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Electrophysiological Studies of Salty Taste Modification by Organic Acids in the Labellar Taste Cell of the Blowfly

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Abstract

Using the labellar salt receptor cells of the blowfly, *Phormia regina*, we electrophysiologically showed that the response to NaCl and KCl aqueous solutions was enhanced and depressed by acetic, succinic and citric acids. The organic acid concentrations at which the most enhanced salt response (MESR) was obtained were found to be different: 0.05–1 mM citric acid, 0.5–2 mM succinic acid and 5–50 mM acetic acid. Moreover, the degree of the salt response was not always dependent on the pH values of the stimulating solutions. The salt response was also enhanced by HCl (pH 3.5–3.0) only when the NaCl concentration was greater than the threshold, indicating that the salty taste would be enhanced by the comparatively lower concentrations of hydrogen ions. Another explanation for the enhancement is that the salty taste may also be enhanced by undissociated molecules of the organic acids, because the MESRs were obtained at the pH values lower than the pKa_1 or pKa_2 values of these organic acids. On the other hand, the salty taste could be depressed by both the lower pH range (pH 2.5–2.0) and the dissociated organic anions from organic acid molecules with at least two carboxyl groups.

Introduction

Salty taste is modified by organic acids in humans. Fabian and Blum showed that the salty taste of 0.1 M NaCl was enhanced by acetic, citric, lactic, malic and tartaric acids at such low concentrations that their taste was not recognized as sour (Fabian and Blum, 1942). Kamen *et al.* also showed by sensory evaluations that, at various concentrations of either NaCl or citric acid, the salty taste of NaCl was enhanced by citric acid (Kamen *et al.*, 1961). On the other hand, the salty taste could be depressed by organic acids because acid seasonings such as vinegar are experientially applied to weaken the salty taste in food. Though salty taste modification by organic acids is being understood psychologically or experientially, it is hardly understood physiologically. Physiological data concerning salty taste modification by organic acids would be valuable for evaluating at least two things: (i) whether salty taste is modified by organic acids peripherally or centrally; and (ii) how salty taste is both enhanced and depressed by organic acids.

Both salty and sour tastes are directly induced via ion channels on taste receptor cells. Recently, the ion channel molecule candidates relevant to salty (Kretz *et al.*, 1999; Lin *et al.*, 1999) and sour tastes (Waldmann *et al.*, 1997; Chen *et al.*, 1998; Ugawa *et al.*, 1998) were identified by immunocytochemical and molecular biological experiments. Additionally, salty as well as sour tastes are generally elicited by electrolytes. These common characteristics between salty and sour tastes prompted us to set up the hypothesis that salty and sour taste stimuli may interact with each other on the same receptor site of a taste cell.

The structural simplicity of the taste organs of flies compared to vertebrates makes them an attractive model system for electrophysiological studies. Each chemosensillum of a fly possesses four functionally differentiated taste receptor cells: the salt, sugar, water and fourth taste receptor cells. Tip (Hodgson *et al.*, 1955) and sidewall (Morita, 1959) recording methods for the labellar chemosensillum of the fly have enabled us to record the electrophysiological responses of a single taste receptor cell without cell isolation. The impulses from four taste receptor cells recorded by these methods are easily distinguished by their amplitude from each other. Therefore, one can expect that salty taste modification by organic acids in the fly salt receptor cell would be easily observed by the tip or sidewall recording methods. Hence, we used the blowfly for elucidating the mechanism behind the organic-acid-induced change of salty taste.

In the present paper, we electrophysiologically show salty taste modification by organic acids at the taste receptor cell level in the fly and discuss the possible roles of hydrogen ions, organic anions derived from the organic acids and undissociated forms of them on this phenomenon. Three kinds of organic acids, chemically different in the number

of carboxyl groups and alkyl chain length, were chosen for this study: acetic acid (CH_3COOH), succinic acid ($HOOCCH_2CH_2COOH$) and citric acid [$HOOCCH_2C(OH)(COOH)CH_2COOH$].

Materials and methods

Fly

Adult blowflies (*Phormia regina*, 5–12 days old) were used for our experiments. They were reared in the laboratory at 24 ± 1°C. They were fed on chicken liver and yeast bait at the larval stage and 100 mM sucrose and water at the adult stage.

Electrophysiological procedures

Impulses from the taste cells of the blowfly were recorded using the tip recording method (Hodgson *et al.*, 1955) and impulses due to organic acid solutions with no salt were recorded by the sidewall recording method (Morita, 1959). Two platinum electrodes were used for the tip recording method: one was an indifferent electrode, inserted into the isolated head of a fly; the other was a recording electrode, inserted into a glass capillary containing a stimulating solution. Impulses, induced by capping the tip of the labellar LL-type chemosensilla with a glass capillary, were recorded on magnetic tape through a band-path filter (100–2000 Hz). LL-type are the largest of the labellar chemosensilla (Wilczek, 1967). The impulses from the salt, sugar and water cells were distinguished from each other by their amplitudes. The number of impulses generated during 0.15–0.35 s after the beginning of stimulation was counted as the magnitude of the response of the taste cells. In some cases, the magnitude of the salt response was represented as the relative response normalized to that of 2 M NaCl. A sucrose solution (50 mM) was used as a sugar stimulus and contained 10 mM NaCl to maintain electrical conductance. Each stimulus was given at an interval of 3 min or more to avoid adaptation effects. During the recordings, the relative humidity in the laboratory was >60% in order to maintain the concentration of the stimulating solution at the tip of the glass capillary. All experiments were performed at room temperature (22–26°C).

