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Authors: Oami, Kazunori, and Takahashi, Mihoko

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Membrane Potential Responses of *Paramecium caudatum* to External Na^+

Kazunori Oami* and Mihoko Takahashi

Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Ibaraki 305-8572, Japan

ABSTRACT—The membrane potential responses of *Paramecium caudatum* to Na^+ ions were examined to understand the mechanisms underlying the sensation of external inorganic ions in the ciliate by comparing the responses of the wild type and the behavioral mutant. Wild-type cells exhibited initial continuous backward swimming followed by repeated transient backward swimming in the Na^+-containing test solution. A wild-type cell impaled by a microelectrode produced initial action potentials and a sustained depolarization to an application of the test solution. The prolonged depolarization, the depolarizing afterpotential, took place subsequently after stimulation. The ciliary reversal of the cell was closely associated with the depolarizing responses. When the application of the test solution was prolonged, the wild-type cell produced sustained depolarization overlapped by repeated transient depolarization. A behavioral mutant defective in the Ca^{2+} channel, CNR (caudatum non reversal), produced a sustained depolarization but no action potential or depolarizing afterpotential. The mutant cell responded to prolonged stimulation with sustained depolarization overlapped by transient depolarization, although it did not show backward swimming.

The results suggest that *Paramecium* shows at least two kinds of membrane potential responses to Na^+ ions: a depolarizing afterpotential mediating initial backward swimming and repeated transient depolarization responsible for the repeated transient backward swimming.

Key words: *Paramecium caudatum*, membrane excitability, Na^+ ion, K^+-induced backward swimming

INTRODUCTION

The swimming behavior of the ciliate *Paramecium* is under the control of membrane electric events (Eckert, 1972; Naitoh, 1974). The ciliate shows backward swimming in response to a Ca^{2+} action potential by reversing the beat direction of the cilia (Naitoh, 1982; Machemer and Ogura, 1979; Oami, 1998), whereas it shows fast forward swimming to the membrane hyperpolarization by augmenting the ciliary beating (Naitoh and Eckert, 1973). The ciliary reversal is produced by activation of the Ca^{2+}-dependent reversal mechanism in the cilia (Naitoh and Kaneko, 1972).

In addition to the action potential-mediated backward swimming, the cells show behavioral responses to external ions, such as K^+, Na^+, Ba^{2+}, or H^+ (Naitoh, 1968; Kung, 1971a, b; Naitoh and Eckert, 1968; Doughty, 1986). Among these ions, the cells show prolonged backward swimming to the increase in K^+ ions. Recently, we identified the membrane electric events controlling the K^+-induced backward swimming of *Paramecium* (Oami and Takahashi, 2002, 2003b). The K^+-induced backward swimming is controlled by a novel Ca^{2+} conductance (K^+-induced Ca^{2+} conductance) distinct from the Ca^{2+} conductance responsible for the action potentials. The novel Ca^{2+} conductance was not activated by current-induced membrane depolarization, suggesting that it is not voltage-dependent, and its inactivation time course is two to three orders of magnitude longer than that of the Ca^{2+} conductance underlying the action potential. These findings indicate that *Paramecium* has a physiological receptor system to K^+ ions. The presence of the receptor system to K^+ ions allows us to expect the existence of receptor systems for other inorganic ions to which *Paramecium* is behaviorally sensitive.

The sensation of external Na^+ seems to be important for freshwater unicellular organisms, since Na^+ is a major ion in the external environment and its concentration changes in a very wide range (almost 0 in some pond water to more than 500 mM in seawater). In this paper, we examined the membrane potential responses of *Paramecium* to external Na^+ ions to understand the receptor systems to the ions and hence the mechanisms underlying the sensation of external inorganic ions in the ciliate. Behavioral mutant cells defective in voltage-gated Ca^{2+} channels (caudatum non

* Corresponding author: Tel. +81-29-853-6684;
FAX. +81-29-853-6614.
E-mail: oami@sakura.cc.tsukuba.ac.jp

reversal; CNR, Takahashi *et al*., 1985) were employed as the negative control of the experiments. CNR cells do not show backward swimming to the applied stimuli.

Some of the results in this study have been presented in abstract form (Oami and Takahashi, 2003a).

