

Large Enhancement of Canine Taste Responses to Sugars by Salts

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ABSTRACT The effects of changed ionic environments on the canine taste responses to sugars were examined by recording the activity of the chorda tympani nerve. *a)* The responses to various sugars were greatly enhanced by the presence of salts having monovalent cations such as Na^+ , K^+ , choline⁺, or Tris⁺. The responses to sugars were suppressed by high concentrations of salts. *b)* The presence of 100 mM NaCl in fructose solution did not affect the maximal response and changed the Hill constant for the concentration-response relationship from 1.3 to 2.4. *c)* CaCl_2 greatly enhanced the response to fructose, while MgCl_2 exhibited practically no effect. The presence of 20 mM CaCl_2 in fructose solution changed the Hill constant from 1.2 to 2.4. *d)* CaCl_2 suppressed the responses to 0.5 M sugars except for fructose and sucrose and enhanced the responses to all sugars examined at 1 M. In the glucose response, the slope of the concentration-response curve was increased by the presence of CaCl_2 . Here the curve in the absence of CaCl_2 intersected with that in the presence of CaCl_2 , indicating that CaCl_2 suppressed the response to glucose of low concentrations and enhanced that of high concentrations. *e)* The enhancement of the sugar responses by salts was not simply explained in terms of ionic permeability at the apical membranes of taste cells. The enhanced and suppressed effects of salts on the sugar responses were interpreted in terms of the cooperativity between receptor molecules for sugars.

INTRODUCTION

It is generally accepted that the initial event of sugar reception in taste cells is the adsorption of sugars on the receptor membranes. To test the possibility that the adsorption of sugars leads to ion permeability changes of the apical membranes of taste cells, it is necessary to examine the effects of salts on the sugar responses. In the rat, which is the most typical experimental animal, salts have essentially no effect on the sugar taste response and hence no electrophysiological study on effects of salts on the sugar response has been carried out with vertebrates.

The enhancing effect of salts on sugar responses was first observed with the fleshfly labellar sugar receptor (Morita, 1969). The fly sugar responses were enhanced by salts such as NaCl, KCl, or NH_4Cl , while the responses were suppressed by LiCl, choline chloride, and CaCl_2 . Recently Kijima et al. (1988) analyzed

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receptor current fluctuation in the fly labellar sugar receptor and suggested that transduction ion channels are present at the tip region of the receptor cells and are operated directly by sugars. The idea that ion channels activated by sugars are present at the tip of the taste cells was also suggested by Mierson et al. (1988) and Simon et al. (1989). They observed that a short-circuit current (I_{sc}) across the canine lingual epithelium evoked by sugars is partially blocked by amiloride and among currents activated by sugars, the current response is greater with Na^+ than with K^+ .

In the above mechanisms, it was postulated that sugar responses are induced by ionic transport through taste cell membranes. In contrast with these mechanisms, another mechanism in which cyclic AMP produced by adsorption of sugars on taste receptor membranes suppresses K^+ channels and depolarizes taste cells of the frog (Avenet et al., 1988) and the mouse (Tonosaki and Funakoshi, 1988) was proposed. Thus it is still unknown which mechanism mainly contributes to the sugar transduction mechanism.

To clarify whether or not ion permeability changes induced by adsorption of sugars on the receptor membranes contribute to the sugar responses, we investigated systematically the effects of changed ionic environments on the canine chorda tympani nerve responses to sugars. It was reported that dogs respond well to various sugars (Funakoshi and Zotterman, 1963; Ferrell, 1984; Beidler and Tonosaki, 1985). Funakoshi and Zotterman (1963) reported that canine chorda tympani nerve responses to sugars are suppressed by salts. In this study, we found that the responses of canine chorda tympani nerves to various sugars are greatly enhanced by relatively low concentrations of salts and suppressed by high concentrations of salts. The sugar response was enhanced not only by NaCl but also by salts having organic cations of relatively large molecular size. CaCl_2 suppressed the responses to sugars of low concentrations and enhanced those of high concentrations. These results suggested that the effects of changed ionic environments are not simply explained in terms of ionic permeability at the apical membranes.

