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PHYSICOCHEMICAL STUDIES OF TASTE RECEPTION

III. INTERPRETATION OF THE WATER RESPONSE IN TASTE RECEPTION

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SUMMARY

The model membrane composed of a Millipore filter paper and the total lipids from bovine tongue epithelium or phosphatidylcholine from egg yolk simulated well the water response of a living taste cell. The water response observed with the model membrane adapted to various salt solutions was interpreted in terms of changes in electric potential at the membrane-solution interface, i.e. the water response was attributed to the e.m.f. change produced by diffusion of the electrolytes dissolved in (or adsorbed on) the membrane surface into the bulk solution.

The water response of the frog tongue was also investigated by measuring the neural response of the glossopharyngeal nerve. The results obtained were consistent with the mechanism proposed in the present paper. The response of the frog to Ca^{2+} was examined under the condition where the water response was suppressed, and it was concluded that the water response of the frog is different from the response to Ca^{2+}.

INTRODUCTION

When distilled water is applied to the gustatory organ adapted to saliva or to Ringer solution, the neural response is increased just as if the tongue was subjected to a high concentration of taste stimuli. This phenomenon can be observed in a variety of animals, and has been referred to as the water response [1]. A number of investigators have paid attention to the water response as a clue to understanding the taste stimulation in general [2–8], but no satisfactory interpretation has been presented as far as the authors are aware.

In a series of papers concerned with physicochemical studies of taste reception [9, 10], we showed that the receptor potential of gustatory organs in response to salt and acid stimuli is well simulated by a model membrane composed of a Millipore filter paper and the total lipids extracted from bovine tongue epithelium. From the results obtained with the model membrane, we proposed a theory of taste reception stating that the reception is initiated by a change in electric potential at the interface between the receptor membrane and the stimulating solution. This paper is devoted to clarify the mechanism of water response, by using the model membrane made of total

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lipids or phosphatidylcholine and the gustatory organ of frog. The change in membrane potential of the model membrane in response to water is compared to the neural activity of the glossopharyngeal nerve of the frog. The results obtained are interpreted in terms of the variation in the potential at the membrane-solution interface.

EXPERIMENTAL

Materials

The total lipids were extracted from bovine tongue epithelium as described in the previous paper [9], and the phosphatidylcholine was prepared from egg yolk with use of a column of cellulose and silicic acid according to the methods employed by Lea et al. [11]. The purity of the sample was established by thin-layer chromatography on silica gel in appropriate solvent systems. As will be shown in a separate paper [12], the model membrane made of single phospholipid exhibits a variety of responses to distilled water, depending on the lipid species used. For example, a phosphatidylethanolamine membrane does not exhibit the water response, but phosphatidylcholine and sphingomyelin membranes respond well to distilled water. Phosphatidylserine membranes respond to water only when the membrane is adapted to 2 : 1 type salts. Therefore, the total lipids membrane and the phosphatidylcholine-membrane are used in the present analysis.

Analytical grade chemicals were used without further purification. Pure water was prepared by distillation of deionized water. The composition of Ringer solution was: 112 mM NaCl, 3.4 mM KCl, 2.5 mM $CaCl_2$, and adjusted pH to 7.1 with 2.5 mM $NaHCO_3/HCl$ buffer.

Preparation of model membrane and measurements of membrane potential

The model membrane was prepared by essentially the same procedure as that described in previous papers [9, 10]. A Millipore filter paper (Type VSWP, the nominal pore size 25 μm) was soaked in the chloroform solution of phosphatidylcholine or of the total lipids. After about 10 min soaking, the filter paper was dried in the air and weighed for determination of the amount of the lipid adsorbed on the filter paper (designated as Q in mg/cm^2). Then the membrane was immersed in 300 mM NaCl solution and conditioned overnight in a cold room. The value of Q for the model membranes used here was higher than 12 mg/cm^2, which was a sufficient amount to plug up the void space of the filter paper with the adsorbed lipids. In fact the membrane resistance did not change in the whole range of salt concentrations examined. In this respect, the model membrane used here is different from that employed by Tobias et al. [13], the inventors of this kind of model membrane, and that used in another series of papers [14].

