The Effects of pH on the Labellar Sugar Receptor of the Fleshfly

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ABSTRACT Reproducible results describing the effects of pH on the response of the labellar sugar receptor of the fleshfly, *Boettcherisca peregrina*, were obtained. The response to sucrose was independent over a wide range of pH (3.0 to 10.0 for sucrose stimulation), but was inhibited fairly sharply on both sides of this range. Similar results were obtained for monosaccharide stimulation. The receptor was excited on stimulation by water above pH 12.0. The effects of high pH, both inhibitory and excitatory, were affected by the presence of salts. In the presence of 0.5 molar NaCl, for example, the pH-inhibition curve was shifted toward lower pH's by about one pH unit. The effects of low pH, on the other hand, were not affected by salts. Following Dixon's theory, it was concluded that at least five ionizable groups (loosing positive charges above pH 10.5) were located at the receptor site.

INTRODUCTION

Several papers dealing with the effects of pH on chemoreceptors have been published. Beidler (1954) studied pH effects on the rat salt receptor and showed that the magnitude of the response to stimulation by 0.5 molar NaCl was constant over a pH range of 3.0 to 11.0. He concluded that the reacting anionic groups of the salt receptor must be strong acidic radicals, probably phosphate or sulfate.

Evans and Mellon (1962) examined pH effects on the salt receptor of the blowfly, *Phormia regina*. They found that the response to NaCl stimulation was unaffected for several pH units below 8.0 when the tests were run in an order of descending pH, but the response was inhibited at a low pH value of about 2.0. They concluded from the steepness of the pH-inhibition curve that the pH effects did not simply reflect the ionization of an acidic group at the receptor site but were the result of indirect processes.

Recently, Gillary (1966) made detailed quantitative experiments on pH effects on the salt receptor in the blowfly, *Phormia regina*, and suggested a similar mode of action of pH on all receptors; i.e., sugar, salt, and water receptors. In the present work, pH effects on the labellar sugar receptor of

the fleshfly were studied in detail, and the properties of ionizable groups related to sugar stimulation were investigated.

MATERIALS AND METHODS

The fleshfly, *Boettcherisca peregrina*, raised in our laboratory, and whose average age was 5 days, was mainly used in the experiments. The methods of preparation, recording, and stimulation were, in principle, the same as those described in previous papers (Morita, 1959; Morita and Shiraishi, 1968).

Only one chemosensory hair located at the outer rim of the labellum was used in each preparation because it afforded greater stability and life time (it took 1 to 2 hr to get the one series of experiments with the single receptor).

Waterhouse's saline (Buck, 1953) was used as the electrolyte solution in the recording electrode. The stimulating glass capillary was 60–100 μ in diameter at the tip. In one series of experiments, the variation in the tip diameter of the stimulating capillary was less than 10 μ, and the changes in effect, if any, on the response by evaporation from the tip were minimized. Molality was adopted for the concentration of test solutions, and molarity was used for convenience only in the experiments concerned with monosaccharide concentration effects on the response of the sugar receptor.

The duration of stimulation was less than 0.5 sec, and the response magnitude was defined as the number of impulses during a period of 0.2–0.3 sec starting at 0.15 sec after the beginning of the stimulus (stationary period of responses). In the sugar receptor of *Lucilia*, the amplitude of the receptor potential has been reported to be proportional to the impulse frequency (Morita and Yamashita, 1966). Recently, Morita and Hori[1] (1969) have studied again the same problem in the fleshfly sugar receptor and ascertained proportionality between the receptor potential and impulse frequency. Therefore, the impulse frequency during the stationary state can be regarded as a measure of the receptor membrane current.

The interval between stimuli was 3 min after stimuli by sucrose solutions lower than 0.2 molal and 5 min after stimuli with concentrations higher than 0.4 molal. In the case of glucose and fructose stimulation, the interval was suitably chosen by comparison with the response to sucrose. The ambient temperature in the course of the experiments was 25°C ± 0.2°C. The relative humidities of the experimental room were maintained at 62–72% throughout this work, and did not change more than 3% during any series of experiments.

The pH of the stimulus solution was adjusted with HCl or NaOH using a Hitachi-Horiba pH meter (accuracy, ±0.03). Although sucrose is stable in an alkaline solution, it hydrolyzes in an acidic one. Therefore, it was necessary to use acidic sucrose solutions within 1 min after they were prepared. The pH value of the remaining test solution was measured immediately after the end of each stimulation. With this procedure, the hydrolysis of sucrose could be limited within 1% in 0.1 N HCl. The monosaccharides, glucose and fructose, are unstable (hydrolyzed) in alkaline pH solutions. The same procedure used in the case of sucrose stimulation at acid pH was applied in the case of monosaccharides at alkaline pH.

[1] Morita, H., and N. Hori. Data to be published.

RESULTS

Identification of Spikes

In the labellar chemosensory hair of the fleshfly, the spike heights of the sugar, salt, and water receptors differ sufficiently to allow classification of a particular spike as belonging to one of these receptors (Morita, Hidaka, and Shiraishi, 1966).

Records at alkaline pH's are shown in Fig. 1, and compared with those at neutral pH stimulation by water (A), sucrose (B), and NaCl (C). The salt receptor discharged impulses on stimulation by water at pH 11.9, but the

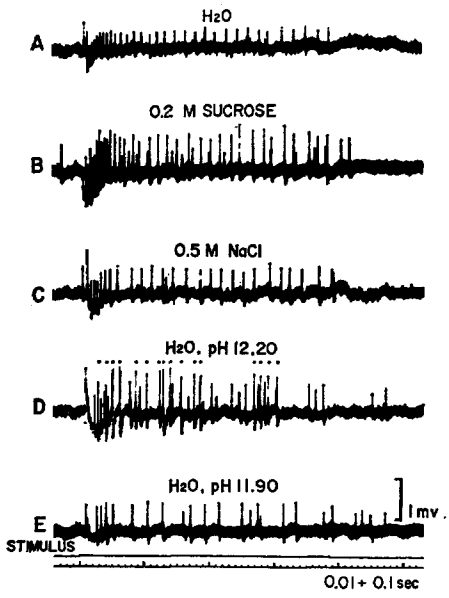

FIGURE 1. Responses of a labellar chemosensory hair of the fleshfly to: (A) H_2O, pH = 5.8; (B) 0.2 molal sucrose, pH = 5.8; (C) 0.5 molal NaCl, pH = 5.8; (D) H_2O, pH = 12.2; (E) H_2O, pH = 11.9. Dots in D indicate the occurrence of spikes in the sugar receptor.

sugar receptor did not (E). The sugar receptor discharged impulses in water at pH 12.2 (D, the dots indicate the occurrence of impulses in this receptor). The relationships of the response to pH in the salt and sugar receptors could, therefore, be plotted without any ambiguity as shown in Fig. 2 (in this and the following figures, the numbers attached to the symbols indicate the order of stimulation). Thus, the response of the sugar receptor to the stimulus solution containing no sugars at high pH was quite independent of that of the salt receptor (cf. Gillary, 1966).