Proboscis extension reflex (PER) test

Flies which had been starved but given water for 36 h were used for the PER tests in order to avoid the effects of blood sugar levels on PER (Dethier and Bodenstein, 1958). The flies were held by the wings with small clothespins. Prior to the test, the flies had been given sufficient water so that they would not respond to water. Test solutions were applied to the labellum with a disposal plastic tip (used for an automatic pipette) having a diameter of ~500 μm. The number of flies showing the feeding response of full proboscis extension within 2 s was counted.

Chemicals

All chemicals were of reagent grade and were purchased from Wako Pure Chemicals Industries Ltd (Osaka, Japan).

Results

Identification of impulses

Figure 1 shows a typical record of impulses in response to 200 mM NaCl (the salt response), 50 mM sucrose (the sugar response), 10 mM NaCl (the water response) and 10 mM NaCl plus acetic acid, in the same labellar LL-type sensillum. Impulses of the salt, sugar, water and fourth chemoreceptor cells of a labellar LL-type sensillum are usually identified by comparing their amplitude. Ten millimolar NaCl plus 30 mM acetic acid (pH 3.05) induced two types of impulses differing in their amplitudes: the smaller one and the larger one (Figure 1Ad). The amplitude of the smaller impulse was the same as that of 10 mM NaCl, clearly indicating that the smaller impulse comes from the water receptor cell. On the other hand, the larger impulse was not clearly identified because its amplitude was significantly different ($P < 0.001$) from 200 mM NaCl, 50 mM sucrose and 10 mM NaCl (Figure 2). The amplitude of the larger impulse was found to be smaller than that of 200 mM NaCl and larger than that of 50 mM sucrose.

Histological observations of a labellar LL-type chemosensillum (Dethier, 1976) along with the generation of four types of injury currents by the chemosensilla tip following treatment with a strong detergent, soyasaponin I (Amakawa, unpublished data) confirmed that no other cells are present except the four taste receptor cells, the so-called sugar, salt, water and fourth chemoreceptor cells in the labellar chemosensillum of the blowfly. Therefore, the larger impulse induced by 10 mM NaCl plus 30 mM acetic acid should be formed from the impulses evoked from any of these four cells. One of the possibilities is that the impulse generated by any of these four cells may be transformed by some effects that ultimately led to the generation of an impulse of larger amplitude (see Discussion).

To identify the origin of the larger impulse induced by 10 mM NaCl plus 30 mM acetic acid, we used another method—the PER test. Figure 3 shows the results obtained by the PER test with 50 mM sucrose, 100 mM NaCl and 10 mM NaCl plus organic acids. Ten millimolar NaCl plus 30 mM acetic acid induced a PER of only 8% in 50 tested flies, indicating that the fly shows a negative feeding to 10 mM NaCl plus 30 mM acetic acid, which means that the larger impulse does not come from the sugar receptor cell. The larger impulse does not come from the water receptor cell either, because the response to 10 mM NaCl plus 30 mM acetic acid in Figure 1Ad consists of the larger impulse and the impulse from the water receptor cell. Neither does the larger impulse come from the fourth chemoreceptor

