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Pentavalent vanadium at concentration of the underground water level enhances the sweet taste sense to glucose in college students

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Abstract

Underground water in volcanic areas contains vanadium when the basalt layer exists among igneous rocks. The concentration of vanadium in drinking water sometimes exceeds 0.8 μM in these areas, however, the physiological effects of vanadium, especially non-toxic effects, at concentrations lower than 1 μM are unknown. In the present experiments, we examined the effect of pentavalent vanadium and tetravalent vanadium at 0.8 and 8.0 μM concentrations on the recognition threshold to taste substances in healthy college students. Pentavalent vanadium, ammonium vanadate, lowered the sweet taste threshold to glucose at 0.8 and 8.0 μM as well. Tetravalent vanadium, vanadium sulfate, did not alter the threshold to glucose either at 8.0 μM or at 0.8 μM. Ammonium vanadate also decreased the sweet taste threshold to L-proline at 8.0 μM. Ammonium vanadate did not influence the sour taste threshold to hydrogen chloride. Neither ammonium sulfate nor ammonium bicarbonate altered the sweet taste threshold to glucose. Therefore, the effect of ammonium vanadate on the sweet taste threshold is attained by vanadium but not by ammonium. It was concluded that pentavalent vanadium at 0.8 μM intensifies the sweet taste sense to glucose rather specifically. We have first shown the physiological effect of vanadium at the concentration of the underground water level.

Introduction

Underground water in volcanic areas contains vanadium when the basalt layer exists among igneous rocks. For example, underground water, also supplied for drinking and other daily use, in the Mt. Fuji area in Japan is enriched with vanadium, the concentration of which varies between 0.5 and 0.8 μM. These concentrations are higher than the average of the underground water level in Japan by 50–100 times. As physiological effects of vanadium, the amplification of Ca^{2+}-induced contraction of smooth muscles (Sunano *et al.* 1988) and the inhibition of cochlear potentials (Nakano & Morimitsu 1988) have been previously reported. However, the concentrations of vanadium used in these experiments were at least 1000 times higher than those in the underground water. As vanadium taken orally has been reported to ameliorate the blood glucose level and hyperglycemia in diabetic patients (Cohen *et al.* 1995; Halberstram *et al.* 1996), an anti-diabetic effect of vanadium is widely expected (Goldwaser *et al.* 2000). The oral dose of vanadium in those clinical studies did not exceed 2 mg/kg/day, to avoid toxic effects. However, if 2 mg vanadium per kg body weight is to be taken only from drinking water which contains 0.8 μM vanadium, at

approximately 0.1 mg/l, the amount of water the patients are expected to intake daily is roughly calculated to be at least 20 l/kg. The concentration of vanadium in underground water is thus too low to reveal anti-diabetic effects.

On the other hand, cations, which bind to the surface membrane of taste cells, generally play an essential role in the perception of taste. For example, in the eel, the reduction of taste responses of the palatine nerve to amino acids after the elimination of ions from the receptor membrane with chelating agent is restored by the presence of cations (Yoshii & Kurihara 1983). In this experiment, the effect of monovalent cations on the activity of gustatory nerves appears at a concentration of the 10^{-5} M order, but that of divalent cations appears at a concentration of the 10^{-7} M order. The effectiveness of divalent cations at a low concentration raises the possibility that vanadium, as a polyvalent cation, also influences the perception of taste also at the low concentrations of the underground water level. In the present experiment, therefore, we examined the possibility that pentavalent vanadium modifies sweet taste sense and sour taste sense in healthy college students. D-glucose and L-proline were chosen as sweet-tasting substances, because the transduction mechanisms of sweet sense to sugar and to amino acid are different (Gilbertson *et al.* 2000; Lindemann 2000). The effects of tetravalent vanadium on sweet taste sense and sour taste sense were also examined.

Materials and methods

Participants

In total, participants were 141 undergraduates from the University of Yamanashi and the Yamanashi Prefectural College of Nursing, aged between 18 and 33 years (53 males and 78 females). None of them had a habit of smoking. The experiment was performed in accordance with the Ethics Committee of Yamanashi Institute of Environmental Sciences on the basis of the Declaration of Helsinki, and the informed consent of all participants was obtained in written form. They were requested not to skip but to finish their breakfast or lunch at least 2 h before the experiment.

Chemicals

D-glucose and L-proline were used to examine the effect of vanadium on sweet taste thresholds. Hydrogen chloride was used as a sour-tasting substance. Ammonium vanadate, NH_4VO_3, as pentavalent vanadium, and vanadium sulfate, $VOSO_4$, as tetravalent vanadium, were used to condition the participants. Ammonium sulfate, $(NH_4)_2SO_4$, and ammonium bicarbonate, NH_4HCO_3, were also used to discriminate the effect of pentavalent vanadium from that of ammonium. All chemicals used were purchased from Wako Pure Chemical Industries, Osaka, Japan.