The membrane potential was measured by the same apparatus described in the previous paper [9]. The e.m.f. arising between the stimulating solution and the reference solution (300 mM NaCl) separated by the membrane was measured with a high input-impedance electrometer (Takeda Riken Co. Type TR-64B) through a pair of saturated KCl salt bridges with calomel electrodes. The variation of the composition and/or concentration of salts in the reference solution did not influence the results obtained.

Recording of activity of glossopharyngeal nerve

Adult bullfrogs, *Rana catesbeiana*, were used in the present experiments. The preparation of the tongue with the glossopharyngeal nerve and the recording of nerve activity were carried out by the method employed by Kusano [15]. The nerve impulses were amplified with a CR-amplifier (Iwatsu Electric Co. Type HP-601), displayed on an oscilloscope and recorded with a tape recorder (Sony Magnescale Inc. Type FR-3215). The amplified impulses were also integrated with an electronic integrator (Nihon Kohden Co. Type RFJ-5), and the results were recorded with a pen-writing recorder. The integrated value of the impulse train which appeared 20 s after the application of a stimulating solution was taken as the magnitude of the response.

Stimulating solutions were applied on the frog tongue with a flow rate of 2 ml/s. Application of Ringer solution with this flow rate to the tongue adapted to Ringer solution did not bring about any appreciable response, which implied that the touch artifact was negligibly small compared with the taste response. After each application of the stimulating solution on the tongue surface, the tongue was rinsed with Ringer solution. About 15 min were interposed between each successive stimulation.

All experiments were performed at 18–20 °C.

RESULTS

Influence of ion species in the adapting solution

Application of distilled water to phosphatidylcholine and total lipids membrane (hereafter abbreviated as T-membrane) adapted to Ringer solution produced a

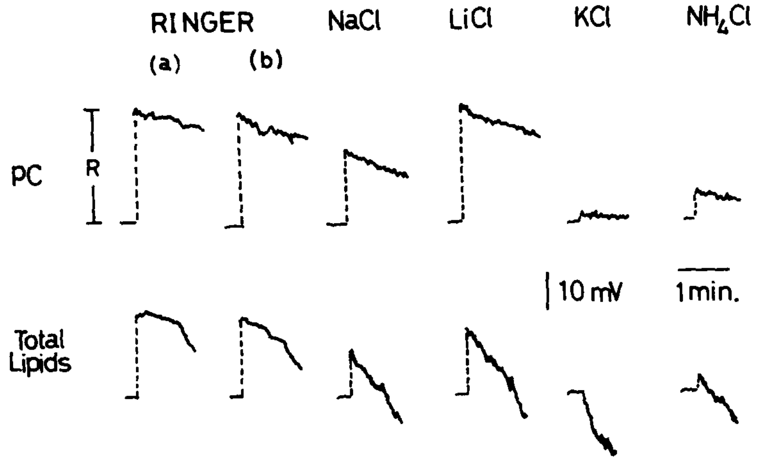

Fig. 1. Potential responses of phosphatidylcholines-(PC-) and T-membranes to water after adaptation to Ringer solution and to various 1 : 1 type salts of 100 mM in concentration. The traces shown in Ringer (a) are obtained by appling pure distilled water to the membrane adapted to Ringer solution, and those in Ringer (b) represent the responses obtained by applying the stimulating water containing 10^{-5} M NaCl to the same membranes as in (a). Other traces show the potential deflections when the stimulating water was applied to the membrane which was adapted in the solution indicated in the figure. *Q* values of the membranes used were 11.4 mg/cm^2 for T-membrane, and 20.3 mg/cm^2 for phosphatidylcholine-membrane.

positive going deflection of the potential ("depolarization" in electrophysiological terminology), which corresponds to the water response in living taste organ. The depolarized membrane potential induced by distilled water decreased with time and approached to a steady level. Note that the water response is a transient response. The peak value of the potential deflection at the initial stage is referred to as the magnitude of the water response and is hereafter denoted by *R* (see Fig. 1). The magnitude of the water response obtained with 10^{-5} M NaCl solution instead of distilled water was essentially the same as that induced by pure water (see Fig. 1). In order to facilitate the theoretical analysis, 10^{-5} M NaCl solution was used as "stimulating water".