MATERIALS AND METHODS

Cells of *Paramecium caudatum* (27a G3 belonging to syngen 3) were cultured in a bacterialized (*Klebsiella pneumoniae*) hay infusion medium. In some experiments, behavioral mutant cells defective in voltage-gated Ca^{2+} channels (caudatum non reversal; *cnrD* (18 D 613); Takahashi *et al*., 1985) were used. Cells in the early stationary growth phase were collected, washed three times with the reference solution, which consisted of 4 mM KCl, 1 mM $CaCl_2$, and a 1 mM Tris-HCl buffer (pH 7.4), and kept in the solution for more than 30 minutes prior to the experimentation. The Na^+-containing test solution was prepared by dissolving NaCl into the reference solution.

To examine Na^+-induced backward swimming, the cells were transferred from the reference solution to the test solution with varying Na^+ concentrations using a pipette, and the duration of the continuous backward swimming was then measured (Oami and Takahashi, 2002).

Conventional electrophysiological techniques were employed for examining membrane potential responses to the test solution (Oami, 1996a, b).

The test solution was squirted over an impaled cell through a pipette (ca. 200 μm in inner diameter) by increasing the hydrostatic pressure (3–5 mm H_2O) inside the pipette, as described previously (Oami, 1996a, b). The external solution was continuously exchanged with a reference solution (flow rate; about 6 ml/min) to minimize the accumulation of the test solution around the cell.

The duration of the ciliary reversal associated with an application of Na^+ was determined by microscopical observation of the cilia at the margin of the cell and indicated on the recorder simultaneously with the membrane potential changes.

All the experiments were performed at room temperature ranging from 20 to 24°C.

RESULTS

Behavioral responses of *Paramecium* cells to Na^+ ions

To examine the behavioral responses of *Paramecium caudatum* to external Na^+ ions, wild-type cells were transferred from the reference solution into the test solution containing Na^+ ions (reference solution + NaCl). When the Na^+ concentration was lower than 16 mM, the cells showed repeated changes in the swimming direction for several seconds and then resumed forward swimming. At Na^+ concentrations higher than 16 mM, the cells exhibited initial continuous backward swimming for several seconds and then changed the swimming direction to forward. When the Na^+ concentration was raised to more than 32 mM, the initial backward swimming came to be followed by repeated transient backward swimming. This repeated transient backward swimming was observed for more than 10 min after the transfer of the cells to a Na^+-containing solution, although there was variability in the frequency and duration of swimming in different sessions of a single cell and among different cells. When the Na^+-concentration was as high as 64 mM, each event of transient backward swimming often continued for more than 1 s.

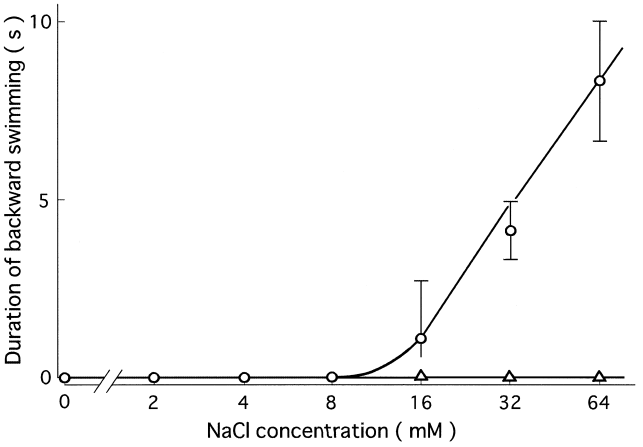

Fig. 1. Effects of the Na^+ concentration on the duration of the initial continuous backward swimming exhibited by the cells of *Paramecium caudatum.* The duration of backward swimming was plotted against the Na^+ concentration in the test solution. Circles, wild-type cells; triangles, CNR mutant cells. Each symbol represents the mean and its standard error of 10–20 measurements with different cells.

Fig. 1 shows the duration of the initial continuous backward swimming of *Paramecium* cells in different Na^+ concentrations. The backward swimming usually appeared at Na^+ concentrations of 16 mM or more, and the duration of backward swimming increased as the Na^+ concentration increased.

CNR mutant cells did not show backward swimming in any Na^+-containing solution used in this study.

Membrane potential responses to an application of Na^+

To examine the membrane potential responses underlying the behavioral responses of *Paramecium* to Na^+, the Na^+-containing solution was applied to cells impaled by a microelectrode (Fig. 2). The wild-type cells showed depolarizing responses, which consisted of an initial depolarizing action potential and a sustained depolarization. The amplitude of the sustained depolarization increased with increasing the Na^+ concentration (Fig. 2 A–D). When the Na^+ concentration was higher than 16 mM, the depolarization continued after the cessation of stimulation (depolarizing afterpotential) (Fig. 2 B–D).

