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Effects of External Cations on Membrane Potential Change of *Opalina*¹⁾

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(With 15 Text-figures)

In *Opalina*, the relationship between the ciliary activity and the membrane potential change was already reported by some investigators (Kinosita, 1954; Ueda, 1961; Yamaguchi and Okumura, 1962). But there are discrepancies among the findings reported by those investigators. According to Kinosita (1954) and Ueda (1961) the inside negativity of the cell decreased accompanied by a change in the direction of the effective stroke of cilia or the reversal of the ciliary beating. On the other hand, Yamaguchi and Okumura (1962) reported the membrane hyperpolarization occurred in the cell instead of the membrane depolarization during activity. It has been well known that an unusual potential change (hyperpolarizing spike) recorded from the vacuole of *Noctiluca* (Hisada, 1957; Chang, 1960; Eckert and Sibaoka, 1967a, b, 1968) and the membrane hyperpolarization was produced by application of mechanical stimulation to the posterior part of *Paramecium* and *Euplotes* (Naitoh and Eckert, 1969a, b). But it was found by Eckert and Sibaoka (1968) that the action potential of *Noctiluca* belongs to the usual type of responses, and the responses have usual polarities, provided the vacuole is designated as an "external" cytoplasmic compartment.

In *Paramecium caudatum*, it was found that the action potential occurred spontaneously accompanied by reversal of direction of ciliary beating in the solution of Ba-Ca (Kinosita *et al.*, 1964a, b) and the action potential was produced by application of outward current (Naitoh and Eckert, 1967, 1968a, b).

In *Opalina*, the action potential (diphasic change in membrane potential) was recorded by application of BaCl₂ solution to the cell, and under some conditions the action potential occurred spontaneously in the adaptation medium. It is a characteristic in *Opalina* that the repeated subthreshold-like depolarization (monophasic change in membrane potential) occurred spontaneously in the adaptation medium. In the present experiments, the effects of external cations

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on these membrane potential change were examined and discussed. Further, it was examined what a cause elicits the monophasic membrane potential change in *Opalina*.

Material and Methods

Opalina, obtained from the rectum of the frog, *Rana japonica*, was used for the experiments. The animals were washed well with the adaptation medium containing 110 mM NaCl, 2 mM KCl, 2 mM CaCl₂ and 5 mM tris buffer (pH=7.4) and kept in the medium for more than one hour before the experiments, and in some cases, the animals were used soon after washing with the adaptation medium.

Glass capillary microelectrodes of tip diameter less than 0.5 μ filled with 2 M KCl solution were used for an intracellular microelectrode and an indifferent electrode. Potential difference between the microelectrode inserted into the central part of the cell and the indifferent electrode outside the cell, both of which were connected with Ag-AgCl electrodes, was measured by the use of a high input impedance amplifier (Nihon Kodens MZ-3B) and of an oscilloscope (Nihon Kodens, VC-6 type).

Mechanical stimuli were applied to selected portions of dorsal side of *Opalina* by means of a glass stylus with a tip diameter of 20–30 μ which was driven by vibrator. For one stimulus, only one cycle of the vibrator was activated by one pulse of duration 50 msec from a pulse generator.

The experiments were carried out at room temperatures ranging from 20° to 24°C.

Results

A. Spontaneous membrane potential change.

In general, the spontaneous monophasic change in membrane potential of comparatively slow time course appeared repeatedly from *Opalina* in the adaptation medium (Fig. 1, A). This type of the membrane potential change seems the same as that already reported by some investigators (Kinosita, 1945; Koshtoyants and

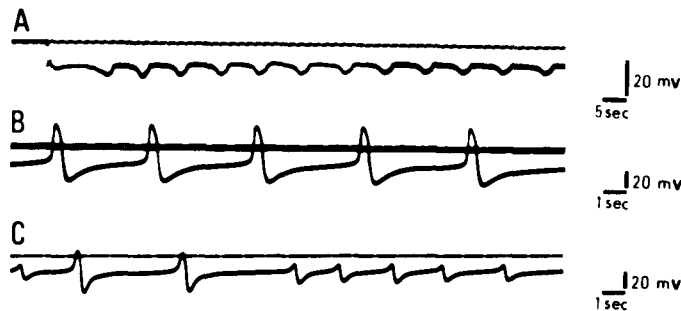


Fig. 1. Spontaneous membrane potential change of *Opalina*. A: Monophasic change in membrane potential recorded from the cell in the adaptation medium. B and C: Diphasic change in membrane potential recorded from the cell collected from the autumn or winter frog, soon after transference of the cell into the adaptation medium.

Kokina, 1958; Yamaguchi and Okumura, 1962). The membrane potential change is closely correlated to the change in the beating direction of effective stroke of cilia. Namely, the inclination of the line of metachronal wave to the lateral axis of the animal begins to increase at the moment when the membrane is hyperpolarized, as reported by Kinoshita (1954) and Yamaguchi and Okumura (1962), the metachronal wave disappears and the direction of effective stroke of cilia is reversed when the membrane is depolarized, as reported by Ueda (1961).

