Perceived 6-n-Propylthiouracil (PROP) Bitterness Is Associated with Dietary Sodium Intake in Female Japanese College Students

Hiroko INOUE1,2, Toshiko KUWANO3, Kimiko YAMAKAWA-KOBAYASHI2, Toshiharu WAGURI2, Teruyo NAKANO2 and Yuichi SUZUKI2,3,*

1Department of Nutrition and Health Sciences, Faculty of Food and Nutritional Sciences, Toyo University, 1–1–1 Izumino, Itakura-machi, Oura-gun, Gunma 374–0193, Japan
2School of Food and Nutritional Sciences, University of Shizuoka, 52–1 Yada, Suruga-ku, Shizuoka 422–8526, Japan
3Department of Health and Nutrition, Faculty of Human Sciences, Sendai Shirayuri Women’s College, 6–1 Honda-cho, Izumi-ku, Sendai 981–3107, Japan

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Summary Despite the negative health consequences of a high sodium consumption, humans consume well above the recommended levels. This study examines whether or not the dietary intake of sodium was affected by individual variation of the perceived bitterness of 6-n-propylthiouracil (PROP), and examines the relationship between the perceived bitterness of PROP and the preferred NaCl concentration of broth. Female students (20–22 y old) were recruited from the university community. Genotypes of A49P and I296V polymorphism of the TAS2R38 bitter taste receptor were determined for each subject. Samples containing NaCl, PROP or broth in 5-mL portions were evaluated by sensory testing. The participants completed a food record for each diet. Our results indicate that the individuals perceiving PROP to be more bitter had consumed a greater amount of dietary sodium. In contrast, there was no significant positive correlation between an individual’s perceived saltiness and the dietary sodium intake. Those who perceived PROP to be more bitter preferred a broth containing a higher concentration of NaCl. All of these correlations were apparent even after those subjects with TAS2R38 AI/AI homozygotes (PROP non-taster) had been excluded. In conclusion, the results of this study suggest that a factor affecting the bitter rating of PROP other than the AI/AI homozygotes of TAS2R38 contributes to the variation in sodium intake and the preference for salty food.

Key Words sodium intake, TAS2R38, bitter taste, salty taste, bitterness inhibition

While sodium is crucial to many physiological processes, the current sodium intake by humans far exceeds the scientific recommendations for good health (1). An elevated dietary salt intake is an established risk factor for increased blood pressure, and salt reduction is associated with a reduced risk of cardiovascular diseases (2–6). An excessive consumption of sodium or salted foods has also been associated with several other negative health effects independent of the blood pressure, including gastric cancer (3, 7–9) and decreased bone mineral density (10, 11). Despite these negative health consequences of a high sodium consumption, humans consume well above the recommended levels in most developed nations, posing a serious threat to public health (1).

Multiple physiological and socio-cultural factors affect an individual’s dietary sodium intake (12). One of the critical factors determining dietary behavior is the sensory control of ingestion. There is some evidence for individual variation in the sensation or liking for salty foods being related to the sodium intake (12–18). In addition, it has recently been shown that the perception of bitterness of 6-n-propylthiouracil (PROP) may also be related to the dietary sodium intake (17). Sodium salts can mask an unpleasant bitter taste, thereby possibly leading individuals who perceives the bitterness more strongly to tend to prefer bitter foods containing more sodium (17, 19–24).

Phenylthiocarbamate (PTC) and PROP are synthetic bitter compounds. A remarkable difference in individual sensitivity to PTC/PROP, i.e., the PTC/PROP tasters and non-tasters, is considerably, but not completely, linked to variation of one of the human bitter taste receptor gene family (hTAS2R). TAS2R38 (25–27). There are three common single-nucleotide polymorphisms in the TAS2R38 gene that result in an amino acid substitution in the protein (A49P, V262A and I296V). These show strong linkage disequilibrium, giving rise to the two common haplotypes, AVI and PAV. The PTC/PROP-taster individuals generally possess one or two PAV haplotypes, whereas non-taster individuals are homozygous for the AVI haplotype. Phenotypic differences in suprathreshold PROP bitterness beyond this TAS2R38-based grouping into the taster and the non-taster have also been recognized (28–30).
Such individual difference in the PTC/PROP taste status or TAS2R38 variation has been demonstrated to result in differences in the liking for some vegetables, fatty foods, spicy foods and alcoholic beverages (27, 31, 32). Previous studies have reported that PTC/PROP-tasters had a lower consumption of glucosinolate-containing vegetables than non-tasters (27). An explanation for this phenomenon is that PROP/PTC contain the thiocyanate moiety (N–C=S), which is responsible for their bitter taste. Isothiocyanates are the breakdown products of glucosinolates that are widely distributed in plants, particularly those of the family Brassicaceae. The relationship of individual difference in the PTC/PROP taste status or TAS2R38 variation to the dietary sodium intake has also been suggested but not yet been established (17, 33, 34).