MATERIALS AND METHODS

Recording of the Canine Gustatory Response

Adult mongrel dogs (7–15 kg) were used in the experiments. The dissection of the chorda tympani nerve was carried out essentially as described by Funakoshi and Zotterman (1963). The dog was anesthetized with an intraperitoneal injection of sodium pentobarbital (25 mg/kg) after ketamine hydrochloride (5 mg/kg) was injected into the muscle of the hind leg, and maintained at a surgical level of anesthesia with supplemental injections of sodium pentobarbital. A tracheal cannula was inserted and the dog was placed on a respirator. The chorda tympani nerve was exposed by removing the chondyloid and cornoid processes of the mandible and retracting the underlying musculature.

The neural activity of the whole chorda tympani nerve was recorded with an Ag-AgCl hook electrode. The impulses were amplified with an AC-amplifier and integrated with an electronic integrator with a time constant of 0.3 s.

Chemical Stimulation

Stimulating solutions were applied to the tongue at a flow rate of 15 ml/s. When the effects of salts on the sugar responses were examined, a salt solution was first applied to the tongue

and after the response to the salt reached a steady level, a sugar solution containing the salt of the same concentration was applied to the tongue. About 5 min were interposed between each stimulation. Deionized water was flowed over the tongue between stimulation.

To examine the effects of salts on the responses to sugars of a fixed concentration, 0.5 M sugars were used unless otherwise noted. To examine the effects of fixed concentrations of salts on the sugar responses, 100 mM salts for those carrying monovalent cations and 20 mM salts for those carrying divalent cations were used. The experiments were carried out at room temperature (20–22°C).

Chemicals

The sources of chemicals are as follows: fructose, sucrose, maltose, and glycine: Wako Pure Chemicals (Tokyo, Japan); galactose, sorbose, xylose, and glucose: Nakarai Chemicals Ltd., (Kyoto, Japan); tris(hydroxymethyl)aminomethane (Tris): Sigma Chemical Co., (St. Louis, MO); choline chloride: Kanto Kagaku Co. (Tokyo, Japan). The pH of stimulating solutions was 5.6–6.0.

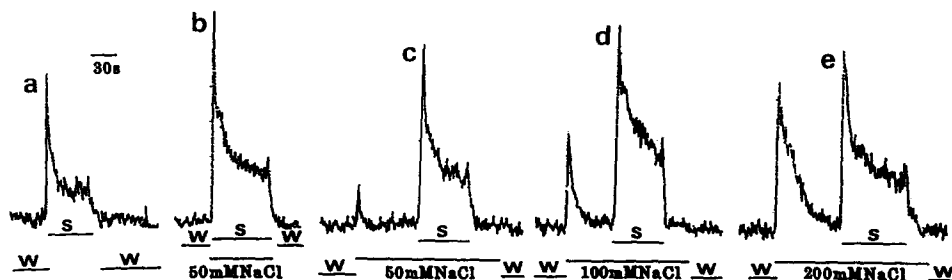


FIGURE 1. The summated responses of the canine chorda tympani nerve to 0.5 M sucrose in the absence of NaCl (*a*) and 0.5 M sucrose solution containing 50 mM NaCl (*b*) after the tongue was adapted to deionized water. Records on the left side of *c*, *d*, and *e* represent the responses to 50, 100, and 200 mM NaCl, respectively, and records on the right side represent the responses to 0.5 M sucrose solutions containing 50, 100, and 200 mM NaCl. Bars at the bottom of each record represent duration of stimulations by deionized water (*w*), 0.5 M sucrose (*s*), and NaCl of concentrations indicated.

RESULTS

Sugar Concentration-Response Relations

Fig. 1 *a* represents the summated response of canine chorda tympani nerves to 0.5 M sucrose. The response is composed of the initial large phasic component and the following tonic component. In the present paper, the magnitude of the tonic component 30 s after onset of stimulation is mostly taken as the magnitude of the response.

