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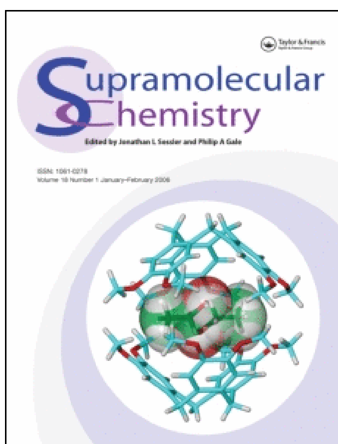

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Molecular recognition and transduction in chemoreceptor systems

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Gustatory and olfactory cells are typical chemical sensors in biological systems, which recognize various species of chemicals in external environments. This paper reviews recent progress in receptor mechanisms of tast and olfaction. It is considered that the initial event of olfactory reception is the binding of odorants to specific receptor proteins in the olfactory cilia. The data presented, however, suggest that lipid layers of the receptor membranes also play an important role in odour reception. For example, membrane fluidity changes induced by a temperature increase of up to 40 °C abolished the ability of olfactory receptors to discriminate odorants having similar structures. Treatment of the olfactory epithelium with phosphatidylserine greatly enhanced the olfactory response to certain species of odorants. The transduction mechanisms of odor reception are also discussed. The paper also deals with characteristics of receptor sites for taste stimuli, the ion dependence of taste responses, and transduction mechanisms.

INTRODUCTION

There are many types of internal receptors in our bodies for detecting chemical substances such as hormones, neurotransmitters, and blood sugar. These internal receptors perceive only such specified substances. On the other hand, the gustatory and olfactory receptors detect chemical substances in the external environment, where there are many types of substances, and hence these receptors perceive multifarious substances including artificial ones.[1,2] The gustatory and olfactory cells are typical chemical sensor, that respond to various types of chemicals in biological systems. This paper reviews recent progress in chemical recognition in olfactory and gustatory cells.

1. CHARACTERISTICS OF OLFACTORY RECEPTION

Olfactory receptors have the following characteristics which distinguish them from other receptor systems. (1) Olfactory cells sense and discriminate multifarious types of substances including artificial substances. (2) In general, the sensitivity of the olfactory systems to stimuli is extremely high. (3) The threshold concentrations of odorants are mainly determined by the partition coefficient between water and oil. (4) Shape and size of molecules as well as functional groups are important factors in determining odour quality. (5) There is no difference in odour intensity between optical isomers. (6) There are many different types of receptor sites on olfactory cell membranes. It is unlikely that there are a limited number of receptor sites for primary odours and variation of odour quality is produced by a combination of the primary odours.

2. ODOUR INTENSITY

Adsorption of odorants on the olfactory receptor membranes induces membrane potential changes in olfactory cells (receptor potential), which leads to generation of nerve impulses. Odour intensity is related to the magnitude of the receptor potential in the cell and to the impulse frequency in the olfactory nerve.

When the Langmuir isotherm is applied, a dynamic range is 2 log units.[3] In usual olfactory systems, the dynamic ranges are much larger than 2 log units. For example, the dynamic range for the response to amyl acetate in the turtle olfactory system is 6 log units.[4] The dynamic range of animals having more sensitive olfactory organs is much larger than that in the turtle.

We compared the odour intensity of six pairs of optical isomers in the turtle olfactory system.[5] The results obtained indicated that with all odorants tested, there was no difference in odour threshold and intensity between optical isomers in the whole concentration range examined.

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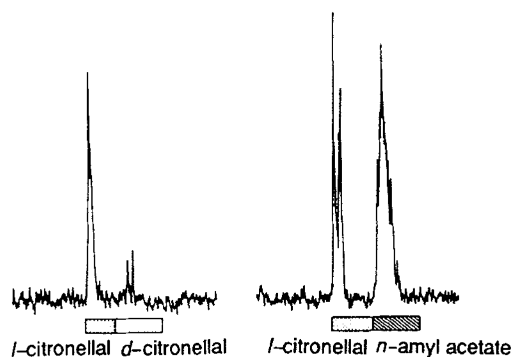

Figure 1 Cross-adaptation in turtle olfactory responses. The patterns represent the summed olfactory responses. The ordinate shows the relative magnitude of the response. After the response to 1 mM L-citronellal was adapted to a spontaneous level, 1 mM D-citronellal (left) or 5 mM *n*-amyl acetate (right) was applied.