The water response was observed with the membrane adapted to different

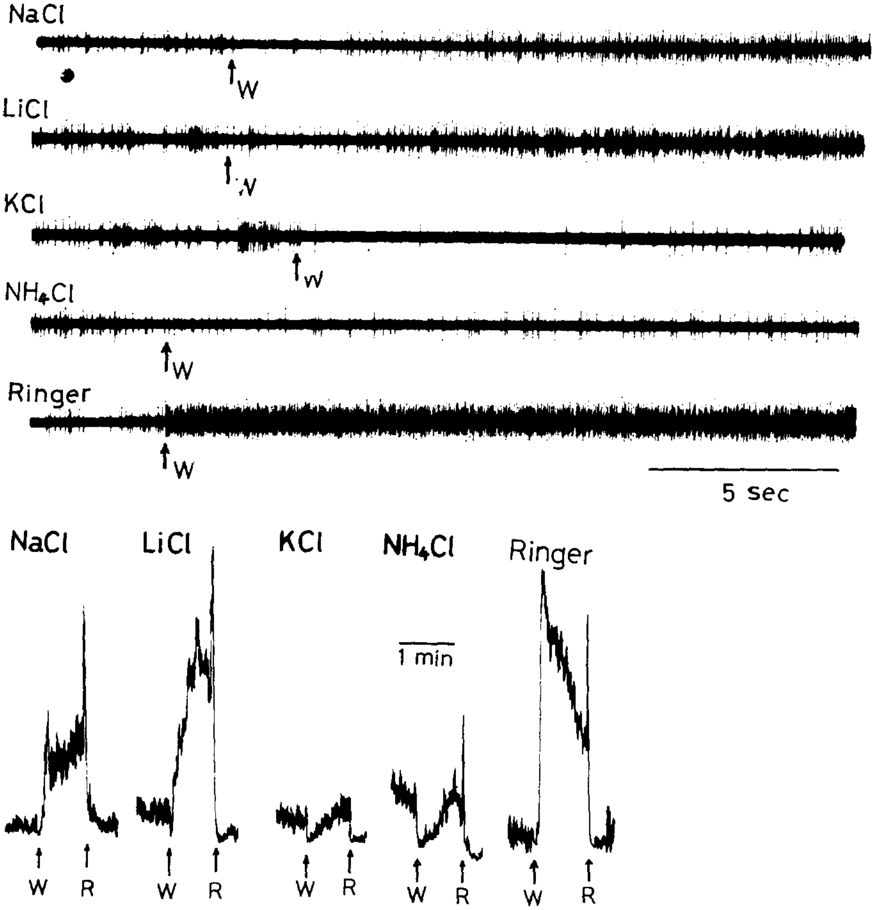

Fig. 2. Water response obtained with the glossopharyngeal nerve of frog whose tongue was adapted to various kinds of salt solutions. The stimulating water was applied to the tongue at the time indicated by *W* in the figure. Then the tongue was rinsed with Ringer solution at the time indicated by *R*. Adapting solutions (100 mM NaCl, LiCl, KCl, NH_4Cl, and Ringer solution) were allowed to flow over the tongue for 2–3 min before application of the stimulating water. (a) Direct spike responses of the glossopharyngeal nerve. (b) Integrated responses of the nerve with time constant of 0.3 s.

kinds of salts. In Fig. 1, the water responses after adaptation to 100 mM of various 1 : 1 type salts are presented in addition to that of Ringer solution. As seen in the figure, adaptation to NaCl or LiCl solution led to a large water response, while that to KCl or NH_4Cl led to none or a small response.

The effect of ion species in the adapting solution on generation of the water response was also examined with frog's tongue. Fig. 2a represents the records of neural response observed with the glossopharyngeal nerve of frog when stimulating water was applied to the tongue adapted to 100 mM of various kinds of 1 : 1 type salts or Ringer solution. In Fig. 2b, the integrated responses are represented. The figure indicates that the tongue adapted to Ringer, LiCl or NaCl solution responds well to water, while that adapted KCl or NH_4Cl exhibits only small response. These results observed with the glossopharyngeal nerve of the frog closely resemble those obtained from measurements of membrane potential of the model membrane shown in Fig. 1. It is noted that there is a linear relationship between taste receptor potential and frequency of impulses in the gustatory nerve [16, 17].

