Biochimica et Biophysica Acta, 367 (1974) 1–10

BBA 76744

PHYSICOCHEMICAL STUDIES OF TASTE RECEPTION

I. MODEL MEMBRANE SIMULATING TASTE RECEPTOR POTENTIAL IN RESPONSE TO STIMULI OF SALTS, ACIDS AND DISTILLED WATER

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(Received March 21st, 1974)

SUMMARY

A Millipore filter paper impregnated with the lipids extracted from bovine tongue epithelium was used as a model for the taste receptor membrane and the membrane potentials arisen between two solutions across the membrane were measured under the presence of salts, acids and distilled water as taste stimuli. The changes in the membrane potential induced by these stimuli paralleled closely the taste receptor potentials observed intracellularly with living taste cells or taste nerve responses as shown in the following.

1. Various salts carrying a common species of anion (NaCl, KCl, NH_4Cl and $CaCl_2$) at a given concentration induced a different magnitude of potential deflections, while sodium salts carrying different species of anion (NaF, NaCl, NaBr and NaI) induced a practically identical potential deflection. The relationship between the magnitude of the potential deflection and the NaCl concentration followed the taste equation. The potential deflection induced by the application of a given concentration of NaCl was independent of pH in a wide range of pH between 3 and 12.

2. A treatment of the membrane with $FeCl_3$ brought about a reversal of the polarity of the steady potential. An application of NaCl to the $FeCl_3$-treated membrane induced a variation of the potential deflection with a polarity opposite to that normally displayed.

3. The relations between pH and the magnitude of the potential deflection induced by HCl and acetic acid closely resemble those derived from the taste nerve responses.

4. The model membrane showed potential deflections corresponding to the "water response"; an application of distilled water to the membrane adapted with tap water, Ringer solution, $CaCl_2$ or NaCl solution brought about a potential deflection as if a high concentration of chemicals were applied to the membrane.

INTRODUCTION

Taste cells, which have resting potentials of −30 to −50 mV being negative

on the inside with respect to the outside, undergo depolarization when the tongue surface is subjected to taste stimuli. These potential changes in taste cells, which were referred to as taste receptor potentials, have been demonstrated by various investigators in rat [1–3], hamster [1] and frog [4–7]. Recently, Eyzaguirre et al. [8] reported that frog tongue epithelial cells other than taste cells also produced, in response to the salt stimuli, a potential change similar to that observed in the taste cells.

The mechanism of generation of taste receptor potential has been worked out by various investigators, but no plausible explanation has been proposed so far as the authors are aware. For example, the sodium theory proposed by Hodgkin and Huxley [9] cannot account for the electrophysiological data concerning the taste stimulus–response relationship in so far as the theory is applied directly to the microvilli membrane in taste cells [10, 11]. The reasons why the sodium theory is not applicable to the microvilli membrane are as follows: (1) the microvilli membrane is, probably, impermeable to taste substances [12, 13]. Hence the diffusion potential may not appear across the membrane; (2) both Na^+ and K^+ applied on the tongue surface induce, more or less, a similar depolarization in taste cells [10]; (3) the application of distilled water brings about a depolarization in the taste cells in spite of a decrease of the ionic concentration in the external solution [4, 6]; (4) the application of non-electrolyte, e.g. some sweet or bitter substances, induces a depolarization in taste cells.

Considering these difficulties, Beidler [10, 11] proposed an idea that the taste receptor potential is generated by a change in permeability of the ions of the membrane situated below the tight junction of the taste cells. This notion has not been proved experimentally, and the question still remains as to why the adsorption of the stimuli on the microvilli membrane induces a change in the permeability of the membrane far apart from the adsorption site. In this series of papers, we intend to make it clear that the change in the phase boundary potential at the interface of the microvilli membrane-stimulating solution is responsible for the taste stimulation.

The receptor elements for salt and acid stimuli have been suggested to be phospholipids in the taste receptor membrane [14–16]. In the present study, a Millipore filter paper impregnated with the lipids is prepared as a model for the taste receptor membrane. The model membrane is found to exhibit a potential deflection similar to the taste receptor potential in response to salts, acids and distilled water.

