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PHYSICOCHEMICAL STUDIES OF TASTE RECEPTION

V. SUPPRESSIVE EFFECT OF SALTS ON SUGAR RESPONSE OF THE FROG

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SUMMARY

The taste responses of frog to various kinds of sugars were measured quantitatively by use of the glossopharyngeal nerve activity under an appropriate condition where the water response was completely suppressed. The concentration dependences of response of frog tongue to D-fructose, D-glucose, and sucrose were almost the same, D-galactose, however, elicited a much larger response in comparison with the other sugars in the whole range of concentrations examined.

The sugar response was suppressed extensively by the presence of small amount of salts in the stimulating sugar solution. The suppressive effects of NaCl, KCl, $MgCl_2$, $MgSO_4$, and $K_4Fe(CN)_6$ were examined with a fixed concentration of sugar. The results obtained with these salts, added in various concentrations, fell on a single curve when the data were plotted against the ionic strength in the stimulating solution. The present results were consistent with the notion that the taste receptor potential for salts or acids is attributable to a change in the phase boundary potential at the membrane-solution interface as proposed in the previous papers of this series.

INTRODUCTION

Stimulation of taste cells results in a change in membrane potential, referred to as the receptor potential [1]. We have proposed that the receptor potentials elicited by salts originate as changes in the phase boundary potentials at the outer surface of the cells' microvilli [2, 3]. The response to water has also been interpreted quantitatively in terms of a change in electric potential at the membrane surface [4]. The present study was begun to explore the possibility that sugars might also stimulate taste cells via a change in the phase boundary potential.

In 1963, Andersen et al. [5] reported that the response of the dog chorda tympani to sucrose was suppressed by 0.2 or 0.5 M NaCl. Ozeki and Sato [6] stated that the receptor potential of the rat taste cell in response to sucrose was diminished by 0.04 M NaCl. Despite these interesting findings, no systematic study on the suppressive effect of salts on the sugar response has been carried out, and the mechanism of the suppression is still unknown.

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The frog, which was first thought to have no sugar sensitivity, was found to respond to sucrose [7]. In the present study, an experimental condition to obtain a reproducible sugar response of the frog is established, and then the responses to various kinds of sugars are examined. It has been found that the sugar response of the frog is suppressed by a very low concentration of added salts, e.g. 1 mM KCl. Since the salts themselves of such a low concentration elicit no response in the frog glossopharyngeal nerve, the suppressive effect of salts on the sugar response can be examined quantitatively by use of the frog. The results obtained are discussed in terms of the phase boundary potential at the receptor membrane-solution interface.

EXPERIMENTAL

Chemicals

The sugars used in the present study were D-fructose, D-glucose, D-galactose and sucrose, and the salts were KCl, NaCl, $MgCl_2$, $MgSO_4$, and $K_4Fe(CN)_6$. These chemicals of analytical grade were used as delivered. The stimulating sugar solutions were prepared by dissolving the sugars in doubly distilled water or in aqueous salt solution. The composition of Ringer solution was: 112 mM NaCl, 3.4 mM KCl, 2.5 mM $CaCl_2$ and adjusted to pH 7.1 with 2.5 mM $NaHCO_3$/HCl buffer.

Recording of activity of the glossopharyngeal nerve

Adult bullfrogs, *Rana catesbeiana*, in autumn and winter seasons were used in the experiments. The preparation of the tongue with the glossopharyngeal nerve and the recording of nerve activity employed were the same as those described in the previous paper [4]. The nerve impulses were amplified with a CR-amplifier (Iwatsu Electric Co. Type HP-601) and integrated with an electronic integrator (Nihon Kohden Co. Type RFJ-5) with a time constant of 0.3 s. Before application of sugars, 5 mM KCl solution was allowed to flow on the tongue surface for 5 min, and then the stimulating sugar solution was applied on the tongue with a flow rate of 2 ml per s. About 15 min were interposed between each successive stimulation, during which time the frog tongue was bathed in the Ringer solution. This procedure has been adopted in the present study for the following reason. The frog glossopharyngeal nerve produces a pronounced response when distilled water is applied to the tongue adapted to the Ringer solution. As pointed out in the previous paper [4], the water response after adaptation to Ringer solution is attributed to the diffusion potential of salts adsorbed on the membrane surface toward the bulk solution. Since KCl produces no diffusion potential, no water response can be elicited with the tongue adapted to KCl solution. Thus the sugar response can be discriminated from the water response by using the procedure described above. The rinsing of the tongue with 5 mM KCl solution is also effective to eliminate the contamination of the stimulating sugar solution with salts, because the ions adsorbed on the tongue surface adapted to the Ringer solution are mostly removed by the rinsing.