Dependence of response on salt concentration in adapting solution

In Fig. 3, the magnitude of the water response observed with T- and phosphatidylcholine-membranes is plotted as a function of the concentration of NaCl in the adapting solution. Similarly, Fig. 4 shows the dependence of the magnitude of water response of the model membranes on the concentration of $CaCl_2$ in the adapting solution. The water response increases with the increase of NaCl or $CaCl_2$ concentration and each approaches a plateau level.

Similar experiments were carried out on the frog tongue. Fig. 5 shows the relation between the magnitude of the water response and NaCl concentration in the adapting solution. The ordinate in Fig. 5 represents the magnitude of water response (R) relative to that obtained with the tongue adapted to 100 mM NaCl (R_{100}). The results closely resemble those shown in Fig. 3.

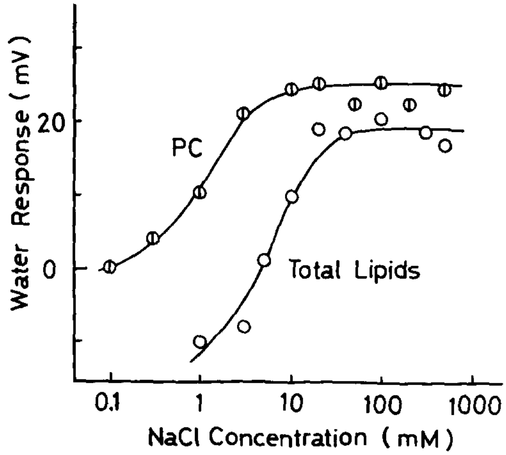

Fig. 3. Magnitude of the water response observed with model membranes as a function of NaCl concentration in the adapting solution. ⦶: phosphatidylcholine-membrane (Q = 12.3 mg/cm^2), ○: T-membrane (Q = 18.1 mg/cm^2) PC, phosphatidylcholine.

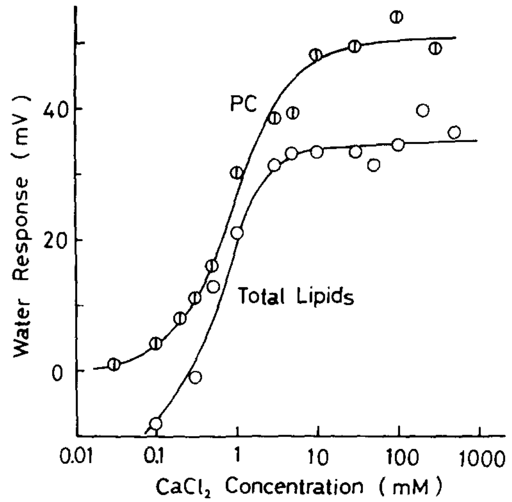

Fig. 4. Magnitude of the water response of model membranes as a function of $CaCl_2$ concentration in the adapting solution. ⦶: phosphatidylcholine-membrane (Q = 12.3 mg/cm^2), ○: T-membrane (Q = 14.3 mg/cm^2) PC, phosphatidylcholine.

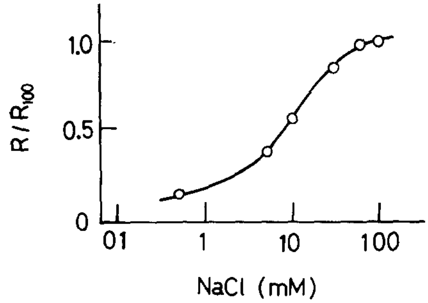

Fig. 5. Reduced magnitude of the water response of the frog as a function of NaCl concentration in the adapting solution. R_{100} represents the magnitude of the water response obtained from the frog tongue adapted to 100 mM NaCl solution.

The effect of $CaCl_2$ in the adapting solution on the water response of the frog could not be examined directly because a solution containing only $CaCl_2$ elicited a too large neural response, and the response was not easily adapted in the solution (cf. Fig. 9). Hence, Ringer solution containing different concentrations of $CaCl_2$ were used as an adapting solution. The increase of $CaCl_2$ in the adapting solution led to an increase of the water response. The relative magnitude of the water response after adaptation to Ringer solutions containing 0, 2, and 20 mM $CaCl_2$ was about 1, 2, and 3.5, respectively.