Reproducibility of Responses

The reproducibility of the response to 0.2 molal sucrose was tested at low and high pH, and the results are shown in Figs. 3 and 4, respectively. Since the response to the control stimulus did not differ before and after the stimuli

at high or low pH, it was concluded that the inhibition effect was reversible. As shown by Figs. 1 and 2, the sugar receptor discharged impulses when stimulated by solutions of pH higher than 12, even though the stimulus solutions did not contain any sugar. Since the response to the control stimulus

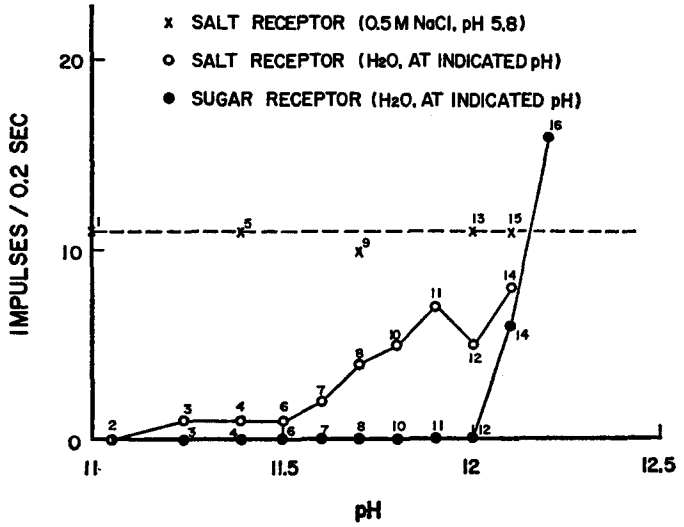

FIGURE 2. Responses of the salt and sugar receptors to water at high pH. The number attached to each symbol shows the order of stimulation (the same in the following figures). Obtained from a single chemosensory hair.

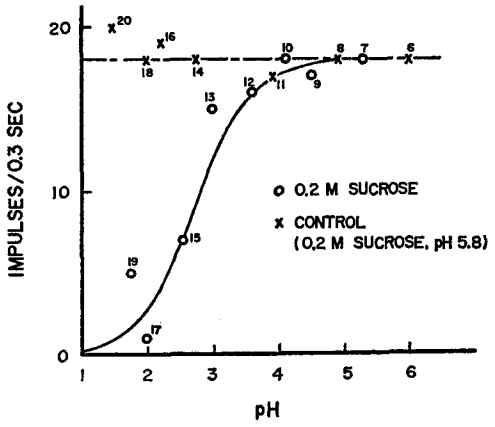

FIGURE 3. Effects of low pH on the response of a single sugar receptor to 0.2 molal sucrose.

did not change even after the application of solutions of extremely high pH, such a discharge of impulses was not due to any irreversible injury.

Examination of the Responses with the Dissociation Curves of Acid and Base

The effect of low pH on the sugar receptor was studied at three different concentrations of sucrose (0.2, 0.4, and 0.5 molal), and the results from three

to four receptors at each concentration are shown in Fig. 5. The response magnitude is normalized in each preparation so that the mean value of the response is unity at neutral pH. The solid line is the dissociation curve of an

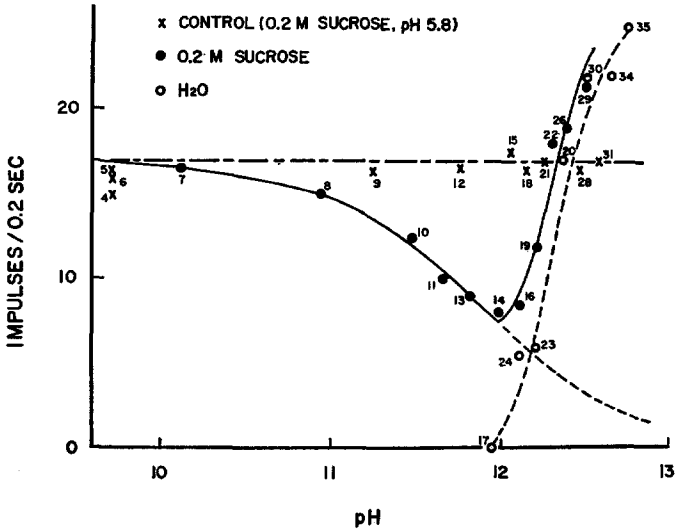

FIGURE 4. Effects of high pH on the response of a single sugar receptor to 0.2 molal sucrose.

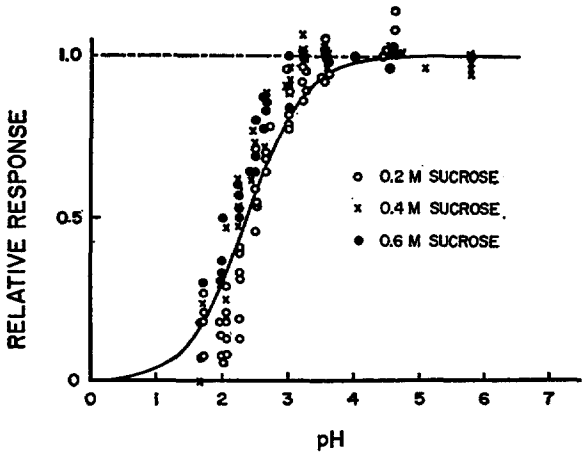

FIGURE 5. Effects of sucrose concentration on inhibition by low pH. Results from three to four receptors for each concentration are expressed relative to the response at pH 5.8 for each receptor. Solid line is the ionization curve of an acid of pK 2.5.

acid of pK = 2.5. The actual inhibition is steeper than the theoretical curve. Fig. 5 also shows that the response to 0.2 molal sucrose is inhibited slightly more strongly than that to 0.4 molal or 0.6 molal sucrose.

Sucrose has a buffering action which occurs especially in the high pH region.

Different amounts of NaOH are required to adjust the pH of different concentrations of sucrose to the same pH value; consequently, the responses to different concentrations of sucrose at a high pH are influenced by different ionic strengths. Effects of ionic strength on the sugar receptor response will be described later.

