Biochimica et Biophysica Acta, 553 (1979) 396—403

BBA 78375

SENSITIZATION OF CHEMOTACTIC RESPONSE BY CHANGING THE LIPID COMPOSITION OF SURFACE MEMBRANE IN *TETRAHYMENA PYRIFORMIS*

HIROSHI TANABE, KENZO KURIHARA and YONOSUKE KOBATAKE

Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo (Japan)

(Received November 20th, 1978)

Key words: Chemotaxis; Sensitization; Lipid composition; Ergosterol; Zeta potential

Summary

Tetrahymanol in *Tetrahymena pyriformis*, which is localized principally in the surface membrane, was replaced by exogenous supplement of ergosterol and the influence of the replacement on the chemotactic responses (negative chemotaxis) to salts and hydrophobic compounds was examined. The results obtained are as follows:

1. The chemotactic responses to various stimuli were greatly sensitized by the replacement. The threshold concentrations of chemotaxis against inorganic salts (NaCl, KCl and $MgCl_2$) in the 'replaced cells' were lowered uniformly to about 1/20 of those of the native cells. The thresholds of hydrophobic compounds were lowered in various degrees; the positively charged compounds such as strychnine and nicotine about 1/1000, the neutral compounds such as skatole and phenylthiocarbamide about 1/10, the negatively charged compounds such as sodium picrate and sodium *m*-nitrobenzenesulfonate slightly lowered or unchanged.

2. The zeta potential of the liposomes made of total lipids extracted from the respective cells was measured in the presence of inorganic salts and strychnine. The results obtained indicated that the liposomes from the 'replaced cells' have stronger affinity to these stimuli than those from the native cells. It was suggested that the changes in the affinity of the lipids to the chemical stimuli contribute to the sensitization of the chemotactic response by the ergosterol replacement.

Introduction

It is generally accepted that the initial event of chemoreception in higher vertebrates is the adsorption of chemical stimuli on the receptor membrane of

sensory cells [1]. This is also true with the chemoreception of most stimuli in the lower organisms [2,3]. Certain chemical stimuli such as sugars and amino acids are considered to be adsorbed on the receptor proteins in the membrane [2,4—6]. On the other hand, the receptor molecules or receptor sites for salts and hydrophobic compounds such as so-called bitter substances and odorants are still unknown. In previous studies [7—9], we suggested that the membrane lipids are possible candidates of receptors for these stimuli. In order to clarify more directly a role of membrane lipids in the reception of these stimuli, it would be a useful approach to modify the chemical composition and physical state of membrane lipids and to examine effect of the modification on the reception of these stimuli. For this purpose, *Tetrahymena pyriformis* is an excellent system, as described below.

Tetrahymena pyriformis exhibits negative chemotaxis to various salts, bitter substances and odorants. The order of threshold concentrations of bitter substances or odorants was quite similar to those in the sensory organs of higher vertebrates [10,11]. The composition of membrane lipids can be easily modified by the exogenous supplement of lipids or lipids analogues [12—14]. Nozawa et al. showed that tetrahymanol localized principally in the surface membrane of *Tetrahymena* was replaced by exogenous supplement of ergosterol and that the replacement induced a profound alteration in the phospholipid class composition and in the membrane fluidity [12]. In the present paper, we report the influence of the replacement of tetrahymanol on the chemotactic response of *Tetrahymena* to salts and a number of typical bitter substances and odorants.

Materials and Methods

Chemicals

Chemicals used throughout the experiments were of analytical grade. NaCl, KCl, $MgCl_2$, strychnine nitrate and sodium *m*-nitrobenzenesulfonate were obtained from Wako Pure Chemical Industries, Ltd. $CaCl_2 \cdot 2H_2O$, $LaCl_3$, nicotine and sodium picrate were purchased from Nakarai Chemicals, Ltd. Phenylthiocarbamide and ergosterol were obtained from Tokyo Kasei Co. Doubly distilled water in the glass vessels was used as solvent.

Measurements of chemotactic responses

Tetrahymena pyriformis (strain w) was kindly furnished by Dr. T. Mita, National Institute of Cancer Research, Tokyo. *Tetrahymena* was grown at 22°C in the medium containing 2% proteose peptone, 1% yeast extracts and 0.6% glucose. The ergosterol replacement was carried out by adding ergosterol to the medium (1 mg/100 ml) according to Nozawa et al. [12]. The cells taken from 3-day culture were used for the experiments. They were collected by gentle filtration through a filter paper (Whatman No. 3) and washed thoroughly with control solution (1 mM Tris-HCl buffer, pH 7.0).