Occasionally, the spontaneous diphasic change in membrane potential was recorded from *Opalina* collected from the frog before hibernation, soon after transference of the cell into the adaptation medium from the frog rectum (Fig. 1, B and C). Such an *Opalina* was obtained only from the frog of which rectum contains less water than the other. This membrane potential change has following characteristics: (1) the membrane is rapidly depolarized and the cell becomes temporarily inside positive (overshoot above the zero level) after the inside negativity of the cell decreased as much as about 10 mV, (2) the overshoot decreases and the cell becomes inside negative again, (3) and at last the membrane potential returns to the resting level. The diphasic change in membrane potential was transformed into the periodical and monophasic membrane potential change about one hour after immersion of *Opalina* in the adaptation medium.

This type of change in membrane potential was rarely produced by electrical current stimulation, while the potential change with the overshoot appeared in *Opalina* in the adaptation medium as mentioned above.

B. Monophasic membrane potential change.

Kinoshita (1954) and Ueda (1961) reported that in *Opalina*, the increase in the inclination of the line of metachronal wave to the lateral axis of the animal occurs in close association with the decrease in inside negative potential. On the other hand, Yamaguchi and Okumura (1962) reported that the membrane potential change of *Opalina* is a hyperpolarized action potential. It seems that the discrepancy of statements among those investigators is due to difference of the base line regard as the resting membrane potential. According to Nakatani (1970), the mean value of the membrane potentials and the maximum membrane potential levels of *Opalina* in the adaptation medium, without the membrane potential change was -25 ± 1.6 mV and that with the membrane potential change -21.8 ± 0.8 mV. This fact may suggest that the minimum membrane potential level is not the resting level, in the case of monophasic change in membrane potential. From this reason, it was examined whether the monophasic membrane potential change is depolarization or hyperpolarization.

The solution contains 110 mM choline chloride, 2 mM CaCl₂ and 5 mM tris buffer (pH=7.4) was suitable for the examination, because *Opalina* in this solution showed comparatively small and stable membrane potential, the metachronal wave disappeared and the beating direction of effective stroke was reversed for about one minute by mechanical shock of impaling electrode, then the inside negativity in-

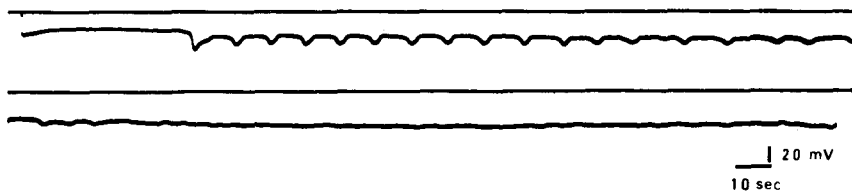


Fig. 2. Membrane potential change of *Opalina* in the solution containing 110 mM choline chloride, 2 mM CaCl_2 and 5 mM tris buffer.

creased and the spontaneous monophasic change in membrane potential appeared. The amplitude of this membrane potential change decreased gradually until the potential change disappeared completely, the inside negativity increased, and the membrane settled in a stable state (Fig. 2). In this case, the mean values of the comparatively small inside negativity soon after insertion of the electrode, the second maximum inside negativity after spontaneous change in membrane potential appeared, the minimum inside negativity soon after the second maximum one and the membrane potential of stable state are -22.1 ± 1.5 mV, -41.5 ± 2.6 mV, -34.6 ± 2.8 mV and -46.9 ± 2.7 mV, respectively.

C. Time change of membrane potential.

Ueda (1961) reported the time change of membrane potential of *Opalina*. But the relationship between the time change and spontaneous change in membrane potential is not clear from his report.

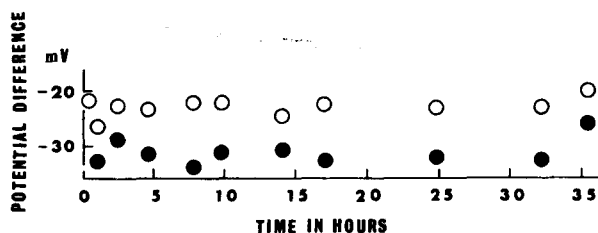


Fig. 3. Time change of the membrane potential (hollow circles) and the maximum membrane potential (solid circles) of *Opalina* during membrane potential change in the adaptation medium after the animal was taken out from the frog's rectum. Each plot was obtained from mean value of 10 individuals. Temperature: 24°C .

Result of the successive measurements of the monophasic membrane potential change is shown in Figure 3. Both of the minimum and maximum membrane potential during the spontaneous membrane potential change of *Opalina*, kept in the adaptation medium, were rather constant for 30 hours, then decreased, and finally fell off.