EXPERIMENTAL

Preparation of model membrane

Lipids were extracted from bovine tongue epithelium with chloroform–methanol (2 : 1, v/v) and the extracts were washed by the method of Folch et al. [17]. The lipids obtained were dissolved in chloroform and a Millipore filter paper composed of cellulose ester (nominal pore size of 25 nm and thickness of 0.1 mm) was soaked in the lipid solution. After about 10 min of soaking, the filter paper was dried in the air and weighed for the determination of the amount of the lipids adsorbed in the filter paper (designated as Q mg/cm^2). Judging from the porosity and the amount of the lipids adsorbed in the membrane (see later), the void space of the filter paper must be fully filled with the lipids. The lipids in the membrane seem to be in the liquid state because the total lipids used for the present study are not solidified at room temperature. The model membrane thus prepared was immersed in 300 mM NaCl and condi-

tioned overnight in a cold room before it is used as a sample membrane. The salt solution does not penetrate into the interior of the membrane because the void space in the membrane must be fully filled with the liquid lipids and then, only the lipids located at the membrane surface seem to change their orientation during the conditioning process so that the hydrophilic groups face into the aqueous bulk solution.

Measurements of membrane potential

Fig. 1 shows a schematic diagram of the cell and apparatus used for measuring the membrane potential. A stimulating solution was placed in compartment A in the figure and 300 mM NaCl was placed in compartment B as a reference solution (see later). The steady potential corresponding to the resting potential of taste cells was obtained by placing tap water in compartment A before and after the application of the stimulating solution to the model membrane. This procedure has been often employed in the measurements of taste receptor potential.

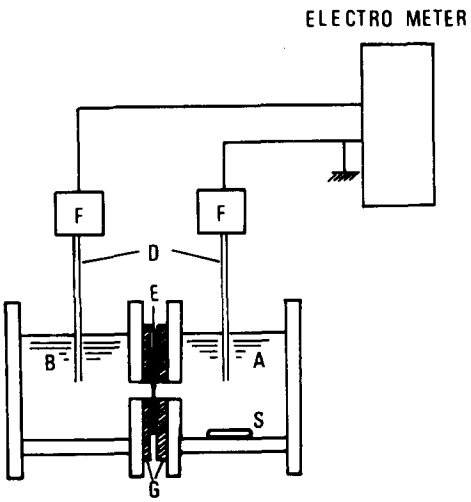

Fig. 1. Schematic diagram of the cell for measurements of membrane potential. A, compartment for stimulating solution; B, compartment for reference salt solution; D, saturated KCl-filled salt bridges; E, model membrane; F, calomel electrodes; G, spacer of silicon rubber gasket; S, magnetic stirrer tip.

The emf arisen between the two sides of the membrane was conducted to an electrometer (Takeda Riken Co., Type TR-8651) through a pair of saturated KCl-filled salt bridges with calomel electrodes, and recorded with a pen-writing recorder. The solution placed in compartment A was grounded through the electrodes as illustrated in Fig. 1. In order to make the potential response hasten, one of the salt bridge tubings was previously soaked in the stimulating solution to be applied. Since any microelectrode was not used in the potential measurements, the tip potential as a possible source of artifact can be safely neglected. The liquid junction potential between the saturated KCl-filled salt bridge and the stimulating solution must not contribute significantly to the observed results [18].

Stimulating solutions were prepared by dissolving reagents of analytical grade in distilled water. The composition of the Ringer solution used was: NaCl 154 mM, KCl 5.6 mM, $CaCl_2$ 2.2 mM and this was adjusted to pH 7.3 with 20 mM $NaHCO_3$–HCl buffer. All measurements were made at 25 °C.

RESULTS AND DISCUSSION

The steady potential of a model membrane adapted to tap water was −35 to −45 mV, being negative on the reference side when 300 mM NaCl was used as a reference solution. As shown in Fig. 2, replacement of the tap water with salt or acid solutions produced a positive-going potential deflection, which corresponds to "depolarization" in electrophysiological terms.

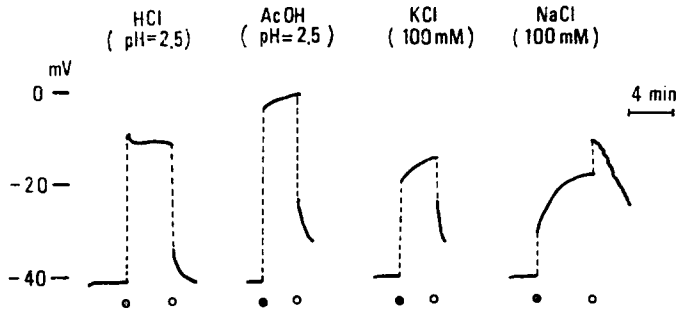

Fig. 2. Potential responses of the model membrane (Q = 15.2 mg/cm^2) to salts and acids. Stimulating solutions were added to the membrane bathed in tap water at the closed circle and were replaced with tap water at the open circle. The dotted line shows the period for the replacement of the solutions. AcOH is acetic acid.

