

Chronic restraint stress in rats suppresses sweet and umami taste responses and lingual expression of T1R3 mRNA

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ABSTRACT

Effects of chronic restraint stress on the taste responses to five basic taste qualities were investigated electrophysiologically in the rat chorda tympani. In addition, the mRNA expression for T1R3, the common G-protein-coupled receptor (GPCR) for sweet and umami tastes, was studied quantitatively by RT-PCR after such stress. Rats were restrained in a small cylindrical restrainer made of steel wire for 8 h daily for 14 successive days. The integrated responses to sweet and umami tastes, as recorded from the chorda tympani, were significantly suppressed after such stress, but the other three basic taste responses were unaffected. Expression of T1R3 mRNA in the fungiform papillae, as estimated by RT-PCR, was slightly reduced by the stress, and a quantitative real time RT-PCR study revealed a significant suppression of T1R3 mRNA expression in the stress group. These results suggest that the observed stress-induced changes in taste sensation could be caused by a peripheral disorder of the transduction mechanism in taste-receptor cells, involving in particular a stress-induced inhibition of T1R3 expression.

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The sense of taste plays several important roles, with sweet taste permitting the identification of energy-rich nutrients, umami allowing recognition of amino acids, salt taste ensuring a proper dietary electrolyte balance, and sour and bitter tastes warning against the intake of potentially noxious and/or poisonous chemicals [2]. Overall, this not only allows evaluation of the nutritious content of food, but also helps to prevent the ingestion of toxic substances.

Recent years have seen an increasing number of people presenting with a taste disorder, which can have an important impact on their quality of life. For some problems of taste recognition, the etiology is known. The causes include: (a) adhesion of dental bacteria or fungi (like candida) to the coated tongue, (b) metabolic suppression of the tongue epithelium by a lack of zinc, and (c) a decline in taste sensation with aging or as a result of mental factors. Many taste disorders can be improved, however. For example, a lack of zinc can be corrected by supplementation with zinc, while adhesion of bacteria to a coated tongue can be dealt with by cleaning the tongue. However, other taste disorders can prove to be refractory, and the mechanisms underlying these disorders are not fully understood. Taste disorders resulting from mental factors are examples of this, and emotional stress is known to be one of the mental factors with effects on taste perception [1].

The relation between stress and taste has already been investigated in many studies. In rodents, several reports have shown that *acute* stress results in a rejection of novel bitter tastes and a desire for sweet ones [3,4,9]. In contrast, it has been reported that the consumption of sucrose is inhibited by *chronic* stress in rats [7,8], while other reports showed that such stress increased sweet intake in the same species [6]. Since most of these studies were designed to assess stress-induced changes behaviorally, the details of the underlying mechanisms remain to be elucidated. In the present study, we made electrophysiological recordings of taste-nerve responses to the five basic taste qualities in rats to investigate whether chronic restraint stress affects the peripheral taste-recognition mechanisms. The possible effects of such stress on the mRNA expressions of some taste-related GPCR were also examined to illuminate the mechanisms involved in any changes in the responses.

Male Wistar rats (11 weeks old) were housed in individual plastic cages (40 cm × 25 cm × 20 cm; length × width × depth) with woodchip bedding in a room maintained at 25 ± 1 °C, with the humidity set at 50%. They experienced a photoperiod of 12 h light:12 h dark (lights on at 07:00). All had ad libitum access to drink (tap water) and standard laboratory rat chow (CE-2; CLEA Japan Inc., Tokyo, Japan). The protocols were reviewed by the Committee on the Ethics of Animal Experiments in Tottori University Faculty of Medicine, and the experiments were carried out in accordance both with the Guidelines for Animal Experiments at Tottori University Faculty of Medicine and with the Federal Law (no. 221) and Notification (no. 6) issued by the Japanese Government.

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Rats were restrained as required in a small cylindrical restrainer made of steel wire (7 cm × 22 cm [diameter × length]) for 8 h (light period, from 09:00 to 17:00) every day for 14 successive days.