D. Effects of external Ca-, and K-ions concentration on the spontaneous change in membrane potential.

External Ca- or K-ions concentration was changed by substituting those ions for an equivalent amount of Na-ions in the adaptation medium. Inside negativity was increased by increase of Ca-ions concentration in the external solution more than that of the normal adaptation medium. And the spontaneous membrane potential change disappeared at the concentration of 80 mM Ca-ions. On the other hand, the change in level of membrane potential by the decrease in external Ca-ions concentration to 0.1 mM, is not conspicuous, compared with that in the normal adaptation medium. Diphasic change in membrane potential with the overshoot was appeared by decrease of external Ca-ions to 0.001 mM, and the amplitude of the membrane potential change increased in the Ca-free medium (Figs. 4 and 5). The monophasic membrane potential change of almost the same grade appeared spontaneously at concentrations of 0.05 mM to 24 mM external Ca-ions. The mean values of the membrane potential (-38.9 mV) of the cell in which the membrane potential change disappeared in the solution contains 80 mM Ca-ions, and those of the maximum membrane potential (-38.0 mV) of the diphasic change in membrane potential in the Ca-free medium, were almost the same. On the other hand, the frequency of membrane potential change was decreased by change of external Ca-ions concentration compared with that in the normal adaptation medium. The frequency increased in the solution of lower concentration than 0.1 mM external Ca-ions and reached the maximum value at 0.001 mM Ca-ions.

Both of the amplitude and the frequency of the membrane potential change were almost unchanged by application of the external solution contains K-ions of lower concentration than that in the normal adaptation medium. Further, almost the same type of spontaneous change in membrane potential with that in the normal adaptation medium, appeared in the K-free medium. On the other hand, both of the values of inside negativity of the maximum membrane potential and the minimum one decreased, in the K-rich (more than 16 mM) medium. Also, the amplitude of membrane potential change decreased in the K-rich

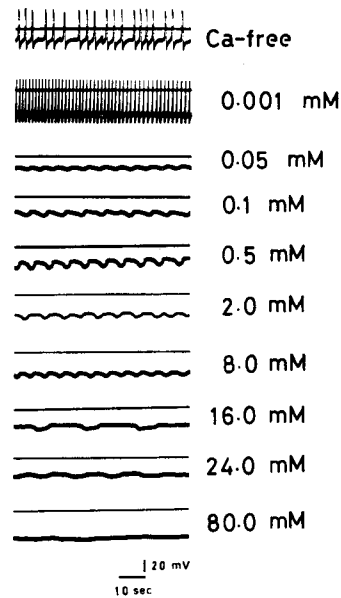


Fig. 4. Effects of external Ca-ions concentration on the membrane potential change. External Ca-ions concentration was changed by substitution of CaCl_2 for an equivalent amount of NaCl in the adaptation medium.

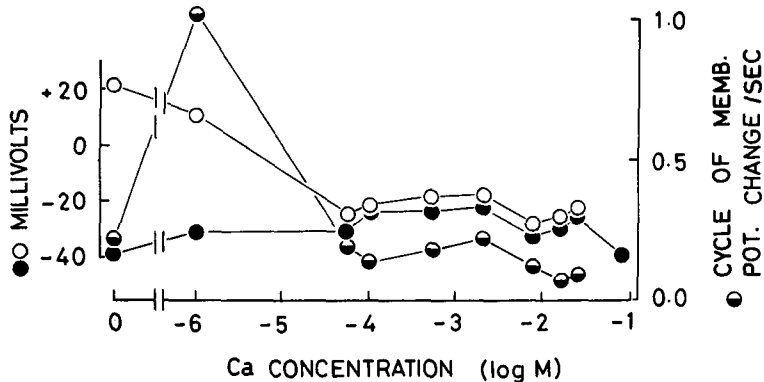


Fig. 5. The relationship among the membrane potential, the frequency of membrane potential change and the external Ca-ions concentration. External Ca-ions concentration was changed by substitution of CaCl_2 for an equivalent amount of NaCl in the adaptation medium. Each plot was obtained from mean value of 5 individuals. ●: Maximum membrane potential. ○: Minimum membrane potential. ●: Frequency of membrane potential change.

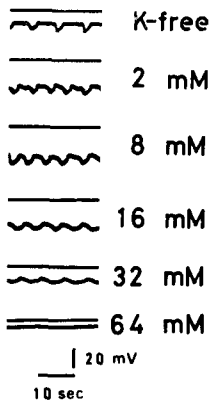


Fig. 6. Effects of external K-ions concentration on the membrane potential change. External K-ions concentration was changed by substitution of KCl for an equivalent amount of NaCl in the adaptation medium.

medium. The spontaneous membrane potential change disappeared and the inside negativity decreased in the solution contains 64 mM K-ions (Figs. 6 and 7). The frequency of the membrane potential change was scarcely changed in the solutions of concentration from K-free to 16 mM KCl, but the frequency rapidly decreased in the solution contains 32 mM K-ions.

E. Mechanical stimulation of *Opalina*.

It is known that in *Paramecium caudatum* and *Euplotes*, the membrane was depolarized accompanied with change in the beating direction of effective stroke of cilia by mechanical stimulus applied to the anterior part of those animals, and the membrane was hyperpolarized accompanied with an increase in the frequency of ciliary beat in the normal direction by mechanical stimulus applied to the posterior part (Naitoh and Eckert, 1969a, b).