All experiments were performed at 18–20 °C.

RESULTS

Fig. 1 represents the integrated responses of the glossopharyngeal nerve when the tongue is subjected to distilled water and to 0.7 M of sucrose D-fructose, D-glucose,

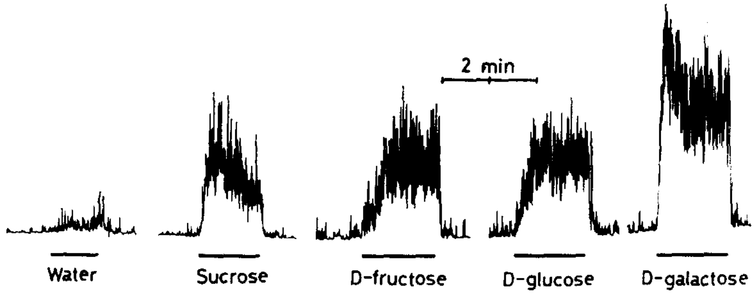

Fig. 1. The integrated responses of the frog glossopharyngeal nerve to 0.7 M of various kinds of sugars. The stimulating solutions were applied after the tongue was rinsed with 5 mM KCl for 5 min. Application of distilled water (the first record in the figure) led to no water response. Each bar at the bottom shows the duration of stimulus application.

and D-galactose. Note that the water response is eliminated almost completely by the procedure adopted in the present study, i.e. 5 min rinsing with 5 mM KCl before stimulation. The responses to the sugars appeared immediately after the application of sugar solutions to the tongue and persisted during the application of a stimulating solution.

In Fig. 2, the magnitude of the responses to sugars is plotted against the molar concentration of sugars applied. Sucrose, D-fructose and D-glucose gave almost the same response curves, while D-galactose brought about a much larger response in comparison with the other sugars.

The responses of the frog to sugars were suppressed by the presence of salts in the stimulating sugar solution. Fig. 3 demonstrates the suppressive effect of KCl on the sugar responses. The first arrow in Fig. 3 indicates where 0.7 M sugar solution was applied to the tongue, and the second arrow where 0.7 M sugar solution con-

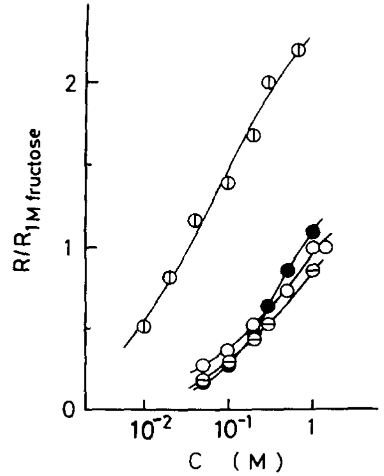

Fig. 2. Magnitude of the integrated responses to various kinds of sugars as a function of molar concentration of sugars. The ordinate represents the relative response when the magnitude of response to 1 M D-fructose is taken as a unit. ⦶, D-galactose; ○, D-fructose; ●, D-glucose; ⊖, sucrose.

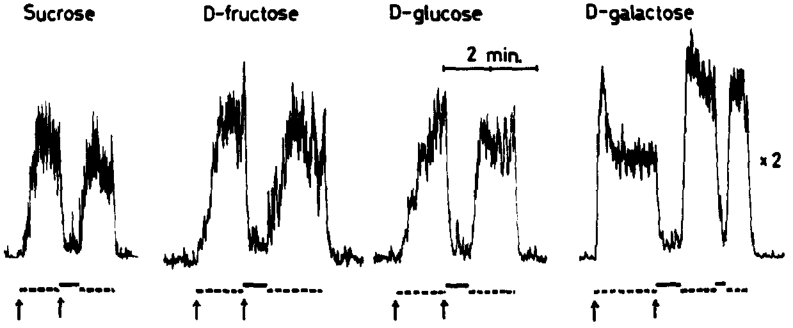

Fig. 3. Suppression of sugar responses by 1 mM KCl added in the stimulating sugar solution. Each dotted bar or solid bar at the bottom shows the duration of application of 0.7 M sugar solutions or of 0.7 M sugar solutions containing 1 mM KCl, respectively. The ordinate scale of the response of D-galactose is reduced to one-half.