Fig. 6 shows the responses of five different receptors to one concentration of sucrose (0.2 molal) at high pH's. The response is expressed as the value relative to that at a neutral pH, and the reversed S-curve represents the theoretical curve whose dissociation constant for an ionizing group is to be

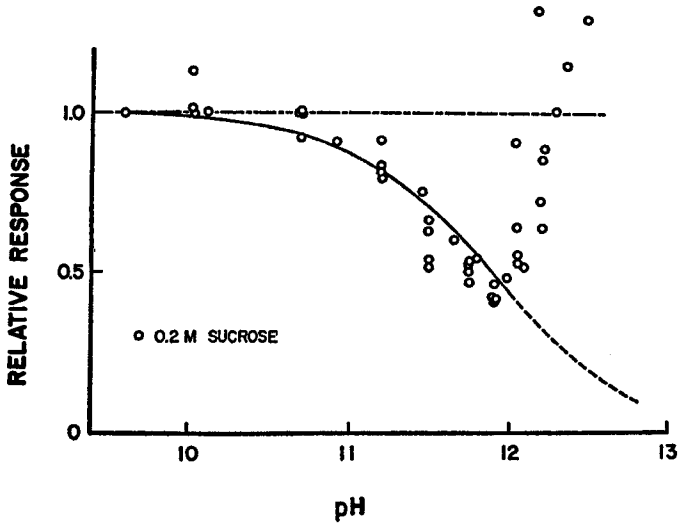

FIGURE 6. Effects of high pH on the response of the sugar receptor to 0.2 molal sucrose Results from five different preparations are expressed as the value relative to that at a neutral pH for each receptor. The line (continuous and broken, respectively, below and above pH 12) is the ionization curve of a base of pK 11.9.

pH = 11.9. The inhibition is steeper than that predicted by the dissociation curve at a high pH region. The conclusion is the same as that in the low pH region. The actual response did not fit the theoretical curve.

Salt Effects

The sugar receptor has been shown to be excited in high pH solutions not containing any sugar. Such excitation occurred irrespective of whether the pH of the solutions was adjusted with NaOH, KOH, or LiOH; however, this type of excitation was markedly affected by the concentration of dissolved neutral salts. The pH-excitation curve is shifted toward lower pH in the presence of neutral salts. Fig. 7 shows such a shift, where the result for each of three different concentrations of NaCl (0, 0.1, and 0.5 molal) came from

one preparation. Detailed studies of the salt effect showed that the shift from the position of the reaction curve obtained without NaCl was approximately one pH unit when 0.4 molal NaCl was dissolved in the stimulus solution. No further shift was observed above 0.4 molal NaCl.

As mentioned above, the pH-excitation curve without sugar (dashed line of steep S-shape in Fig. 4) was independent of different alkaline hydroxide metals (NaOH, KOH, and LiOH) used in the pH adjustment. The shift by neutral salts was also independent of the cations of the salts dissolved in the stimulus solution. Fig. 8 shows one of these results, where the results for three preparations using 0.1 molal choline chloride are compared with those

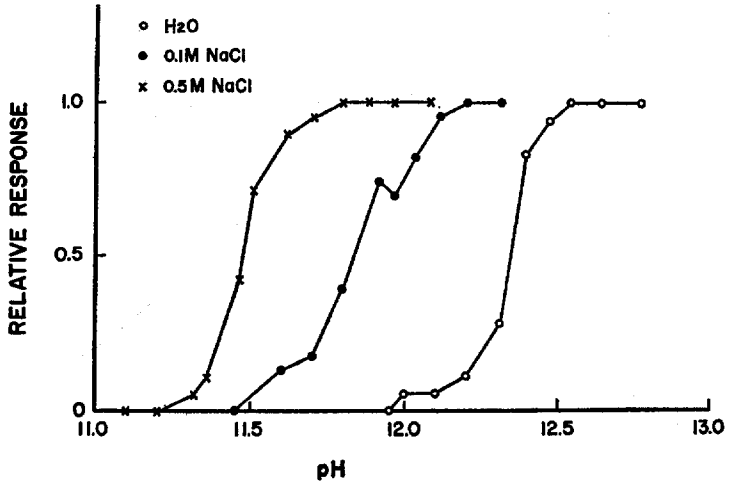

FIGURE 7. The effect of salt on the excitation of the sugar receptor at high pH. Results from one receptor for each concentration of NaCl (0, 0.1, and 0.5 molal) are expressed relative to the maximum response for each receptor.

for 0.1 molal NaCl in three other preparations. The two salts have many different chemical properties, yet both shifted the excitation curve to the same extent, about 0.5 pH unit toward lower pH.

The pH-inhibition curve for sucrose stimulation was also shifted toward lower pH in the presence of salt. This situation is shown by Fig. 9. In the presence of 0.5 molal NaCl (closed circles), the reaction to 0.2 molal sucrose (solid line) and to a pH-adjusted solution without sugar (broken line) was recorded from one receptor (receptor II). In the absence of NaCl in the stimulus solution (open circles), the reaction to 0.2 molal sucrose (solid line) and a pH-adjusted solution without sugar (broken line) was recorded from another receptor (receptor I). The values represented by the closed or open circles are relative to the responses to 0.2 molal sucrose solutions of neutral pH with or without 0.5 molal NaCl, respectively. The open triangle on the pH-inhibition curve of receptor I represents the response of receptor II to

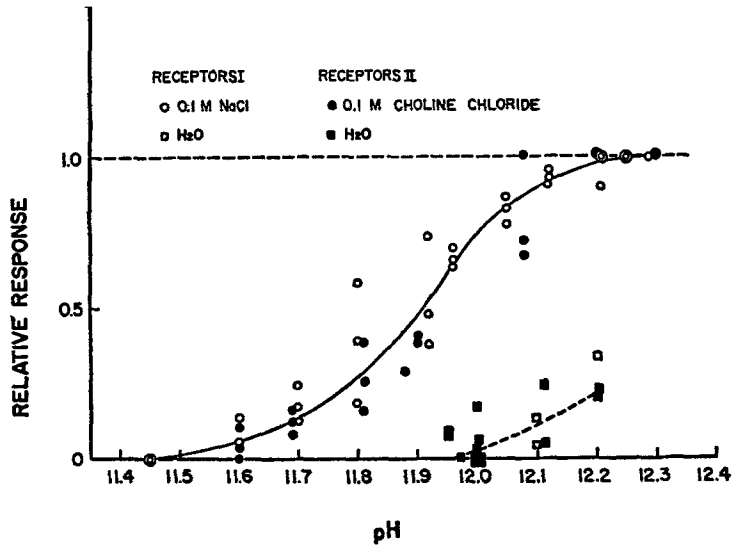

FIGURE 8. Comparison of the salt effect between NaCl and choline chloride, the concentration being 0.1 molal for both salts. Results from three different receptors for each stimulus solution are expressed relative to the maximum response for each receptor. The solid and broken curves for the two stimulus solutions (0.1 molal salts and H_2O, respectively) were fitted by eye.