Procedures

Vanadates and ammoniates were diluted with distilled water, and prepared as 0.8 and 8.0 μM solutions. These solutions together with distilled water were used for conditioning the participants. The participants were requested to rinse their mouths with conditioning solution every time before testing thresholds. The volume of conditioning solution was 10 ml. The recognition thresholds of glucose and L-proline were determined as weight per volume percent, and those for hydrogen chloride as volume to volume percent. The thresholds were tested in downward order from the higher concentrations to the lower concentrations by 0.1% or by 0.01%, when the threshold concentration was suspected to be lower than 0.1%. The participants were asked to report whether they felt a given test solution sweet for glucose and L-proline, or sour for hydrogen chloride. When they felt a test solution neither sweet nor sour, one step higher concentration of the test solution was regarded as detection threshold. Thresholds were first examined with distilled water, then with 0.8 μM conditioning solution and finally with 8.0 μM conditioning solution. The volume of the test solution was 5 ml.

In 38 of the 141 participants, the effects of ammonium vanadate on recognition thresholds to glucose and L-proline were examined. The effects of ammonium vanadate on sour taste threshold to hydrogen chloride were determined in 22 participants. The effects of vanadium sulfate and ammonium sulfate on sweet taste threshold to

glucose were tested in 21 participants, and those of ammonium bicarbonate in 15 participants.

In one experiment, participants sequentially judged the recognition threshold. Therefore, we examined whether the threshold concentration of glucose varies, during one experiment using 22 participants conditioned only with distilled water. In 24 participants, we examined whether the effect of vanadate on the threshold concentration of glucose is reversible. In this experiment, participants judged the threshold concentration of glucose three times, first conditioned with distilled water, next with 0.8 μM vanadate, and with distilled water again.

All experiments were performed in a room in which air temperature was kept at 24 °C. The temperatures of test solutions and conditioning solutions were also 24 °C. The pH of the ammonium vanadate solution was 7.41 at 0.8 μM and 7.40 at 8.0 μM. The pH of the vanadium sulfate solution was 6.52 at 0.8 μM and 6.21 at 8.0 μM. The pH of the solutions of ammonium sulfate and ammonium bicarbonate were 6.89 and 6.78 at 0.8 μM, and 6.65 and 6.43 at 8.0 μM, respectively.

Statistics

Statistical significance among threshold concentrations was examined by one-way ANOVA. If statistical differences were confirmed by ANOVA, data was further analyzed by Fishers PLSD. P values lower than 0.05 were regarded as significant.

Results

Effects of ammonium vanadate on taste thresholds to glucose, L-proline and hydrogen chloride

Ammonium vanadate significantly decreased the threshold concentration of glucose ($F(2, 37) = 5.557$, $P = 0.0056$). Threshold concentrations of glucose were significantly lower under the conditions with 0.8 μM and 8.0 μM ammonium vanadate (Figure 1A). Ammonium vanadate significantly decreased the threshold concentration of L-proline ($F(2, 31) = 4.157$, $P = 0.0202$), however Fisher's PLSD showed that the effective concentration of ammonium vanadate was 8.0 μM but not 0.8 μM (Figure 1b).

Ammonium vanadate at the concentration of the underground water level, 0.8 μM, intensified sweet taste sense to glucose but not to amino acid. In this experiment, threshold concentration was sequentially tested., Therefore, we examined whether the threshold concentration of glucose varies, in the experiment using participants conditioned only with distilled water (Figure 1c). There were no significant differences among threshold concentrations of glucose sequentially determined three times ($F(2, 21) = 1.538$, $P = 0.2267$). Therefore, it was concluded that the changes in the threshold concentration in Figures 1a and b are due to the action of vanadium. Figure 1d further shows that the effect of 0.8 μM vanadium on sweet taste threshold to glucose is reversible ($F(2, 23) = 5.702$, $P = 0.0061$). Figure 2a shows that sour taste threshold to hydrogen chloride was not affected by ammonium vanadate ($F(2, 21) = 0.513$, $P = 0.6023$).