taining 1 mM KCl was applied. The figure indicates that the responses to all sugars examined were almost completely suppressed by the presence of 1 mM KCl. The sugar responses recovered to the original level when the added KCl was removed from the stimulating sugar solution. In the case of D-galactose, the second application of sugar solution containing no KCl always gave a larger response than that of the first application after adaptation to 5 mM KCl solution. The subsequent applications of salt free D-galactose solution led to no appreciable change in the magnitude of response compared with that of the second application (see Fig. 3). Hence, to quantitate the suppressive effect of salt, D-galactose solution containing various concentrations of salts was applied after the second application of salt free sugar solution, and the response was expressed as the relative value against the magnitude of the second response. In Fig. 4, the response to 0.5 M D-galactose in the presence of various kinds

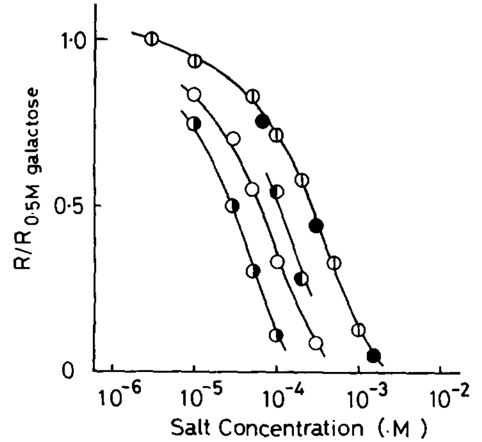

Fig. 4. Relative magnitude of the integrated response to 0.5 M D-galactose solution containing various kinds of salts as a function of salt concentration. The ordinate represents the integrated responses relative to that of the salt free sugar solution. ⦶, NaCl; ●, KCl; ◐, $MgCl_2$; ○, $MgSO_4$; ◑, $K_4Fe(CN)_6$.

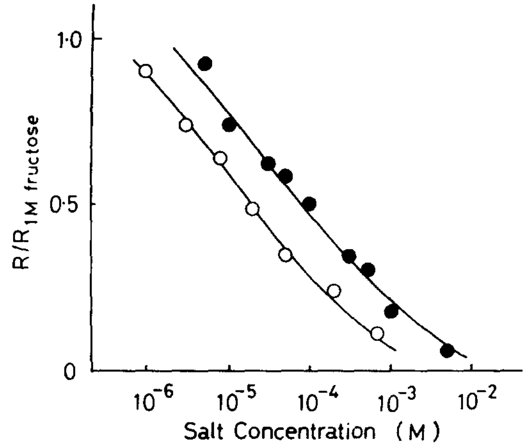

Fig. 5. Magnitude of the integrated response to 1 M D-fructose solution containing KCl (●) or $MgSO_4$ (○) as a function of salt concentration. The ordinate represents the responses relative to the magnitude of response to the salt free sugar solution.

of salts are plotted against the salt concentration. The salts used were NaCl, KCl, $MgCl_2$, $MgSO_4$, and $K_4Fe(CN)_6$. These salts were selected for the reason that the response of these salts was negligibly small in the concentration range examined here and application of these salts led to no irreversible effect on the sugar response. As seen in the Fig. 4, NaCl and KCl brought about a similar suppressive effect on the sugar response. The suppressive effect of these salts appeared when the concentration exceeded 10^{-5} M in the stimulating D-galactose solution and the response vanished when the concentration of salt rose to about 3 mM. The effectiveness of the suppressive effect of salts depended strongly on the valences either of cations or of anions involved. The following order for the suppressive effect has been obtained for salts examined: KCl, NaCl < $MgCl_2$ < $MgSO_4$ < $K_4Fe(CN)_6$. A similar sequence is observed for the suppressive effect of salts on 1 M D-fructose, partly shown in Fig. 5. The measurements of electric conductance of the salt solutions indicated that the salts used were completely dissociated into free ions in the concentration range examined above [8].

DISCUSSION

As noted above, the frog was first thought to have no sensitivity to sugars. This probably stemmed from the failure of reproducible observations of the sugar response. As seen in the above, the response of the frog to sugars was suppressed easily by the presence of a small amount of salts in the stimulating sugar solution. Therefore, it was hard to observe a reproducible sugar response by application of sugars after adaptation of the frog tongue to the Ringer solution without rinsing with 5 mM KCl solution. An adequate flow of stimulating solution is also necessary to obtain quantitative data of sugar response.