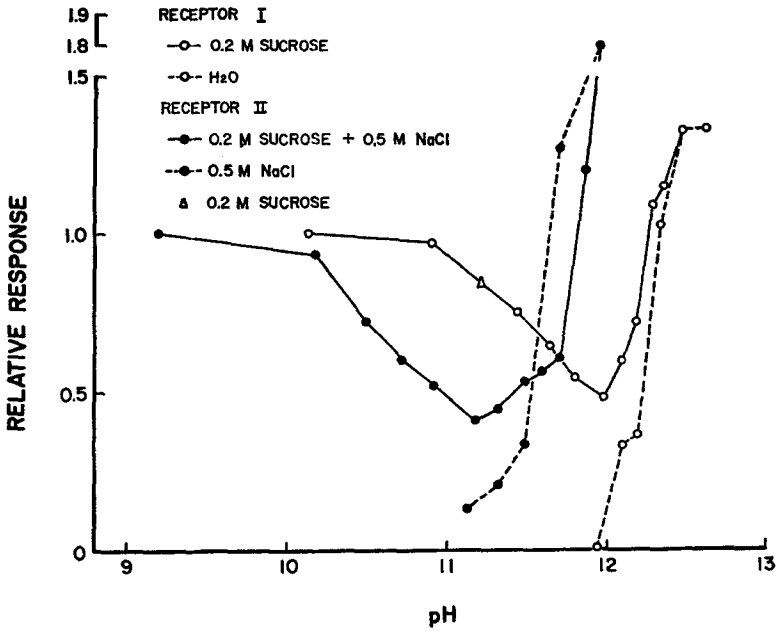

FIGURE 9. Effects of salts on the pH-reaction curve, with or without sugar. Results from two different receptors (represented by closed and open circles, respectively) are expressed relative to the response to 0.2 molal sucrose at neutral pH with or without 0.5 molal NaCl, respectively. The value of the open triangle was obtained from the same receptor represented by closed circles and is relative to the response of this receptor to plain 0.2 molal sucrose at neutral pH.

pure 0.2 molal sucrose. This value, however, is relative to the response of the same receptor (receptor II) to pure 0.2 molal sucrose at neutral pH. Therefore, the pH-inhibition curve of receptor II can be assumed to be the same curve as the one obtained from receptor I for sucrose stimulation without NaCl. As shown in Fig. 9, the shift of the pH-response curve by salt was greatest for the inhibitory stage with sugar, less for the excitatory stage without sugar, and least for the excitatory stage with sugar. In general, the response to a high concentration of sucrose was higher in the presence of 0.5

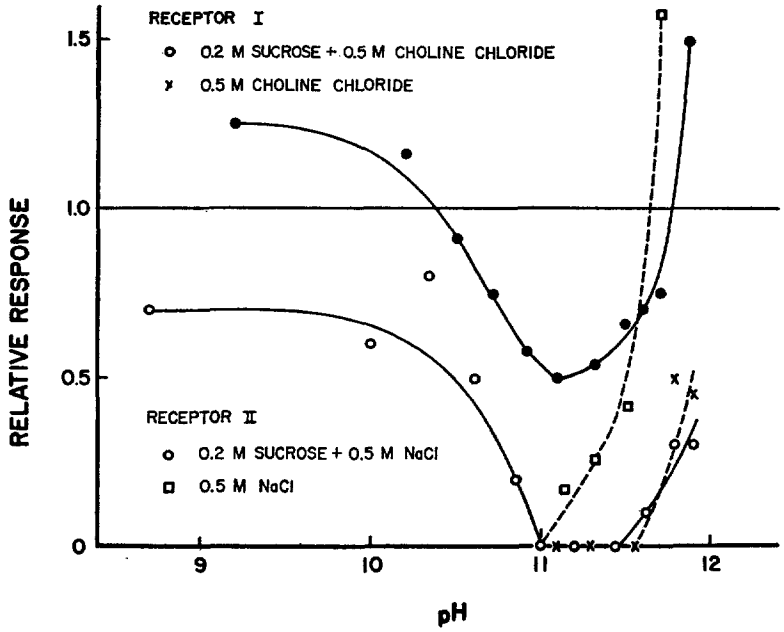

FIGURE 10. Comparison of the effects of NaCl and choline chloride on the pH-reaction curve. The response to a plain 0.2 molal sucrose solution at neutral pH was chosen to be unity in each preparation. The results for 0.5 molal NaCl are the same as those shown in Fig. 9.

molal NaCl than that in the absence of NaCl (cf. Morita, Hidaka, and Shiraishi, 1966).

The shift of the inhibition curve of sucrose caused by the presence of salts is also independent of the species of salts. In Fig. 10 the shift caused by 0.5 molal choline chloride is compared with that caused by 0.5 molal NaCl. The data for receptor II in Fig. 9 are displayed again in this figure relative to a different standard. The standard was taken to be the response to a 0.2 molal sucrose solution at a neutral pH and the relative responses are higher by about 20 % in this figure than those in Fig. 9. This figure shows that the presence of different salts in the stimulating solutions produced different

vertical shifts (magnitude of response) but not horizontal shifts (cf. Morita et al., 1966).

The maximum response evoked at an extremely high pH without sugar depends on the concentration of neutral salts. The maximum response to an extremely high concentration of sucrose at neutral pH was approximately equal to the maximum response to an extremely high pH solution containing no sucrose if both solutions had an equal salt concentration. This suggests

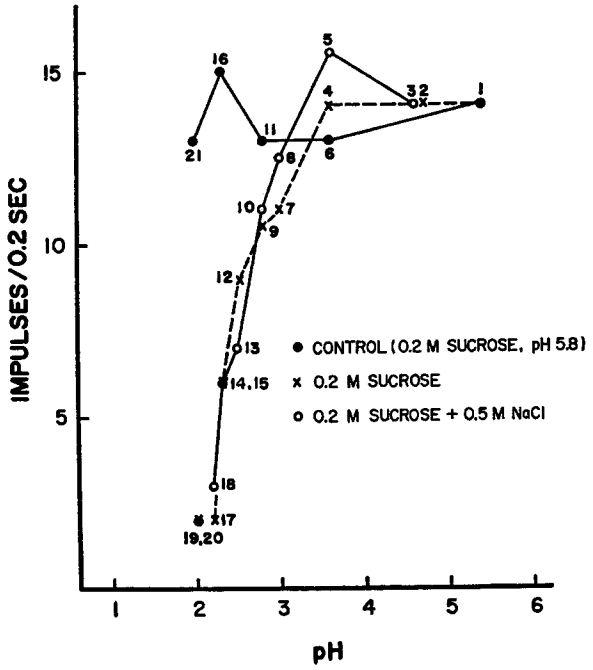

FIGURE 11. The effect of salt on the pH-inhibition curve in a single receptor at low pH.

that the ionizable groups responsible for the excitation at high pH are directly related to the final step in the gating mechanism for ions to pass through the receptor membrane.