3. ODOUR QUALITY

Differences in odour quality between one pair of odorants can be evaluated by a cross-adaptation method.[5] In Figure 1 (left), after the turtle olfactory bulbar response to L-citronellal had been adapted to a spontaneous level, D-citronellal was applied. As seen from the Figure, only a small response to *d*-citronellal appears, suggesting that the turtle cannot discriminate these two odorants. On the other hand, the response to amyl acetate appears independently of the previous application of L-citronellal (Fig 1, right), suggesting that the turtle olfactory system completely discriminates between these two odorants. In other words, L-citronellal and D-citronellal are adsorbed on the same site in the membrane, and amyl acetate is adsorbed on a different site from that of L-citronellal. There were many combinations of odorants which gave intermediate responses; the response induced by one odorant applied after another was partly suppressed by the first odorant.

We examined differences in odour quality between six pairs of optical isomers in the turtle olfactory system.[5] D- and L-isomers of some odorants (e.g. carvone) were well discriminated, but the optical isomers of other odorants (e.g. limonene) were little discriminated. The magnitude of the difference in odour quality between optical isomers greatly varies with species of odorants. The above experiments were carried out at room temperature, but at 40 °C, even D- and L-carvone could not be discriminated, which is discussed later.

4. PUTATIVE RECEPTOR PROTEINS AND TRANSDUCTION MECHANISMS

In 1972, Kurihara and Koyama[6] demonstrated that rabbit olfactory epithelium exhibits high adenylate cyclase activities. In 1985, Pace *et al.*[7] demonstrated that a preparation of cilia from frog olfactory epithelium contained adenylate cyclase at nearly 15 times higher specific activity than the whole olfactory epithelium, and that odorants activate the enzyme in the cilia in the presence of GTP, Nakamura and Gold[8] showed that excised patches of ciliary plasma membrane contain a conductance which is gated directly by cAMP and cGMP. Injection of the cyclic nucleotides into isolated olfactory cells of the frog,[9] salamander[10] and newt[11] led to depolarization of the cells. Kurahashi and Shibuya[12] pointed out that the characteristics of cAMP-sensitive conductance in isolated cells of the newt are similar to those of odorant-sensitive conductance. These results suggested that cAMP played a role as a second messenger in olfactory reception.

Sklar *et al.*[13] assayed more than 60 odorants for their potential to stimulate olfactory adenylate cyclase, and found that certain odorants were ineffective. Breer *et al.*[14,15] found that certain odorants which did not activate the cAMP pathway caused a rapid and transient increase of inositol 1,4,5-triphosphate (IP_3). For example, lyral, which belongs to the fruity odorants, did not activate adenylate cyclase and increased the IP_3 level, while citralva, which also belongs to the fruity odorants, activated adenylate cyclase and did not increase the IP_3 level. They concluded that compounds with similar odour do not all activate the same second messenger, and alternative reaction cascades can be activated by chemicals with similar odour quality.

In 1991, Buck and Axel[16] cloned and characterized 18 different members of an extremely large multigene family that encoded seven transmembrane domain proteins whose expression was restricted to the olfactory epithelium. They suggested that the members of this novel gene family were likely to encode a diverse family of odorant receptors. In 1992, Parmentier *et al.*[17] found that the gene family also existed in human sperm cells and suggested that a common receptor gene family encoded olfactory receptors and sperm cell receptors that could be involved in chemotaxis during fertilization.

5. ROLE OF LIPIDS OF OLFACTORY RECEPTOR MEMBRANES IN ODOUR RECEPTION

Responses of non-olfactory systems to odorants

It has been shown that non-olfactory systems such as *Tetrahymena*,[18] the turtle trigeminal nerve,[19] the *Helix* ganglion,[20] the fly taste nerve,[21] the frog taste cell[22] and the neuroblastoma cells,[23,24] respond to various

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odorants. These non-olfactory systems do not seem to provide specific receptor proteins for odorants. In general, odorants are hydrophobic and the interaction of odorants with lipid layers of olfactory receptor membranes seems to play an important role in the generation of olfactory responses, at least in these systems.

