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Neural impulse amplitude of taste nerve fiber branches depends on taste receptor sites in bullfrogs

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The branches of each single taste nerve fiber selectively innervate the same taste receptor site on different taste receptor cells. Here we show that the impulse amplitude and firing pattern of nerve branches depends on taste receptor sites in bullfrogs. L-Threonine, L-serine, and galactose elicited larger impulses in amplitude than quinine HCl. L-Leucine, L-phenylalanine, and papaverine HCl elicited both impulses, where the train of the small impulses preceded that of the large ones. The large impulses were suppressed in the presence of salts, but the small impulses were not. Washing subjects' tongues with an alkaline solution facilitated the firing of the large impulses. The firing of the small impulses stopped shortly, but that of the large impulses kept firing during taste stimulation. The present results showed that taste receptor sites examined could be classified into salt-sensitive and salt-insensitive sites, and that the nerve branches generating the large impulses innervated salt-sensitive sites while the nerve branches generating the small impulses innervated salt-insensitive sites. These results suggest that the excitability of taste nerve branches contributes to the selective innervation. We discuss the firing of the large and small impulses.

Key words : taste nerve responses; suction electrodes; impulse amplitude; antidromic impulses; alkaline treatment

1 Introduction

Single taste nerve fibers of mammals, frogs, and fishes more or less selectively respond to taste substances.[1–8] Notwithstanding that single taste nerve fibers ramify and innervate different taste receptor cells, each single taste nerve fiber comprising the coherent response of its branches showed selective responsiveness. These results showed that the branches of each single taste nerve fiber innervate the same taste receptor site on different taste receptor cells, and suggest that these branches differentiate for selective innervation. The differentiation may affect the electrophysiological properties of the taste nerve fiber branches, and the neural impulses of branches may reflect the differentiation.

Frog taste receptors are taste disks innervated by ~10 myelinated fibers and a few unmyelinated fibers on the tip of fungiform papillae.[9, 10] They are homologous with mammalian taste buds, though they are much bigger than taste buds. In bullfrogs, the branch of each single taste nerve fiber innervates 6.6 taste disks.[6] Therefore, action potentials of taste nerve fibers generated in response to taste substances are not only transmitted to the brain but also antidromically transmitted to neighboring taste disks on different fungiform papillae. These antidromic action potentials have been recorded extracellularly by sucking the fungiform papillae with suction electrodes.[11–14] The amplitude of antidromic neural impulses thus recorded depends on the amplitude of action currents, and hence shows the electrophysiological properties of the taste nerve fiber branches.

Our previous study showed that although the instantaneous component (phasic response) of integrated taste nerve responses of bullfrogs to hydrophobic amino acids, L-leucine, L-phenylalanine, etc. was insensitive to salts, the subsequent long-lasting component (tonic response) was suppressed in the presence of salts.[15] Bullfrog taste nerve responses to galactose, a hydrophilic substance, were suppressed in the presence of salts.[16] These results showed that these taste receptor sites could be classified as salt-sensitive or salt-insensitive.

In the present study, we took advantage of bullfrog taste receptors and investigated the neural impulses of taste nerve fiber branches innervating the salt-sensitive and salt-insensitive sites by recording these antidromic impulses with suction electrodes. In addition to amino acids and galactose, we chose papaverine HCl and quinine HCl as

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hydrophobic substances, in order to examine the relations among the hydrophobicity/hydrophilicity of taste substances, the salt-sensitivity of taste receptor sites for them, and the height of neural impulses they elicited. The present results showed that taste nerve branches innervating the salt-sensitive sites generated larger impulses in amplitude than taste nerve branches innervating the salt-insensitive sites. Also we showed that the small impulses formed the phasic responses and the large impulses formed the tonic responses. These results suggest that taste nerve fiber branches differentiate or adjust to selective innervation, and that the differentiation or adjustment affects the impulse amplitude. We discuss the firing of these impulses.

2 Materials and Methods

All experimental protocols were conducted in compliance with the Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences approved by the council of the Physiological Society of Japan, and were permitted by the Animal Institutional Review Board of Saitama Institute of Technology in accordance with the guidelines of the U.S. National Institutes of Health.