When 10 mM NaCl, 300 mM NaCl and Ringer solution were used as the reference solution, the observed steady potentials of the membrane bathed in tap water were −6, −42 and −41 mV, respectively. If the magnitude of the potential deflections induced by the application of NaCl to the membrane is plotted against the NaCl concentration, we obtain a single curve as illustrated in Fig. 3. This indicates that the magnitude of the potential deflections induced by the chemical stimuli is independent of the concentration and/or the composition of salt in the reference solution. Therefore, 300 mM NaCl solution was used as the reference solution in the subsequent experiments.

Beidler [19] pointed out that the following equation adequately describes the relationship between the concentration of NaCl (C) and the magnitude of the neural response (R)

$$C/R = C/R_m + 1/KR_m \quad (1)$$

where R_m is the maximum response and K is the equilibrium constant. Eqn (1) is usually called the taste equation. Kimura and Beidler [1] found that Eqn (1) also satisfies the relationship between the magnitude of the receptor potential evoked by NaCl in rat taste cells and NaCl concentration. In order to examine whether or not the relationship between the potential deflection (R) induced by the application of

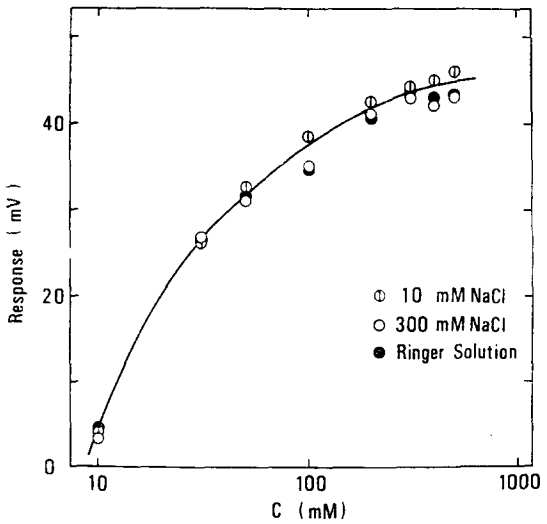

Fig. 3. Effect of concentration and composition of salt in reference solutions on the magnitude of the potential deflections induced by various concentrations of NaCl. Data were obtained when 10 mM NaCl, 300 mM NaCl and Ringer solution were used as reference solutions. The ordinate represents the magnitude of the potential deflections induced by the application of NaCl to the membrane bathed in tap water. $Q = 14.4$ mg/cm^2.

NaCl to the model membrane and the NaCl concentration (C) follows Eqn (1), the values of C/R calculated from data given in Fig. 3 are plotted against C (line 1 in Fig. 4). Line 2 in the figure shows plots of the data observed with a different membrane preparation. From the straight line in Fig. 4, it is seen that the relationship between the NaCl concentration and the magnitude of the potential deflection observed with the present model membrane also satisfies Eqn (1). The slope of the straight line varied to some extent from one membrane preparation to another.

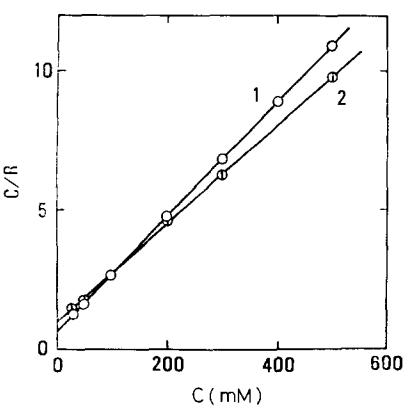

Fig. 4. Plots of the magnitude of the potential deflections induced by NaCl according to Eqn (1). R, magnitude of potential deflection induced by NaCl; C, NaCl concentration. The data for line 1 was taken from Fig. 3 (the data obtained when 300 mM NaCl was used as reference solution). The data for line 2 was obtained with a different membrane preparation ($Q = 14.7$ mg/cm^2).