Liposomes having high sensitivity to odorants

We found that liposomes sensitively respond to various odorants,[25-28] and the sensitivity varies with the species of odorant. The minimum concentration of amyl acetate needed to induce the response (referred to as the threshold) in phosphatidylcholine (PC) liposomes was approximately 10^{-4} M. Addition of 10% or 20% phosphatidylserine (PS) lowered the threshold to approximately 10^{-9} M and increased the magnitude of the response. The olfactory thresholds for amyl acetate were determined to be 10^{-4} M for the frog, and 10^{-7} M for the turtle. Hence, the threshold of the PC–PS liposomes (PS/PC = 0.2) to amyl acetate was comparable to or lower than the olfactory thresholds in these animals. It was calculated that adsorption of less than a few molecules of amyl acetate on a single liposome elicits detectable changes in the membrane potential.

The specificity of liposomes to odorants was greatly dependent on the lipid composition. The responses to fatty acids such as valeric acid, isovaleric acid and butyric acid became remarkably large when PS was added to PC. The specificity of liposomes to odorants was also affected by addition of proteins or peptides. For example, incorporation of concanavalin A to PS/PC = 0.2 liposomes greatly increased the response to citral and decreased that to amyl acetate.[28]

Detailed analysis of the adsorption sites for various odorants in lipid membranes was carried out using various fluorescent dyes which monitor the membrane fluidity changes in different regions of the membrane.[29] It was shown that different odorants are adsorbed on different regions of the membranes that have a complex lipid composition, whereas different odorants are adsorbed on similar regions in membranes having simple lipid compositions.

Enhancement of turtle olfactory responses to fatty acids by treatment of the epithelium with PS-liposomes

As described above, addition of PS to PC-liposomes enhanced the response to fatty acids. In order to examine whether or not this occurs in the olfactory system, PS-containing liposomes were applied to the turtle olfactory epithelium and its effects on the olfactory responses were observed[30]. PS treatment enhanced the response to fatty acids such as valeric acid, isovaleric acid and butyric acid by a factor of 4–5 times. The threshold concentration of valeric acid was lowered from 10^{-4} M to 10^{-6} M by PS treatment. The treatment did not significantly affect the response to other odorants examined.

Enhancement of olfactory response to fatty acids by PS treatment closely resembles that observed with the liposomes. It seems that PS is incorporated into the olfactory receptor membranes and modifies the receptor site for the fatty acids. These results suggest that lipids are important for the reception of certain odorants.

Effects of membrane fluidity changes on the ability of turtle olfactory receptors to discriminate odorants

If the lipid layer in the olfactory receptor membrane plays an important role in odour reception, changes in temperature, which cause membrane fluidity changes, may affect the structure of the receptor sites. We examined the effects of temperature changes on the ability of the turtle olfactory receptors to discriminate odours using a cross-adaptation method.[31] Temperature was changed by perfusing the olfactory epithelium with Ringer solution of different temperatures. The cross-adaptation experiment (see Fig 1) was carried out as follows. For example, first 1 mM *trans*-3-hexenol was applied to the olfactory epithelium and after the response to the *trans* isomer was adapted to a spontaneous level, 1 mM *cis*-3-hexenol was applied. These experiments were carried out at 5, 18 and 40 °C. At 5 °C, the response to the *cis* isomer applied secondarily was not suppressed by previous application of the *trans* isomer, suggesting that the receptor site for the *trans* isomer is different from that for the *cis* isomer. The response to the *trans* isomer was partly suppressed at 18 °C and greatly suppressed at 40 °C. It is noted that both responses to the *trans* and the *cis* isomer when applied alone increased with an increase in temperature.

Similar results to those obtained with *trans*-3-hexenol and *cis*-3-hexenol were obtained with other pairs of odorants having similar odours (L-carvone and D-carvone, *n*-amyl acetate and isoamyl acetate, camphor and cineole). On the other hand, suppression of the response to odorants applied secondarily at 40 °C was not appreciably suppressed when pairs of odorants having different odours (e.g. L-limonene and cineole, anisol and cineole) were used.