The objective of our study was to explore the relation between PTC/PROP taste status and sodium intake. To this end, we examined in a group of young Japanese women whether or not individual variation in the perceived bitterness of PROP would be correlated with the dietary intake of sodium. We also examined the relationship between the perceived bitterness of PROP and the preferred NaCl concentration in broth. Meanwhile, we explored whether individual variation of the NaCl saltiness was involved as a confounding factor to these correlations.

**METHODS**

**Subjects.** This research consisted of two separate studies (study 1 and study 2) and was conducted at the University of Shizuoka. Fifty and 40 female students were respectively recruited in study 1 and study 2 from the university community with an age range of 20–22 y. The ethics committee of the University of Shizuoka approved the study protocol (nos. 21-9 and 22-31), according to principles laid out in the Declaration of Helsinki, and all participants provided written informed consent.

**Genetic analysis of TAS2R38.** Genomic DNA was isolated from buccal mucosa cells by using a high-purity PCR template preparation kit (Roche Diagnostics, Tokyo, Japan). We determined the genotypes of A49P and I296V polymorphism for each subject by the PCR restriction fragment length polymorphism (PCR-RELP) method. We unfortunately were unable to determine the genotypes of V262A polymorphism.

**Sensory testing.** The NaCl and PROP taste intensity levels were evaluated. The participants were asked to refrain from eating for 0.5 h prior to sensory testing. All sensory testing was conducted during the 8:30–10:00 period in partitioned booths in the sensory laboratory of the University of Shizuoka.

**Study 1.** A series of three NaCl solutions (10, 100 and 1,000 mM), and a series of four PROP solutions (0.032, 0.32, 1 and 3.2 mM) were prepared by using deionized water. NaCl and PROP were purchased from Sigma (St. Louis, MO). Samples were served in 5-mL portions in glass cups, two for each solution. The NaCl session was performed first, and then the PROP session was performed first, and then the PROP session.
Of the 40 participants in study 2, one with a mean daily energy of 882 kcal was rejected as an outlier by applying the Smirnov-Grubbs test \((p<0.01)\). The data obtained from the remaining 39 participants were subsequently analyzed. Their BMI values were 20.2 ± 2.7 kg·m\(^{-2}\) (SD, 16.4 minimum and 30.0 maximum).

Statistical analysis. A statistical analysis was performed by using SPSS software (version 18.0J, SPSS, Tokyo, Japan). A one-way repeated-measure analysis of variance and Tukey HSD comparison were used to assess if there were significant differences among the three \(TAS2R38\) genotypes, i.e., PV/PV, PV/AV and AV/AV. Pearson’s product moment correlation was used to determine if there was any association between the taste intensity rating and dietary intake, and if there was any association between the taste intensity rating and liking for NaCl in the broth. The statistical criterion for all tests was set at \(p<0.05\).

RESULTS

Study 1

Perceived PROP bitterness and NaCl saltiness as a function of the \(TAS2R38\) genotype. Of the 47 participants included in the analysis, there were 14 PV homozygotes (PV/PV), 25 heterozygotes (PV/AV), and 8 AV homozygotes (AV/AV). The PROP solution was significantly less bitter for the AV/AV homozygotes than for the carriers of the PV haplotype at 0.32–3.2 mM of PROP, but not at 0.032 mM (Fig. 1A). In contrast, the perceived saltiness of the NaCl solution did not differ among the three different \(TAS2R38\) genotypes at NaCl concentrations of 10 mM, 100 mM or 1 mM (Fig. 1B).