Effects of tetravalent vanadium and ammonium on sweet taste threshold to glucose

The effects of tetravalent vanadium on sweet taste threshold to glucose were examined (Figure 2b). The threshold concentration of glucose was not influenced by vanadium sulfate at 0.8 or 8.0 μM ($F(2, 11) = 0.787$, $P = 0.4677$). This result shows that pentavalent vanadium sensitizes the sweet taste of glucose, but tetravalent vanadium does not. In the present experiment, we used ammonium vanadate as pentavalent vanadium. However, ammonium is reported to stimulate taste cells and to activate the chorda tympani nerve in rats, especially in juvenile and aged groups (McBride & Mistretta 1986). Therefore, the possibility remains that an increase in nervous activity of the chorda tympani generally influences the sensitivity to taste substances applied orally. To examine this possibility, we investigated the effects of ammonium sulfate on sweet taste threshold to glucose (Figure 2c). The threshold concentration of glucose was not influenced by ammonium sulfate at 0.8 or 8.0 μM ($F(2, 20) = 0.817$, $P = 0.4491$). Ammonium bicarbonate (Figure 2d) also failed to affect the threshold concentration of glucose ($F(2, 12) = 0.367$, $P = 0.6963$). It was therefore concluded that the effect of ammonium vanadate of sensitizing the sweet taste to glucose (Figure 1) is due to pentavalent vanadium and not ammonium.

Discussion

The present results showed that pentavalent vanadium at the concentration of the underground water level, 0.8 μM, enhanced the sweet taste sense to glucose. Facilitation of the sweet taste sense to L-proline by vanadium occurred at a concentration 10-times higher than underground water level. Responses of the chorda tympani nerve to sugars given orally are generally enhanced by the coexistence of monovalent cations like Na^+ and K^+, however, the effects of divalent cations are different depending on the concentration of sugar (Kumazawa & Kurihara 1990). For example, responses of the chorda tympani nerve to glucose are inhibited by Ca^{2+} when the glucose concentration is 0.5 M, but are enhanced when the concentration of glucose is 1.0 M. The recognition threshold to glucose determined in the present experiment remained at around 0.056 M. Therefore, we found that pentavalent vanadium intensifies the sweet taste sense to glucose at quite a low concentration.

To provoke sweet taste sense, L-proline is considered to rely on receptor proteins and transduction mechanisms different than those glucose relies on. Among the taste receptors coupling with G-proteins, a combination of T1R2 and T1R3 is found to be specific for sweet taste by sugars (Nelson *et al.* 2001), and a combination of T1R1 and T1R3 for L-amino acid, including L-proline, eliciting sweet taste (Nelson *et al.* 2002). Different combinations of receptor proteins probably recruit different mechanisms of signal transduction within taste cells, and thus sensitivity to pentavalent vanadium differs among taste cells.

Sour taste sense to hydrogen chloride was not affected by ammonium vanadate. This result coincides with the preceding study in hamsters showing that responses of taste cells to acidic stimulation by citrate are not influenced by pentavalent vanadium (sodium orthovanadate) and NH_4Cl (Gilbertson *et al.* 1992). Amiloride-sensitive Na^+ channels and/or proton-channels on the taste cell membrane are essentially involved in signal transduction for sour taste