Also, in *Opalina* graded mechanoreceptive potentials were recorded. The membrane was depolarized accompanied with change in the beating direction of

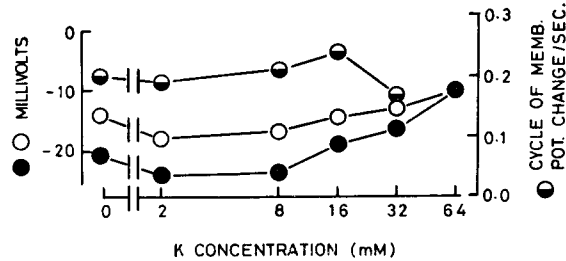


Fig. 7. The relationship among the membrane potential, the frequency of membrane potential change and the external K-ions concentration. External K-ions concentration was changed by substitution of KCl for an equivalent amount of NaCl in the adaptation medium. Each value was obtained from mean value of 5 individuals.

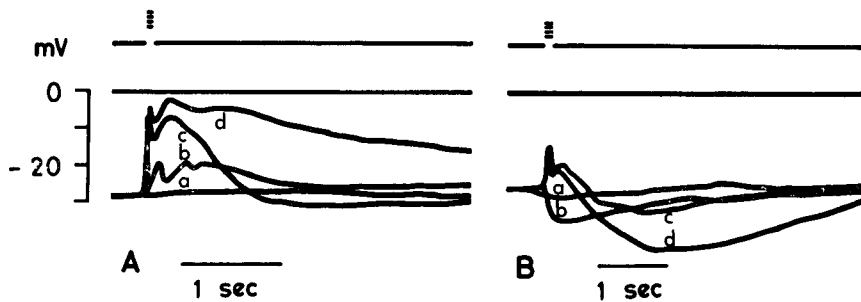


Fig. 8. Mechanoreceptor potentials of *Opalina* in the adaptation medium. A: Anterior receptor potentials elicited by mechanical stimulation of the anterior part. B: Posterior receptor potentials elicited by mechanical stimulation of the posterior part. Deflections in the upper trace shows the relative intensity of current pulses to drive the small glass rod mechanically.

cilia by application of mechanical stimulus to the anterior part of the animal (Fig. 8, A). When mechanical stimulus of comparatively lower intensity applied to the posterior part of the cell, the membrane was hyperpolarized, but to the strong stimulus the membrane was depolarized and successively hyperpolarized (Fig. 8, B, c and d). It is known that in *Paramecium caudatum* the electrical response is not generated when the mechanical stimulus applied to the central part of the cell (Naitoh and Eckert, 1969a). In the posterior part, electrical responses were generated by lower stimulus than that in the case of the anterior part, as reported by Naitoh and Eckert (1969a) in *Paramecium caudatum*. But it is not clear whether in *Opalina* there is a neutral zone or not, because comparatively strong stimulus as much as the cell vibrates violently was needed for generation of the electrical response.

F. Membrane potential change produced by Ba-ions.

It is well known that *Paramecium caudatum* elicits the spontaneous change in membrane potential with the overshoot in the solution containing Ba-ions (Kinosita *et al.*, 1964a, b, 1965), or action potentials were generated by application of electric current (Naitoh and Eckert, 1967, 1968a, b).

The repeated diphasic change in membrane potential was produced by application of 8 mM BaCl₂ (dissolved in the adaptation medium) to the cell in which no active change in the membrane potential was observed. In this, case the inside negativity of the cell was decreased as much as about 10 mV by application

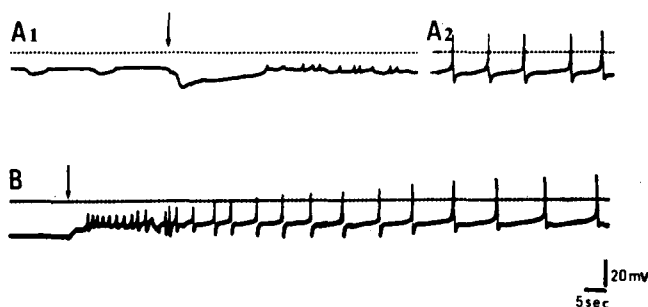


Fig. 9. Diphasic change in membrane potential produced by application of BaCl₂ solution to *Opalina*. Arrows show the beginning of application of the BaCl₂ solution. A: The animal eliciting the monophasic change in membrane potential spontaneously in the adaptation medium. A₁: Comparatively small amount of the BaCl₂ solution was applied to *Opalina*. A₂: Enough amount of the BaCl₂ solution was applied to the cell after about 5 minutes from A₁. B: The animal elicited no membrane potential change in the adaptation medium.

of the BaCl₂ solution (Fig. 9, B). When BaCl₂ solution was applied to the cell in which the spontaneous monophasic change in membrane potential was produced, the membrane was further hyperpolarized once, then the inside negativity decreased, and the repeated diphasic change in membrane potential appeared spontaneously (Fig. 9, A). The hyperpolarization accompanied with application of the BaCl₂ solution may be due to a mechanical stimulus caused by the current of the external solution in the trough. It is known that the augmentation of ciliary activity of *Opalina* was produced by the current of external solution (Naitoh, 1963).