A correlation between individual perceived NaCl saltiness and PROP bitterness was then examined in each of 12 pairs (3 different concentrations of NaCl×4 different concentrations of PROP). The perceived PROP bitterness was significantly correlated with the perceived NaCl saltiness in two pairs (1.0 mM NaCl vs. 1.0 mM PROP, \(r=0.293\), \(p=0.045\); and 1.0 mM NaCl vs. 3.2 mM PROP, \(r=0.338\), \(p=0.020\)). However, among the individuals carrying the PV haplotype (i.e. excluding the AV/AV homozygotes), there was no significant correla-

| Daily dietary intake | Concentration of PROP (mM) |
|----------------------|---------------------------|
| NaCl (g)             | 0.335* 0.400** 0.075 -0.032 |
| NaCl/Energy (g/kcal) | 0.311* 0.379** 0.152 0.071 |
| Total vegetables (g) | -0.002 0.095 -0.052 -0.035 |
| Brassicaceae vegetables (g) | 0.072 -0.137 -0.258 -0.186 |

\(\text{Table 1. Correlation between perceived PROP bitterness and dietary intake including all (PV/PV, PV/AV, AV/AV) genotypes (}\ n=47, \text{ study 1).}\)

\(\text{Table 2. Correlation between perceived PROP bitterness and dietary intake among individuals carrying the PV haplotype (i.e. excluding AV/AV homozygotes) (}\ n=39, \text{ study 1).}\)

\(\text{Table 1. Correlation between perceived PROP bitterness and dietary intake including all (PV/PV, PV/AV, AV/AV) genotypes (}\ n=47, \text{ study 1).}\)

| Daily dietary intake | Concentration of PROP (mM) |
|----------------------|---------------------------|
| NaCl (g)             | 0.344* 0.436** 0.084 -0.039 |
| NaCl/Energy (g/kcal) | 0.315* 0.352* 0.080 0.019 |
| Total vegetables (g) | -0.005 0.088 -0.121 -0.095 |
| Brassicaceae vegetables (g) | 0.088 -0.150 -0.377 -0.297 |

\(\text{Table 2. Correlation between perceived PROP bitterness and dietary intake among individuals carrying the PV haplotype (i.e. excluding AV/AV homozygotes) (}\ n=39, \text{ study 1).}\)
tion between NaCl saltiness and PROP bitterness in any of 12 pairs.

Relationship between the perceived PROP bitterness or NaCl saltiness and dietary intake. We then examined whether phenotypic variation of the perceived PROP bitterness was associated with dietary intake (Table 1). The individuals perceiving PROP to be more bitter consumed a greater amount of dietary NaCl with 0.032 and 0.32 mM PROP although not with 1 or 3.2 mM PROP (Table 1, NaCl). This correlation was not due to any greater food intake by those individuals perceiving PROP as being more bitter; the NaCl intake was still correlated with the perceived PROP bitterness after the intake had been normalized by the energy intake (Table 1, NaCl/Energy). It is notable that these positive correlations were preserved among the individuals carrying the PV haplotype (i.e. excluding the AI/AI homozygotes; Table 2, NaCl and NaCl/Energy). A factor affecting the bitter rating to PROP other than the AI/AI homozygotes of TAS2R38 was thus mainly responsible for the variation in sodium intake.

The perceived PROP bitterness was not correlated with the total vegetable intake, regardless of whether or not AI/AI homozygotes were excluded (Tables 1 and 2, Total vegetables). We then specifically examined the intake of vegetables belonging to Brassicaceae family which contain bitter compounds, glucosinolates, as previous studies have reported that PTC/PROP-tasters had a lower consumption of glucosinolate-containing vegetables than non-tasters (27). Indeed, the dietary intake of brassicaceae vegetables in particular was negatively correlated with 1 mM PROP bitterness, this being significant only for the group excluding AI/AI homozygotes (Table 1 and 2, Brassicaceae vegetables).

In contrast, there was no significant correlation between the individual perceived saltiness and dietary intake of either NaCl or NaCl/Energy, total vegetables or brassicaceae vegetables, whether examined for the group including all TAS2R38 genotypes or for the group excluding AI/AI homozygotes (data not shown).

Study 2

Relationship between the perceived PROP bitterness or NaCl saltiness and the dietary NaCl intake. Of the 39 participants included in the analysis, there were 11 PV homozygotes (PV/PV), 21 heterozygotes (PV/Al), and 7 AI homozygotes (AI/AI). The taste intensity levels for two NaCl solutions (100 and 330 mM) and two PROP solutions (0.32 and 1 mM) were rated by using the modified scale (see “Methods” for details). A phenotypic variation of the perceived PROP bitterness was not significantly correlated with the perceived NaCl saltiness in any of the 8 pairs, i.e. 4 pairs including all genotypes and 4 pairs including genotypes with PV/PV and PV/Al (data not shown).