Chemotactic response was observed in a thin glass vessel (2 × 6 cm, 1 mm thick). One side of the vessel was filled with the control solution and the other side with a test solution, both containing an equal number of the organisms. A test solution was prepared by dissolving chemical stimuli in the control solu-

tion. Meanwhile, the organisms began to gather to one side of the vessel when the concentration of chemical stimuli in the test solution exceeded a certain value. After 25 min, the number of *Tetrahymena* in both halves was counted by taking microscopic photographs. The details of the experimental setup have been reported [10]. The chemotactic response, R, is expressed by the following equation;

$$R = (n_1 - n_2)/(n_1 + n_2)$$

where n_1 and n_2 stand for the density of *Tetrahymena* in the test solution and that in the control solution, respectively. $R < 0$ corresponds to negative chemotaxis. Measurements of the chemotactic response to each chemical were carried out three times or more and typical data are shown in the figures. All the measurements were made at 22°C.

Preparation of liposomes

The total lipids were extracted from the respective cells of 3 days culture by the method of Bligh and Dyer [15]. No difference was found in electrophoretic mobility between the liposomes made of the total lipids extracted by the method of Bligh-Dyer and those by that of Folch et al. [16]. The lipids obtained were dissolved in chloroform and stored at −20°C in N_2 until use. Dispersions of lipids were prepared in the control solution by agitation of the flask with lipid film by a Vortex mixer until all lipids were freed from the wall. The liposomes thus prepared were added to the solution containing stimulus chemicals of varying concentrations. The final concentration of the liposomes in the solution was 0.6 mg lipid/ml.

Measurement of the zeta potential

The zeta potential of the liposomes was calculated from the electrophoretic mobility with the aid of the Helmholtz-Smoluchowski equation [17]. The electrophoretic mobility was measured by a microelectrophoretic apparatus (Cytopherometer, Carl Zeiss, F.R.G.). The measurement was made by the direct observation of the velocity of the liposomes at the 'stationary layer' in the flat cell at 22°C. The details of the measurement are the same as those in a previous paper [18].

Results and Discussion

In Fig. 1A, the magnitude of chemotactic response of native cells to various inorganic salts is plotted as a function of logarithmic concn. of salts. Above respective threshold concentrations, the cells exhibit negative chemotaxis. The magnitude of the response increases almost linearly with a similar slope for various salts. Hereafter the chemotactic sensitivity is represented by the threshold concentration. The threshold concentrations of NaCl and KCl are $1 \cdot 10^{-3}$ and $1 \cdot 10^{-4}$ M, respectively. The threshold concentrations of salts of polyvalent cations are lowered with an increase of valence of cations; those of $MgCl_2$, $CaCl_2$ and $LaCl_3$ are $7 \cdot 10^{-6}$, $1 \cdot 10^{-5}$ and $5 \cdot 10^{-8}$ M, respectively. Note that there is no significant difference in the threshold between $MgCl_2$ and $CaCl_2$.

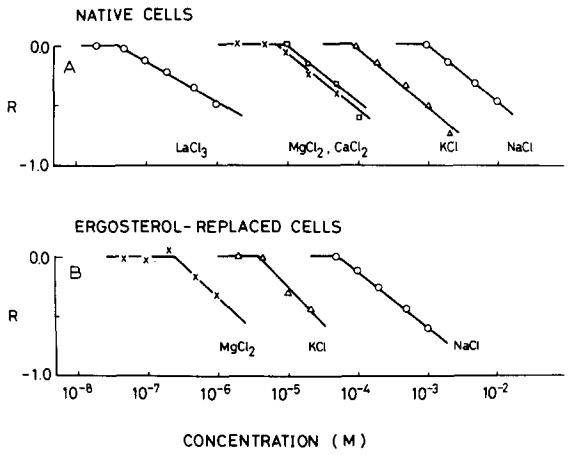

Fig. 1. The magnitude of chemotactic responses of native and ergosterol-replaced cells to inorganic salts. A, the responses of the native cells; B, the responses of the ergosterol-replaced cells.