In Fig. 5, the magnitude of the potential deflections induced by various kinds of salts carrying a common anion species (chloride in this case) is plotted as a function of salt concentration. In order to examine the effect of salt of low concentration on the membrane potential, the salt solutions were applied to the membrane adapted to distilled water instead of tap water in this experiment. As seen from the figure, KCl and NH_4Cl induced a larger potential change than NaCl in a low concentration range. The curve for $CaCl_2$ rose steeply in a low concentration range and declined

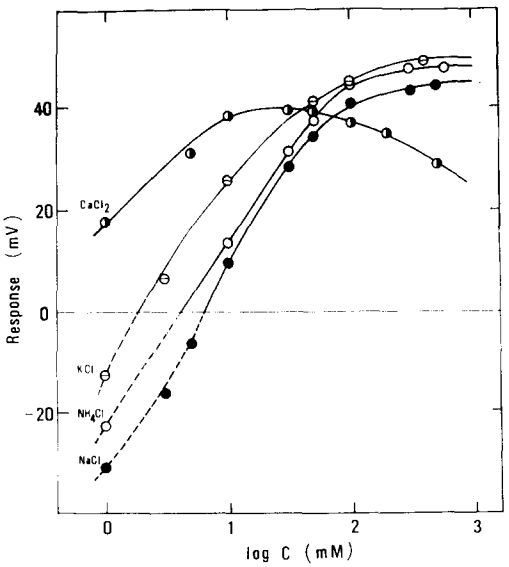

Fig. 5. Potential deflections induced by various kinds of salt as a function of the molar concentration of the salts. $Q = 18.3$ mg/cm^2. Salt solutions were applied to the membrane adapted to distilled water. The potential level of tap water is taken as zero in the ordinate.

with an increase of the concentration. The curves in Fig. 5 show that the salts carrying the same species of anion brought about different potential deflections, while the potential deflections induced by sodium salts carrying different species of anions such as NaF, NaCl, NaBr and NaI were essentially the same as each other. These results are in accordance with the fact that the magnitude of the taste response depends predominantly on the cation species [19, 20].

It is known that the taste response to a given concentration of NaCl is almost independent of pH between 3 and 11 [20]. Fig. 6 shows that the potential change induced by the application of 100 mM NaCl to the model membrane is independent of pH between 3 and 12. Below pH 3, the binding of proton to the negative groups of the phospholipids in the membrane seems to interfere with the electrostatic interaction between Na^+ and the lipid molecules.

Tateda and Beidler [2] found that the steady potential of the taste cell may be made positive with respect to the outside by applying cocain or $FeCl_3$ to the tongue surface. Increasing the steady potential to a level of about 30 mV, no concentration of NaCl applied to the tongue produced a potential response. Above this level, a potential response is obtained but with a polarity opposite to that normally displayed.

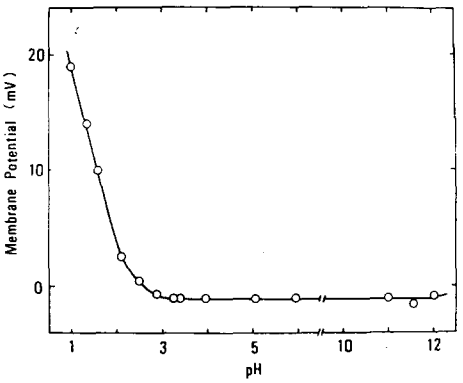

Fig. 6. pH dependence of the magnitude of the potential deflections induced by 100 mM NaCl. The pH of the solution was adjusted by HCl and NaOH.

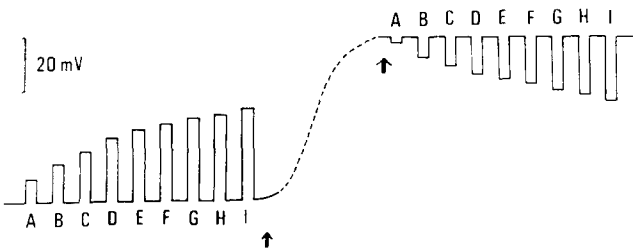

Fig. 7. Histogram of the potential deflections induced by various concentrations of NaCl before and after the application of $FeCl_3$. The potential deflections induced by NaCl were measured and then 60 mM $FeCl_3$ solution was applied to the membrane at the first arrow. After 12 min, the $FeCl_3$ solution was replaced with tap water at the second arrow and the potential deflections induced by NaCl were measured again. A, 10 mM NaCl; B, 20 mM; C, 30 mM; D, 50 mM; E, 75 mM; F, 100 mM; G, 200 mM; H, 300 mM; I, 500 mM. $Q = 17.7$ mg/cm^2.