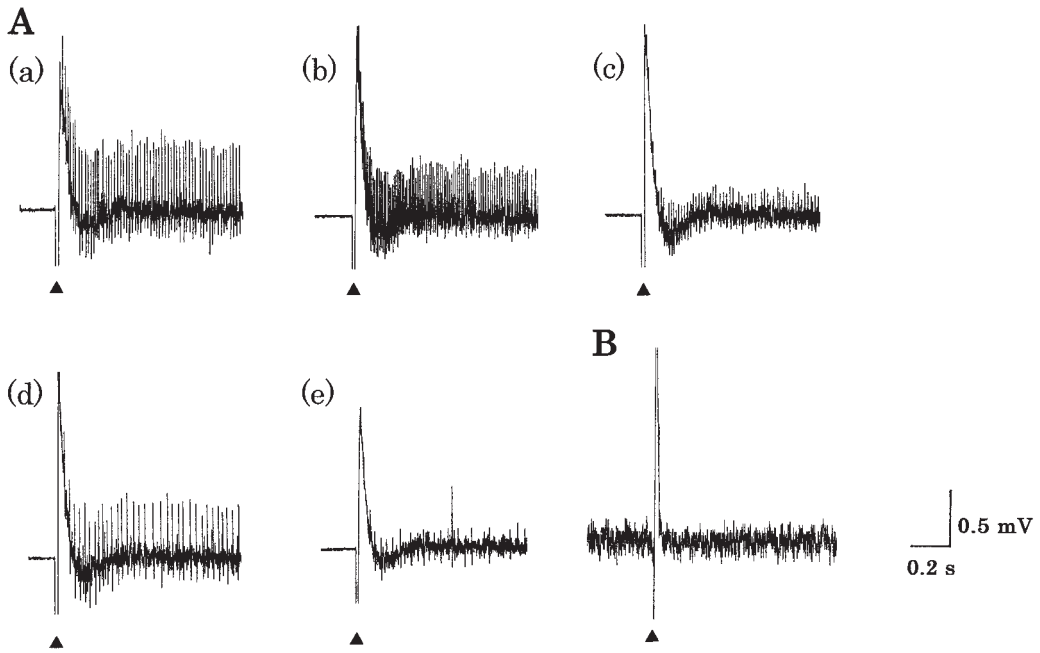

Figure 1 A typical record of impulses from a labellar LL-type chemosensillum of *Phormia regina* **(A)** to 200 mM NaCl **(a)**, 50 mM sucrose **(b)**, 10 mM NaCl **(c)**, 10 mM NaCl + 30 mM acetic acid **(d)** and 10 mM NaCl + 500 mM acetic acid **(e)** by the tip recording method and **(B)** to 30 mM acetic acid by the sidewall recording method. The triangles in the record indicate the beginning of stimulation.

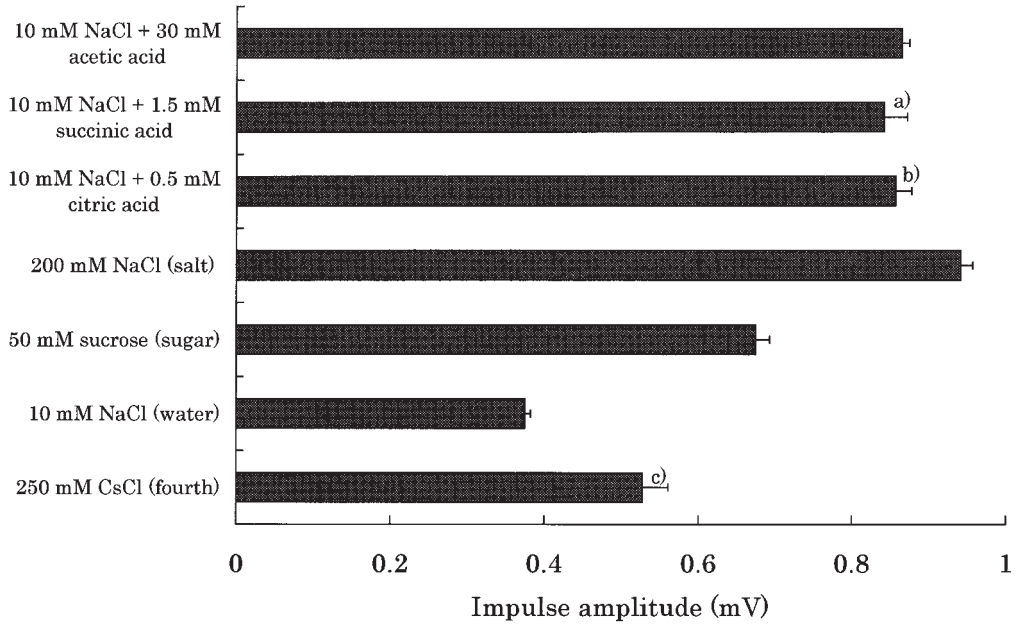

Figure 2 Amplitude of impulses induced by application of various test solutions to a labellar LL-type chemosensillum. The amplitudes of the impulses from 10 mM NaCl + 30 mM acetic acid, 200 mM NaCl, 50 mM sucrose and 10 mM NaCl belonged to one fly ($n = 20$) and those from 10 mM NaCl + 1.5 mM succinic acid ($n = 20$), 10 mM NaCl + 0.5 mM citric acid ($n = 20$) and 250 mM CsCl ($n = 10$) to another. **(a–c)** To compare the amplitudes from one fly with those from the other, they were corrected using the amplitude of the impulses from the water chemoreceptor cell as a standard measure.

cell, because the impulse from the fourth chemoreceptor cell, induced by 250 mM CsCl (Gillary, 1966b), was also observed in the response to 10 mM NaCl plus 30 mM acetic acid mixed with 250 mM CsCl different to the larger impulse (data not shown). These results strongly disagree with the likelihood that the larger impulse induced by 10 mM NaCl plus 30 mM acetic acid could come from the sugar, water or fourth chemoreceptor cells; hence, we conclude that the larger impulse is the salt response. We also conclude, according to the same procedures as in the case of acetic acid, that the larger impulse induced by the salts plus succinic or citric acid is the salt response.

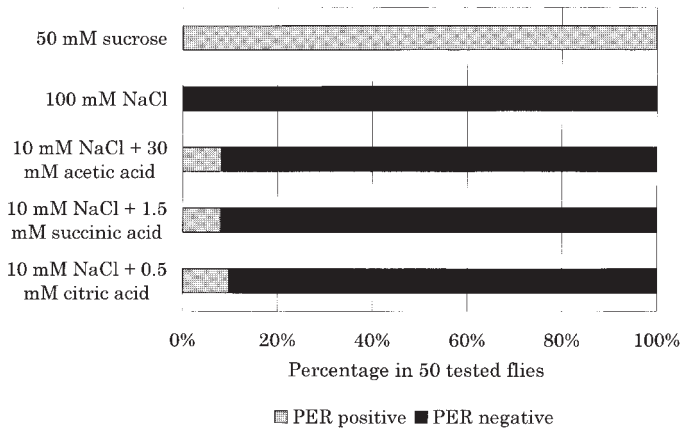

Figure 3 Proboscis extension reflex (PER) to 100 mM NaCl, 50 mM sucrose and 10 mM NaCl + organic acids (n = 50). The organic acid concentrations at which the MESR was obtained in 10 mM NaCl were added to 10 mM NaCl solutions.