The ergosterol-replaced cells showed motile activity quite similar to that of native cells. The chemotactic responses of the replaced cells to various salts are shown in Fig. 1B. As seen from the figure, the thresholds of the inorganic salts in the replaced cells are lower than respective thresholds in the native cells by a factor of 1/20—1/35, i.e., the replaced cells respond to the salts 20—35 times more sensitively compared with the native cells.

Fig. 2A and 2B showed the chemotactic responses of the native and the replaced cells to a number of hydrophobic compounds. The most responses are more or less sensitized by the ergosterol replacement. It is striking that the

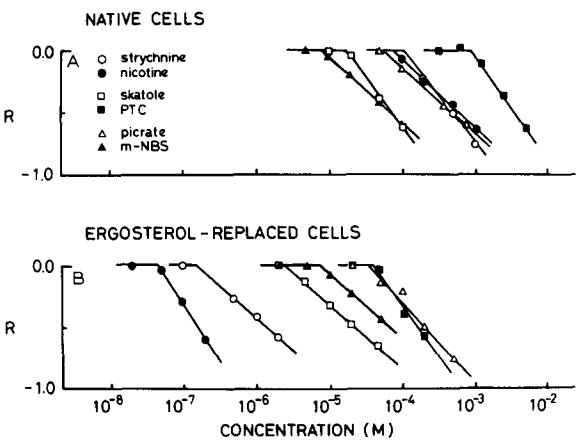

Fig. 2. The magnitude of chemotactic responses of native and ergosterol-replaced cells to hydrophobic compounds. A, the responses of the native cells; B, the responses of the ergosterol-replaced cells. The data for PTC and sodium picrate in the native cells shown in Fig. 2B were taken from a previous paper [11]. Abbreviations: PTC, phenylthiocarbamide; *m*-NBS, sodium *m*-nitrobenzenesulfonate.

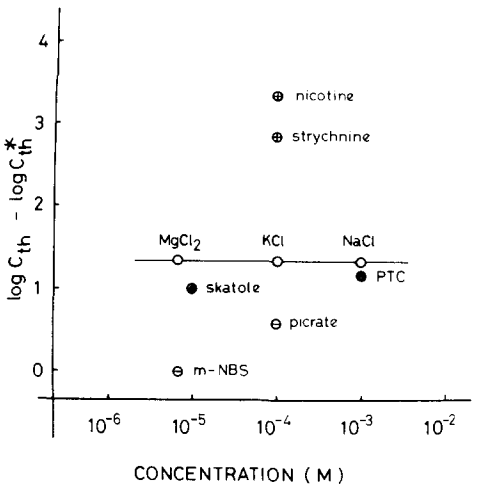

Fig. 3. Shift in the chemotactic threshold caused by the ergosterol replacement (log C_{th} − log C^*_{th}) plotted against C_{th}, where C_{th} and C^*_{th} stand for the threshold in the native cells and in the replaced cells, respectively.

replaced cells can recognize strychnine nitrate or nicotine 1000 times more sensitively than can the native cells.

In Fig. 3, the difference in the thresholds between the native and replaced cells (a shift of threshold caused by the replacement) is plotted against the thresholds of the native cells. As seen from the figure, the responses to the inorganic salts are sensitized to a similar degree. In contrast to the salt reception, the degree of sensitization in the responses to hydrophobic compounds is not uniform. The compounds are classified into three groups, based on the degree of sensitization. The responses to the negatively charged compounds (sodium *m*-nitrobenzenesulfonate and sodium picrate) are not or slightly sensitized. Those to the neutral compounds (phenylthiocarbamide and skatole) are sensitized about 10 times. Those to the positively charged compounds (strychnine nitrate and nicotine) are sensitized about 1000 times. The above results, showing that the degree of sensitization depended on the electrical charge of the stimulus chemicals imply that the electrostatic affinity of stimulus chemicals to the surface membrane was affected by the ergosterol replacement. Furthermore, the findings that the responses to the neutral compounds were also sensitized and that the responses to the positively charged hydrophobic compounds were sensitized to much larger degree than were those to the inorganic salts suggest that non-electrical part of the affinity was also affected by the replacement.