After chronic restraint stress, rats were deeply anesthetized with an intraperitoneal injection of pentobarbital sodium (50 mg/kg). The chorda tympani, which innervates the taste buds on the anterior part of the tongue, was exposed by the conventional approach. The whole-nerve responses of the chorda tympani to various taste stimuli were led via a pair of Ag–AgCl electrodes to a pen-recorder, and recorded as integrated responses (time constant: 0.5 s), with digital recording being performed simultaneously through a personal computer equipped with an AD converter (Powerlab). Height above the baseline was measured at 20 s after the onset of stimulation. The taste stimuli used in the present experiment were 0.03 M NaCl for salty taste, 0.5 M sucrose for sweet taste, 0.05 M monosodium glutamate (MSG) for umami, 0.003 M HCl for sour taste, and 0.01 M quinine hydrochloride (QHCl) for bitter taste [10,13,14]. Each taste stimulus (10 ml) was applied through a polyethylene tube by gravity flow (at about 0.5 ml/s) to the anterior part of the tongue, which was inserted into a glass chamber.

The mRNA expressions for T1R3 (the common receptor for sweet and umami tastes) and T2R5 (a receptor for bitter taste) were analyzed by RT-PCR. After chronic restraint stress, rats were deeply anesthetized with an intraperitoneal injection of pentobarbital sodium (50 mg/kg). The apex of the tongue was pulled forward with the aid of mosquito forceps, and fifteen fungiform papillae were collected from the anterior tongue epithelium using tweezers. Total RNA was extracted using a commercial RNeasy Mini kit (Qiagen Inc., Valencia, CA, USA) according to the manufacturer's instructions. To ensure no cross-contamination with genomic DNA, samples were treated for 30 min with 10 units of RNase-free DNase (Qiagen Inc.) at room temperature. Then, 0.5 µg of total RNA was reverse-transcribed, to synthesize cDNA, using a First Strand cDNA Synthesis Kit (Fermentas, Burlington, Canada). The primer [11] sequences for each PCR were as follows:

T1R3	Forward	5'-GATCAGTGGTCCCGAGAAA-3'
	Reverse	5'-TAAGCTAGCATGGCGAAGGT-3'
T2R5	Forward	5'-TGGCAAATCCACATGAAGAA-3'
	Reverse	5'-GCAGGGATAGAGGAATGCAA-3'
β-actin	Forward	5'-TCATGTTTGAGACCTTCAA-3'
	Reverse	5'-GTCTTGGCGATGTCCACG-3'

For PCR, using Taq DNA polymerase (Roche Diagnostics, Indianapolis, IN, USA), all transcripts were amplified by 35 cycles of annealing (53 °C, 1 min), extension (72 °C, 1 min), and denaturation (94 °C, 2 min). Amplification products were analyzed on 2% agarose gels and visualized with ethidium bromide. DNA bands were photographed under UV light. The PCR products were expected to be of the following sizes: T1R3, 682 bp; T2R5, 330 bp; β-actin, 493 bp.

The mRNA expressions for T1R3 and T2R5 in fungiform papillae were measured by real-time quantitative PCR using a LightCycler (Roche Diagnostics, Mannheim, Germany). Fungiform papillae were obtained as described above. Total RNA was extracted from the tissues by means of a commercial RNeasy Lipid Tissue Mini Kit (Qiagen K.K., Tokyo, Japan) according to the manufacturer's instructions. To ensure no cross-contamination with genomic DNA, samples were treated for 30 min with 10U of RNase-free DNase (Qiagen K.K.) at room temperature. For cDNA synthesis, 0.5 µg of total RNA was reverse-transcribed, using a First Strand cDNA Synthesis Kit (Fermentas). TaqMan probes and primers for the T1R3 and T2R5 genes were designed using the primer-design software ProbeFinder (Roche Diagnostics), and optimized for use in this study. TaqMan probes (Roche Diagnostics) were labeled at the 5' end with the reporter-dye molecule FAM, and at the 3' end with