Suppression of water response by electrolytes

The water response is suppressed by adding an electrolyte to the distilled water to be applied. Fig. 6 represents the suppression of the water response in T- and phosphatidylcholine-membranes adapted to Ringer solution as a function of concentration of added salts such as NaCl, Na_2SO_4, and sodium benzoate. Similar suppression

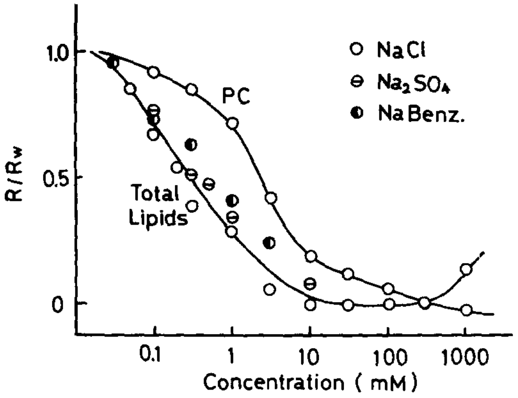

Fig. 6. The relative magnitude of water response of model membranes as a function of salt concentration added in the stimulating water. The membranes were adapted to Ringer solution. R, magnitude of response induced by salt solution; R_W, magnitude of water response induced by stimulating water. ○, NaCl; ⊖, Na_2SO_4; ◐, Sodium-benzoate. Q values were 17.4 mg/cm^2 for phosphatidylcholine-membrane and 16.5 mg/cm^2 for T-membrane. PC, phosphatidylcholine.

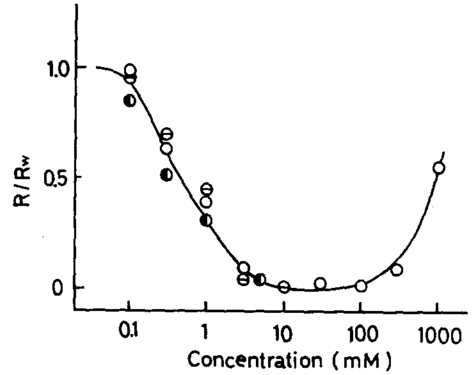

Fig. 7. The relative magnitude of the water response of frog tongue as a function of salt concentration added in the stimulating water. The frog tongue was adapted to Ringer solution. Notations are the same as those in Fig. 6.

curves were also obtained when KCl, NH_4Cl and LiCl were added to the stimulating water.

The water response of the frog was also diminished by adding electrolytes to distilled water. As seen in Fig. 7, the suppression curve of the water response of the frog closely resembles the curves given in Fig. 6, especially the curve observed with T-membrane.

Theoretical interpretation of water response

When distilled water or the stimulating water are applied to the membrane adapted to a salt solution, electrolyte components dissolved in or adsorbed on the membrane surface will diffuse out into the bulk solution. This, of course, is not a steady process. Even if the potential observed is a transient one as is encountered in the present study, it is not unreasonable to consider that the membrane potential is expressed by the sum of three parts as assumed by Teorell [18] and Meyer and Sievers [19], i.e. two electric potential differences at the membrane-solution interfaces and the intramembrane diffusion potential. As shown in previous papers [9, 10], the intramembrane diffusion potential scarcely contributes to the observed membrane potential for the model membrane used here and the taste receptor membrane. Hence the potential deflection observed in the present system, where one side of the membrane is contiguous to the reference solution of a fixed salt composition, can be attributed to the change in potential at the interface between the membrane and the stimulating solution. In principle, both the phase-boundary potential (the Donnan potential) and the diffusion potential in the solution phase outside the membrane are observed as the variation of the membrane potential in response to water. In this connection, it is worthwhile to note the results obtained with phosphatidylcholine-membrane. The Donnan potential of the phosphatidylcholine-membrane, which has no net charge at the surface, is independent of the salt species (1 : 1 type electrolyte) used and of its concentration applied [12], but a pronounced water response is induced in the phosphatidylcholine-membrane as seen in Fig. 1 and 3. When the membrane surface has fixed charges as in the case of T-membrane, the phase boundary potential may vary in response to water. However, as will be shown below, the magnitude of the water response which is defined as the potential deflection at the initial stage of the response (see Fig. 1) can be represented quantitatively in terms of the diffusion potential in the bulk solution outside the membrane phase. This fact suggests that the process of desorption of salts from the membrane phase is much slower than that of diffusion in the bulk solution. Therefore, the e.m.f. produced by diffusion of the electrolyte components from the membrane surface to the bulk solution is mainly responsible for the generation of the water response for all cases examined. For the sake of illustration, we will show below a theoretical base of the diffusion potential in the bulk phase.