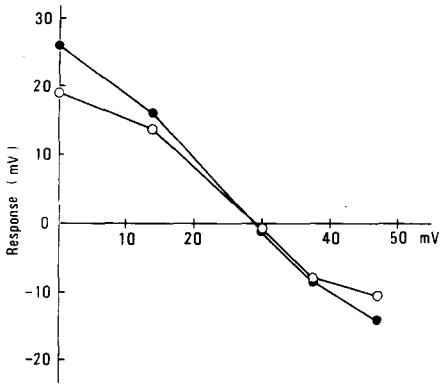

Fig. 8. The magnitude of the potential responses to 250 mM NaCl (closed circle) and 100 mM NaCl (open circle) as a function of the potential change produced by $FeCl_3$. $Q = 17.7$ mg/cm^2.

Figs 7 and 8 show that the treatment of the model membrane with $FeCl_3$ brought about effects similar to those observed by Tateda and Beidler [2]. The histogram in Fig. 7 shows the magnitude of the potential deflections induced by various concentrations of NaCl before and after the application of $FeCl_3$. In Fig. 8, the sizes and polarities of the potential deflections caused by NaCl are plotted against the change of the steady potential elicited by various concentrations of $FeCl_3$. The reversals of the polarities of the potential deflections induced by NaCl occurred when the potential elicited by $FeCl_3$ reached about 30 mV.

As shown in Fig. 2, the model membrane responds to acids. The curves in Fig. 9 show the relationship between pH and the magnitude of the potential deflection induced by HCl and acetic acid. The curves closely resemble those derived from the chorda tympani responses of the rat [11]. The result that the potential deflections induced by acetic acid are larger than those of HCl at the same pH is in accordance with the electrophysiological results [11].

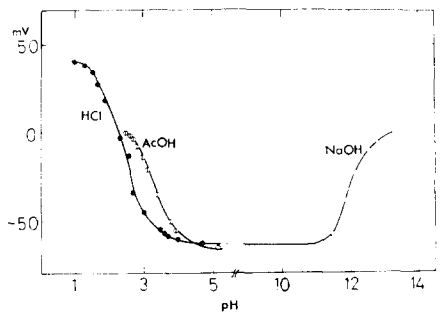

Fig. 9. Potential deflections induced by HCl, acetic acid (AcOH) and NaOH as a function of pH. Each solution contains 1 mM NaCl. Q = 15.1 mg/cm^2.

It is known that the application of distilled water to the tongue surface elicites taste nerve responses [21] and depolarization [4, 6] in taste cells. This so-called "water response" was also observed when distilled water was applied to the model membrane. Fig. 10 shows the records of the "water response" produced by the application of distilled water to the membrane bathed in tap water, Ringer solution, 1 mM $CaCl_2$ and 100 mM NaCl. As seen from the figure, the magnitude of the "water response" and its time course of decay depended on the salt composition and concen-

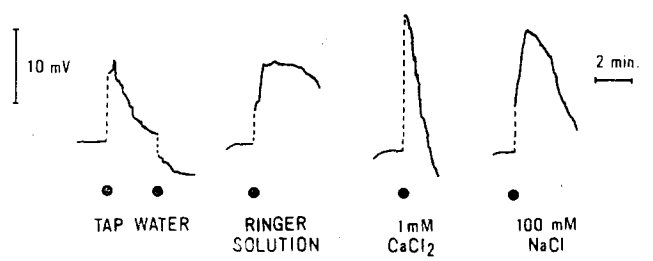

Fig. 10. The "water response" observed with the model membrane. Distilled water was applied at the closed circle to the membrane bathed in various kinds of solution. Q = 12.1 mg/cm^2.

tration of the solution in which the model membrane was being bathed before the application of distilled water.