We then re-examined the relationship between the PROP bitterness or NaCl saltiness and the daily NaCl/Energy intake (Table 3). There was no significant correlation between the PROP bitterness and NaCl/Energy intake when all genotypes were included (Table 3, PV/PV, PV/Al, AI/AI). However, among the individuals carrying the PV haplotype (i.e. excluding the AI/AI homozygotes; Table 3, PV/PV, PV/Al), those perceiving PROP to be more bitter consumed a greater amount of dietary NaCl/Energy in the case with 1 mM PROP although not with 0.32 mM. The finding in study 1 was therefore basically reproduced, that a factor affecting the bitter rating of PROP other than the AI/AI homozygotes of TAS2R38 was positively associated with the dietary sodium intake. Again, the perceived NaCl saltiness was not correlated with the daily NaCl/Energy intake, whether examined for the group including all TAS2R38 genotypes or for the group excluding AI homozygotes (Table 3).

Relationship between the perceived PROP bitterness and the preferred NaCl concentration in the broth. The participants reported the strongest preference for one of the five concentrations of NaCl in the broth made from dried bonito stock. Twenty-one participants liked the 0.4% NaCl content, 11 liked the 0.6% NaCl content and 7 liked the 0.8% NaCl content. No participants preferred either 1.0% or 1.2% NaCl in the broth. The result of exploring a reciprocal relation between the perceived bitterness of PROP and the preferred NaCl concentration in the broth shows that, among carriers with the PV haplotype, the individuals perceiving PROP to be more bitter preferred the broth containing more NaCl (Table 4, PV/PV, PV/Al). This correlation was only significant at 1 mM PROP, although tending to be apparent at 0.32 mM PROP (p=0.08). This correlation was not apparent among those with all genotypes included (Table 4, PV/PV, PV/Al, AI/AI). We anticipated from the

Table 3. Correlation between perceived bitterness (PROP) or saltiness (NaCl) and daily NaCl/Energy intake (study 2).

| Genotype (n) | PROP (mM) | NaCl (mM) |
|-------------|-----------|-----------|
| PV/PV, PV/Al | r: -0.120, p: 0.468 | 0.054, -0.243 |
| Al/Al (39)   | r: -0.062, p: 0.736, 0.746, 0.913, 0.137 |
| PV/PV, PV/Al | r: 0.347, 0.025 |
| (32)         | r: 0.314, 0.025 |
| PV/PV, PV/Al | r: 0.314, 0.025 |
| (32)         | r: 0.314, 0.025 |

* 0.01<p<0.05.

Table 4. Correlation between perceived PROP bitterness and preferred NaCl concentration in broth (study 2).

| Genotype (n) | Concentration of PROP (mM) |
|-------------|---------------------------|
| PV/PV, PV/Al | r: 0.203, 0.216, 0.314, 0.080, 0.396 |
| Al/Al (39)   | r: 0.182, 0.266, 0.396* |
| PV/PV, PV/Al | r: 0.182, 0.266, 0.396* |
| (32)         | r: 0.182, 0.266, 0.396* |

* 0.01<p<0.05.
present results and the previous findings that those individuals consuming a higher amount of NaCl/Energy would prefer the broth containing a higher NaCl concentration (17). However, there was no significant correlation between daily NaCl/Energy intake and the favoured NaCl concentration in the broth, whether examined for the group including all TAS2R38 genotypes \((r=0.007, p=0.967)\) or for the group excluding AI homozygotes \((r=0.020, p=0.912)\).

In contrast to the PROP bitterness, the perceived NaCl saltiness of 0.1 or 0.33 m were not associated with the preferred NaCl concentration in the broth, whether examined for the group including all TAS2R38 genotypes or for the group excluding AI homozygotes (data not shown).

**DISCUSSION**

The results of the present study with a group of female college students show that those perceiving more bitterness with PROP took in more dietary sodium. Although a different bitter response for PROP has been believed to be mainly affected by the TAS2R38 genotype, the present association between supra-threshold PROP bitterness and dietary NaCl intake was apparent even after those subjects with TAS2R38 AI/AI homozygotes (PROP non-tasters) had been excluded, suggesting that the phenotypic variation caused by factors beyond the AI/AI homozygotes of TAS2R38 was a major contributor to this association.