Let C_i^{I} and C_i^{II} be the concentration of i-ion in the bulk solution and at the membrane surface, respectively. Assuming that the solution composing the boundary phase between the membrane surface and the bulk solution is a series of mixtures of solutions II and I and that mobilities of ions stay constant through the boundary phase, the diffusion potential, $\Delta\psi$, is represented as follows [20]

$$\Delta\psi = -(RT/F)\frac{\Sigma_i(u_i/z_i)(C_i^{II}-C_i^{I})}{\Sigma_i u_i(C_i^{II}-C_i^{I})}\ln\frac{\Sigma_i u_i C_i^{II}}{\Sigma_i u_i C_i^{I}} \tag{1}$$

where u_i and z_i stand for the mobility and the valence of i-ion, respectively, and R, T, and F have their usual thermodynamic meanings.

When distilled water is applied to the membrane, C_i^{I} should be zero theoretically. However, as seen in Figs. 1 and 6, the magnitude of the responses evoked by the distilled water and the stimulating water containing 10^{-5} M NaCl are practically identical each other. This probably stems from the contamination of distilled water by carbonate ions dissolved and/or by the electrolytes coming from the experimental chamber used. Therefore, the stimulating water is used in place of pure distilled water in order to reduce the ambiguity of C_i^{I} in the present experiments and to facilitate the following analysis.

Under the experimental condition that the stimulating water is applied to the membrane adapted to a solution composed of a single salt, Eqn. 1 is simplified to give

$$\Delta\psi = (RT/F)\,\Delta\tau\,\ln\,(C^{\mathrm{II}}/C^{\mathrm{I}})$$

with

$$\Delta\tau = -[(u_+/z_+)+(u_-/z_-)]/(u_+ + u_-) \tag{2}$$

where C^{I} and C^{II} are the concentrations of the electrolyte component in the stimulating water and at the membrane surface, respectively, and z_+ and z_- stand for the valencies of cation and anion. Figs. 3, 4, and 5 show that the magnitude of the water response increases with the concentration of the adapting solution, which is in line with Eqn. 2. Assuming that C^{II} is approximately constant irrespective of salt species used, (the concentration in the adapting solution is the same) Eqn. 2 predicts that the diffusion potential, and hence, the magnitudes of the water response is proportional to $\Delta\tau$. In Fig. 8, magnitudes of the water response observed with the model membranes and with frog tongue both adapted to 100 mM of various kinds of 1 : 1 type salts is plotted against $\Delta\tau$. As seen in the figure, a linear relationship between the magnitude of the water response and $\Delta\tau$ holds for all species of salts examined.

Comparison of the curves presented in Figs. 3 and 4 indicates that adaptation to $CaCl_2$ solution leads to a larger water response than that to NaCl solution. The value of $\Delta\tau$ for NaCl and $CaCl_2$ in the bulk solution are 0.21 and 0.35, respectively [21]. This difference of $\Delta\tau$ values between NaCl and $CaCl_2$ is partially responsible for the difference of magnitude of the water responses given in Figs. 3 and 4.