2.1 Recording of antidromic impulses

We purchased adult bullfrogs, *Rana catesbeiana* from a local commercial source, and anesthetized them as described in a previous paper.[17] In brief, we anesthetized bullfrogs of 180~250 g with intraperitoneal injection of urethane (0.2 g/100 g body weight), recorded the taste responses from the branch of glossopharyngeal nerves innervating fungiform papillae with suction electrodes as the train of antidromic neural impulses as described in previous papers.[11–14] The impulses were amplified, band-pass filtered (300-3000 Hz) with homemade devices, and stored in a computer. A small amount of urethane was added to maintain anesthetization. These animals were killed by decapitation under anesthetization after the experiments.

Since each fungiform papilla contained ~10 myelinated nerve fibers and a few unmyelinated fibers,[9, 10] suction electrodes recorded antidromic impulses from these myelinated fibers. Also suction electrodes might record the antidromic impulses of unmyelinated fibers, if they were detectable.

2.2 Stimulation

All taste substances were dissolved in deionized water or salt solutions, and applied to the whole ventral surface of tongues to simulate whole fungiform papillae acclimated to their respective vehicles through a silicon tube. Although bullfrog taste disks respond to deionized water,[18–20] we irrigated taste disks with deionized water until the water response in the firing frequency became negligible. All chemicals used were of analytical grade purchased from Wako Pure Chemical Industries, Ltd, and all experiments were carried out at room temperature.

3 Results

3.1 Alkaline treatment

The application of taste substances to the tongue elicited antidromic impulses (Figure 1). We previously showed that a wash of the tongue with an alkaline solution (2.5 mM $NaHCO_3$-Na_2CO_3 buffer, pH 10.0) for ~5 min enhanced the tonic responses to amino acids.[15] In the present study, the alkaline treatment increased the frequency of the large antidromic impulses generated by the application of 50 mM L-leucine. Similar results were found in response to the other amino acids, papaverine HCl, and galactose. In the following experiments we performed alkaline treatment before all tongue examinations.

3.2 Current amplitude

Several hydrophilic amino acids, L-threonine, L-serine, and glycine and galactose primarily generated higher-amplitude antidromic impulses, whereas quinine HCl primarily generated smaller antidromic impulses (Figure 2). We classified these impulses obtained from the same fungiform papillae into two groups by their amplitude. When the amplitude of given impulses was larger than one-third of the maximum impulse in amplitude, we referred to them as large impulses. Impulses smaller than the criteria but 1.5-times larger than the fluctuation amplitude of the current traces in the absence of stimulation were referred to as small impulses. The other impulses were neglected as noises. We stopped the recording when the amplitude of the maximum impulses was changed by 20%.

Hydrophobic amino acids such as L-leucine, L-phenylalanine, and L-tryptophan generated both large and small impulses (Figure 2A). Hydrophilic amino acids such as L-threonine, L-serine, and glycine typically generated large impulses (Figure 2B). Galactose generated large impulses,

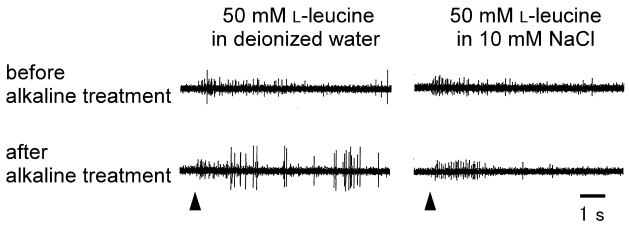

Fig. 1 Antidromic impulses in response to 50-mM L-leucine before (upper traces) and after alkaline treatment (lower traces) obtained from the same fungiform papilla.

L-Leucine dissolved in deionized water (left traces) or 10 mM NaCl (right traces) was applied to the whole tongue surface acclimated to respective vehicles before and after an alkaline treatment. Arrowheads show the onset of the stimulation in this and following figures.