Salt response to NaCl or KCl plus organic acids

Figure 4 shows the change in salt responses to NaCl plus acetic, succinic and citric acids. The salt responses to 10, 50 and 100 mM NaCl were enhanced by acetic, succinic and citric acids, respectively. The organic acid concentrations giving the most enhanced salt response (MESR) were different and found to be in the order citric acid < succinic acid < acetic acid. The MESR to 10 mM NaCl was obtained by 0.5 mM citric acid, 1.5 mM succinic acid and 30 mM acetic acid (Figure 4a); for 50 mM NaCl, the values were 0.5 mM citric acid, 1.0 mM succinic acid and 40 mM acetic acid (Figure 4b); and for 100 mM NaCl, the required concentrations were 0.1 mM citric acid, 0.5 mM succinic acid and 5 mM acetic acid (Figure 4c). At the MESRs, the organic acids enhanced the relative response to NaCl by 0.20–0.30 in 10 and 50 mM NaCl and by 0.10–0.15 in 100 mM NaCl.

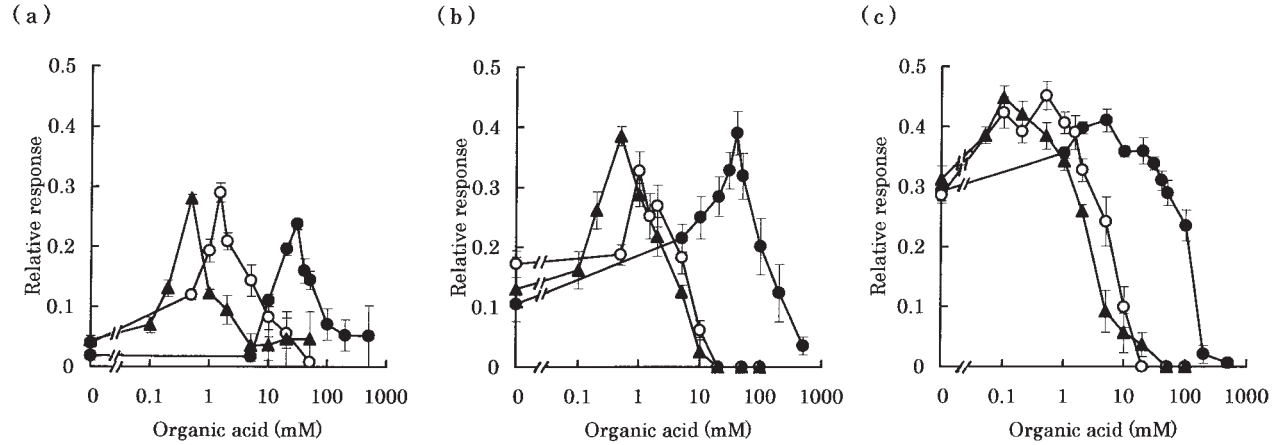

Figure 4 Electrophysiological responses from salt receptor cells to **(a)** 10 mM, **(b)** 50 mM and **(c)** 100 mM NaCl with various concentrations of citric acid (solid triangles), succinic acid (open circles) and acetic acid (solid circles). Ordinates represent the ratios of magnitude of salt responses to 2 M NaCl. The values are means ± SEM (n = 7–9).

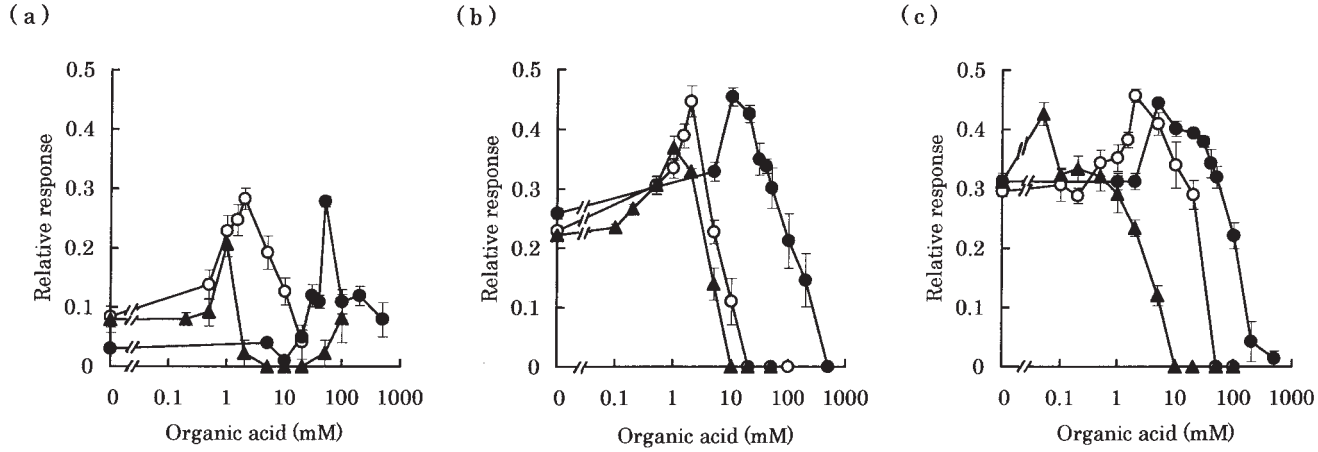

Figure 5 Electrophysiological responses from salt receptor cells to **(a)** 10 mM, **(b)** 50 mM and **(c)** 100 mM KCl with various concentrations of citric acid (solid triangles), succinic acid (open circles) and acetic acid (solid circles). Ordinates represent the ratios of magnitude of salt response to 2 M KCl. The values are means ± SEM (n = 7–9).