The response of the frog to sugars can not be explained in terms of the osmotic pressure of sugar solution applied, because various sugar solutions of the same molar concentration induce the responses of different magnitudes as seen in Fig. 1. Also a small amount of added salts, which does not change the osmotic pressure of sugar solution, leads to an extensive diminution of the sugar response. One may consider that a reception of sugar induces a change in the ionic permeability of microvilli membrane and then the receptor potential is produced by a change in diffusion potential of ions across the membrane. This hypothesis, however, is not able to account for the fact that the sugar response of the frog is eliminated by the presence of either NaCl or KCl in the external solution. On the other hand, the following possibility may not be denied; the binding of ions on the receptor membrane induces a conformational change in the membrane, which in turn, interferes with the binding of sugar molecule to the receptor site. However, an ion species is bound, in general, to the membrane surface whose charge has the opposite sign to that of the ion. Since the suppressive effect of salts on the sugar response depends on the valences both of cation and of anion, it is unlikely that a specific binding of ions to the membrane surface contributes to the suppressive effect of the salts on the sugar response.

The molecular mechanism of sugar reception in higher vertebrates is not known at present. The results of sugar reception obtained with a primitive organism may offer a hint for basic understanding on the mechanism of sugar reception in higher vertebrates. The plasmodia of the true slime mold exhibit chemotaxis against various kinds of sugars as well as salts, acids or nucleotides. As shown in a previous paper concerned with the true slime mold, the chemotaxis of the slime mold was closely correlated to the change in the membrane potential [9]. Furthermore, the electrophoretic experiments on the spherical microplasmodia of the slime mold indicated that the magnitude of the change in membrane potential in response to sugars was identical with that of the zeta-potential of the slime mold (ref. 10). This implies that a change in the membrane potential of the slime mold in response to sugar is attributed to a change in the phase boundary potential at the membrane-solution interface. Calculation of the surface change density from the data of zeta-potential indicated that a depolarization induced by sugar was accompanied by a decrease in negative charge or an increase of positive charge exposed on the membrane surface of the mold. Since the sugar is a non-electrolyte, the change in the surface charge density may probably be attributed to a conformational change of the receptor membrane. It is not unreasonable to assume that a similar mechanism is involved in the sugar reception of the frog taste cell. A conformational change of the taste membrane induced by binding of sugar molecules causes the surface charge in the region of the receptor to become more positive. The change in the phase boundary potential is a function both of the charge density at the membrane surface and of the ionic strength in the solution. An increase of ionic strength leads to a diminution of the phase boundary potential. When a stimulating solution contains a fixed concentration of sugar and various concentrations of a salt, the phase boundary potential (or the taste receptor potential) is reduced with an increase of the ionic strength, provided that the surface charge density in a given concentration of sugar stays constant. Fig. 6 illustrates the response of the frog to D-galactose and D-glucose as a function of ionic strength in the stimulating sugar solution. The data are taken from Figs. 4 and 5. As seen in Fig. 6, the data fall on a single curve for respective sugars irrespective of the salt species involved.

We showed that the suppressive effect of salts on sugar response is reasonably interpreted in terms of a change in the phase boundary potential at the taste cell membrane. The phase boundary potential at the membrane-solution interface plays

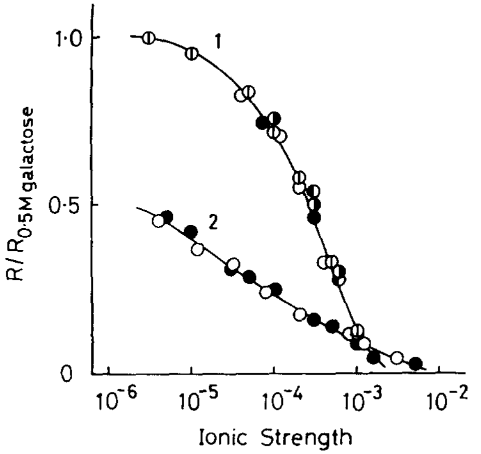

Fig. 6. Magnitude of the integrated response to 0.5 M D-galactose (curve 1) and to 1 M D-fructose (curve 2) containing various kinds of salts as a function of the ionic strength in the stimulating sugar solutions. Data were taken from Figs. 4 and 5. The notations are the same as in Figs. 4 and 5.

an unmistakable role in various chemoreceptions of the taste cells as has been demonstrated in this series of papers. Further study will be needed, however, to establish that the receptor potential of the taste cell in response to non-electrolyte stimuli is solely attributed to a change in the surface charge density caused by a conformational change in membrane structure.

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