Similar to the wild-type cells, CNR mutant cells showed sustained depolarization in response to Na^+ stimulation. However, neither the action potential nor the depolarizing afterpotential was observed (Fig. 3). The cell repolarized rapidly, and the time course of the repolarization was not affected by changes in the duration of the stimulation. The half-decaying time of the depolarization after the termination of the application of 64 mM Na^+ was 4.06±1.53 s (mean± SE, n=7) in the wild-type and 1.5±0.23 s (mean±SE, n=4) in the CNR.

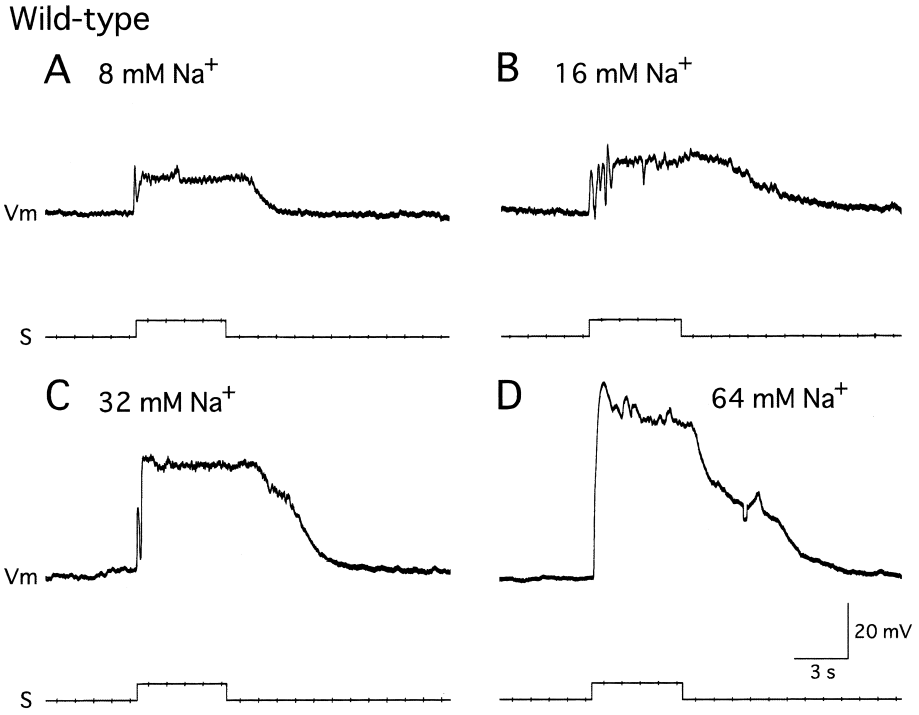

Fig. 2. Membrane potential responses of wild-type *P. caudatum* in response to an application of the test solution with varying Na^+ concentrations. The upper trace in each pair of recordings shows the membrane potential, and the lower trace, the timing and duration of the application of the test solution.