The potential responses of the model membrane shown above were measured with filter paper impregnated with the lipids of 12–21 mg/cm^2. When the adsorbed quantity of the lipids is small, the model membrane responds to the chemical stimuli in quite a different manner. For example, the membrane with the lipids of 0.3 mg/cm^2 produced a negative-going potential deflection in response to 100 mM KCl or 100 mM NaCl, while the application of HCl at pH 2.5 and distilled water produced positive-going deflections (upper record in Fig. 11). The potential of this kind of membrane,

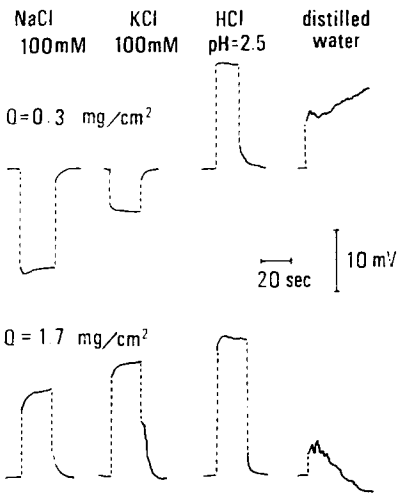

Fig. 11. The effect of the amount of the adsorbed lipids on the membrane function. The stimulating solutions were applied to the membrane bathed in tap water.

which is permeable to both anion and cation, can be attributed to the diffusion potential of ions across the membrane. In fact, a similar potential deflection was observed with the Millipore filter alone containing no lipids. The membrane with the lipids of of 1.7 mg/cm^2 produced a potential deflection similar to that shown in Fig. 1 and Fig. 10 in response to the various stimuli, but the magnitude of the potential deflection was small. If the membrane with the lipids of more than about 8 mg/cm^2 was used, the magnitude of the potential deflections was essentially independent of the quantity of the adsorbed lipids. It is noted that the membrane with the lipids of 78 mg/cm^2 reproduced all the functions shown in the present paper.

As described above, the model membrane responded to salts, acids and distilled water quite similarly to the taste receptor membrane. Therefore, the studies on the mechanism of the generation of the potentials across the model membrane, which will be performed in the following paper, would offer a clue to clarify the mechanism of the generation of receptor potential in taste cells.

ACKNOWLEDGEMENT

This work was supported in part by a grant from the Ministry of Education, Japan.

REFERENCES

1 Kimura, K. and Beidler, L. M. (1961) J. Cell. Comp. Physiol. 58, 131–139
2 Tateda, H. and Beidler, L. M. (1964) J. Gen. Physiol. 47, 476–486
3 Ozeki, M. (1971) J. Gen. Physiol. 58, 688–699
4 Sato, T. (1969) Experimentia 25, 709–710
5 Nomura, H. and Sakada, S. (1969) Olfaction and Taste (Pfaffman, C., ed.), Vol. 3, pp. 345–351, Rockefeller University Press, New York
6 Sato, T. and Beidler, L. M. (1973) Brain Res. 53, 455–457
7 Akaike, N., Noma, A. and Sato, M. (1973) Proc. Japan Acad. 49, 464–469
8 Eyzaguirre, C., Fidone, S. and Zapata, P. J. (1972) J. Physiol. 221, 515–532
9 Hodgkin, A. L. and Huxley, A. F. (1952) J. Physiol. 117, 500–544
10 Beidler, L. M. (1967) Olfaction and Taste (Hayashi, T., ed.), Vol. 2, pp. 506–534, Pergamon Press
11 Beidler, L. M. (1971) Handbook of Sensory Physiology (Beidler, L. M., ed.), Vol. 4-2, pp. 200–220, Springer-Verlag
12 Beidler, L. M. (1965) Cold Spring Harbour Symp. Quant. Biol. 30, 191–200
13 Mistretta, C. M. (1971) Am. J. Physiol. 220, 1162–1167
14 Kurihara, K., Koyama, N. and Kurihara, Y. (1972) Olfaction and Taste (Schneider, D., ed.), Vol. 4, pp. 234–240, Wissenschaftliche Verlagsgesellschaft mbH, Stuttgart
15 Koyama, N. and Kurihara, K. (1972) Nature 239, 459–460
16 Kurihara, Y. (1973) Biochim. Biophys. Acta 306, 478–482
17 Folch, J., Ascoli, I., Meath, J. A. and Lebaron, F. N. (1951) J. Biol. Chem. 191, 833–841
18 Guggenheim, E. A. (1930) J. Am. Chem. Soc. 52, 1315–1337
19 Beidler, L. M. (1954) J. Gen. Physiol. 38, 133–139
20 Beidler, L. M. (1961) Progress in Biophysics and Biophysical Chemistry, Vol. 12, pp. 109–151, Pergamon Press
21 Cohen, M. J., Hagiwara, S. and Zotterman, Y. (1955) Acta Physiol. Scan. 33, 316–332