The water response was suppressed by an addition of electrolyte to stimulating water (see Figs. 6 and 7). Since the model membrane or the tongue surface is adapted to Ringer solution in the experiments given in Figs. 6 and 7, the term $\Sigma_i u_i C_i^{\mathrm{I}}$ may be negligibly small compared with $\Sigma_i u_i C_i^{\mathrm{II}}$ in Eqn 1. Hence, an increase of C_i^{I}, which is brought about by adding electrolyte to stimulating solution, diminishes the value of $\Delta\psi$ according to Eqn 1. This implies that the water response is suppressed more or less by adding any species of electrolyte in the stimulating solution. Actually, the results

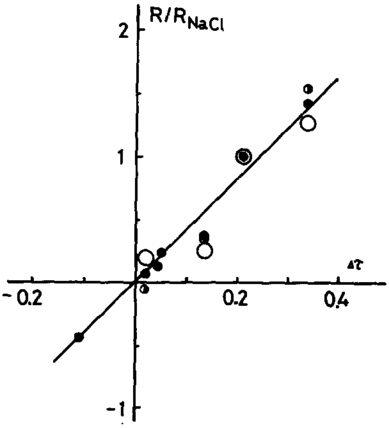

Fig. 8. The relative magnitude of water response (R/R_{NaCl}) after adaptation to various 1 : 1 type salts of 100 mM in concentration as a function of $\Delta\tau$, where R_{NaCl} stands for the magnitude of the water response after adaptation to 100 mM NaCl solution. The salts used were NaCl, NaCl, NH_4Cl, NaF, sodium formate, sodium-acetate, and KCl. ◑: T-membrane, ●: phosphatidylcholine-membrane, ○: frog tongue.

depicted in Figs. 6 and 7 show that the effect of added salts on the water response was practically independent of ion species added, at least in the systems studied here.

DISCUSSION

Nomura and Sakada [6], and Casella and Rapuzzi [22] reported that trace amounts of Ca^{2+} elicit a large neural response in the glossopharyngeal nerve of frog, and so questioned the existence of the water response of the frog. In order to clarify the relation between the water response and the response caused by Ca^{2+}, the following experiments were performed; $CaCl_2$ of varying concentrations was applied to frog's tongue under the conditions that the water response is completely suppressed, i.e., under the presence of 5 mM NaCl (see Fig. 7). Fig. 9 illustrates that Ca^{2+} induces a large response under this condition. This implies that the frog responds both to distilled water and to Ca^{2+} separately. Fig. 9 also indicates that the response to calcium benzoate was quite different from that to $CaCl_2$. This result contrasts to the fact that sodium benzoate added to the stimulating water led to no appreciable difference from that of NaCl as illustrated in Figs. 6 and 7.

Casella and Rapuzzi [22] stated that distilled water itself was not able to stimulate the taste organ of the frog. Their failure in observing a response to distilled water seems to be attributed to their experimental procedures employed, i.e. they immersed the tongue in a vial of water and did not flow the water over the tongue surface. In fact, the water response could be observed only when the water was flowed over the tongue. This observation is consistent with the results obtained with cat by Cohen et al. [2]. According to the mechanism of the water response proposed in the present paper, the water response is generated by the process of removal of the salts

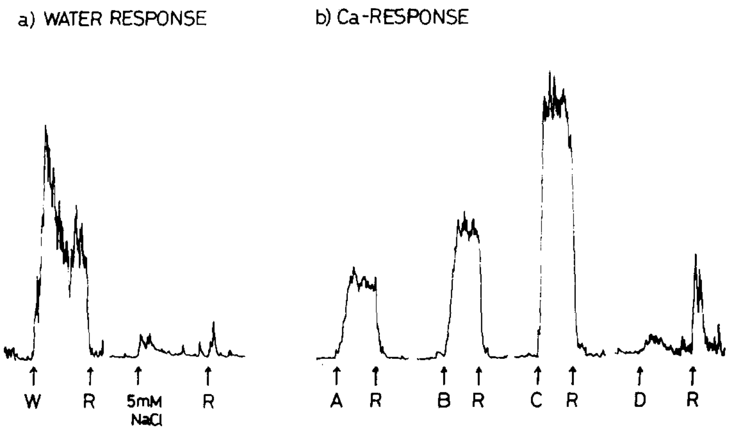

Fig. 9. Traces of integrated water response and Ca-response observed with frog tongue which was adapted to Ringer solution. (a) The water response and its suppression caused by 5 mM NaCl in the stimulating solution. Arrows with *W*, 5 mM NaCl, and *R* stand for the times when stimulating water (10^{-5}M NaCl), 5 mM NaCl solution, and Ringer solution were applied, respectively. (b) Ca-response under the presence of 5 mM NaCl in stimulating solutions. The following stimulating solutions (A, B, C, and D) were applied at the time shown by the arrows with the respective letters, and returned to Ringer solution at *R*. A, 0.1 mM $CaCl_2$+5 mM NaCl; B, 0.3 mM $CaCl_2$+5 mM NaCl; C, 3 mM $CaCl_2$+5 mM NaCl; D, 3 mM Ca-benzoate+5 mM NaCl. The vertical scale in Fig. 9a is three time larger than that in Fig. 9b.