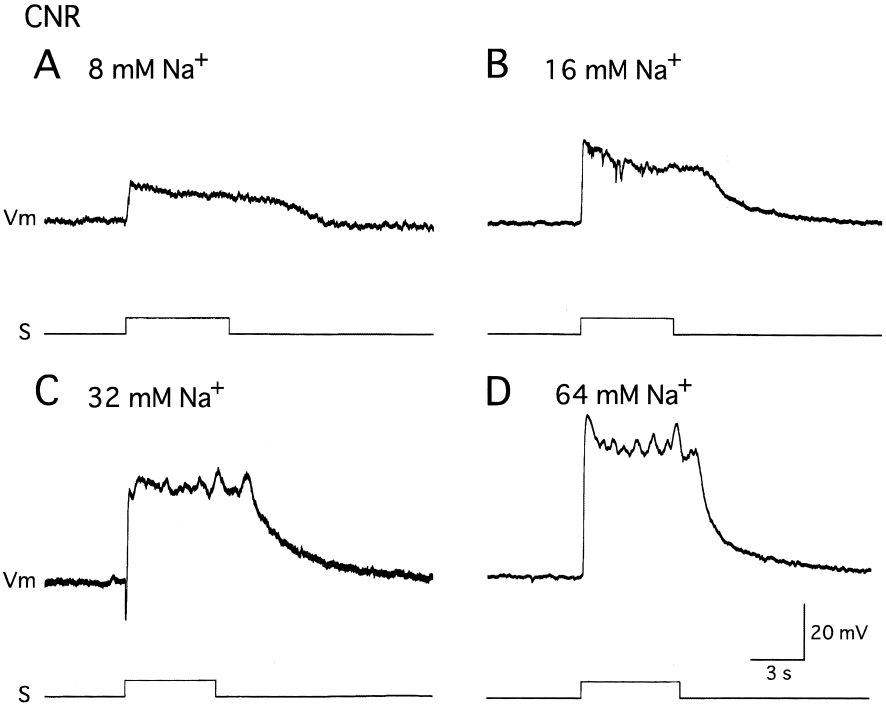

Fig. 3. Membrane potential responses of CNR mutant cells of *P. caudatum* in response to an application of the test solution with varying Na^+ concentrations. The upper trace in each pair of recordings shows the membrane potential, and the lower trace, the timing and duration of the application of the test solution.

Relationship between Na^+-induced membrane potential responses and ciliary reversal of the cell

The temporal relationships between Na^+-induced membrane potential changes and the ciliary reversal were examined. Fig. 4 shows representative responses of a wild-type cell to 32 mM Na^+ of different duration. When the duration

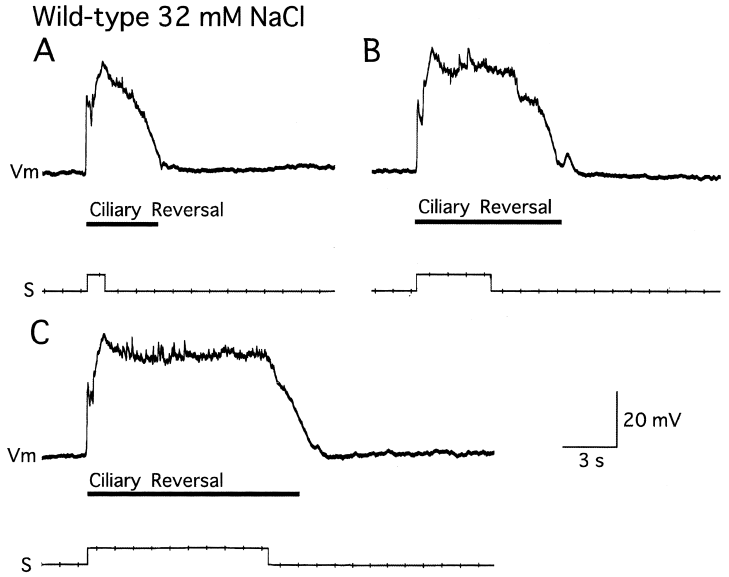

Fig. 4. Temporal relationships between the Na^+-induced membrane potential responses and the ciliary reversal of the wild-type cell of *P. caudatum*. The records were obtained with varying the duration of the application of 32 mM Na^+. The upper trace in each pair of recordings shows the membrane potential, and the lower trace, the timing and duration of the application of test solution. The bar below the voltage trace indicates the timing and the duration of the ciliary reversal exhibited by the cell.