According to Nozawa et al. [12], the replacement of tetrahymanol induced a profound alteration in phospholipid class composition, in the degree of saturation of fatty acids and in the fluidity of the surface membrane. These results suggest that the sensitization of chemotactic responses by the ergosterol replacement comes from an alteration in the chemical and/or physical properties of the membrane lipids. In order to examine the effect of the ergosterol replacement on the interaction between the membrane lipids and stimulus chemicals in a simple system, liposomes were prepared from the native and

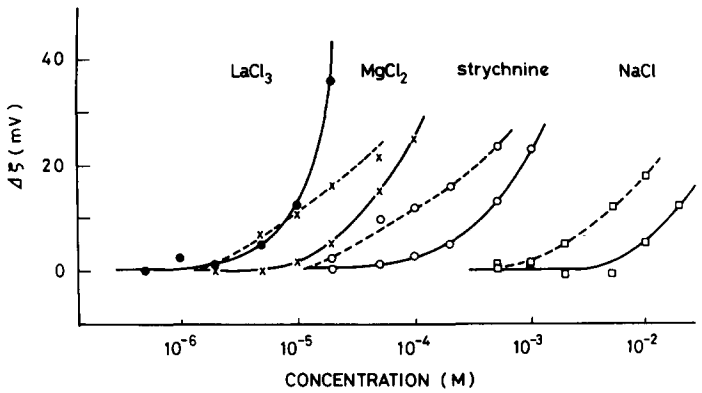

Fig. 4. Changes in the zeta potential, $\Delta\zeta$, of liposomes of total lipids from the native cells (solid curves) and the replaced cells (dotted curves) as a function of concentration of chemical stimuli. Measurements were performed at least three times, and gave almost identical results. The data shown here are typical data thus obtained.

replaced cells. The interaction between the membrane lipids and chemical stimuli was examined by measuring the zeta potential of the liposomes in the presence of chemicals of varying concentrations with the aid of microelectrophoretic apparatus.

Solid curve in Fig. 4 show the zeta potential of the liposomes made of lipids from the native cells as a function of concentration of NaCl, $MgCl_2$, $LaCl_3$ and strychnine nitrate. The zeta potential of the liposomes in the control solution (1 mM Tris-HCl buffer, pH 7.0) was -74 ± 2 mV. The zeta potential changed to the positive direction with an increase of concentration of the chemicals applied above a certain concentration for respective chemicals. Since it is hard to determine exactly the critical concentration where the zeta potential begins to change, we use the concentration required for a rise of 5 mV (referred to as C_5) as a measure for the effectiveness of chemicals on the zeta potential. The values of C_5 for NaCl, $MgCl_2$, $CaCl_2$ (data is not shown) and $LaCl_3$ are $1 \cdot 10^{-2}$, $2 \cdot 10^{-5}$, $4 \cdot 10^{-5}$ and $5 \cdot 10^{-6}$ M, respectively. The above results imply that C_5 is decreased with an increase of valence of cations of the inorganic salts. The value of C_5 of strychnine nitrate which is monovalent cation is $2 \cdot 10^{-4}$ M. This value is much lower than C_5 of NaCl. The above relation about the effectiveness of various inorganic salts and strychnine nitrate on the zeta potential resembles those on the chemotatic responses.

Dotted curves in Fig. 4 show the zeta potential of the liposomes made of lipids from the replaced cells as the function of concentration of inorganic salts and strychnine nitrate. The zeta potential in the control solution is -74 ± 2 mV, which is identical to the value of the liposomes from the native cells. The values of C_5 of NaCl, $MgCl_2$ and strychnine nitrate are $2 \cdot 10^{-3}$, $4 \cdot 10^{-6}$ and $3 \cdot 10^{-5}$ M, respectively. The values of C_5 of inorganic salts for the liposomes from the replaced cells are approximately five times less than respective values for the liposomes from the native cells. The values of strychnine nitrate concentration for the liposomes from the replaced cells are about 7—8 times less than that for the liposomes from the native cells. These results imply that the changes in the zeta potential of the liposome partly simulate the changes in

chemotaxis caused by the ergosterol replacement, no matter how the lipids extracted from the whole cells are different from those of the surface membrane [19]. The above results suggest that the liposomes from the ergosterol-replaced cells have stronger affinity to the inorganic salts and strychnine nitrate than do those from the native cells and that the changes in the affinity of the lipids to the stimuli contribute to the sensitization of the chemotactic responses by the ergosterol replacement, although the changes in the affinity of strychnine nitrate to the liposomes were not so remarkable as the chemotactic responses. Interaction between the liposomes and the neutral compounds could not be examined by the present method, since the neutral compounds did not induce a change in the zeta potential of the liposomes.