G. Effects of external BaCl₂ concentration on the membrane potential change.

It is clear from the above mentioned result that the change in the external Ba-ions concentration has effects on the membrane potential change of *Opalina*. Prior to investigate what kind of cations are involved in the components of the depolarization and the hyperpolarization of the diphasic change in membrane potential, and in the monophasic one, the effects of external Ba-ions concentration

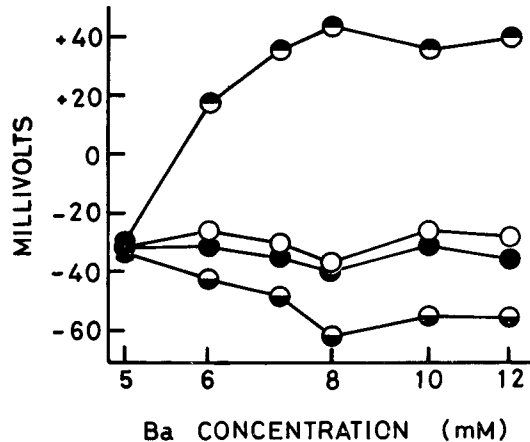


Fig. 10. Relationship between the membrane potential and the Ba-ions concentration in the adaptation medium. ○: Firing level. ●: Subthreshold-like depolarization. ◐: Overshoot. ◑: Undershoot. Each plot was obtained from mean value of 5 individuals.

on the membrane potential change were examined. The result is shown in Figure 10. Repeated diphasic changes in membrane potential were produced by application of the solution which contains Ba-ions more than 5 mM. And its amplitude was increased by increase of the external Ba-ions concentration and reached to the maximum amplitude at the Ba-ions concentration above 8 mM.

Now, each level of membrane potentials is defined as follows; the levels of the stable membrane potential before firing and of smaller inside negativity than the resting level, the abrupt decrease in inside negativity of the cell, the peak potential of depolarization, and the maximum inside negativity succeed the depolarization, are "subthreshold-like depolarization", "firing level", "maximum depolarization" (overshoot; inside positive at this moment) and "maximum hyperpolarization" (undershoot), respectively. According to this definition, the effect of the external concentration of Ba-ions within 5 mM to 8 mM on the maximum depolarization and the undershoot was large, but both of the subthreshold-like depolarization and the firing level were scarcely changed by change of external Ba-ions concentration.

H. Effects of K- and Ca-ions concentrations on the membrane potential change produced by Ba-ions.

Effects of Ba-ions concentration on the membrane potential change of *Opalina* in the solution which K-ions were increased from 2 mM to 8 mM substituted by Na-ions in the adaptation medium, are shown in Figure 11. The amplitude of membrane potential change was increased by increase of Ba-ions concentration and its magnitude reached to the maximum at 20 mM Ba-ions. The concentration of Ba-ions produced the diphasic change in membrane potential with the maximum

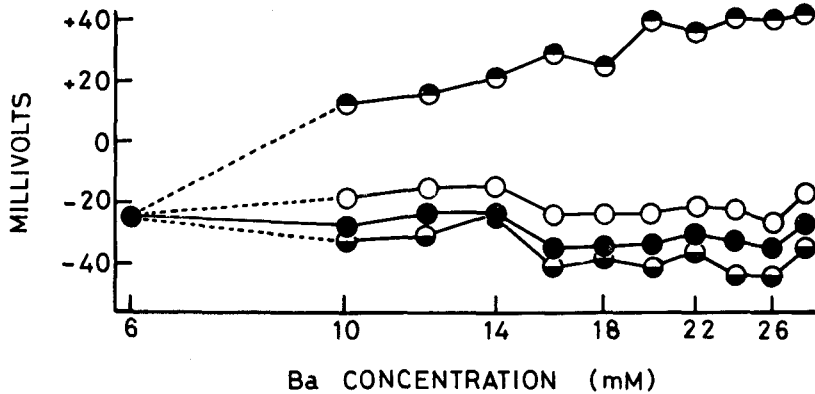


Fig. 11. Relationship between the membrane potential and the Ba-ions concentration in the high K-ions medium (8 mM KCl, 104 mM NaCl, 2 mM CaCl₂ and 5 mM tris buffer). ○: Firing level. ●: Subthreshold-like depolarization. ●: Overshoot. ○: Undershoot. Each plot was obtained from mean value of 5 individuals.

