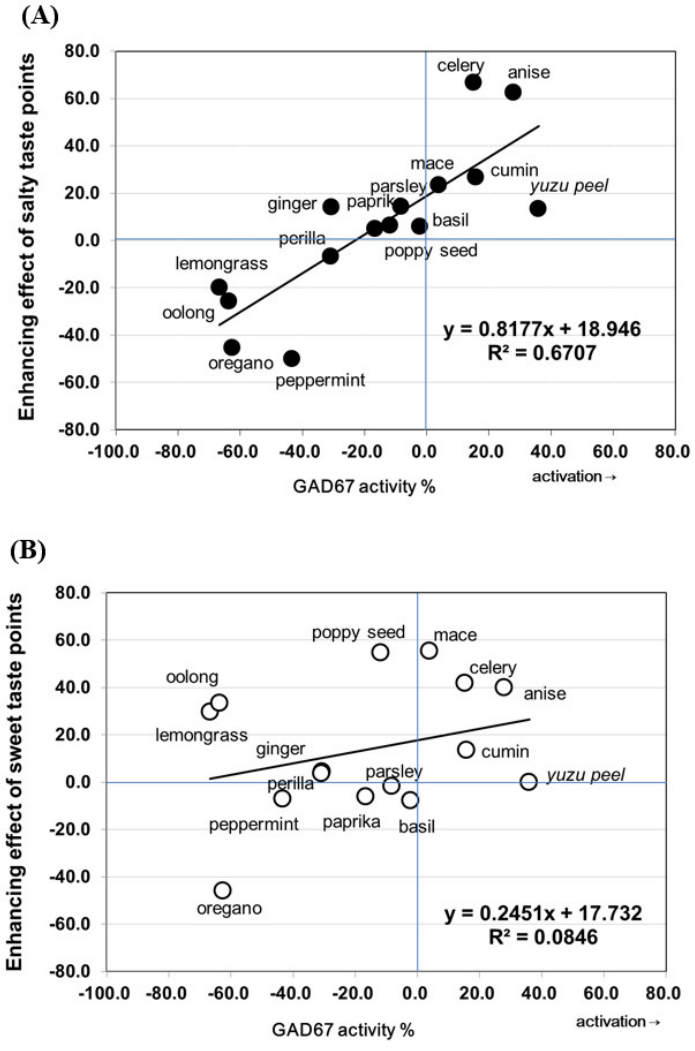

Fig. 2 Effect of the Taste Sensation by the Food Extracts Which Affected GAD67 Activity
(A) Enhancing effect of salty taste points by the food extracts.
(B) Enhancing effect of sweet taste points by the food extracts.
X-axis represents GAD67 activity in % scale (Table 2) and Y-axis represents the enhancing effect of salty taste and/or sweet taste in points (Table 3).

Based upon our preliminary results, it was clear that GABA plays a role in salty taste transduction, possibly via GABA-gated chloride ion channel or any other unknown pathways that may have some contacts with the taste nerves. Designing an experiment directly proof the involvement of GABA in the taste signal pathway is difficult; thus, we have chosen indirect ways to examine the role(s) of GABA in taste by utilizing food extracts. Effects of these food extracts on both GAD67 activity and the taste sensation tests were compared.

Fifteen food extracts, most of them are classified as spices and herbs, were chosen for GAD67 activity tests (Table 1). Our results indicate that the effect of food extracts on GAD67 activity varied widely; some activated and others inhibited. It was surprising that such food extracts as yuzu peel, anise, celery, and cumin, activated the enzyme activity since, in general, activation of enzyme by natural products is quite rare. To make sure of the elimination of possible contamination of GABA included in the food extracts, we have carried out the assays by using proper controls. At the time zero, PCA was added to the assay mixture that contained all the components including GAD67 and the food extract. The obtained GABA level was subtracted from the analyzed data where the assay reaction was terminated by the addition of PCA at the end of the reaction time. Therefore, any residual GABA presented in the food samples was not counted in the assay results. This means that if there is any increased GABA production in the assay conditions, it would be due to the results of enzymatic reactions. Those *in vitro* results encouraged us to advance to the next stage, an examination of food extracts with human subjects.

We have considered that the effects of food extracts could be due to the endogenous GABA present in the extracted samples. Since any residual GABA was not counted in the GAD67 activity assay system, residual GABA possibly present in the sample was determined (Table 1). Those samples exhibiting significantly high levels of GABA, such as celery, peppermint, and ginger, do not necessarily show the salt enhancing effects. Poppy seed, mace, basil, cumin, and oregano showed medium level salty taste enhancing effects, but these extracts do not contain significant amount of GABA. Hence, we have concluded that endogenous GABA in the extracted samples do not affect our taste sensation tests.

The presence of GABA in NaCl solution showed the enhancement of the salty taste [25, 26]. When those food components that activated GAD67 were present in the salty solution, our subjects expressed changes in saltiness. It is noted that those food extracts tend to inhibit GAD67 gave less salt enhancing effects and those to enhance GAD67 activity showed the enhancement of the saltiness. Our present results indicate that food extracts have a direct effect on the salty taste signal transduction.

Results of the 15 food extracts examined for their activities on GAD67 and the human taste test are shown in Fig. 2A. X-axis represents GAD67 activity in % scale and Y-axis represents effects on salty taste in points. There is a positive correlation between the elevated GAD67 activity by food extracts and the enhancement of the salty taste. The results imply that GABA synthesized in type III taste buds by GAD67 is likely to activate the salty taste signal transduction.

The taste contrast effect is known for sweet taste enhanced by the presence of a small amount of salt. If GABA is involved in the salt signaling mechanism, it might be possible to influence sweet taste according to the principle of the taste contrast effect. Hence, we have carried

out the experiment how GABA is effective on sweet taste. Fig. 1 represents the effect of two different concentrations of GABA on sweet taste as judged by the human taste tests. It suggests that the addition of GABA to sugar does not affect the sweetness. Additionally, 15 types of food extracts examined were plotted in order to examine the correlation between GAD67 activity and sweet taste (Fig. 2B). The results suggest there seem to be no correlation between GAD67 activity and sweet taste when various food extracts were added to sugar. It is reasonable to exclude the idea that GABA might be involved in the sweet taste signal pathway via the taste contrast effect as expected for the action of salt.

We have demonstrated that yuzu peel, anise, cumin and celery are capable of enhancing salty taste. The detailed mechanism of the salty enhancement is not clear at the moment, but it is most likely that those food extracts raise GABA level by acting on GAD67. We should be able to develop a dietary menu by using yuzu peel, anise, cumin or celery as salty taste enhancers for those who need low salt diet.

In the present study, a possible regulation of GAD67 activity in type III taste buds by the food extracts from common foods, such as spices, herbs and teas. The food extracts which increase GAD67 activity can enhance salty taste, in other words, it can be effective for prevention of hypertension and useful to prevent lifestyle-related diseases such as strokes. These food extracts can also be used in the diets of patients with dialysis. Among 15 types of foods that are analyzed in this study, the extracts from anise, celery, cumin, and yuzu peel may be promising additions to our diets to increase GAD67 activity and to raise the intensity of the salty taste; therefore, they may be useful for reducing salt intake.

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