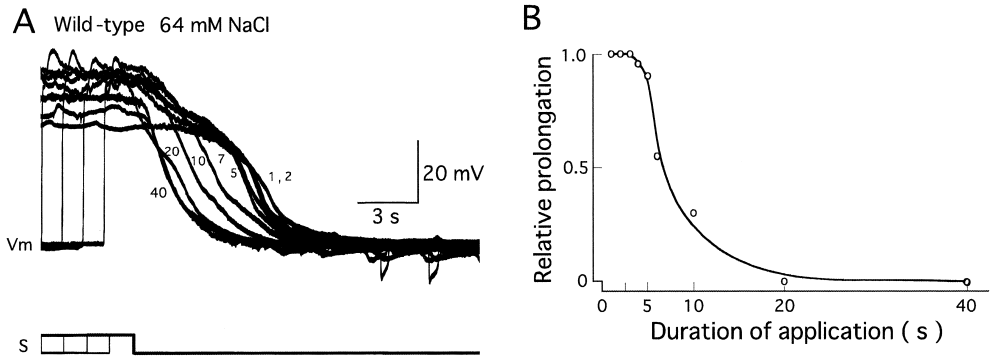

Fig. 5. Effects of the duration of application of the Na^+-containing solution on the recovery phase of the Na^+-induced membrane potential responses of the wild-type cell of *P. caudatum* (A). The duration of the application was changed from 1 s to 40 s, while the Na^+ concentration was kept constant at 64 mM. Records were overlapped so that the timing of the termination of the application became identical. Numbers besides the traces indicate the duration of the application. Duration of depolarizing afterpotential plotted against the duration of the application of the test solution (B). The duration of the afterpotential was defined as the half-decaying point and is expressed as a relative value normalizing the maximum response as 1 and the minimum value as 0.

of the application was short (e.g., within 10 s), the ciliary reversal always coincided with the duration of the Na^+-induced depolarizing responses involving the depolarizing afterpotential (Fig. 4 A–C). With an application of longer duration, the ciliary reversal terminated during the application, and the cell did not produce a depolarizing afterpotential.

Effects of the duration of stimulation on the depolarizing afterpotential

We next examined the effects of the duration of the application of a Na^+-rich solution on the relaxation process of the responses. Fig. 5 A shows overlapped recordings of the relaxation process at different durations of the application of 64 mM Na^+. The records are overlapped so that the

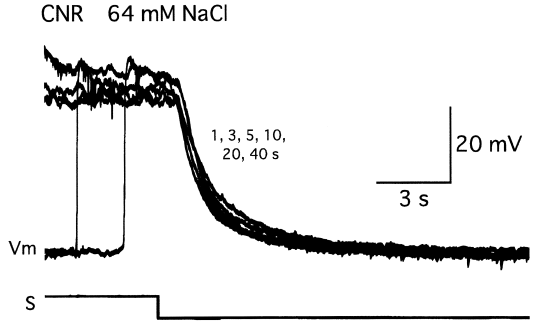

Fig. 6. Effects of the duration of the application of the Na^+-containing solution on the recovery phase of the Na^+-induced membrane potential responses of the CNR mutant cell of *P. caudatum*. The duration of the application of a test solution containing 64 mM Na^+ was changed from 1 s to 40 s. Other explanations are as in Fig. 4 A.