One possible explanation of the results is as follows. The temperature change induces a conformational change of a specific receptor protein for an odorant, which leads to a change in the selectivity of the protein to the odorant. In general, the selectivity of a protein is not, however, changed by a small temperature change from 18 to 40 °C as far as we know. For

example, the receptor protein for an L-amino acid does not accept a D-amino acid even when the temperature is increased. Hence the present results are not simply explained in terms of a conformational change of protein.

Another possible explanation of the abolishment of the odor-discriminating ability is as follows. Odorants are assumed to be adsorbed onto hydrophobic pockets composed of lipids and proteins in the receptor membranes. At lower temperature, the lipid structure is rather rigid and different odorants are adsorbed onto different pockets. At higher temperature, the fluidity of the lipid layers is increased and then the pockets for the odorants become flexible. At 40 °C, the receptor pocket for *cis*-3-hexenol, for example, accepts *trans*-3-hexenol and hence is desensitized by previous application of *trans*-3-hexenol.

6. RESPONSES OF OLFACTORY SYSTEMS TO GUSTATORY STIMULI

As will be described later, typical gustatory stimuli are salts, acids, bitter substances, sweet substances and umami substances. Among these stimuli, salts, acids and bitter substances elicited the responses in the turtle olfactory system.[32] These stimuli were applied to the turtle olfactory epithelium perfused with a salt-free solution (200 mM mannitol). All the salts, acids and bitter substances tested elicited responses in the olfactory system. The threshold concentrations for the salts and bitter substances in the olfactory system were much lower than those in the rat and frog gustatory systems. The responses of olfactory systems to salts, acids and bitter substances had similar properties to those in the gustatory systems as will be described later. The above results suggest that the basic structure of the biological membranes is concerned with the reception of salts, acids and bitter substances and that the olfactory system carries the transduction function for these non-odorous stimuli.

7. ROLE OF CILIA IN RECEPTION OF ODORANTS

As described before, the cAMP-gated channels are located at olfactory ciliary membranes. We eliminated the carp olfactory cilia by 'ethanol/–calcium shock' and examined its effects on olfactory responses to amino acids.[33] The results obtained indicated that deciliation did not meaningfully affect the response to amino acids, suggesting that the olfactory cilia may not be necessary for receptor neuron function in the carp.

The vomeronasal organ is a chemoreceptor which is different from the main olfactory organ. Vomeronasal cells lack cilia and possess microvilli, while olfactory cells have long cilia. We compared the sensitivities of the turtle vomeronasal organ to various odorants with those of the olfactory organ.[4] The threshold concentrations for various odorants in the vomeronasal organ were similar to those of olfactory organs, despite the fact the vomeronasal organ receptor cells lack cilia. These results suggested that cilia are not essential for the reception of odorants at least in the systems tested.

8. EFFECTS OF IONIC ENVIRONMENT ON OLFACTORY RESPONSES

In order to explore the mechanism of generation of olfactory receptor potentials, we tested the possibility that activation of ionic channels at the apical portion including cilia and olfactory knobs contributes to the receptor potentials. The turtle olfactory epithelia were perfused with a salt-free solution or solutions containing various concentrations of salts and the effects of different ionic environments on the olfactory responses were examined.[34] The olfactory responses to all odorants examined were little affected by perfusing the olfactory epithelium with the salt-free mannitol solution. It was evident that salts on the epithelium were sufficiently eliminated since the olfactory system responded to very low concentrations of salts after perfusion with the salt-free solution. The above results suggested that activation of ion channels at the apical membrane including ciliary membranes does not contribute to generation of the olfactory response in the turtle. The results obtained with the frog[35] and the carp[36] olfactory systems also supported the above idea.

9. PROBLEMS IN SECOND MESSENGERS-MEDIATED TRANSDUCTION MECHANISMS

As described before, the following transduction mechanism is generally accepted; odorants bind to the receptor proteins located at olfactory cilia, increase cAMP or IP_3 levels and activate cAMP- or IP_3-gated channels at the ciliary membranes. Many data supporting the above mechanism have been obtained by *in vitro* experiments. However, data not consistent with the above mechanism were obtained from experiments *in vivo* as described above. In addition, recently we found that application of forskolin, a powerful adenylate cyclase activator of the turtle olfactory epithelium, only affected a little the olfactory responses to various odorants.[37] Further study will be needed to clarify the transduction mechanism of odour reception in *in vivo* system.