Figure 5 shows the change in the salt responses to KCl plus acetic, succinic and citric acids. The results for KCl were similar to those obtained with NaCl. The salt responses to 10, 50 and 100 mM KCl were enhanced by the three organic acids. Although the order of the organic acid concentrations for the MESR was the same (i.e. citric acid < succinic acid < acetic acids), the organic acid concentrations were different than in case of NaCl. The MESR for 10 mM KCl was obtained by 1 mM citric acid, 2 mM succinic acid and 50 mM acetic acid (Figure 5a), for 50 mM KCl by 1 mM citric acid, 2 mM succinic acid and 10 mM acetic acid (Figure 5b) and for 100 mM KCl by 0.05 mM citric acid, 2 mM succinic acid and 5 mM acetic acid (Figure 5c). At the MESRs, the organic acids enhanced the relative response to KCl by the same degrees as observed in NaCl.

Each organic acid itself evoked no salt response, a typical record of impulses of which is shown in Figure 1B: the salt response induced by 10 mM NaCl plus 30 mM acetic acid was not observed when 30 mM acetic acid was applied. The salt response to NaCl and KCl was enhanced by the low concentrations of organic acids, while it was depressed by high concentrations.

Figure 1Ae shows a typical record of impulses for the salt response depression: the salt response induced by 10 mM NaCl plus 30 mM acetic acid was hardly observed when 10 mM NaCl plus 500 mM acetic acid (pH 2.42) was applied. The salt response to 50 or 100 mM NaCl and KCl was also greatly depressed by the large amounts of organic acids. Moreover, in the case of 50 mM NaCl and KCl, the salt response was exponentially depressed by each organic acid (Figures 4b and 5b).

pH values at MESR in NaCl

Table 1 shows the pH values of NaCl plus various concentrations of organic acids giving the MESR. The pH values were different among the three kinds of organic acids at all concentrations of NaCl. Especially in the case of 10 and 50 mM NaCl, the pH values with acetic acid were lower than those with succinic and citric acids. The pH values of NaCl plus acetic or succinic acid shown in Table 1 were less than the pK_{a1} values of each organic acid, indicating that higher proportions of undissociated acetic and succinic acid molecules exist at chemical equilibrium in NaCl solutions than in dissociated ones. For citric acid, the pH values were within the range between pK_{a1} and pK_{a2}, indicating that the citric acid molecules dissociated at one carboxyl group exist in the highest proportion at chemical equilibrium in the NaCl solutions (see Table 2).

Salt response to NaCl plus HCl

Figure 6 shows the effects of pH on the salt response to NaCl aqueous solutions when mixed with HCl. The salt response to 10 mM NaCl was hardly changed from pH 5.4 through pH 3.0 and enhanced only at pH < 2.5, which was consistent with the results on the fleshfly (Shiraishi and Morita, 1969). The salt response to 50 mM NaCl was enhanced at pH 3.0. The enhanced salt response was depressed at pH 2.0, though it recovered to some extent at pH 1.5. The salt response to 100 mM NaCl was also enhanced at pH 3.0. The enhanced salt response was strongly depressed at pH < 2.0.

Table 1 pH values of the salt solutions at the various concentrations of organic acids that gave the MESR, and the pK_a values of organic acids

	Citric acid	Succinic acid	Acetic acid
NaCl (mM)			
10	3.30	3.45	3.05
50	3.39	3.58	2.88
100	3.78	3.75	3.56
pK_{a1}	2.87	4.00	4.56
pK_{a2}	4.35	5.24	
pK_{a3}	5.69		

The pK_a values are calculated under the conditions that the ionic strength is 0.1 at 25°C (Martell and Smith, 1977).

Table 2 Existing concentrations and percentages of the molecular species derived from the organic acids in 10 mM NaCl giving the MESR

Number of dissociated carboxyl group(s) in an organic acid molecule	Acetic acid (30 mM, pH 3.05)	Succinic acid (1.5 mM, pH 3.45)	Citric acid (0.5 mM, pH 3.30)
0	29.3 (97.6)[a]	1.2 (80.0)	0.2 (40.6)
1	0.7 (2.4)	0.3 (20.0)	0.3 (57.4)
2	–	0.0 (0.0)	0.0 (1.9)
3	–	–	0.0 (0.0)

[a]Units are mM (%). These values were calculated by the Debye–Hückel equation.