At acidic pH the pH-inhibition curve was not shifted in any direction in the presence of salts. One of the examples of this is shown in Fig. 11. This figure shows that the presence of 0.5 molal NaCl had a negligible effect on the pH-inhibition curve.

Monosaccharides

The pH-inhibition curve for the sugar receptor with monosaccharides, glucose and fructose, generally showed the same tendency at low pH as with sucrose; however, the pH-inhibition curve at pH values above 10 showed variations with different sugars. Concentrations of 0.2, 0.4, and 0.6 molal

were used for sucrose, fructose, and glucose stimulation, respectively, in experiments to investigate the action of these sugars at high pH values. At these concentrations, each sugar evoked responses of 60–80 % of the maximum which it could produce, and the magnitude of the response to fructose and glucose stimulation was about 0.6 and 0.8, respectively, relative to that to sucrose at the given concentrations. Since the buffering action differed for each sugar, different amounts of NaOH were required to adjust the stimulus solutions to the same alkaline pH. For example, the amounts of NaOH which were necessary to prepare the above stimulating solutions of pH = 11.0 were 0.004, 0.03, and 0.04 mole per liter solution for sucrose, fructose, and glucose,

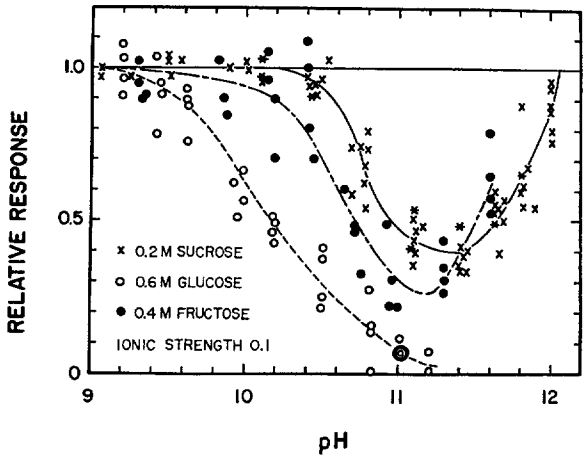

FIGURE 12. Effects of pH in three different sugar solutions under an ionic strength of 1.0. Results from four to eight preparations for each sugar are expressed relative to the response at neutral pH for each receptor.

respectively. To avoid the salt effects described in the previous section, the stimulus solutions were adjusted to a constant ionic strength of 0.1 by adding NaCl. Fig. 12 illustrates the results of such experiments with four to eight preparations for each sugar. The response magnitude was standardized in each preparation so that the mean value of the response was unity at neutral pH. The inhibition of response to 0.6 molal glucose started at approximately pH 9.0 and was almost complete at pH 11.0. The pH-inhibition curves for 0.4 molal fructose and 0.2 molal sucrose showed similar tendencies but were different in degree.

Concentration Effects

In the salt receptor of the blowfly, it has been reported that the pH-inhibition curve in the acidic pH region is shifted toward lower pH with application of stimuli of higher concentrations (Evans and Mellon, 1962; Gillary, 1966).

Similar tendencies for the sugar receptor in fleshfly are revealed by Fig. 5. Therefore, the concentration effect of sucrose on inhibition at low pH was studied under more drastic conditions. Stimulus solutions of 0.5 and 0.05 molal sucrose were prepared and adjusted to an ionic strength of 0.1 by adding NaCl. The response to these stimuli was recorded in one preparation and plotted in Fig. 13 relative to the response at neutral pH. Fig. 13 indicates that the inhibition curve was shifted toward lower pH by about 0.8 pH unit with a 10-fold increase of sucrose concentration. The shift is saturated above 0.4 molal sucrose, as shown by the results in Fig. 5. The concentration effect

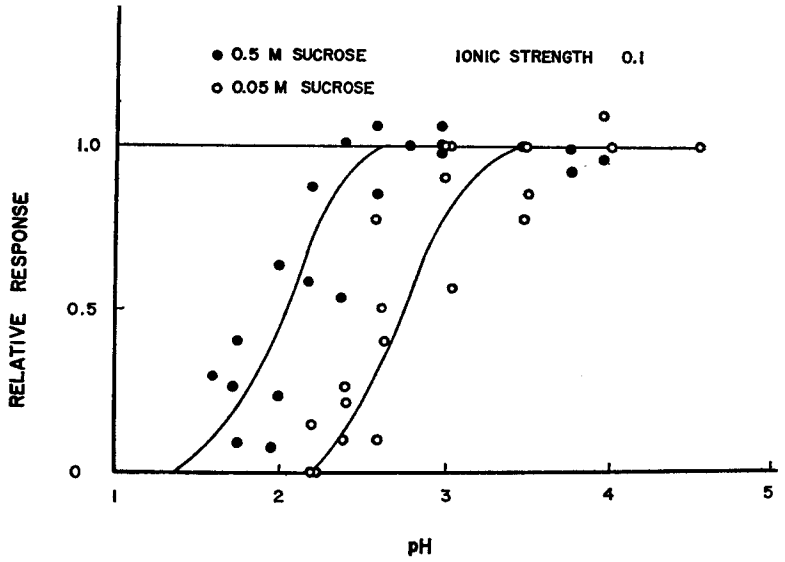

FIGURE 13. The effect of sucrose concentration on inhibition by low pH's. Results from three preparations for each concentration (0.5 and 0.05 molal) of sucrose are expressed relative to the response at a neutral pH. The solid curves were fitted by eye.

of sugar was much more fully analyzed in the alkaline pH region, and is described below in terms of pK_m and log R_m.

Ionizable Groups at the Receptor Site

RESPONSE TO SUCROSE

Dixon (1953) presented a method for the evaluation of the pK values of ionizable groups involved in a system of enzymatic reactions which are described by the Michaelis-Menten equation (cf. Dixon and Webb, 1961). As pointed out by Morita and Shiraishi (1968), the response of the sugar receptor of the fleshfly to sucrose is described by the theory of Beidler (1954), which is completely consistent with the Michaelis-Menten formulation. If

Beidler's taste theory can be accepted, the pK values of the ionizable groups at the sugar receptor site can be evaluated using Dixon's treatment. There are, however, at least two other prerequisites when Dixon's method is applied to studies on chemoreceptors. First, the response must have a stationary phase at any pH value. This was satisfied both at acidic and alkaline pH's. To eliminate the salt effects previously described, all experiments shown in the following figures were performed at an ionic strength of 0.1, adjusted with NaCl. Second, Beidler's relation must hold for all pH values. This was satisfied at alkaline pH's (cf. Fig. 15), but not at acidic pH's (Fig. 14). Consequently,