Fig. 2 shows the relative magnitude of the tonic component of the responses to various sugars as a function of logarithmic sugar concentration. Here the magnitude of the response to 0.5 M sucrose is taken as unity. The relative magnitudes of the responses \pm SE ($n = 3$) to 1 M sugars when the magnitude of the response to 1 M sucrose is taken as unity are 1.04 ± 0.38 (fructose) = 1 (sucrose) $> 0.59 \pm 0.14$

(maltose) $> 0.45 \pm 0.12$ (galactose) $> 0.25 \pm 0.11$ (sorbose) $= 0.23 \pm 0.08$ (xylose) $> 0.11 \pm 0.09$ (glucose).

Effects of Monovalent Cations on Sugar Responses

In Fig. 1, effects of NaCl on the response to 0.5 M sucrose are represented. Fig. 1 *b* shows the response to 0.5 M sucrose solution containing 50 mM NaCl after the tongue is adapted to deionized water. This record indicates that the tonic response to sucrose is greatly enhanced by the presence of 50 mM NaCl since the tonic response to 50 mM NaCl alone is negligibly small (see Fig. 1 *c*). The tonic responses to higher concentrations of NaCl cannot be, however, neglected (see Fig. 1, *d* and *e*). Hence, to eliminate experimentally the response to NaCl itself from the response to a solution containing sucrose and NaCl, sucrose solutions containing NaCl were applied after the response to NaCl applied previously reached a steady level. As shown by Fig. 1, *c* and *d*, the responses to sucrose thus obtained are greatly enhanced by the presence of 50 and 100 mM NaCl. The enhancement is especially remarkable in the tonic component. The fact that the magnitude of the response to

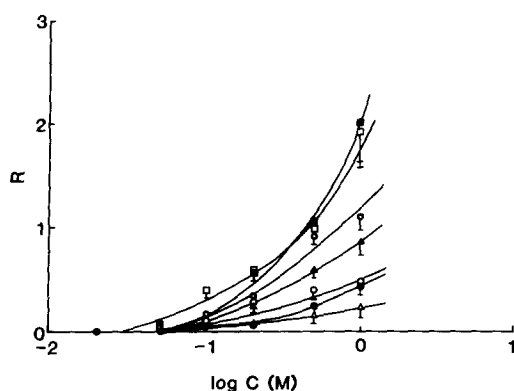


FIGURE 2. The relative magnitude of the tonic component of the responses to various sugars (■, fructose; □, sucrose; ⊙, maltose; ▲, galactose; ○, sorbose; ●, xylose; Δ, glucose) as a function of logarithmic sugar concentration ($\log C$). Plotted response (R) was calculated relative to the magnitude of the response to 0.5 M sucrose. The data are means \pm SE of three preparations.

0.5 M sucrose in the presence of 50 mM NaCl after preadaptation with water (Fig. 1 *b*) is similar to that after preadaptation with 50 mM NaCl solution (Fig. 1 *c*) indicates that preadaptation with salt solution is not needed for enhancement of the sugar response by the salt. The response is decreased by the presence of high concentration (200 mM) of NaCl (Fig. 1 *e*) compared with the enhanced response by 100 mM NaCl.

In Fig. 3, the magnitudes of the phasic and tonic components of the response to 0.5 M sucrose plus salt of varying concentrations after the salt response is adapted are plotted as a function of logarithmic concentration of NaCl. Here the magnitude of the response to 0.5 M sucrose alone is taken as unity. The phasic component is practically unchanged with an increase of NaCl concentration up to 100 mM and decreased with a further increase in NaCl concentration. The magnitude of the tonic component is greatly increased with an increase of NaCl concentration up to 100 mM and the enhanced response is decreased with a further increase of NaCl concentration. The suppression by high concentration of salts was also observed with the responses to other sugars. Fig. 4 shows the suppressive effect of sodium