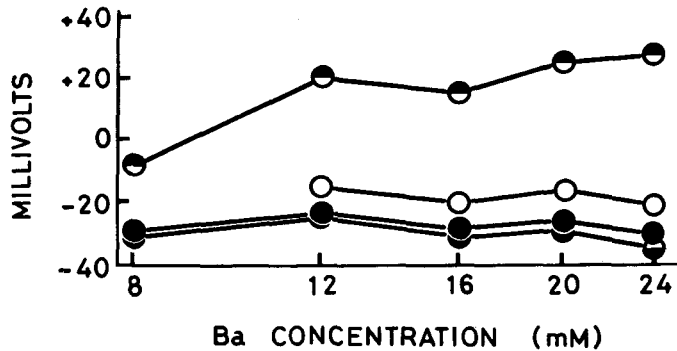


Fig. 12. Relationship between the membrane potential and the Ba-ions concentration in the high K-ions medium (containing 16 mM KCl, 96 mM NaCl, 2 mM CaCl₂ and 5 mM tris buffer). ○: Firing level. ●: Subthreshold-like depolarization. ●: Overshoot. ○: Undershoot. Each plot was obtained from mean value of 5 individuals.

amplitude in this solution was greater than that in the adaptation medium. When the maximum membrane potential change in the solution containing 8 mM K-ions was compared with that in the adaptation medium, the levels of the overshoot, the firing level and the subthreshold-like depolarization were scarcely changed, but the level of the undershoot decreased as much as about 15 mV.

In the solution which contains 16 mM K-ions, the excitability was rather decreased as compared with that in the solution containing 8 mM K-ions, and

spontaneous diphasic change in membrane potential was disappeared by Ba-ions above 28 mM. The result is shown in Figure 12. In this case, the level of the undershoot decreased remarkably as compared with that of the control. Further, the value of the overshoot was rather smaller as compared with that in the solution containing 8 mM K-ions, but it may be thought that the spontaneous change in membrane potential disappeared in the solution of lower concentration of Ba-ions than the solution of Ba-ions in which the maximum overshoot was produced.

Effects of Ba-ions concentration on the membrane potential change of the animal in the solution in which Ca-ions increased from 2 mM to 8 mM or 16 mM substituted Na-ions in the adaptation medium, are shown in Figure 13. In the solution contains 8 mM Ca-ions, the spontaneous change in membrane potential was produced by only application of Ba-ions concentration ranged in 16 mM to 28 mM. In this case, the amplitude of the membrane potential change reached to the maximum at the concentration of Ba-ions from 20 mM to 24 mM. The levels

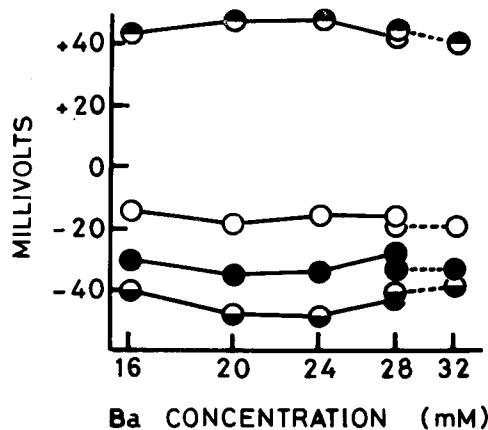


Fig. 13. Relationship between the membrane potential and the Ba-ions concentration in the high Ca-ions medium. Solid lines: 8 mM CaCl_2 , 101 mM NaCl, 2 mM KCl and 5 mM tris buffer. Broken lines: 16 mM CaCl_2 , 89 mM NaCl, 2 mM KCl and 5 mM tris buffer. ○: Firing level. ●: Subthreshold-like depolarization. ●: Overshoot. ○: Undershoot. Each plot was obtained from mean value of 10 individuals.

of the undershoot and the firing level were slightly decreased as compared with that of the control, but the magnitude of the overshoot was more increased than that in the control.

In the solution containing 16 mM Ca-ions, the range of the Ba-ions concentration which produced the diphasic change in membrane potential was narrower than that in the solution containing 8 mM Ca-ions. Namely, the membrane potential change was produced by application of Ba-ions at high concentrations than 28 mM,

I. *Effects of change in the external Na-ions concentration (and osmotic pressure) on the frequency of the membrane potential change.*

It is interesting to examine what a cause produces the repeated spontaneous monophasic change in membrane potential of *Opalina* in the normal frog Ringer's solution or the choline chloride solution. *Amoeba*, *Paramecium* and many other protozoa have one or a few contractile vacuoles in the cell, which regulate the internal osmotic pressure with its periodic pulsation. But, *Opalina* used in these experiments has no contractile vacuole or the excretory organella. It may be thought that the animals regulate the internal osmotic pressure through the cell surface. So, the effect of change in the external Na-ions concentration (accompanied with change in the osmotic pressure of external medium) on the frequency of the periodic spontaneous monophasic change in membrane potential was examined.