Dose–response curves of NaCl and sodium salts of organic acids

Figure 7 shows the relationship between sodium salt concentrations and the salt responses to them. The salt response to NaCl was almost proportional to the log scale of its concentrations, which followed the results of previous experiments (Evans and Mellon, 1962; Gillary, 1966a). Although the level of saturated salt response to sodium acetate was slightly depressed compared to NaCl, the dose–response curve of sodium acetate was shifted to the left of that of NaCl, suggesting that acetate ions raise the sensitivity of salt receptor cells to sodium ions. On the other hand, the dose–response curve of sodium succinate or sodium citrate was shifted to the right of that of NaCl. The level of saturated salt response to sodium succinate or

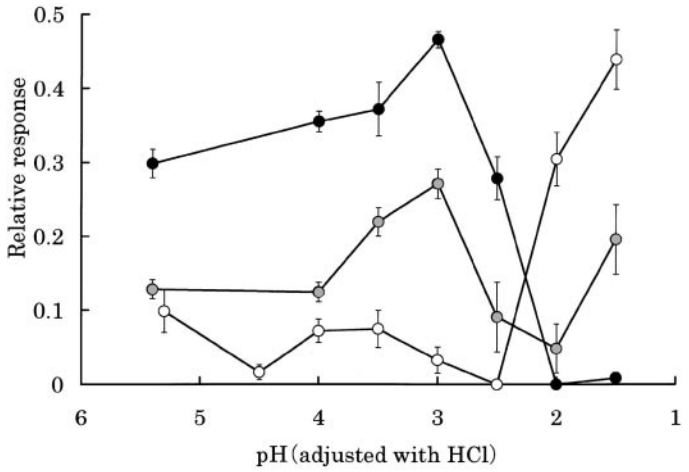

Figure 6 pH response curves of salt receptor cells for 10 mM (open circles, $n = 7$), 50 mM (stippled circles, $n = 8$) and 100 mM (solid circles, $n = 5$) NaCl. Ordinates represent the ratios of magnitude of salt responses to 2 M NaCl. The values are means ± SEM.

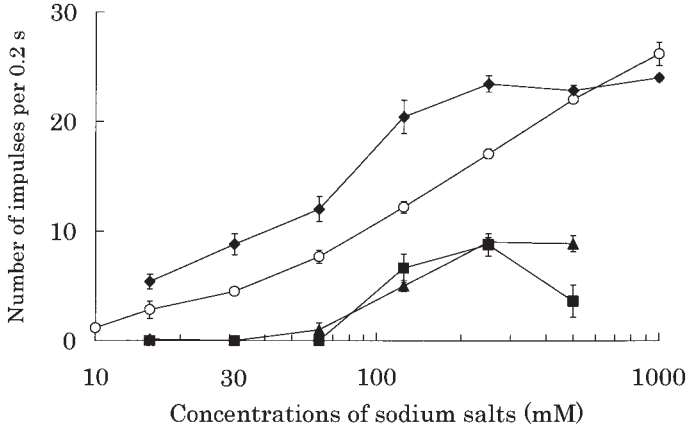

Figure 7 Dose–response curves of salt receptor cells for NaCl (open circles, $n = 6$), sodium acetate (solid diamonds, $n = 5$), sodium succinate (solid triangles, $n = 7$) and sodium citrate (solid squares, $n = 8$). The values represent means ± SEM.

sodium citrate was strongly depressed compared to NaCl response.

Salt response to NaCl plus sodium salts of organic acids

We also investigated whether the sodium salts of organic acids at the concentrations giving the MESR (Figure 4a) could also enhance the salt response to 10 mM NaCl. The results were clearly different between acetate and the others, as shown in Figure 8. Thirty millimolar sodium acetate in 10 mM NaCl evoked almost the same frequency of salt impulses as 100 mM NaCl. On the other hand, 1.5 mM sodium succinate and 0.5 mM sodium citrate in 10 mM NaCl evoked as few salt impulses as 10 mM NaCl. These results are in agreement with the previous idea that acetate ions increase the sensitivity of the salt response to sodium ions.

Discussion

Organic acids modify the salty taste at the taste receptor cell

The salty taste modification by organic acids seen in humans was also observed at the taste cell of the blowfly. Salt response enhancements by the comparatively lower concentrations of the organic acids in the fly taste receptor cell were consistent with the results in humans measured by sensory evaluations (Fabian and Blum, 1942). The salt response depression by the comparatively higher concentrations of the organic acids also seemed to be consistent with the phenomenon in humans that the strong salty taste would be depressed by acid seasonings such as vinegar. Additionally, the salt concentrations affected the degree of enhancement in the salt response by the organic acids in the fly as well as in humans. It has been shown (Kamen *et al.*, 1961) in the case of citric acid that the degree of enhancement in salty taste intensity declined in proportion to NaCl concentration. We also found that the degrees of enhancement in the salt response by organic acids varied with different salt concentrations (Figures 4 and 5). In both NaCl and KCl, the response to 10 and 50 mM salt solutions was more enhanced compared to 100 mM salt solutions. These correspondences suggest that this salty taste modification by organic acids in humans could be determined peripherally, at the taste cell, though the concentrations of the organic acids giving the MESR were different between fly and human. Additionally, both the fly and humans might possess a common mechanism for salt reception at the taste cell.