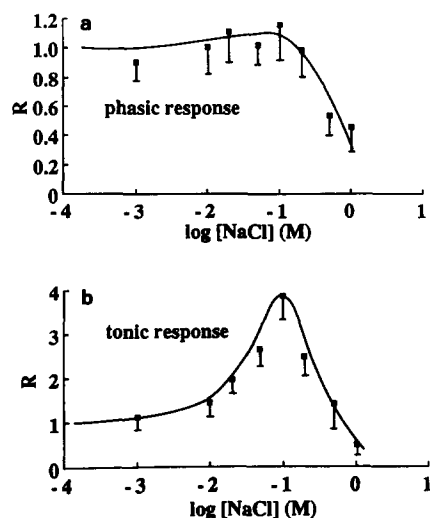


FIGURE 3. The relative magnitude of the phasic (*a*) and tonic (*b*) components of the response to 0.5 M sucrose as a function of logarithmic NaCl concentration. Plotted response (*R*) was calculated relative to the magnitude of each component of the response to 0.5 M sucrose alone. The data are means \pm SE of three preparations.

salts carrying different species of anions on the response to fructose. Here 0.2 M fructose was used since its response in the presence of relatively high concentrations of salts is large enough. As seen from the figure, the suppressive effect is a function of ionic strength. The reason why the suppressive effect is a function of ionic strength is discussed later.

Fig. 5 shows the effects of various salts having monovalent cations on the magnitude of the tonic response to 0.5 M sucrose. Here concentrations of cations are fixed at 100 mM. The enhancing effect of sodium salts carrying different species of anions (NaCl , Na_2SO_4 , $\text{Na}_4\text{Fe}(\text{CN})_6$) is similar to each other, indicating that a difference in species of anion does not affect the enhancing effect. Not only Na^+ but also K^+ , choline $^+$, and Tris $^+$ exhibit the enhancing effect, although the enhancing effect of K^+ is less than that of Na^+ ($P < 0.05$). Fig. 6 shows the effects of 100 mM NaCl on the responses to various species of sugars. The responses to all sugars examined are enhanced by NaCl. The enhancement of the response to glucose is less than that to sucrose or galactose ($P < 0.05$).

Fig. 7 shows the magnitude of the response to fructose of varying concentrations as a function of fructose concentration in the absence and the presence of 100 mM

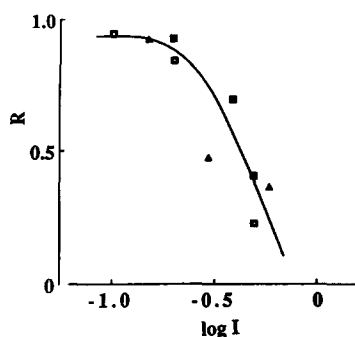


FIGURE 4. The relative magnitude of the tonic component of the response to 0.2 M fructose in the presence of NaCl (\blacksquare), Na_2SO_4 (\blacktriangle), and $\text{Na}_4\text{Fe}(\text{CN})_6$ (\square) as a function of ionic strength (*I*). Plotted response (*R*) was calculated relative to the magnitude of the response to 0.2 M fructose alone. The data are the means of at least three preparations.

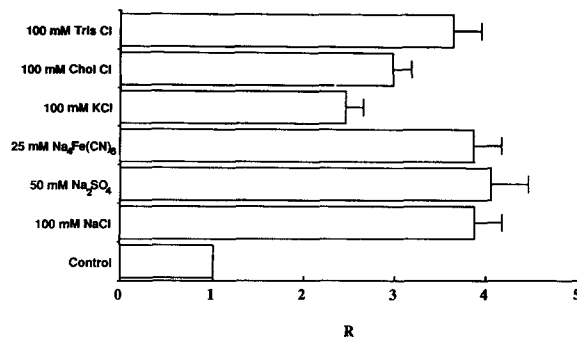


FIGURE 5. Effects of various salts carrying monovalent cations on the tonic response to 0.5 M sucrose. The magnitude of the response to 0.5 M sucrose alone (control) is taken as unity. Concentrations of cations used were 100 mM. The data are means \pm SE of at least four preparations.