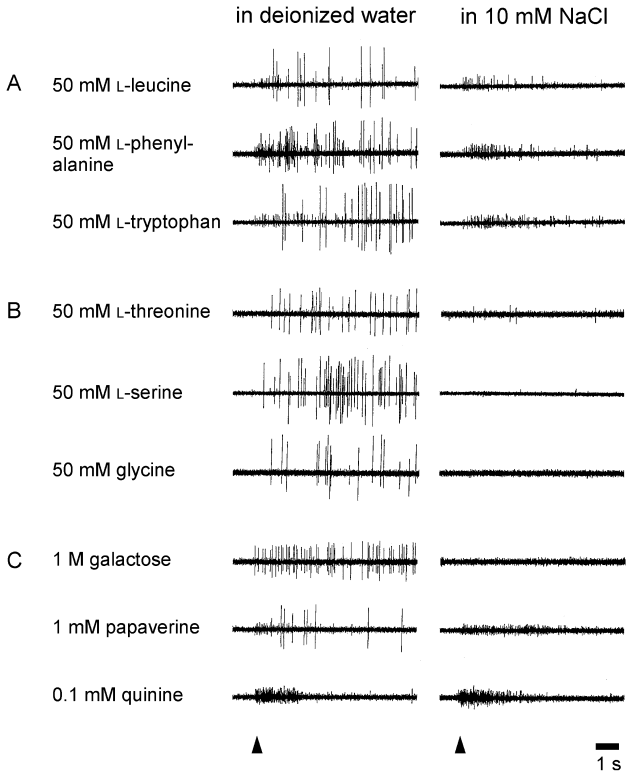

Fig. 2 Antidromic impulses in response to hydrophobic amino acids, L-leucine, L-phenylalanine, and L-tryptophan (A), hydrophilic amino acids, L-threonine, L-serine, and glycine (B), and other taste substances (C) in the absence (left traces) and presence of 10 mM NaCl (right traces).

Taste substances were dissolved in deionized water (left traces) or 10 mM NaCl (right traces). We recorded from the same fungiform papilla to compare the effect of NaCl. All stimuli were applied to the whole tongue surface acclimated to respective vehicles after alkaline treatment in this and all subsequent figures.

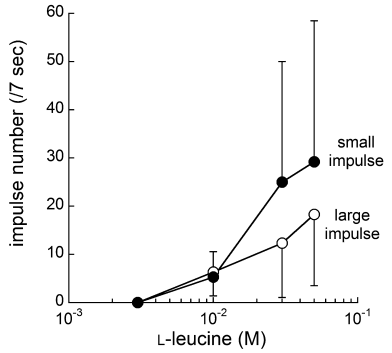

Fig. 3 The number of large (open circles) and small (closed circles) impulses in response to L-leucine in the first 7 s after the onset of stimulation as a function of L-leucine concentration.

L-Leucine was dissolved in deionized water and applied to the whole tongue surface. Plotted are means and SDs (3 fungiform papillae of different frogs).

Table 1 Salt-sensitivity of neural impulses in response to amino acids and galactose

	deionized water		10 mM NaCl	
taste substances	large impulses	small impulses	large impulses	small impulses
50 mM L-leucine	17.6 ± 13.2	16.6 ± 6.7	0.0 ± 0.0	20.2 ± 8.9
50 mM L-threonine	17.0 ± 12.6	2.7 ± 2.4	0.0 ± 0.0	2.3 ± 2.2
1 M galactose	18.6 ± 11.0	4.2 ± 3.2	0.0 ± 0.0	2.4 ± 3.8

The numerals are means and SDs (6 fungiform papillae of different frogs) of summated impulse number over 7 s after the onset of the stimulation with the indicated taste substances. There were no significant differences between the number of small impulses in the absence and presence of 10 mM NaCl ($p > 0.05$, two-tailed t-test).

papaverine HCl generated large and small impulses, and quinine HCl generated small impulses (Figure 2C).

The concentration-response curves for L-leucine showed that the threshold concentration of the large impulses was between 3 mM and 10 mM (Figure 3). The threshold concentration of the small impulses was in the same range. The number of large and small impulses fired in the first 7 s after the onset of stimulation was similar at 10 mM.