Hydrogen ions and undissociated molecules of organic acids are involved in the enhancement of salty taste

Comparatively lower concentrations (3.2×10^{-4}–1.0×10^{-3} M) of hydrogen ions are required to enhance the salty taste. As shown in Figure 6, HCl proportionally enhanced salt responses to 50 and 100 mM NaCl from pH 5.4 to pH 3.0. The effect of the same pH range in the enhancement of the salt response by organic acids suggests that hydrogen ions may enhance the salt response to some extent. However, HCl did not enhance the salt response to 10 mM NaCl that elicits little salt response in the blowfly in the same pH range. This result indicates that hydrogen ions have no ability to elicit the salt response by themselves; rather, they act on the enhancement of the salt response only when the salt receptor cells are excited. Additionally, the pH values of NaCl plus the organic acids giving the MESR did not correspond to each other as shown in Table 1, indicating that the enhancement could not be explained by pH dependence. This raises the possibility of the involvement of other molecular species than hydrogen ions in the enhancement of salt responses in the blowfly.

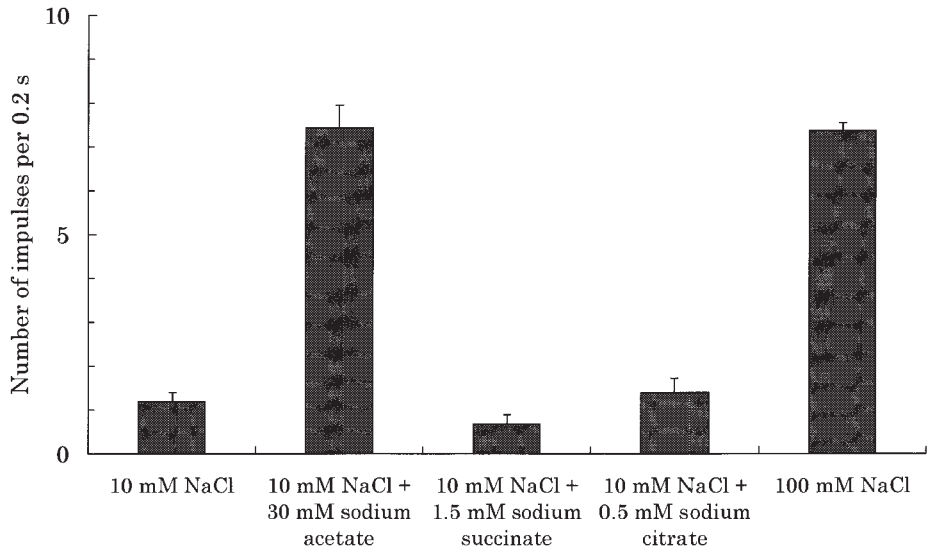

Figure 8 Effects of the sodium salts of organic acids on the salt response to 10 mM NaCl (n = 7–36). The values are means ±SEM.

From our experimental results, we predict that undissociated molecules of the organic acids may be probable candidates. Table 2 shows the existing concentrations and percentages of undissociated and dissociated organic acid molecules in 10 mM NaCl plus the organic acid solutions, calculated from the pH and pK_a values. In the concentration of organic acids at which the MESR was obtained in 10 mM NaCl, most molecules of each organic acid existed undissociated: 97.6% of 30 mM acetic acid; 80.0% of 1.5 mM succinic acid; and 40.6% of 0.5 mM citric acid. It has been reported (Ogiso *et al.*, 2000) that undissociated weak acids can be a stimulant for the taste receptor cells, based on investigations of the chorda tympani nerve response of the rat. Although their report was related to sour taste reception, the sour taste reception mechanism is generally proposed to be similar to that of salty taste and mediated directly via ion channels. Therefore, undissociated organic acids may be involved in the enhancement of the salt response. However, acetic, succinic or citric acid did not induce the salt response by itself, which was recorded by the sidewall recording method (Figure 1B), showing that the presence of the salts is necessary for the organic acids to activate the salt receptor cell.

The concentration differences of acetic, succinic and citric acid giving the MESR shown in Figures 4 and 5 could depend on the chemical structure of the organic acids, as their undissociated molecules are involved in the enhancement of the salt response. It has been suggested (Hatano *et al.*, 2000) that the salt response stimulatory capacity of the benzene sulfonic acid analogs is structurally specific in the taste cells of the fleshfly. The differences in the chemical structures of the organic acids could be one of the factors determining the ability of organic acids to activate the salt receptor cell.

Hydrogen ions and organic anions are involved in the depression of salty taste

Unlike the role of the hydrogen ions in the enhancement of salty taste, comparatively higher concentrations of hydrogen ions (that is to say, the lower pH of the stimulating solution) depresses salty taste. As shown in Figure 6, HCl depressed the enhanced salt response to 50 and 100 mM NaCl from pH 3.0 through pH 2.0. These results suggest that the depression of the salt response by organic acids in the same pH range may be due to the lower pH range. Especially, the pH values of 10 mM NaCl plus acetic acid and 50 mM NaCl plus acetic acid at the MESR (Table 1) almost correspond to pH 3.0, at which the salt response was most enhanced by HCl (Figure 6). This result suggests that hydrogen ions alone might depress the salt response in the case of acetic acid. Hydrogen ions would inactivate the salt receptor cell owing to the denaturation of ion channels in the receptor membrane by the lowering of pH values. However, the pH values of NaCl plus succinic and citric acids at the MESR (Table 1) did not correspond to pH 3.0. In the case of these organic acids, the salt response tended to be depressed over pH 3.0. These results raise the possibility of involvement of other molecular species than hydrogen ions in the depression of salty taste.