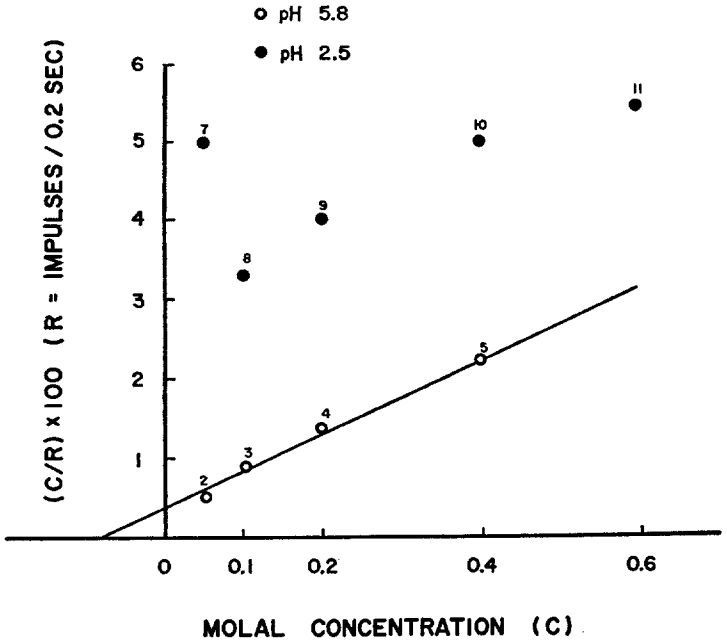

FIGURE 14. Beidler's plot of the response to sucrose at pH's 5.8 and 2.5.

the ionizable groups at the receptor site were analyzed for the inhibition at high pH only.

Fig. 15 illustrates the procedure to obtain K_m (reciprocal of the equilibrium constant in Beidler's theory) and the maximal response to sucrose at pH 11.4 in one receptor. In the first run, the responses to sucrose at a neutral pH were recorded (stimulation Nos. 2–5), and in the second run the responses at pH 11.4 were obtained (Nos. 7–11). In the third run the responses at the neutral pH were again recorded (Nos. 13–17). The intercept of the straight line on the X-axis gives $-K_m$, and the slope gives the reciprocal of the maximal response ($1/R_m$), according to Beidler's taste equation,

$$C/R = C/R_m + K_m/R_m .$$

Denoting the values of K_m and R_m at the neutral pH by K_m^0 and R_m^0, respectively (when the values were different in the first and third runs, the mean values were taken as K_m^0 and R_m^0), the values of K_m and R_m relative to those at the neutral pH were defined as

$$K'_m = K_m/K_m^0 \text{ and } R'_m = R_m/R_m^0.$$

These relative values were necessary and useful in the following treatments,

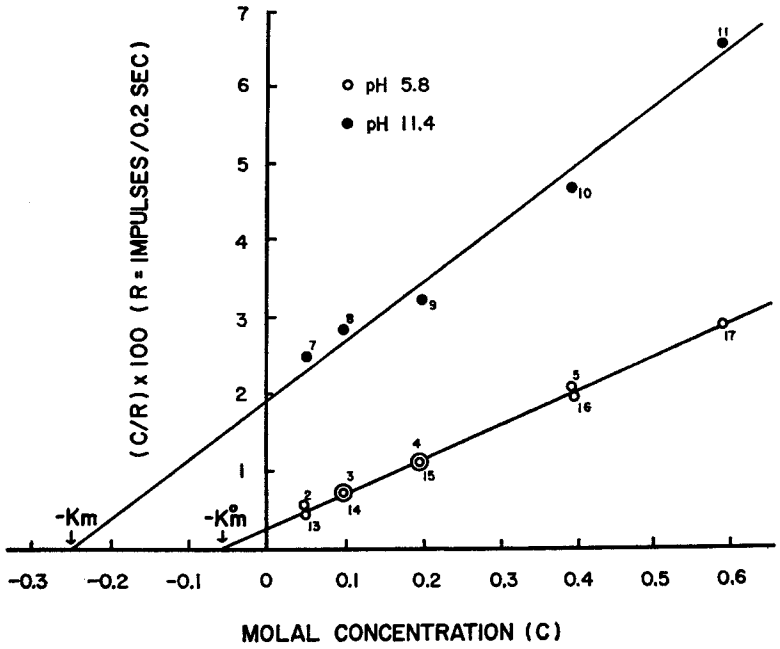

FIGURE 15. Beidler's plot for determining constants for the combination of sucrose with the receptor site.

since the values of K_m^0 and R_m^0 varied with the preparations and only changes with pH values were of interest.

According to Dixon (1953), we obtain the relations as

$$\text{pK}'_m = \log f_{as}(\text{pH}) - \log f_a(\text{pH}) - \log f_s(\text{pH}), \quad (1)$$

and

$$\log R'_m = -\log f_{as}(\text{pH}), \quad (2)$$

where f_a (pH), f_s (pH), and f_{as} (pH) are the functions of pH for the stimulant, the receptor site, and the stimulant-receptor site complex, respectively.

Consequently for the analysis of ionizable groups at the receptor site, it was convenient to plot pK'_m and log R'_m against pH. Since the effect of ionization of sucrose in the pH region under discussion can be neglected (sucrose pK = 12.6 and 13.54, Urban and Shaffer, 1932), it is sufficient only to consider the terms, log f_{as}(pH) and log f_s(pH). Fig. 16 shows the results of the above analytical procedures.

The pK'_m-pH relation revealed in Fig. 16 suggests that at least eight ionizable groups are related to the excitation mechanism of the sugar receptor at alkaline pH. Five ionizable groups are related to the unoccupied

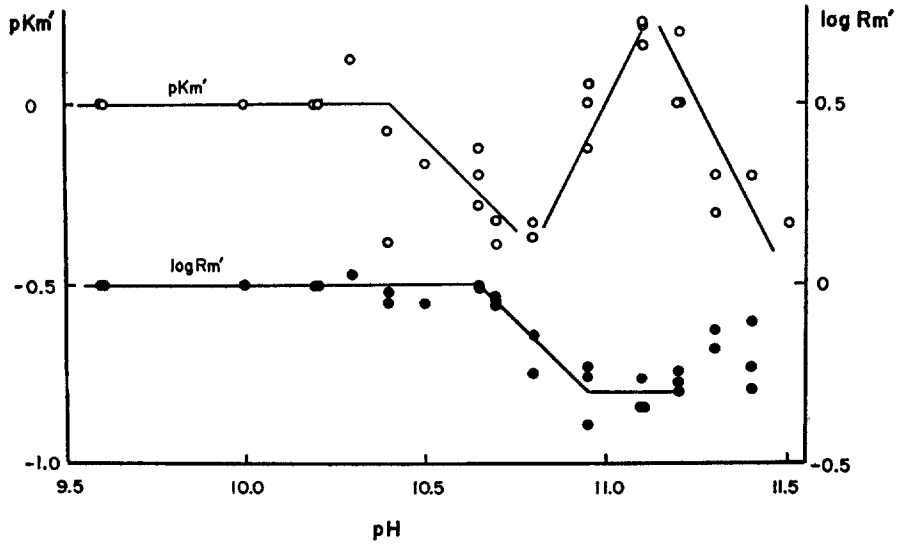

FIGURE 16. Effects of pH on the Michaelis constant, K_m, and on the maximum response, R_m, for sucrose stimulation. $K'_m = K/K^0_m$ and $R'_m = R_m/R^0_m$, where K^0_m and R^0_m are the values at pH 5.8.