Phenotypic differences in supra-threshold PROP bitterness beyond the AI/AI homozygotes of TAS2R38 have been recognized (28–30). It has been reported that those perceiving PROP to be more bitter also exhibited an enhanced response to many other prototypical tastants (salty, sweet, sour and bitter) (28, 36–38). Hayes and Keast have consequently proposed adopting the term “hyperguesia” to describe these broadly-tuned, heightened taste responders, rather than “supertaster” which had been widely used to describe a PROP bitterness variation beyond the TAS2R38 genotype (38). Several molecular mechanisms underlying hyperguesia have been proposed. Polymorphism in the gene for carbonic anhydrase VI, which is highly expressed in the salivary glands and secreted into saliva, has been demonstrated to be associated with individual differences in PROP bitterness and also with fungiform papilla development and maintenance (17, 39–42), although a recent report has failed to confirm this correlation (43). Salivary peptides belonging to the basic proline-rich protein family have also been implicated in contributing to PROP responsiveness (44, 45). It has recently been reported that the mRNA expression level of the taster allele of the TAS2R38 gene was correlated with the sensory perception of bitterness for PROP and broccoli juice (TAS2R38 ligands), and that it was also correlated with the taste intensity for such non-TAS2R38 ligands as quinine and denatonium, although no such relationship was apparent for other non-TAS2R38 ligands (urea and caffeine) (46). Consequently, it might be interesting to examine in the future a correlation between the perceived bitterness of such other compounds as quinine and denatonium and the dietary sodium intake (31).

It is conceivable that individual variation of judging saltiness from NaCl would affect the dietary sodium intake: low saltiness is likely to lead high sodium intake (12, 14). This begs the question that individual variation of the perceived NaCl saltiness could then be a major confounding factor for the positive correlation between PROP bitterness and a high sodium intake observed by the present participants. This possibility is, however, unlikely: Firstly we failed to demonstrate any significant correlation between the perceived saltiness and dietary sodium intake. Secondly, the perceived PROP bitterness was not negatively associated with perceived NaCl saltiness. In some cases the PROP bitterness was rather positively associated with NaCl saltiness (1.0 mM or 3.2 mM PROP vs. 1.0 mM NaCl: the genotype with PV/PV and PV/AI in study 1), in agreement with the previous reports (17, 46). Individual variation of saltiness to NaCl would consequently not be a major confounding factor for the positive correlation between PROP bitterness and a high sodium intake observed with the present participants.

One possible explanation for the association between PROP bitterness and the dietary sodium intake is the masking of bitterness, an aversive taste, by sodium salts (19–24). Previous reports have shown that the response to PROP was associated with increased bitterness and therefore reduced the liking for such vegetables as Brassicaceae (cruciferous) (27, 32, 47). The present participants, however, despite the apparent negative correlation for Brassicaceae vegetables, reported that the perceived PROP bitterness was not negatively correlated with the total vegetable intake (Tables 1 and 2). It is thus conceivable that individuals who perceived PROP to be more bitter tended to prefer the vegetables containing more sodium, and took a higher amount of sodium unconsciously while preserving health-promoting vegetable consumption (48).

The results of this present study have also demonstrated that those individuals who evaluated PROP as being more bitter liked a broth containing a higher concentration of NaCl. This was apparent among carriers of the PV haplotype, suggesting again that a phenotypic variation caused by factors beyond the TAS2R38 genotype contributed to this association. Although the underlying mechanism remains to be explored, this relation is at least consistent with the higher dietary sodium intake by those individuals who evaluated PROP as being more bitter. This relationship could be the consequence of a habitual preference for high sodium-containing foods resulting from an established high dietary sodium intake (12, 14).

In summary, our findings raised the possibility that a factor affecting the bitter rating of PROP other than the genotypes of TAS2R38 contributes to the increased sodium intake by the present group of young Japanese women. Further studies are needed to confirm the present correlation, since the relationship between taste intensity and food preference is likely to be influenced by age, sex and socio-cultural factors (17, 49, 50).
tion, the mechanism linking the perceived PROP bitterness to an elevated sodium intake remains to be further explored in the future.

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