NaCl. The magnitude of the response at the saturation level is practically unchanged by the presence of NaCl, but the slope of the curve in the presence of NaCl is larger than that in the absence of NaCl. The Hill plot of the data shown in Fig. 7 showed that the Hill constants for the curves in the absence and the presence of NaCl are 1.3 and 2.4, respectively, indicating that the Hill constant is increased by the presence of NaCl.

Effects of Divalent Cations on Sugar Responses

Fig. 8 shows the summated responses to 0.5 M fructose in the absence and the presence of various concentrations of CaCl_2 and MgCl_2 . As seen from the figure, the magnitude of the response to fructose is greatly enhanced by the presence of CaCl_2 , while the fructose response is practically unchanged by the presence of MgCl_2 . The response to high concentration of CaCl_2 does not decline to a spontaneous level during adaptation (see Fig. 8) and hence the response to a sugar in the presence of CaCl_2 was measured from the tonic level to the immediately previous CaCl_2 presentation. In Fig. 9, the magnitude of the tonic response to 0.5 M fructose is plotted as a function of concentrations of CaCl_2 and MgCl_2 . The response is greatly enhanced with an increase in CaCl_2 concentration, while it is practically unchanged by an increase in MgCl_2 concentration. This suggests that the enhancing effect of CaCl_2 on the sugar response is not due to an increase in ionic strength.

Fig. 10 A shows the effects of 20 mM CaCl_2 on the responses to various sugars where the magnitude of the tonic response to each stimulus in the absence of the salt is taken as unity. Here 0.5 M sugars are used. Only the response to fructose is greatly enhanced by CaCl_2 , and the responses to other sugars except for sucrose are suppressed by CaCl_2 ($P < 0.001$). Fig. 10 B shows the effects of 20 mM CaCl_2 on the

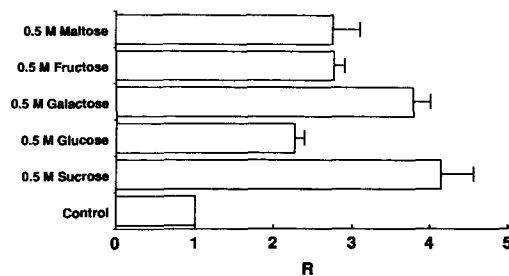


FIGURE 6. Effects of 100 mM NaCl on the responses to various species of sugars. The response shown (R) was calculated relative to the magnitude of each response to sugars. The data are means \pm SE of four preparations.

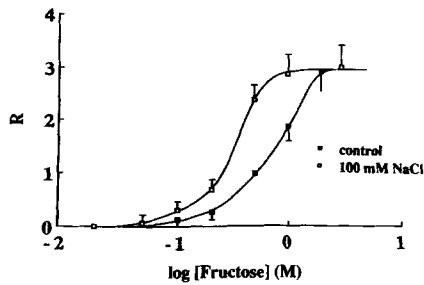


FIGURE 7. The relative magnitude of the response to fructose of varied concentrations as a function of fructose concentration in the absence and presence of 100 mM NaCl. The plotted response (R) was calculated relative to the magnitude of the response to 0.5 M fructose alone. The data are means \pm SE of three preparations.