3.3 Salt-sensitivity

The addition of 10 mM NaCl to acclimating solutions and taste-stimulating solutions suppressed the firing of the large impulses (Figure 2). The responses to galactose were remarkably suppressed, and the responses to quinine HCl remained unchanged. The quantitative comparison showed that the addition of 10 mM NaCl significantly decreased the number of large impulses generated in response to 50 mM L-threonine and 1 M galactose, and that the number of the small impulses generated remained unchanged (Table 1). Similar results were obtained from the tongue untreated with the alkaline solution (Figure 1), showing that the salt-sensitivity did not result from the alkaline treatment.

The number of large impulses in response to 50 mM L-leucine was decreased with increasing NaCl concentration (Figure 4A). The addition of $Na_4Fe(CN)_6$ and K_2SO_4 also decreased the number of large impulses with increasing concentration, and no large impulses appeared at 10 mM (Figure 4B).

The large impulses elicited in response to 50 mM L-leucine disappeared in the presence of 1 mM K_2SO_4 and 10 mM $Na_4Fe(CN)_6$ and NaCl (Figure 4B). Although the number of the large impulses might decrease with increasing salt concentration, the large SDs prevented further analyses.

3.4 Other differences of the large and small impulses

The train of small impulses always preceded that of the large impulses by ~1 s in response to hydrophobic amino acids, L-leucine, L-phenylalanine, and L-tryptophan,

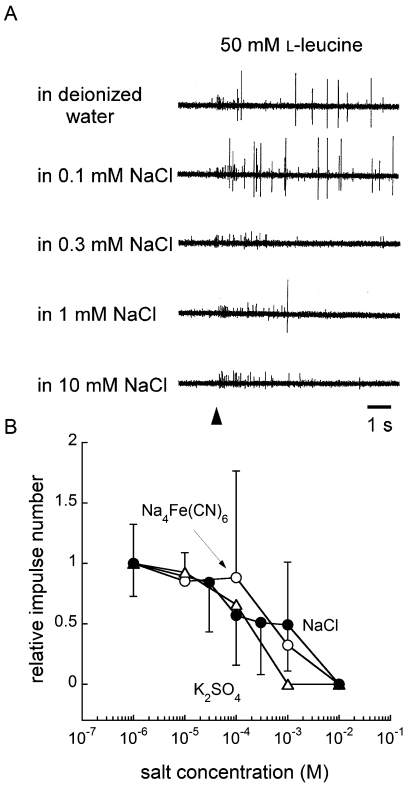

Fig. 4 Salt-dependent suppression of antidromic impulses in response to 50 mM L-leucine.

A, antidromic impulses in different NaCl concentrations.
B, relative number of large impulses in the frist 7 s after the onset of stimulation as a function of salt concentrations added to the L-leucine solution. Plotted are the means and SDs for NaCl and $Na_4Fe(CN)_6$ (3 fungiform papillae of different frogs) and means for K_2SO_4 (2 fungiform papillae of different frogs). The total large impulse number in the 7 s was calculated relative to the total impulse number elicited in the same duration in the presence of 10^{-6} M respective salts. We applied stimulating solutions to the whole tongue surface.

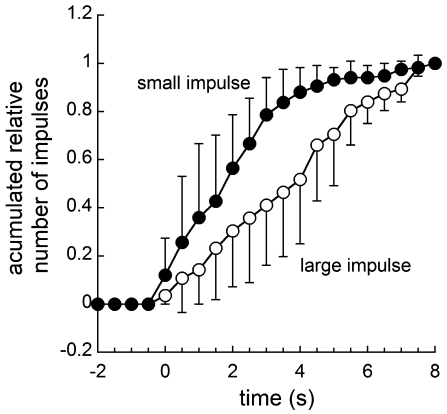

Fig. 5 Accumulated relative number of impulses as a function of time after the onset of stimulation with 50 mM L-leucine.