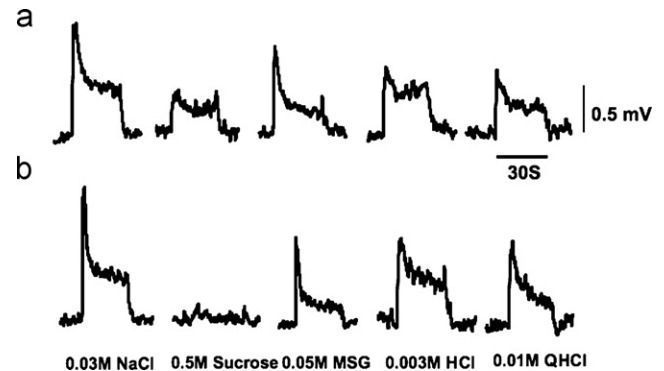


Fig. 1. Integrated whole-nerve responses of chorda tympani to lingual application of 0.03 M NaCl, 0.5 M sucrose, 0.05 M MSG, 0.003 M HCl, and 0.01 M QHCl in the control (a) and stress (b) groups. Each integrated curve shown here is an example of non-averaged experimental records.

a quencher-dye molecule. The primer (Operon Biotechnologies, Tokyo, Japan) sequences were as follows:

T1R3	Forward	5'-ATGTAGTGGCCAGGCAACC-3'
	Reverse	5'-ACCTGGCCATCTTGGCACT-3'
T2R5	Forward	5'-GGTATTACCATCAATGTCATTGTCT-3'
	Reverse	5'-ATCGTCAAACCAACACGAA-3'
β-actin	Forward	5'-CTGGCTCTAGCACCATGA-3'
	Reverse	5'-TAGAGCCACCAATCCACACA-3'

Then, cDNA samples (20 ng in a total volume of 20 µl) were mixed with the probe, primers, and LightCycler TaqMan Master Mix (Roche Diagnostics). The reaction program was as follows: denaturation at 95 °C for 10 min, followed by 50 thermal cycles of denaturation (at 95 °C for 10 s) and extension (at 60 °C for 30 s) (i.e., 2-step PCR cycles). The fluorescent signal in each cycle generated by the release of the reporter (FAM) from the quencher (by the 5'-exonuclease activity of AmpliTaq polymerase) was plotted versus the cycle number. Standard curves for each individual target amplicon were constructed using the PCR product. All mRNA expression levels were normalized to the β-actin mRNA content, and non-template controls were incorporated into each PCR run.

All results are expressed as mean ± S.E.M. Taste-response and real-time RT-PCR data were analyzed for statistical significance by ANOVA, followed by Fisher's PLSD test (post hoc test) (Macintosh, StatView 4.0). Differences were considered significant at $P < 0.05$.

Fig. 1 shows typical integrated chorda tympani responses to various taste solutions (0.03 M NaCl, 0.5 M sucrose, 0.05 M MSG, 0.003 M HCl, and 0.01 M QHCl) in control (a) and chronically

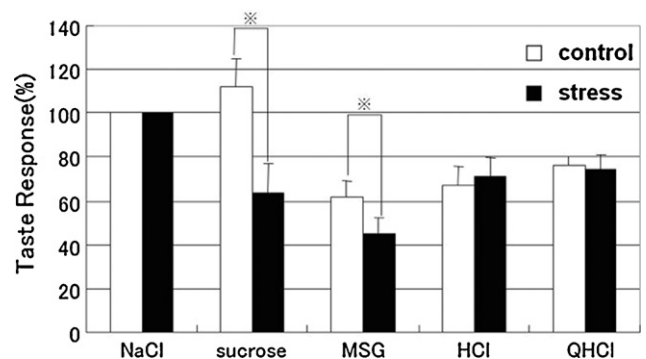


Fig. 2. Relative responses to stimulation by five taste qualities (as in Fig. 1) in control (open bars) and stress (closed bars) groups (mean ± S.E.M., $n = 9$ for control and $n = 11$ for stress group). Responses are expressed as a percentage of the response to 0.03 M NaCl. The range of individual values for the taste response to 0.03 M NaCl was 0.25–1.0 mV. * $P < 0.05$.