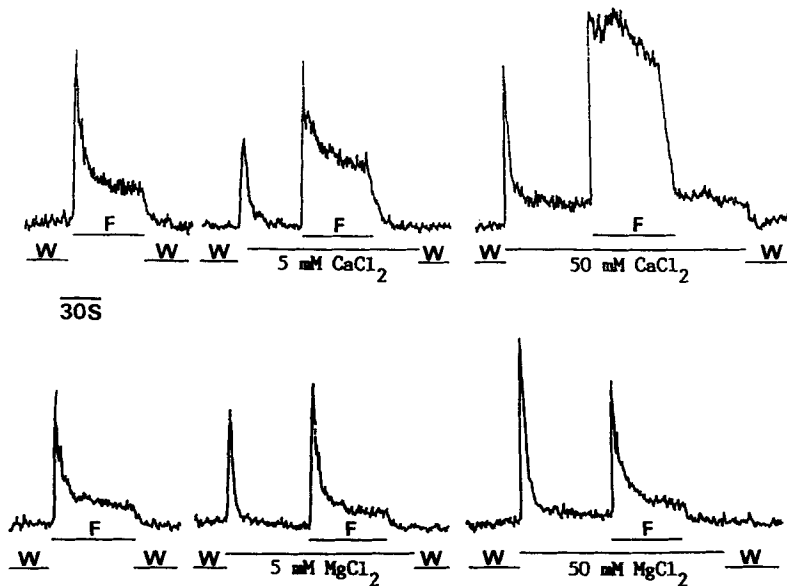


FIGURE 8. The summated response to 0.5 M fructose in the absence and presence of 5 and 50 mM CaCl_2 and MgCl_2 . In the control, deionized water (w) was first applied and then 0.5 M fructose was applied. To see the effects of CaCl_2 or MgCl_2 , a solution of CaCl_2 or MgCl_2 was applied to the tongue and after the response reached a steady level, 0.5 M fructose solution containing CaCl_2 or MgCl_2 was applied. Bars at the bottom of each record represent the durations of stimulations by deionized water (W), fructose (F), and CaCl_2 , respectively.

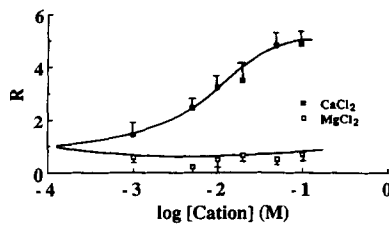


FIGURE 9. The relative magnitude of the tonic response to 0.5 M fructose in the absence and presence of CaCl_2 and MgCl_2 . The plotted response (R) was calculated relative to the magnitude of the response to 0.5 M fructose alone. The data are means \pm SE of three preparations.

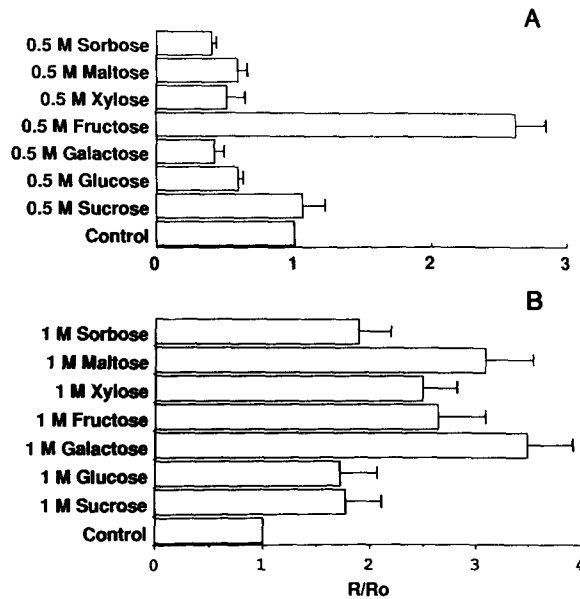


FIGURE 10. Effects of 20 mM CaCl_2 on the responses to 0.5 M sugars (A) and on those to 1 M sugars (B). The response shown (R) was calculated relative to the magnitude of the response to each stimulus alone. The data are means \pm SE of at least four preparations.

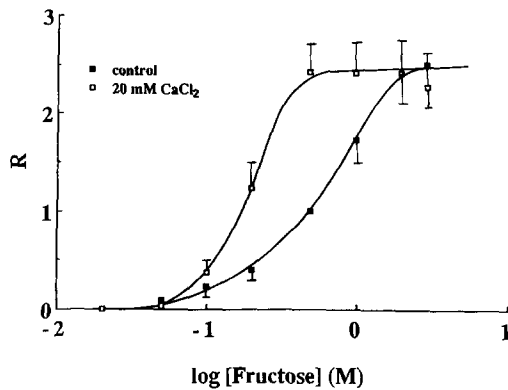


FIGURE 11. The relative magnitude of the response to fructose of varied concentrations as a function of fructose concentration in the absence and presence of 20 mM CaCl_2 . The plotted response (R) was calculated relative to the magnitude of the response to 0.5 M fructose alone. The data are means \pm SE of three preparations.