Organic anions may also work as effective molecules to depress the salt response in the case of succinic and citric acids. Sodium succinate and citrate generally elicited less salt response than NaCl, while sodium acetate elicited more than NaCl (Figure 7), consistent with the results that the salt response to 10 mM NaCl was not increased by sodium succinate or citrate, though it was increased by sodium acetate (Figure 8). The test solutions used in these experiments covered a wide range of pH values, i.e. pH 2–8. We can predict that the ionization state of groups on the membrane

surface is affected by the pH of the stimulating solution. Considering that both succinic and citric acids, beginning to depress the MESR in NaCl (Figures 4 and 5), were dissociated in the same order of concentrations—i.e. 0.2–0.5 mM, which values were obtained by calculation (data not shown)—the depressing effect of the succinate and citrate ions on the salt response to sodium ions can also be explained, though the pH values of the stimulating solutions were pH 2–4 in Figures 4 and 5, different from those containing sodium salts of the organic acids as shown in Figures 7 and 8. The effects of the organic anions on salty taste at the taste cell have previously been reported (Ye *et al.*, 1991). Their report suggested that, in the rat, the reception of salty taste stimuli involves the paracellular pathway via the tight junctions between the taste cells, as well as the transcellular pathway via ion channels in the apical membrane. They also showed that the anions derived from salty taste stimuli affect the paracellular pathway dependent on their permeability via the tight junctions. This explanation is not in agreement with our results, since acetate ions enhance the salt response in the blowfly (Figure 8). Because of their larger ionic size, acetate ions are not expected easily to permeate the tight junction compared to chloride ions. Based on our results, we suggest that succinate and citrate ions could simply interact with the surface of the distal membrane related to the transcellular pathway. One can expect that they may electrically interact with the positively charged groups, such as $-NH_3^+$ groups, on the membrane surface to depress the salt response.

The response difference between acetate ions and the succinate and citrate ions could depend on the chemical structure of the organic anions, as only succinate and citrate ions are involved in the depression of the salt response. The number of carboxyl groups in the respective organic acid molecules could explain this: acetate, one; succinate, two; citrate, three. Organic anions possessing at least two carboxyl groups would depress the salt response. Acetate ions did not depress the salt response but enhanced it, because acetate ions possess only one carboxyl group.

Inward current may reduce the amplitude of the impulse from the salt receptor cell

The amplitude of the impulses induced by the salts plus organic acids was significantly smaller than that induced by 200 mM NaCl, though these impulses came from the salt receptor cell. To investigate this difference in more detail, the shapes of the impulses were compared with each other by enlarging and superimposing the impulses obtained in Figure 1 (Figure 9). This analysis indicated that the impulse induced by 10 mM NaCl plus 30 mM acetic acid was slightly shorter than that induced by NaCl at the former part of a positive phase (Figure 9d), though the impulses induced by 10 mM NaCl plus 30 mM acetic acid (Figure 9a) and 200 mM NaCl (Figure 9b) were commonly biphasic.

The biphasic salt impulse of the fly, recorded by the tip-recording method, is mediated by the summation of the orthodromic and antidromic phases, which is the backfiring of the orthodromic phase through a dendrite in a labellar chemosensillum (Morita and Yamashita, 1959). The biphasic salt impulse has been reported to become smaller because of conduction delaying (Morita and Yamashita, 1959) or the decrement (Fujishiro *et al.*, 1984) of the antidromic phase. However, these two effects cannot completely explain the result where the negative phase of the biphasic salt impulse was unchanged. Conduction delaying of the antidromic phase would reduce the negative phase of the salt impulse as well as the positive phase. The decrement of the antidromic phase would also reduce the negative phase of the salt impulse. To reduce only the positive phase of the biphasic salt impulse, the newly generated negative phase different from the backfiring of the orthodromic phase is required at the beginning of impulse formation. Considering that the antidromic phase depends on the excitation of the membrane at the tip, inward current generated by some ion channels at the tip could be a candidate for the newly generated negative phase. Further experiments using the patch-clamp method, as in the fleshfly (Murakami and Kijima, 2000), would clarify more elaborately the mechanism of ion channels in the taste cells of the fly.

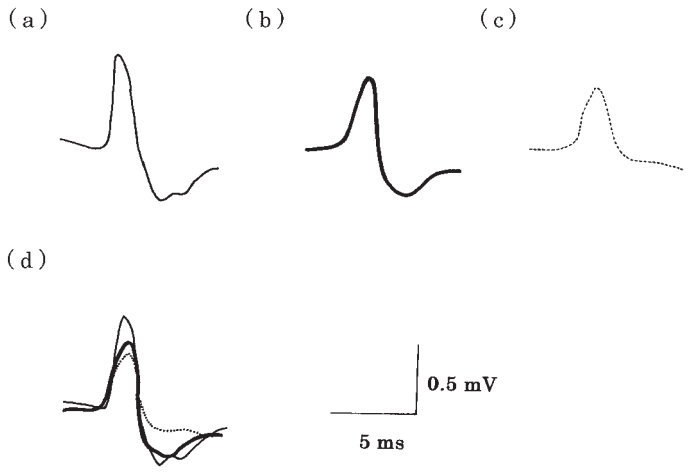

Figure 9 A typical trace of impulses from the taste receptor cell to **(a)** 200 mM NaCl, **(b)** 10 mM NaCl + 30 mM acetic acid and **(c)** 50 mM sucrose. **(d)** Superimposed traces of (a), (b) and (c).

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