As described above, the zeta potentials of the liposomes from the native and replaced cells in the control buffer solution were practically the same as each other. This implies that the surface charge densities of both liposomes in the control solution are similar to each other since the zeta potential is a function of ionic strength and surface charge density. Nevertheless, cations showed stronger affinity to the liposomes from the replaced cells than those from the native cells. These results cannot be explained in terms of the effect of ionic strength in the bulk solution on electrical double layer at the membrane surface. Probably the cations bind to the surface of the liposomes. The increase of the binding affinity in the liposomes from the replaced cells may come from changes in the chemical composition and/or physical state such as cluster formation or fluidity of the membrane lipids by the ergosterol replacement. Further study will be needed to elucidate the detailed mechanism for the sensitization of the chemotactic response by the ergosterol replacement.

In a separate paper (unpublished results), we show that the membrane potential of *Tetrahymena* measured by a fluorescence dye, rhodamine 6G, changes in parallel with the chemotactic response. In addition, we show that the fluorescence of rhodamine 6G reflects changes in the surface potential (or the phase boundary potential) and those in the intramembrane diffusion potential, which in turn reflects the total membrane potential across the membrane. Hence it has been concluded that both the surface potential and the intramembrane diffusion potential contribute to the changes in the membrane potential in *Tetrahymena* associated with the chemotactic responses. The results observed with the liposomes in the present study suggested that the adsorption of the chemicals to the membrane lipids plays an important role in the initiation of chemotactic response. These results can be understood under the assumption that a change in the phase boundary potential at the membrane/solution interface induced by the adsorption contributes directly to the membrane potential.

References

1 Beidler, L.M. (1971) in Handbook of Sensory Physiology (Beidler, L.M., ed.), Vol. IV—2, pp. 200—220, Springer-Verlag, Berlin

2 Adler, J. (1974) in Biochemistry of Sensory Functions (Jaenicke, L., ed.), pp. 107—131, Springer-Verlag, Berlin

3 Hato, M., Ueda, T., Kurihara, K. and Kobatake, Y. (1976) Biochim. Biophys. Acta 426, 73—80

4 Koshland, D.E., Jr. (1974) in Biochemistry of Sensory Functions (Jaenicke, L., ed.), pp. 133—155, Springer-Verlag, Berlin

5 Dastoli, F.R. and Price, S. (1966) Science 154, 905—907
6 Hiji, Y. (1975) Nature (Lond.) 256, 427—429
7 Koyama, N. and Kurihara, K. (1972) Nature (Lond.) 236, 402—404
8 Koyama, N. and Kurihara, K. (1972) Biochim. Biophys. Acta 228, 22—26
9 Kamo, N., Miyake, M., Kurihara, K. and Kobatake, Y. (1974) Biochim. Biophys. Acta 367, 1—10
10 Ueda, T. and Kobatake, Y. (1977) J. Membrane Biol. 34, 351—368
11 Ataka, M., Tsuchii, A., Ueda, T., Kurihara, K. and Kobatake, Y. (1978) Comp. Biochem. Physiol. 61A, 109—115
12 Nozawa, Y., Fukushima, H. and Iida, H. (1975) Biochim. Biophys. Acta 406, 248—263
13 Fukushima, H., Watanabe, T. and Nozawa, Y. (1976) Biochim. Biophys. Acta 436, 249—259
14 Kasai, R., Kitajima, Y., Martin, C.E., Nozawa, Y., Skriver, L. and Thompson, G.A. (1976) Biochemistry 15, 5228—5233
15 Bligh, E.G. and Dyer, W.J. (1959) Can. J. Biochem. Physiol. 37, 911—917
16 Folch, J., Lees, M. and Stanley, G.H.S. (1957) J. Biol. Chem. 226, 497—509
17 Davies, J.T. and Rideal, E.K. (1961) Interfacial Phenomena pp. 312, Oxford
18 Kamo, N. Miyake, M., Kurihara, K. and Kobatake, Y. (1974) Biochim. Biophys. Acta 367, 11—23
19 Nozawa, Y. and Thompson, G.A., Jr. (1971) J. Cell Biol. 49, 712—721