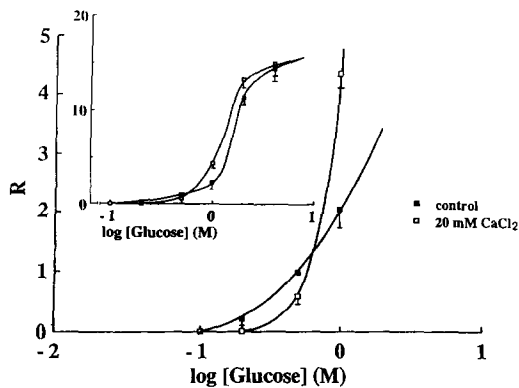


FIGURE 12. The relative magnitude of the response to glucose of varied concentrations as a function of glucose concentration in the absence and presence of 20 mM CaCl_2 . The plotted response (R) was calculated relative to the magnitude of the response to 0.5 M glucose alone. The main figure shows the data in an expanded scale and the figure in the inset shows data in the whole concentration range examined. The data are means \pm SE of three preparations.

responses to 1 M sugars. As seen from the figure, the responses to all sugars examined are more or less enhanced by CaCl_2 ($P < 0.01$). These results indicate that whether the sugar responses are suppressed or enhanced by CaCl_2 depends on the concentrations of the sugars used.

Fig. 11 shows the magnitude of the response to fructose of varying concentrations in the absence and the presence of 20 mM CaCl_2 . As is similar to the effect of NaCl shown by Fig. 7, the presence of CaCl_2 does not affect the saturation level of the response and makes the concentration-response curve steep. The Hill plot of the data shown in Fig. 11 indicated that the Hill constants in the absence and presence of CaCl_2 are 1.2 and 2.4, respectively.

Fig. 12 shows the magnitude of the response to glucose of varying concentrations in the absence and the presence of 20 mM CaCl_2 . The main figure shows the data in an expanded scale, while the data in the whole concentration region examined are shown in the inset. Similarly to the case of fructose, the presence of CaCl_2 does not affect the saturation level and makes the concentration-response curve steep. The curve in the presence of CaCl_2 intersects with the curve in the absence of CaCl_2 , which implies that CaCl_2 suppresses the response to glucose of low concentrations and enhances that of high concentrations.

DISCUSSION

In the present study, we recorded the canine chorda tympani responses to various sugars. The order of the relative magnitudes of the responses to various sugars at 1 M (fructose = sucrose > maltose > galactose > sorbose = xylose > glucose) was consistent with those reported by Andersen et al. (1963). It is noted that there is a very good conformity between the canine sugar responses and the human sugar responses (Andersen et al., 1963; Zotterman, 1971).

The enhancing effects of salts on the sweet responses are very important in food science, since we often taste foods containing both salts and sugars. We investigated whether or not the enhancing effect of NaCl is seen in animals such as rat and frog, but these animals did not exhibit the enhancing effect (Yoshii, K., and K. Kurihara, unpublished). The present results demonstrated that the canine sugar responses are greatly enhanced by relatively low concentrations of salts.

The enhancing effects of salts on the sugar responses were first reported with the fly (Morita, 1969). The enhancing effects of salts on the sugar responses in the fly were explained in terms of cation permeability; a transport of monovalent cations across the apical membranes of taste cells activated by sugars leads to depolarization of taste cells. In the fly, LiCl, choline chloride, and CaCl_2 suppressed the sucrose response. The canine taste responses to sugars were enhanced not only by Na- and K-salts, but also by salts carrying cations of large molecular size such as choline⁺ or Tris⁺. Note that the magnitude of the enhancing effect of TrisCl on the sugar response is similar to that of NaCl, although Tris⁺ seems to be much less permeable to taste cell membrane than Na⁺. In addition, Ca²⁺ greatly enhanced the responses to high concentrations (e.g., 1 M) of sugars, while it suppressed the responses to low concentrations (e.g., 0.5 M) of sugars except for sucrose and fructose. These results suggest that the enhancing effect of the salts on the sugar responses in the dog cannot be simply explained in terms of ionic permeability.