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10. CHARACTERISTICS OF TASTE RECEPTION

The typical taste stimuli are salt, acid, sweet, bitter and umami. Among these stimuli, salt, acid and bitter substances elicited responses in non-gustatory cells such as neuroblastoma cells and olfactory cells as described earlier. These results suggest that the basic structure of biological membranes are concerned with the reception of these stimuli. On the other hand, sweet and umami substances did not elicit responses in the non-olfactory cells, suggesting that the receptor proteins unique to the gustatory cells are concerned with the reception of the stimuli. We are now cloning the genes for putative receptor proteins unique to gustatory cells.[38]

Salts

Various inorganic salts elicited the gustatory responses. Salts carrying trivalent cations such as Fe^{3+} and La^{3+} gave a much greater response than those carrying divalent or monovalent cations.[39] The order of magnitude of the response to salts carrying monovalent cations varied with species of animal. Not only inorganic salts but also organic salts such as salts carrying choline, Tris, bis–Tris, glycylglycineamide, tetraethylammonium and glucosamine gave responses in the gustatory systems that were similar to inorganic salts.[39] The magnitude of the response to salts also varied with species of anions. In the rat, the order of the response to Na salts carrying different anions was NaCl, NaBr > $NaNO_3$, NaSCN > NaF > Na_2SO_4 > Na_2HPO_4.[40] Not only taste intensity but also taste quality greatly varied with species of anion. For example, the taste of Na_2SO_4 is quite different from that of NaCl. The properties of the salt response described above (e.g. dependence of cation and anion and the responses to orgaic cations) were also observed with the response of turtle olfactory systems to salts.

In 1954, Beidler[41] observed that the magnitude of the rat chorda tympani nerve response to NaCl follows the Langmuir isotherm and proposed the mechanism that taste response is induced by adsorption of chemical stimuli on the receptor membranes. In 1984, DeSimone and his coworkers[42] reported that amiloride, an inhibitor of Na^+ transport, reduces the short-circuit current (Isc) in the presence of mucosal hyperosmotic NaCl in an *in vitro* preparation of canine or rat dorsal lingual epithelium. It was also shown that amiloride selectively blocks the rat chorda tympani nerve response to hyperosmotic NaCl with a small suppression of the KCl response. On the basis of these results, it was suggested that an initial event in gustatory transduction with respect to NaCl is the passage of Na^+ through specific transport pathways in the apical region of the taste cells.

Nakamura and Kurihara[43] examined the effects of amiloride on the canine chorda tympani nerve response to salts. The results obtained indicated that amiloride non-specifically inhibits the canine taste nerve response to various salts such as NaCl, KCl and NH_4Cl and that the suppression is competitive between amiloride, which carries a positive charge, and cations of the salts. These data suggested that suppression of the salt response by amiloride is not always brought about by suppression of ionic channels.

It was proposed that the response to KCl is also induced by ion flux at the receptor membrane. Kinnamon and Roper[44] proposed that the response to KCl is induced by an influx of K^+ through voltage-dependent K-channels on the apical membranes of mudpuppy taste cells.

The results described above are not, however, consistent with the above ionic permeability hypothesis. Various salts including salts carrying impermeable cations such as organic cations induced taste responses in the rat and frog.[45] The polyvalent cations such as Fe^{3+} or La^{3+} induced much greater responses than monovalent cations, although the polyvalent cations are less permeable to the membranes.

Acids

Various acids including inorganic and organic acids elicit sour taste. Differences in species of anions does not greatly affect the quality of sourness, which is different from the salt response. The species of anions, however, affects the magnitude of the response.[46] In general, organic acids elicit greater responses than inorganic acids at equal pH.