We accumulated the number of the large impulses every 0.5 s from 2 s before to 8.5 s after the onset of stimulation, divided them with the total number of the large impulses elicited during the stimulation period of 8.5 s, and plotted this value as a function of stimulation time. The small impulse number was similar normalized. Plotted are the means and SDs (10 fungiform papillae of different frogs) of the relative number of small (closed circles) and large impulses (open circles). Stimulating solutions were applied to the whole tongue surface.

and papaverine HCl (Figs. 1, 2 and 4). The firing of the small impulses generated by 50 mM L-leucine stopped shortly in ~4 s after the onset of firing, but the large ones continued firing during stimulation (Figure 5). Similar inactivation in the firing of the small impulses was found in response to the other hydrophobic amino acids, quinine HCl, and papaverine HCl (Figure 2).

4 Discussion

In the present study, we classified the antidromic impulses of taste nerve fiber branches into large and small impulses. It is likely that thicker branches generate the large impulses and thinner branches generate the small impulses, if both branches are equal in membrane properties including voltage-gated channel molecules and their densities.[21] In brief, impulse amplitude depends on the local circuit current magnitude of taste nerve fiber branches. The current magnitude is proportional to the axon radius, the second derivative of the membrane potential with respect to time, and is inversely proportional to the square of the conduction velocity and the axoplasm resistivity. The axoplasm resistivity is similar among axons. When the kinetics and the density of voltage-gated channels are the same between the branches, the second derivative depends on the branch radius. The conduction velocity would also depend on the branch radius. Therefore, the branch radius primarily decides the impulse amplitude under these assumptions.

The firing of the small impulses stopped shortly, but that of the large impulses was long-lasting. These results suggest that taste receptor cells that respond to hydrophobic substances inactivate easily. It is also possible that taste nerve fibers that conduct the response to hydrophobic substances by firing small impulses inactivate easily. We previously investigated integrated taste nerve responses to hydrophobic amino acids recorded from the trunk of the glossopharyngeal nerve, and showed that salt-insensitive sites yielded phasic responses and salt-sensitive sites yielded tonic responses.[15] The present results not only agreed with the previous study but also showed that the small impulses yielded the phasic responses and the large impulses yielded the tonic responses. The transient firing of the small impulses suggest that salt-insensitive taste receptor cells or the small impulse-generating taste nerve fibers easily inactivate.

The train of the small impulses in response to hydrophobic amino acids and papaverine HCl always preceded that of the large impulses by ~1 s. Since the distance between stimulated and recorded fungiform papillae was a few centimeters, even the slowest, unmyelinated nerves conduct impulses between them in 10 ms. Therefore, the difference in the conduction velocity of taste nerves never produces this lag time. We assume that the small impulses are fired before the large impulses. In other words, taste receptor cells expressing salt-insensitive sites may more rapidly trigger the neurotransmitter releases than those expressing salt-sensitive sites.

The application of quinine HCl, a hydrophobic substance, only fired the small impulses. Although hydrophobic amino acids and papaverine HCl fired the large responses, the small impulses always preceded the large impulses. Since many hydrophobic substances are bitter and toxic,[22] the small impulses may stop swallowing toxic hydrophobic substances. By contrast, the large impulses may urge or allow swallowing. Therefore, it is likely that taste nerve fibers firing the small impulses and the large impulses may have different roles.

The firing of large and small impulses showed that hydrophilic amino acids and galactose primarily stimulated salt-sensitive sites; hydrophobic amino acids and papaverine HCl stimulated both the salt-sensitive and salt-insensitive sites; and quinine HCl primarily stimulated the salt-insensitive sites. Mammalian taste receptors express two taste receptor molecule families, T1Rs and T2Rs.[23–25] T1Rs consist of three subtypes[1–3]. The complex of T1R1 and T1R3 forms taste receptor sites for amino acids, and that of T1R2 and T1R3 for sweet substances.[26, 27] T2Rs are taste receptor sites for bitter substances,[24] and are expressed in different taste receptor cells from those expressing T1Rs.[23] So far, a few reports have detected genes for T2Rs but not T1Rs in frogs.[28, 29] However, we assume that bullfrog taste receptors express their functional counterparts, and suggest that the counterparts of frog T1Rs are salt-sensitive and frog T2Rs are salt-insensitive. Since hydrophobic amino acids are bitter substances, it is likely that they stimulate both the counterparts of frog T1R1/T1R3 complex and some frog T2Rs.

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