receptor site (the term, f_s(pH)), one at about pK 10.4, four at about pK 11.1 and the other three ionizable groups are related to the occupied receptor site (the term, f_{as}(pH)) at pK's 10.7–10.8. The log R'_m plot reveals only one ionizable group of pK value about 10.7 at the occupied receptor site, and therefore, it must be assumed that the effects of the two other ionizable groups suggested by the pK'_m plot were cancelled by two ionizable groups responsible for the excitation in the absence of sugars at extremely high pH's. Since the log R'_m plot shows a tendency to bend upward near pH 11.3, at least four ionizable groups per receptor site must be present which are not directly related to the complex formation between the receptor site and the stimulus molecule but are presumably related to the excitation itself (gating mechanism of the receptor cell).

The number of ionizable groups estimated above is a minimum. The straight line of a predicted slope can be obtained only in the pH regions

sufficiently removed from any of the pK'_s of all ionizable groups. The straight lines in Fig. 16 are all limited to small ranges of pH, except for the pH region below 10.4 for pK'_m and that below 10.7 for log R'_m. Therefore, the actual number of the ionizable groups may be seven or more at the unoccupied receptor site.

Response to Monosaccharides

As pointed out by Morita and Shiraishi (1968), the response of the sugar receptor of the fleshfly to monosaccharide stimulation is not described by

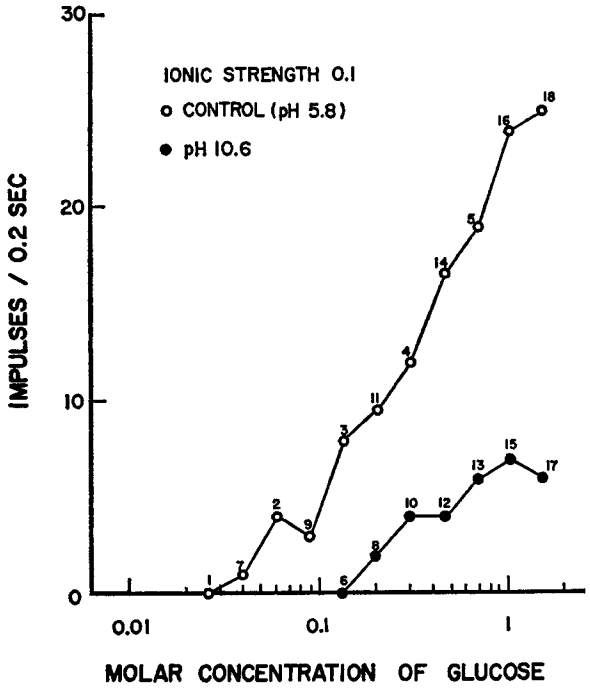

FIGURE 17. Comparison of responses of a single sugar receptor to glucose at pH's of 5.8 and 10.6.

the original equation of Beidler (1954), but rather by an equation based on the assumption that the response is proportional to the number of receptor sites, each occupied by two molecules of monosaccharides. The straight line–fitting technique as used in Beidler's theory cannot be applied to the analysis of the responses to monosaccharides. Therefore, the response over an entire range of concentration of monosaccharides (from zero to the maximum response) at every fixed pH must be measured in order to obtain the same type of information about ionizable groups as in the case of sucrose. If the results derived from sucrose and monosaccharide stimulation agree with each other, support for the applicability of Dixon's theory to the sugar

receptor would be provided. However, the results for monosaccharide stimulation were unsatisfactory due primarily to technical difficulties.

Responses of one receptor to glucose of various concentrations, in steps increasing serially by a factor of 1.5 times up to 1.5 molar, were recorded at a neutral and a fixed alkaline pH (Fig. 17). The ionic strength was adjusted to 0.1 with NaCl. The value of the response relative to the maximum (No. 18, for example, in Fig. 17) was then calculated at each pH in each receptor. The response was studied at nine different pH values from 9–11. Among them, the results at two alkaline pH's (the mean value in 3 preparations for

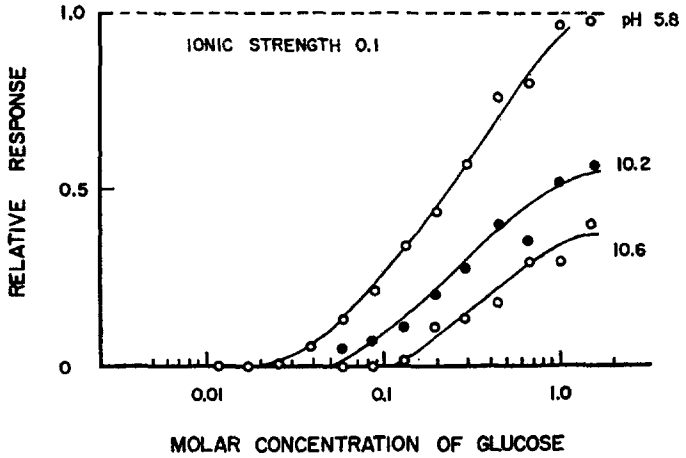

FIGURE 18. Responses to glucose at pH's of 5.8, 10.2, and 10.6 with an ionic strength of 0.1. Each point represents the average of responses in several preparations, relative to the maximum at pH 5.8 for each preparation.

each alkaline pH, and the mean in 20 preparations for the neutral pH) are shown in Fig. 18. The maximum response decreases, and the response-concentration curve has a tendency to move to the right at high pH's.

Experiments with fructose were carried out with the same procedure as with glucose, but the concentrations of fructose were increased serially by a factor of 2 up to 1.0 molar. The qualitative tendencies were almost the same as observed in the glucose experiments, but there were quantitative differences as shown by Fig. 19. The plots in Fig. 19 are the same as that for sucrose (log R'_m curve in Fig. 16). The value of pK, however, cannot be estimated as accurately as in Fig. 16, since the maximum response was not obtained at all alkaline pH's as shown in Fig. 18.