The osmotic pressure of the external solution was changed by change of NaCl concentrations in the adaptation medium (concentrations of KCl and CaCl₂ were kept constant at 2 mM). The frequency of the membrane potential change decreased, when the external osmotic pressure increased, and *vice versa*. The relationship between the frequency of the membrane potential change and the external concentration of NaCl, is shown in Figure 14. As clear from this Figure,

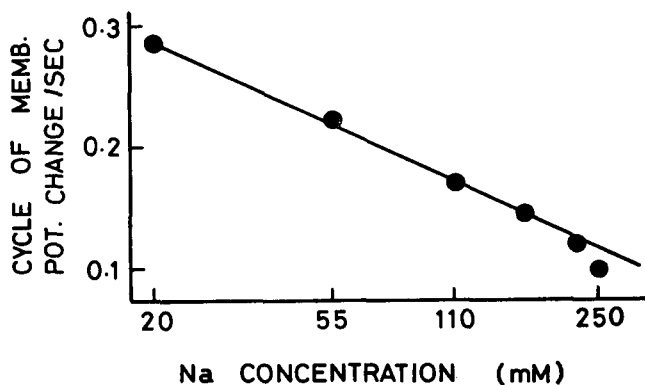


Fig. 14. Effects of change in the external osmotic pressure on the frequency of spontaneous change in membrane potential in *Opalina*. The concentration of NaCl was changed in the adaptation medium, but the concentration of KCl and CaCl₂ kept constant at 2 mM. Each plot was obtained from mean value of 10 individuals.

there is a linear relationship between the frequency and the external concentration of NaCl within a range from 20 mM to 165 mM. Further, it is a noticeable phenomenon that the repeated monophasic change in membrane potential was produced in the individual without change in membrane potential in the adaptation medium by application of the solution (contains 220 mM NaCl) with twice osmotic pressure as compared with that in the adaptation medium (Fig. 15).

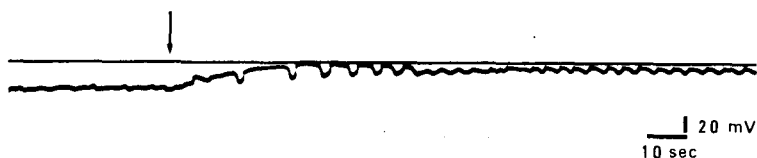


Fig. 15. Effect of the hypertonic solution on the membrane of *Opalina*. Arrow shows the beginning of application of the solution, having the twice osmotic pressure as compared with the adaptation medium, to the cell.

Discussion

In general, the repeated monophasic change in membrane potential was observed in *Opalina*. Kinoshita (1954), Koshtoyants and Kokina (1957) and Ueda (1961) reported that the membrane potential was decreased, *i.e.* a depolarized membrane potential change. On the other hand, Yamaguchi and Okumura (1962) described that the membrane potential change was a hyperpolarized action potential.

The mean value of inside negativity of *Opalina*, no active change in membrane potential was observed, was slightly larger than the maximum inside negativity during the monophasic change in membrane potential (Nakatani, 1970). In the solution composed of 110 mM choline chloride, 2 mM CaCl_2 and 5 mM tris buffer, the inside negativity of the cell after spontaneous monophasic change in membrane potential for few minutes and in the inactive state, was larger than the maximum membrane potential during the active membrane potential change. Further, the mean values of the firing level of the spontaneous diphasic change in membrane potential in the adaptation medium, and the minimum membrane potential of spontaneous monophasic change in membrane potential were -15.7 ± 0.6 mV and -17.3 ± 0.7 mV, respectively (Nakatani, 1970), and there is almost no difference between those values. The membrane potential was increased once, then the inside negativity decreased and the membrane action potential was fired, by application of BaCl_2 solution to the cell in which spontaneous monophasic change in membrane potential was produced (Fig. 9, A). On the other hand, the inside negativity was decreased immediately and the action potential was fired by application of the BaCl_2 solution to the cell in which no active change in the membrane potential was observed in the adaptation medium (Fig. 9, B). From those facts, it may be thought that there are two levels of the membrane potential in *Opalina* which is in comparatively stable state. The first is the level of large inside negativity with no active change in the membrane potential, and the second is the level of the minimum membrane potential during spontaneous monophasic change in membrane potential. In the present experiments, by increase of external Ca-ions concentration, the inside negativity was increased and the membrane potential

change was disappeared (Fig. 4), and this case may belong to the former. The inside negativity was decreased and the membrane potential change was disappeared by application of K-rich solution. In this case the inside negativity may decrease to the level of the latter. Namely, it may be thought that the effects of the Ca-rich and K-rich solution are the inhibition of the depolarized membrane potential change and the hyperpolarized one, respectively. If the level of the large inside negativity of the cell in which no active change in the membrane potential was observed, is considered as a base line, the monophasic change in membrane potential is a depolarized membrane potential change as reported by Kinoshita (1954), Koshtoyants and Kokina (1958) and Ueda (1961), but if the level of the minimum membrane potential during the membrane potential change is considered as a base line, the monophasic change in membrane potential is a hyperpolarized one as reported by Yamaguchi and Okumura (1962).