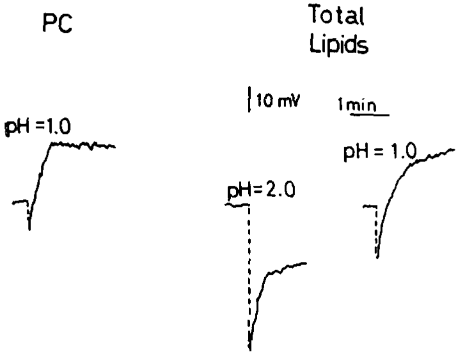

Fig. 10. Time courses of potential response observed with phosphatidylcholine- and T-membranes when stimulating water was applied to the membrane adapted to HCl solution. Q values were 20.3 mg/cm^2 for phosphatidylcholine-membrane, and 14.8 mg/cm^2 for T-membrane. PC, phosphatidylcholine.

from the tongue surface, and is suppressed by the addition of electrolytes in the stimulating water to be applied. Then, flowing water over the tongue is an indispensable requisite for observing an appreciable water response, because the stagnation of water on the tongue surface prevents the removal of salts from the surface.

It is reported that the neural activities are increased when water is applied after stimulation with acids [23, 24]. This phenomenon resembles the water response in a sense that application of water increases neural activities. The underlying mechanism, however, seems to be different from that observed with a tongue adapted to Ringer solution as will be shown below. Fig. 10 shows the change in the membrane potential when distilled water is applied to T- and phosphatidylcholine-membranes adapted to HCl solution. As illustrated in the figure, the membrane potential decreases temporarily, and is followed by an increase in the direction of depolarization. Since $\Delta\tau$ of HCl is negative, the e.m.f. produced by diffusion of HCl from the membrane into the bulk solution must lead to a decrease in the membrane potential. The initial decrease in the potential seems to be attributed to the diffusion potential of HCl. The following gradual depolarization is attributed to the change in the surface charge of the membrane adapted to an acid solution. As shown by the electrophoretic study on the liposomes made of the total lipids [10], the surface charge of the liposome became positive in a HCl solution, and NaCl or KCl did not reverse the sign of the surface charge. This implies that protons bind strongly to the negatively charged group on the liposome surface. When distilled water is applied to the positively charged membrane, the phase-boundary potential at the membrane-solution interface changes in the direction of depolarization [9]. A similar effect contributes partially to the water response when the membrane is adapted to a high concentration of $CaCl_2$, since the surface charge of T-membrane becomes positive in high Ca^{2+} media [10].

It is also reported that water induces a depolarization after adaptation of the tongue to certain organic salts, e.g. sodium butyrate, sodium benzoate, which have a suppressive effect on the taste response [25]. The mechanism of depolarization after organic anions such as butyrate and benzoate seems to be quite different from that of the water response dealed with in the present study, since the depolarization induced by water after adaptation to the organic salts is not suppressed by the presence of electrolytes in the water.

As shown in the present paper, the water response obtained with the model membrane made of total lipids closely resembles that observed with frog. We do not insist, however, that the phospholipids are the sole species of receptor molecules for the water response. The discussion presented here holds irrespective of species of molecules constituting the receptor membrane. The water response can be observed more or less with a wide variety of animals, but there is a great difference in their sensitivity of response. The reason for the difference of sensitivity among various kinds of animals is not known to us, but one of the possible reasons to be considered is the difference in the mechanism of transduction of a change in the electric potential at the surface of the receptor membrane into the nerve endings. In this connection, it is noted that the water response recorded from the rat chorda tympani is very small in spite of an appreciable depolarization in the taste cell of rat [26]. It is also noted that the rat glossopharyngeal nerves have fibers responsive to distilled water, while the chorda tympani has few such fibers [27].

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