DISCUSSION

Effects of pH on Other Receptors

The present results indicate that the pH inhibition in the labellar sugar receptor of the fleshfly is similar to that reported by Gillary (1966) in the salt

receptor of *Phormia*. However, the excitation at extremely high pH's is somewhat different between salt and sugar receptors when examined in detail as shown in Fig. 2. The results from the salt receptor shown in Fig. 2 agree with that from *Phormia regina* reported by Gillary. Since detailed results of the salt effect on the excitation of the salt receptor have not been reported at extremely high pH's, this problem was studied with the blowfly, *Phormia regina*.

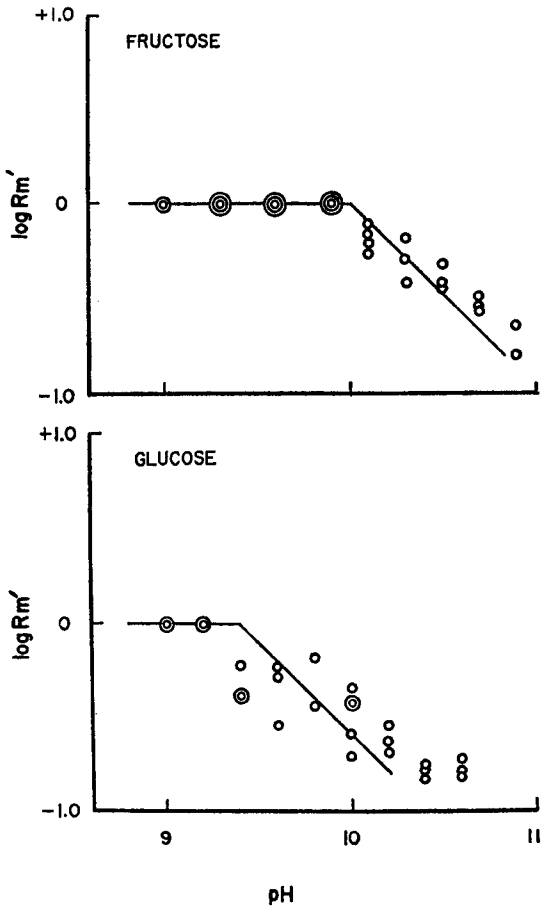

FIGURE 19. Effects of pH on the maximum response, R_m, to fructose and to glucose. $R'_m = R_m/R^0_m$, where R^0_m is the value at pH 5.8.

In this species of fly, the salt receptor discharged the largest spikes among the receptors, thus making this species of blowfly a much more suitable material for studies on the salt receptor. Fig. 20 summarizes the results with four to six preparations for each of 0, 0.1, and 1.0 molal NaCl stimulations at high pH's. The excitation occurred above pH 11.0, and there was no definite tendency to shift to the left at high concentrations of NaCl, while the shift by salts was dominant in the sugar receptor as shown in the Results. This suggests that there are different mechanisms or different structures for permeability regulation in sugar and salt receptors.

Applicability of Dixon's Theory

The original treatment by Dixon (1953) was concerned with an equilibrium constant (the dissociation constant of an enzyme-substrate complex), but Dixon and Webb (1961) have proved that this treatment can be used in the steady state. Therefore, Dixon's equation can be applied to our results in the form of equation (1), though there is no assurance that the value of K_m

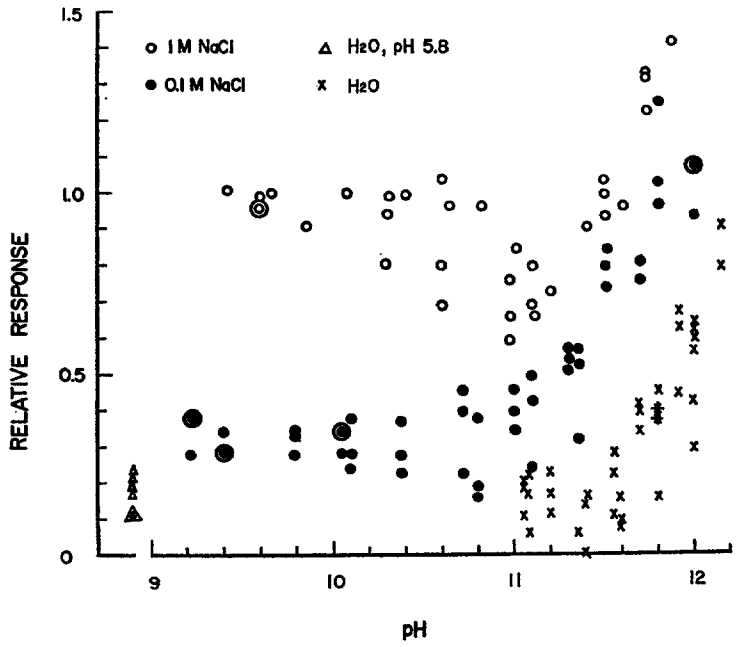

FIGURE 20. Effects of pH on the response of a salt receptor to different concentrations of NaCl (0, 0.1, and 1 molal).

obtained for the sugar receptor represents an equilibrium constant. The same equation can be used for K_1 and K_2 in a 2:1-complex model proposed for monosaccharides (Morita and Shiraishi, 1968), if the changes in K_1 and K_2 with the pH variation can be determined. The results in Fig. 16, however, suggest some mechanisms different from those originally assumed by Dixon. If it is assumed that only one form of ionization in the complex (stimulant-receptor site) is responsible for the excitation, an upward trend in log R'_m at high pH cannot be expected.

At least two explanations are possible. The first is to assume the existence of ionizable groups which are not directly coupled with the response to sugars, as already discussed under Results. The second is to assume that there are many forms of ionization in the complex connected with the response. In

this case the interpretation of the pK curve will be different from that given in the Results. Actual mechanisms for stimulation of the sugar receptor might be quite different from those considered here (for example, an allosteric model proposed by Morita and Shiraishi, 1968). At present no sufficient data exist to decide which is the most likely.

The first assumption mentioned above would be the best as a tentative hypothesis because it is the simplest. Under this assumption, the steepness of rise in the pH-excitation curve without sugar (Fig. 7) cannot be explained without assuming the cooperation of three to four ionizable groups, which are presumed to exist in the interpretation of the results of Fig. 16.

Salt Effects

It has been generally observed in pH-titration curves of proteins that an increase in salt concentration shifts the curve toward the isoelectric point of the protein (*q.v.* Alberty, 1953). The same relation holds for the titration curves of ion-exchange resins (Helfferich, 1962). According to these general observations, the results shown by the effects of salts on the sugar receptor suggest that the receptor site and its neighborhood have an isoelectric point at about pH 2–3. This means that the locus of the receptor site bears a net negative charge at neutral pH.

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