The presence of NaCl or CaCl₂ in fructose solution changed the Hill constant from 1.2–1.3 to 2.4. The fact that the Hill constant for the fructose response in the absence of salts is close to 1 suggests that one fructose molecule is bound to one taste receptor protein. The fact that the Hill constant increased to ~2 in the presence of the salts implies that the cooperativity between different receptor molecules seems to occur, probably through conformational changes of the receptor membranes. An increase in the slope of the concentration-response curve by the presence of CaCl₂ was also observed in the response to glucose. The curve in the presence of CaCl₂ intersects with that in the absence of CaCl₂. Hence the results that the response to glucose was suppressed at low concentrations and enhanced at high concentrations are also explained in terms of an increase in the cooperativity between the receptors. Similarly to glucose, the responses to other sugars of low concentrations except for sucrose were suppressed and those of high concentrations were enhanced by CaCl₂. The response to 0.5 sucrose was practically unaffected by CaCl₂ probably because the suppressed and enhanced effects happened to be balanced at 0.5 M. Thus all data on the effects of monovalent cations and Ca²⁺ on the sugar responses are explained in terms of cooperativity between receptor molecules for sugars, although the effectiveness of salts on the sugar responses varies with species of salts and sugars. Adsorption of cations on the receptor membrane seems to induce conformational changes of the receptor membranes. This will bring about clustering of the receptor molecules, which results in an increase in interaction between the receptor molecules. As mentioned above, there are several levels of integration between the responses of the nerves and the interaction of receptors and hence there is also a possibility that the enhancement of the sugar responses by salts occurs at other levels than the receptor membrane level.

The present results indicated that Mg²⁺ had no enhancing effect on the response to fructose, although Ca²⁺ showed a large enhancing effect. In this connection, it is interesting to note that the presence of CaCl₂ greatly increased the tonic component of the frog taste nerve responses to bitter substances, while MgCl₂ had no such effect (Kato and Kurihara, 1989). One may consider that Ca²⁺ penetrates taste cells and enhances a transmitter release from taste cells. This possibility is, however, excluded because Ca²⁺ suppressed the responses to low concentrations of sugars, which is shown in Fig. 10 A.

The relative magnitudes of I_{sc} in the presence of 1 M fructose, sucrose, and glucose in the *in vitro* preparation of the canine lingual epithelium (Mierson et al., 1988) were 1:0.28:1.2, while those of the chorda tympani nerve responses to these sugars of 1 M observed in the present study were 1:0.95:0.11. Thus I_{sc} and the chorda tympani nerve responses are not closely related to each other. Mierson et al. (1988) also demonstrated that amiloride selectively suppressed I_{sc} which was carried out by Na⁺ in the presence of sugars. Amiloride, however, did not practically suppress the canine chorda tympani response to sucrose (Nakamura and Kurihara, 1990). Thus I_{sc} and the whole chorda tympani nerve response are two different measurements and they may not reflect the same thing.

The canine sugar responses were suppressed by high concentrations of salts. Similar suppression of the sugar responses by salts was also reported with the fly (Morita, 1969), the dog (Andersen et al., 1963; Funakoshi and Zotterman, 1983)

and the frog (Miyake et al., 1976). The suppression curve of the canine fructose response by sodium salts was a function of ionic strength (Fig. 4). The frog taste nerve responses to sugars were also suppressed by salts as a function of ionic strength (Miyake et al., 1976), although the frog sugar responses were suppressed at much lower ionic strength. There are two possible mechanisms for the suppression of the sugar response by salts. One possibility is that binding of the sugar to the receptor molecule may be inhibited by the presence of salts. Another possibility is that the suppression of the surface potential, which was proposed to be the origin of the receptor potential (Kurihara et al., 1986), by salts leads to the suppression of sugar responses. This possibility was supported by the fact that the suppressive effect of the sugar responses by salts was a function of ionic strength, because the surface potential is suppressed by a function of ionic strength. Further study is needed to clarify an exact mechanism of the suppression.

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