Effects of external Ca-ions on the membrane properties have been examined and discussed by many investigators. Frankenhaeuser and Hodgkin (1957) explained the effects of increase of external Ca-ions concentration, as that the membrane resistance and the resting potential are increased by Ca-ions and they result in the decrease of K- and Na-ions activation. Frankenhaeuser (1957) explained the same relation for the effects of Ca-ions on the myelinated nerve fibre of frog. Ito, Kuriyama and Tashiro (1970) reported that Ca-ions control potassium and sodium permeability in the longitudinal somatic muscle of earthworm. They explained the role of calcium in the longitudinal muscle membrane of lobworm. Ca-ions were tightly bound to the membrane and stabilized the membrane by reduction in the sodium permeability. Further, Ettiene (1970) investigated the relationship between the role of calcium and the contractility in *Spirostomum*, and came to conclusion that calcium plays the same role in the control of contractility in the animal that it does in striated muscle, *i.e.* Ca-ions are stored in vesicles in the inactive organism and that, on contraction they are released.

In the present experiments, the diphasic change in membrane potential occurred in the solution of low calcium or Ca-free and it changed into the monophasic change in membrane potential with increasing the external calcium concentration. The frequency of the membrane potential change was decreased, the inside negativity increased and the membrane stabilized by increase of the external calcium concentration. This fact may show that Na-ions permeability of the cell membrane was decreased by increase of external Ca-ions, as found by Ito and Tashiro (1970) in the longitudinal muscle membrane of lobworm. Further, from those effects of Ca-ions and the effects of external potassium concentration on the spontaneous monophasic change in membrane potential or the diphasic change in membrane potential was produced by Ba-ions, it may be thought that Na-ions acts as a current carrier during activity of the membrane in the adaptation medium.

Both of the overshoot and the undershoot of Ba-spike were decreased by increase of external K-ions concentration. This fact may show that the internal

K-ions concentration is increased with increase of external K-ions concentration, because it has been well known that K-ions easily permeate through the cell membrane. On the other hand, the diphasic change in membrane potential was not produced completely by comparatively low concentration of Ba-ions, in the Ca-rich solution. As pointed out by Naitoh and Eckert (1968b) in *Paramecium caudatum*, the increase of external calcium may increase surface-bound calcium and anionic binding sites on the membrane are saturated with calcium. Amounts of the bound calcium and the bound barium may depend on the rate of calcium and barium in the external solution. So, if barium increases in the external solution, calcium is released from the membrane.

What is the chief cause which produces the periodical and spontaneous monophasic change in membrane potential of *Opalina* in the adaptation medium? It may be possible that the regulation of the osmotic pressure is related to the change in the membrane potential, considered from the results of the relationship between the external osmotic pressure and the frequency of the membrane potential change summarized in Figures 14 and 15. It is known that the change in potential difference synchronous with pulsation of the contractile vacuole can be recorded from *Paramecium caudatum*, if the tip of electrode is inserted into the contractile vacuole or near it (Yamaguchi, 1960). According to Herfs (1922) the interval of pulsation of contractile vacuole was increased by increase of external osmotic pressure, in *Paramecium caudatum* or *Nyctotherus cordiformis*. It is thought enough that *Opalina* has a mechanism by which the internal osmotic pressure was actively regulated, because the animals live in the frog rectum and the osmotic pressure may change usually in the rectum. But the animals used in the present experiments have no contractile vacuole. Therefore, it is possible that the animals regulate the osmotic pressure through the cell membrane. And it may be thought that the change in membrane potential occurs accompanied with the excretion of water which entered into the cell, because the frequency of the membrane potential change is the higher, the medium the more hypotonic.

Summary

1) Periodical and spontaneous membrane potential change of *Opalina*, and the effects of external cations on the membrane potential change were examined.

2) The membrane potential change is correlated to the change of ciliary activity. The depolarization and repolarization of the membrane are accompanied with ciliary reversal and augmentation of normal stroke, respectively.

3) Repeated spontaneous monophasic change in membrane potential (sub-threshold-like depolarization) occurred in the adaptation medium. Spontaneous diphasic change in membrane potential was recorded from *Opalina* collected from autumn and winter frogs.

4) Diphasic change in membrane potential was produced by application of 8 mM BaCl₂ solution to the cell. When the BaCl₂ solution was applied to the

cell in which the spontaneous monophasic membrane potential change was produced, the inside negativity was further increased, and repeated diphasic change in membrane potential appeared. The inside negativity was decreased about 10 mV by application of the BaCl₂ solution to the cell in which no active change in the membrane potential was observed.

5) The frequency of the monophasic membrane potential change was decreased and the membrane was stabilized by increase of external calcium concentration.

6) The monophasic membrane potential change disappeared and the inside negativity was decreased by increase of external potassium concentration.

7) The magnitudes of the depolarization and the undershoot of Ba-spike were decreased by increase of external potassium concentration. When the external calcium concentration was increased, the diphasic change in membrane potential was not produced by application of comparatively low concentration of barium solution.

8) The monophasic change in membrane potential was produced by change of external osmotic pressure of the cell in which no active change in the membrane potential was observed in the adaptation medium. The frequency of the membrane potential change was the higher, the external NaCl concentration the lower (the osmotic pressure of the medium the lower).

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