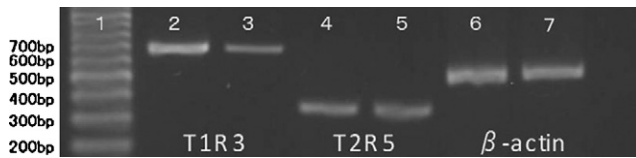


Fig. 3. Presence of mRNAs coding for T1R3, T2R5, and β -actin in the fungiform papillae of the anterior tongue. Photographs of DNA bands stained with ethidium bromide (RT-PCR experiment). (Bands are as follows: 1 is a marker; 2, 4 and 6 represent control groups; and 3, 5 and 7 represent stress groups.)

stressed (b) rats. This figure shows a marked reduction in the integrated sweet response to sucrose and a more modest reduction in the umami response to MSG after chronic stress exposure.

In Fig. 2, the responses to 0.5 M sucrose, 0.05 M MSG, 0.003 M HCl, and 0.01 M QHCl are quantified and compared between the control and stressed groups, with the response to 0.03 M NaCl in each rat being given the value 100%. In the stressed rats, significant reductions were observed in the responses to 0.5 M sucrose [ANOVA, $F(1, 90) = 8.68$, $p < 0.05$; post hoc test, $p < 0.05$ versus control rats] and 0.05 M MSG [ANOVA, $F(1, 90) = 8.68$, $p < 0.05$; post hoc test, $p < 0.05$ versus control rats], but not in those to 0.003 M HCl and 0.01 M QHCl.

Fig. 3 shows results from the RT-PCR study. This demonstrated that the fungiform papillae of the anterior tongue express mRNAs coding for T1R3, T2R5, and β -actin. The sizes of the PCR products, which were estimated by comparison with a size marker, were found to correspond to the calculated ones (682 bp for T1R3, 330 bp for T2R5, 493 bp for β -actin). T1R3, T2R5 and β -actin mRNAs were all expressed in both the control and stress groups. However, the expression of T1R3 mRNA in the stress group (band 3) appeared to be weaker than that in the control group (band 2).

To try to confirm the RT-PCR results, a quantitative analysis of the mRNA expressions of taste GPCRs was performed using real time RT-PCR. In other words, we visualized each mRNA expression as a “band” by RT-PCR and it was quantified by real-time RT-PCR. Fig. 4 shows that T1R3 mRNA expression was significantly suppressed in the stress group versus the controls [ANOVA, $F(1, 22) = 16.325$, $p < 0.05$; post hoc test, $p < 0.05$ versus control rats]. On the other hand, the mRNA expression for T2R5 (which recognizes the bitter taste of cycloheximide) showed no change after chronic stress. These results support those obtained in the above electrophysiological and RT-PCR studies.

In the present study, on rats, we carried out electrophysiological recordings of taste responses to investigate whether chronic restraint stress induces peripheral disorders of taste-reception mechanisms. The results showed that both sweet and umami taste

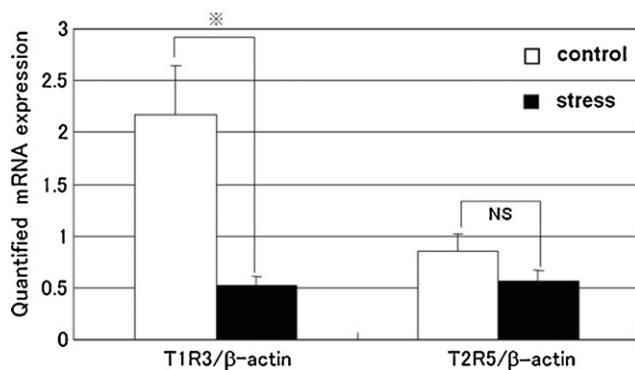


Fig. 4. Relative expressions of T1R3 mRNA and T2R5 mRNA in the fungiform papillae of the anterior tongue. Mean \pm S.E.M. (control, $n = 6$; stress, $n = 7$) values for mRNA expressions. The level of β -actin mRNA expression was used as the internal control. * $P < 0.05$; NS, not significant.

responses were specifically suppressed by such stress. For the present analysis, we elected to set the integrated taste responses to 0.03 M NaCl as the standard for the evaluation of any stress-induced changes in the responses to all other taste stimuli. Although we had no specific reason for applying this standardization procedure, the conclusion that sweet and umami responses were selectively reduced by the imposed stress is well in line with the results of our subsequent study of taste receptor mRNA expressions, apparently justifying the present form of electrophysiological analysis.