In the mudpuppy taste cells, acids depolarize the cells by inactivation of the voltage-dependent K-channels at the apical membranes.[47] This does not seem to be applicable to mammalian taste cells, since tetraethylammonium, an inhibitor of the K-channels, does not elicit a sour taste in humans. It was also proposed that acids depolarize frog taste cells by activation of Ca-channels[48] at the apical membranes, based on the fact that the presence of Ca^{2+} on the tongue surface enhances the response to HCl.[49]

Bitter substances

The structures of bitter substances are extremely diverse and it is difficult to find a chemical structure common to bitter substances. Kumazawa *et al.*[50] found that the mouse neuroblastoma cell (N-18 clone) is depolarized by various bitter substances although the N-18 cell is unrelated to taste cells. It was

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also found that various bitter substances depolarize liposomes.[51] The concentration of each substance needed to depolarize azolectin liposomes was similar to the concentration needed to induce taste response in humans. These results suggest that an hydrophobic interaction between bitter substances and the receptor sites contributes to the generation of the responses.

Bitter substances carrying a positive charge at neutral pH such as quinine or strychnine elicit the gustatory responses at very low concentrations. The responses to these stimuli were suppressed by the presence of salts.[52] The above results suggested that an electrostatic interaction between the positive bitter substances and negative sites on the gustatory receptor membranes, as well as an hydrophobic interaction, contribute to the binding of the bitter substances to the receptor sites. Similar phenomena were observed with the turtle olfactory system.[32]

Sweet substances

A sweet taste in humans is elicited by many species of substances such as sugars, glycosides, peptides, proteins and artificial sweeteners. Gymnemic acid[53] and ziziphin[54] selectively suppress the sweet taste in humans. Kurihara and her coworkers[54] showed that ziziphin suppressed the sweet taste of all sweeteners tested, while it had no suppressive effect on tastes induced by salt, acid, bitter and umami substances. There are a number of different types of receptor proteins (or sites) for different sugars,[55] but the sweet receptors have a common structure to which ziziphin binds.

Miraculin[56,57] and curculin,[58] which are proteins isolated from plants, have an unusual property of changing a sour taste into a sweet taste. These proteins are tightly bound to the surface of the gustatory membranes and stimulate sweet receptor sites when acids are present on the tongue.

Umami substances

Monosodium glutamate (MSG), disodium 5′-guanylate (GMP) and disodium 5′-inosinate (IMP) elicit a unique taste and are referred to as 'umami substances'. It is known that there exists a remarkable synergism between MSG and the 5′-nucleotides.[59] Electrophysiological studies indicate that the synergism between MSG and the nucleotides occurs in taste systems of various animals such as the rat,[60] mouse[61] and dog.[62,63] The most remarkable synergism was observed in the dog. In the presence of GMP, a large response appeared at low concentrations of MSG, while response at high concentrations of MSG was unchanged. The results can be explained by an allosteric model. That is, the receptor proteins for umami substances have two binding sites; one for MSG and another for 5′-nucleotides. Binding of the nucleotide to one site leads to an increase in the affinity of another site for MSG or *vice versa*.

Both MSG and the nucleotides are Na salts and hence Na ions in the umami substances stimulate salt receptors. In the rat, hamster and cat, single fibres of chorda tympani nerve which were sensitive to MSG or mixtures of MSG and the nucleotides were also sensitive to NaCl. Hence it has been argued whether or not the umami substances stimulate only the salt receptor. In the mouse glossopharyngeal nerve,[61] the chimpanzee chorda tympani nerve[64] and the macaque brain neuron,[65] there were MSG-specific units which responded little to other stimuli such as HCl, NaCl, sucrose and quinine.

In the dog, the chorda tympani response to NaCl and MSG alone was suppressed by amiloride,[63] but the response to GMP alone or a mixture of GMP and low concentrations of MSG was not suppressed by amiloride. These results suggest that in the dog, the response to MSG alone is mainly composed of the salt component and the response to GMP alone or that induced by the synergism is composed of the umami component. Hence, GMP acts as an agonist and MSG acts as a modulator in the dog. In humans, MSG alone elicits a distinct umami taste and the umami taste induced by GMP or IMP is much weaker than that induced by MSG. It seems that MSG acts as a main agonist and GMP acts mainly as a modulator in humans. Hence whether MSG acts as a modulator or an agonist varies with species of animal.

The above results obtained with the mouse and the dog indicate that the umami substances induce a response unique to the umami substances, suggesting that the umami taste is independent of any of the other primary tastes.