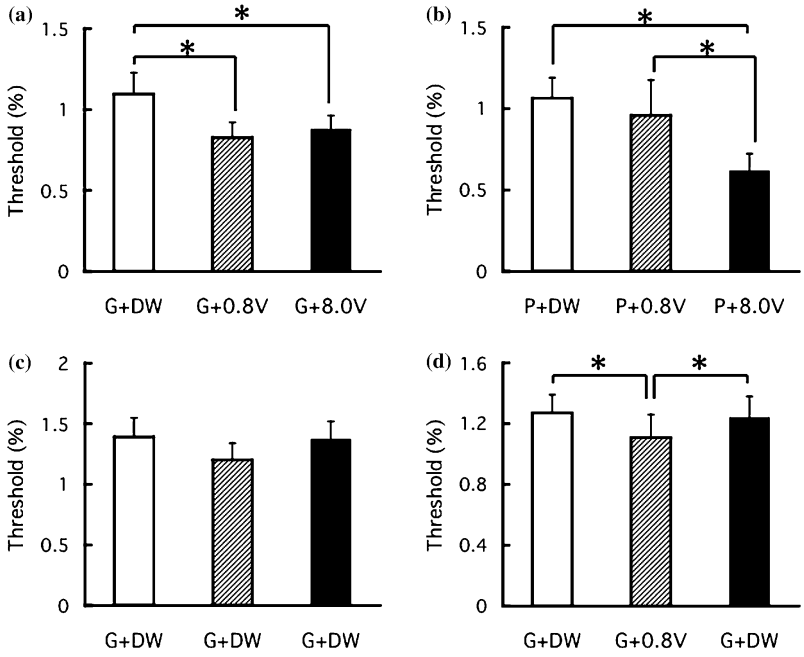

Figure 1. The effects of ammonium vanadate on the recognition thresholds of sweet taste to glucose (a) and to L-proline (b). Threshold concentrations of glucose and L-proline were sequentially determined three times, before testing thresholds, participants rinsed their mouths with distilled water (G + DW, P + DW), conditioning solution containing 0.8 μM vanadium (G + 0.8V, P + 0.8V), or conditioning solution containing 8.0 μM vanadium (G + 8.0V, P + 8.0V). (c) shows the reliability of three-times determination of thresholds, and (d) shows that the effect of ammonium vanadate was reversible. Means and SEMs are shown. (a) $F(2, 31) = 5.557$, $P = 0.0056$; (b) $F(2, 31) = 4.157$, $P = 0.0202$; (c) $F(2, 21) = 1.538$, $P = 0.2267$; (d) $F(2, 23) = 3.732$, $P = 0.0151$. *: $P < 0.05$ (Fisher's PLSD).

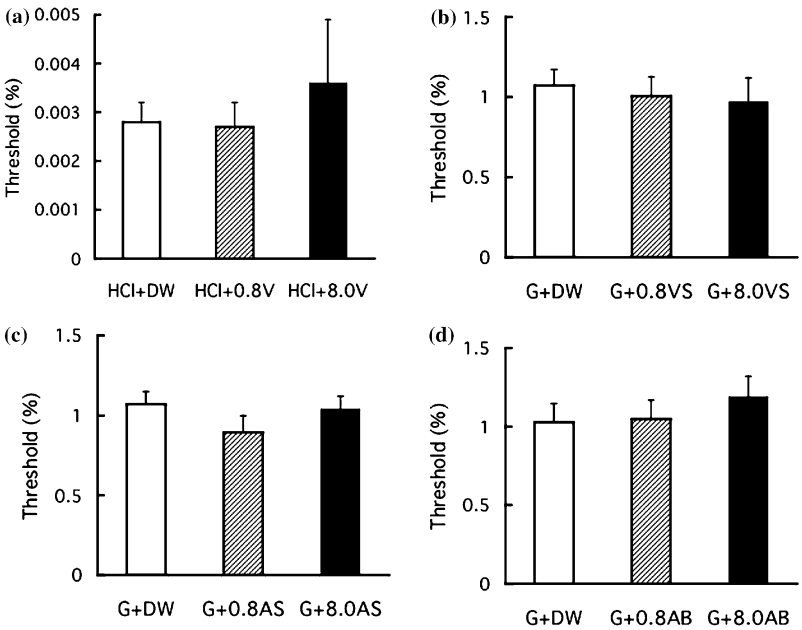

Figure 2. The effect of ammonium vanadate on the recognition threshold of sour taste to hydrogen chloride (a) and the effects of vanadium sulfate (b), ammonium sulfate (c), and ammonium bicarbonate (d) on the recognition threshold of sweet taste to glucose. Threshold concentrations were sequentially determined three times. Before testing thresholds, participants rinsed their mouths with distilled water (HCl + DW, G + DW), conditioning solution containing 0.8 μM vanadium (HCl + 0.8V, G + 0.8VS) or ammonium (G + 0.8AS, G + 0.8AB), or conditioning solution containing 8.0 μM vanadium (HCl + 8.0V, G + 8.0VS,) or ammonium (G + 8.0AS, G + 8.0AB). Means and SEMs are shown. (a) $F(2, 21) = 0.513$, $P = 0.6023$; (b) $F(2, 11) = 0.787$, $P = 0.4677$; (c) $F(2, 20) = 0.817$, $P = 0.4491$; (d) $F(2, 12) = 0.367$, $P = 0.6963$.

(Gilbertson *et al*. 2000). It is likely that pentavalent vanadium does not influence these transduction mechanisms.

Tetravalent vanadate, vanadium sulfate, did not influence the sweet taste sense to glucose (Figure 2b). Vanadium as a phosphate analog penetrates the plasma membrane by simple diffusion. It was formerly considered that pentavalent vanadium, after entering the cell, is reduced to tetravalent vanadium, and tetravalent vanadium tightly binds to proteins (Smith *et al*. 1980; Degani *et al*. 1981). However, it has recently been found that the function of glutathione of reducing vanadate is not as strong as previously considered, and that oxidation of tetravalent vanadium to pentavalent vanadium occurs at physiological pH and temperature (Li *et al*. 1996). Therefore, we deduce that pentavalent vanadium, after entering the taste cell, acts on the intracellular mechanism independent of tetravalent vanadium, and sensitizes the sweet taste to glucose.

It was concluded that pentavalent vanadium at 10^{-7} M intensifies the sweet taste sense to glucose rather specifically. We have first shown the physiological effect of vanadium at the concentration of the underground water level.

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