Next, we used RT-PCR to investigate the effects of chronic restraint stress on the mRNA expressions for T1R3, the common receptor for sweet and umami taste transduction, and T2R5, one of the bitter-taste receptors. Real time RT-PCR showed that the expression of T1R3 mRNA was significantly suppressed in the stress group, while that of T2R5 mRNA was unchanged. These results are in good agreement with those of the electrophysiological study. By these observations the present study is the first to suggest that chronic stress may actually cause a peripheral disorder of taste transduction through an inhibition of the expressions of the sweet and umami taste receptors.

One of the mechanisms responsible for stress-induced taste disorders is thought to be a drying of the oral cavity due to decreased salivation. During stress, the sympathetic nervous system dominates the reactions of the body, resulting in a decrease in salivation among other effects. This causes dryness of the oral cavity and taste-bud atrophy, leading to abnormal taste discrimination. In addition, a decrease in blood flow may occur around taste cells when the sympathetic nervous system becomes dominant, perhaps leading to decreased oxidative metabolism within the peripheral tissue. However, in that scenario all, or almost all, of the taste qualities would be expected to be affected in a non-selective manner. The present observation that only sweet and umami taste receptors were affected suggests that more specific effects of chronic restraint stress on taste cells need to be invoked to explain the present results.

Among the many previous reports on the relation between taste sensation and stress, some papers have focused on the stress-induced change in the consumption of, or preference for, sucrose and/or a bitter tastant. For example, Gronli et al. [7,8] and Willner [12] noted that consumption of sweet solution is decreased by chronic mild stress in rats, while Ely et al. [6] showed that such stress resulted in increased sweet intake. Dess and Edelhait [5] found evidence that in humans, stress leads to higher bitter-taste ratings and lower sweet-taste ratings, although the mechanism remains unknown. In the present study, we found that chronic restraint stress reduced the expressions of the mRNAs for the taste receptors involved in sweet and umami recognition. Taste perception not only depends on the chemical and physical properties of tastants, but may also depend on the physiological and psychological status of those who do the tasting. It would be interesting to know whether the present stress-induced changes in receptor mRNA expressions might underlie the changes in taste behavior reported previously. However, at this stage we must resist directly connecting our findings with the previous reports on taste behavior. Thus, behavioral experiments need to be performed in the near future to show whether the present changes in taste responses and mRNA expression are associated with differences in sweet-solution intake. Furthermore, the central mechanism mediating the effects exerted by stress on taste reception remains unknown, and would be useful to investigate in the future.

In conclusion, the present results represent the first evidence that in rats, chronic restraint stress reduces sweet and umami responses in the chorda tympani, an effect probably due to a peripheral disorder of the transduction mechanism in taste-receptor cells, involving in particular a stress-induced inhibition of T1R3 expression. An interesting question is whether acute stress elicits similar

changes in taste-related responses (i.e., as detected by electrophysiological recordings and quantification of the expression of T1R3 mRNA). In a preliminary experiment, we found that a single period of acute restraint stress lasting 8 h (from 9:00 to 17:00) on one day had a non-significant ($p > 0.05$) effect on T1R3 mRNA expression (quantified mRNA expression) [stressed group ($n = 6$), 1.56 ± 0.24 ; control group ($n = 6$), 1.82 ± 0.32] (unpublished observation). On that basis, it seems unlikely that taste-related responses of the type observed in the present study are induced by such acute stress. However, we still need to examine the effects of acute stress on electrophysiological recordings from the chorda tympani. Finally, the activity of the HPA axis and/or the level of sympathetic nerve activity need to be examined in chronic-restraint rats to determine whether adaptation or habituation to the stress might be occurring in rats repeatedly receiving the same stressor.

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