11. TEMPERATURE DEPENDENCE OF GUSTATORY RESPONSE

The magnitude of the gustatory response depends on the temperature of stimulating solutions. In the rat gustatory system, the gustatory response to most stimuli showed peaks at approximately 30 °C.[66] The response to glycine, sucrose, and quinine showed sharp temperature dependence, while that to acids and salts showed relatively linear dependence. The temperature change did not practically affect the thresholds for these stimuli and affected the magnitude of the responses to higher concentrations of stimuli. Interestingly, the temperature dependence of the response to salt, acid and bitter substances in the turtle olfactory system[32] was similar to that observed with

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the gustatory system, while the response to odorants did not show peaks but increased with an increase of temperature. It seems that the conformation of the receptor membranes is changed with temperature and leads to exposure and embedding of available receptor sites.

12. EFFECT OF SALTS ON THE GUSTATORY RESPONSE

Figure 2 shows the effects of ionic environment on the gustatory response to various stimuli.[67] The response to neutral bitter substances are independent of salt concentration on the tongue surface (Fig 2*a*). The response to sugar and amino acids in the rat are also independent of salt concentration. The response to sugars[68] and amino acids[69] in the dog are enhanced by the presence of lower concentrations of salts and the enhancement is suppressed by higher concentrations of salts (Fig 2*b*). These phenomena are also observed with humans.[70] That is, it is well known in Japan that addition of NaCl to watermelon or to adzuki bean soup increases the sweetness of these foods.

The response to sugars[71] and amino acids[72] in the frog is suppressed by an increase in salt concentration (Fig 2*c*). The suppression by higher concentrations of salts shown in Figure 2(*b*) seems to correspond to the type shown in Figure 2(*c*). The water response is suppressed by the presence of salts.[73,74] The water response is observed in gustatory cells of various animals such as fly, fish, frog, and rabbit. The surface of the taste organs of these animals is usually covered with mucus which contains certain concentrations of salts. Application of deionized water to the organs decreases the salt concentration, which leads to depolarization of taste cells. The mechanism of the water response will be discussed later.

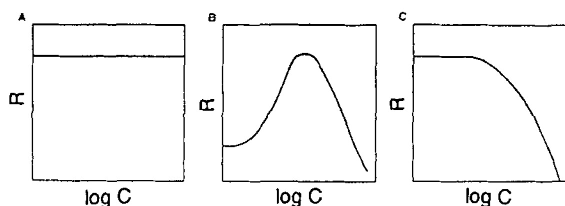

Figure 2 Schematic representation of the relationship between the magnitude of the gustatory response to various stimuli (R) and the logarithmic salt concentration (log C). (A) Responses to neutral bitter substances such as caffeine or theophyline in the frog and the rat, responses to amino acids in the rat and the eel, responses to sugars in the rat. (B) Responses to sugars and amino acids in the dog. (C) Responses to sugars and amino acids in the frog, and the water responses.

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13. ION FLUX AT THE RECEPTOR MEMBRANES: CONTRIBUTION TO RECEPTOR POTENTIALS

As shown in Figure 2, gustatory response to certain stimuli is independent of salt concentration on the tongue surface and the responses to some stimuli are suppressed by the presence of salts. In responses of these types, ion flux at the receptor membranes of gustatory cells does not contribute to the generation of receptor potential. On the other hand, the gustatory responses of the type in Figure 2(*b*) seem to show that ion flux at the receptor membranes contributes to the receptor potential. The following facts are, however, not consistent with this mechanism. Salts carrying impermeable cations contributed to the enhancement of the response to sugars[68] and amino acids[69] in the dog. Not only species of cations but also species of anions greatly affected the enhancement of the response to amino acids in the dog.[69] Thus it is unlikely that ionic permeability at the apical membranes of taste cells is concerned with the reception of these stimuli.

It was reported that injection of cAMP into mouse[75] and frog[76] taste cells led to depolarization of the cells. It was also reported that application of sugars to the rat tongue increased cAMP in the tongue epithelial tissue.[77] On the basis of these results, it was proposed that the taste receptor potentials in response to sugars are induced by inactivation of K-channels caused by the action of cAMP-dependent protein kinase. The following fact, however, is not consistent with the above hypothesis: fructose, which is one of the most potent sweeteners, did not activate adenylate cyclase in the rat tongue epithelium.[77]

14. CONTRIBUTION OF PHASE BOUNDARY POTENTIAL TO THE MEMBRANE POTENTIAL OF LIPID BILAYERS AND NEUROBLASTOMA CELLS

Membrane potential is composed of the diffusion potential within the membrane and the two phase boundary potentials at both sides of the membrane. It has been considered that the phase boundary potential changes do not contribute to the membrane potential of the living cells. In the following examples demonstrating this, the phase boundary potentials are shown.

We measured the membrane potential changes of the planar lipid bilayers in response to various salts.[78] The order of magnitude of the membrane potential at equimolar concentration was Fe^{3+}, $La^{3+} > Ca^{2+}$, $Mg^{2+} > Na^{+}$, K^{+}, which was similar to that of the

rat taste nerve response as described earlier. The concentration–response curves for the trivalent cations had much larger slopes than the Nernst slope. Hence it is evident that the membrane potentials induced by these polyvalent cations do not come from the diffusion potential but from the phase boundary potential.

The neuroblastoma cells also exhibited membrane potential changes in response to various polyvalent cations.[79,80] Here it is also true that the polyvalent cations induced much larger responses than the monovalent cations at equimolar concentrations. The surface potential of the neuroblastoma cells can be estimated by measuring the zeta potential under a microscope. There was a close correlation between the membrane potential changes and the zeta potential changes in response to various polyvalent cations and protons, suggesting that the membrane potential changes of the neuroblastoma cells in response to polyvalent cations and protons come from the phase boundary potential changes.

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15. THE PHASE BOUNDARY POTENTIAL CONTRIBUTION TO TASTE RECEPTOR POTENTIALS

The phase boundary potentials are composed of the 'surface potential' (electric double layer potential) and the 'boundary potential' (potential at the 'boundary region', which is located within the membrane interior near the membrane–solution interface).[81] The surface potential is suppressed by an increase in ionic strength in bulk solution, whereas the boundary potential is independent of ionic strength.

We proposed that changes in the membrane potential in response to salts and acids are brought about by changes in the surface potential.[2] This hypothesis well explains the following facts: (1) The membrane potential changes in response to polyvalent cations and protons are accompanied with little or no membrane resistance change. (2) The polyvalent cations induce a much larger taste response than the monovalent cations. (3) Various inorganic cations which are impermeable to the membrane induce a large taste response.

The results of the ion dependence of the taste response suggested that the above hypothesis is also applicable to the responses to other stimuli than salts. As shown in Figure 2, the taste response to sugars in the frog[71] and the dog[68] and those to amino acids in the frog[72] were suppressed by an increase in ionic strength, which suggested that the receptor potentials in response to these stimuli stem from changes in the surface potential. The taste response to sugars in the rat, to amino acids in the eel[82] and to bitter substances in the frog and the rat, which were independent of ionic strength, seem to stem from changes in the boundary potential.

As described above, the water response is induced by a decrease in ion concentration in an external solution and is suppressed by an increase in ionic strength. This implies that taste cells are depolarized by a decrease of salt concentration in the external medium. Hence the water response is the most typical example that cannot be explained by the ionic permeability theory. The mechanism of the water response is well explained in terms of surface potential changes.[73,74]

16. TRANSDUCTION OF TASTE RECEPTOR POTENTIALS INTO NERVE IMPULSES

As described above, the initial depolarization induced by adsorption of stimuli seems to occur at the apical membranes of taste cells. The depolarization is propagated electronically to the synaptic region. A current carrier across the apical membranes of taste cells may be Cl^- since taste response occurs even when no permeable cation is present in a solution perfusing the apical membranes.

Depolarization of the synaptic area leads to activation of the voltage-dependent Ca-channels,[83] which triggers the release of a transmitter from taste cells. In the frog taste cells, the transmitter seems to be norepinephrine.[84] (The effect of Ca^{2+} on the release of norepinephrine was examined by varying Ca^{2+} concentrations in a solution perfusing the lingual artery.[84] The taste nerve response to all species of stimuli (salt, amino acid, sugar, bitter and deionized water) showed similar Ca dependence.

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