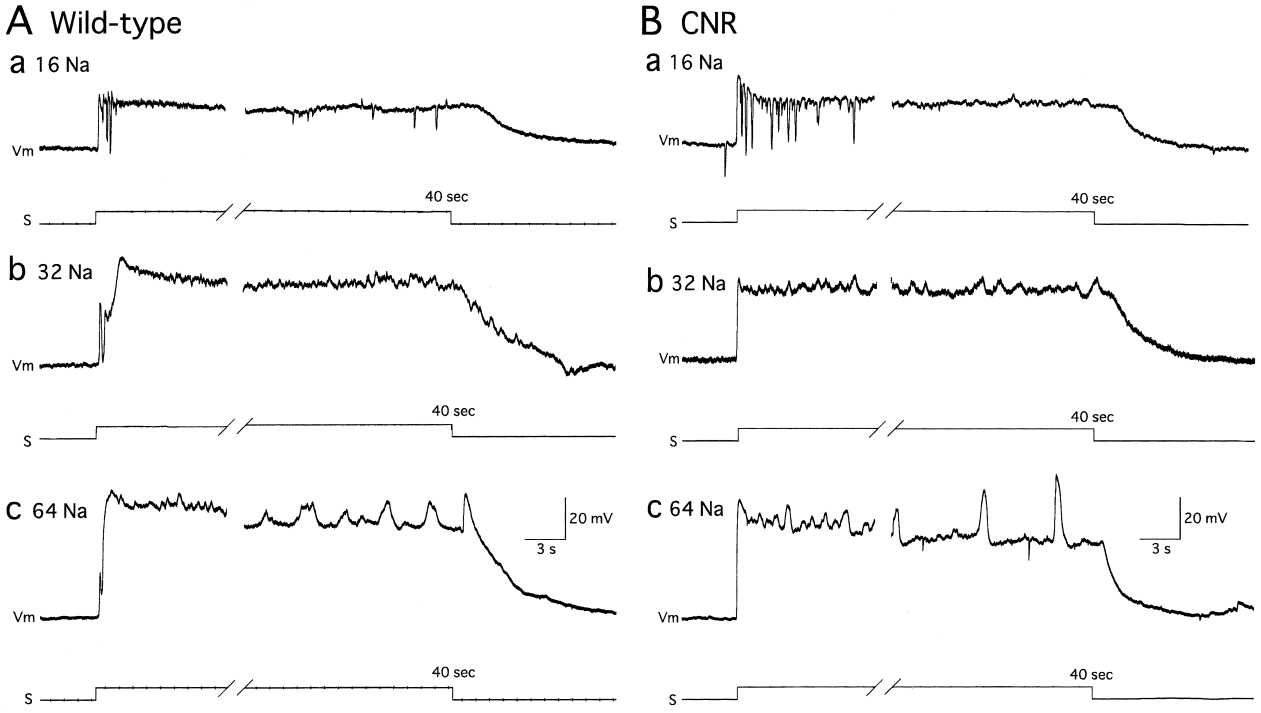

Fig. 7. Membrane potential responses of wild-type (A) and CNR mutant (B) cells to the prolonged (40 s) application of Na^+-containing solution with varying concentrations. Upper traces, membrane potential responses; lower traces, timing and duration of the application of the Na^+-containing solution.

timing of the termination of each application became identical. When the duration was shorter than 3 s, depolarization continued for about 7 s after the termination of stimulation. The extent of the afterpotential prolongation decreased with increasing the duration of the application.

To assess the extent of the decrease in the prolongation quantitatively, we measured the duration of the depolarizing afterpotential. The duration was measured at the half-decaying point and expressed as a value relative to the maximum. The minimum stable value obtained with the application longer than 40 s was taken as 0. As shown in Fig. 5 B, the duration of the afterpotential declined more or less exponentially with increasing the duration of application of the test solution.

Similar experiments were conducted on the CNR mutant cells that did not show backward swimming in the Na^+-containing test solution. CNR cells responded to the test solution with sustained depolarization. However, the repolarization occurred rapidly after the stimulation, and the depolarizing afterpotential was not observed irrespective of the duration of the application (Fig. 6).

Membrane potential responses to prolonged application of the Na^+-containing solution

In the test solution with higher Na^+ concentrations, the wild-type cells showed repeated transient backward swimming following the initial continuous backward swimming. To relate the membrane potential responses to the repeated backward swimming, the Na^+-containing solution was applied to the cell impaled by a microelectrode for a longer period.

Typical responses of wild-type cells are shown in Fig. 7 A. The cell exhibited more or less sustained depolarization in response to an application of 16 mM Na^+ (Fig. 7 A-a). When the applied Na^+ concentration was raised to 32 mM, the cell exhibited small transient depolarization overlapping the sustained depolarization (Fig. 7 A-b). The transient depolarization became conspicuous at 64 mM Na^+ (Fig. 7 A-c). Ciliary reversal of the cell occurred in association with the transient depolarization.

Similar experiments were done using CNR mutant cells (Fig. 7 B). CNR cells exhibited sustained depolarization. When the Na^+ concentration was 32 mM or more, the sustained depolarization was overlapped by transient depolarization, which lasted for 1–2 s. The mutants did not show ciliary reversal.

DISCUSSION

The present study revealed that *Paramecium* shows, at least, two distinct modes of behavioral responses to Na^+ ions. These are initial continuous backward swimming and repeated transient backward swimming. Kung (1971a) reported that the wild-type cells of *P. aurelia* showed repeated short avoiding reactions that faded into a normal forward movement. These responses were observed in the present study when the applied Na^+ concentration was low (cf. 4–8 mM). In contrast to these responses, the behavioral

responses in this study were only observable at Na^+ concentrations higher than those employed by Kung (1971a).

The cell of *Paramecium* impaled by a microelectrode produced prolongation of the depolarization after stimulation. The Na^+-induced depolarizing afterpotential seems to represent an ionic conductance that had been activated by Na^+ ions. Similar to the K^+-induced depolarizing afterpotential, the Na^+-induced depolarizing afterpotential most probably represents the Ca^{2+} conductance (Na^+-induced Ca^{2+} conductance), since only the equilibrium potential for Ca^{2+} ion is expected to be positive. It is probable that the initial continuous backward swimming is controlled by the Na^+-induced Ca^{2+} conductance because the depolarizing responses, including the prolongation, closely correspond to the ciliary reversal of the cells (Fig. 4).

The duration of the depolarizing afterpotential declined with time, indicating that the inactivation of the Na^+-induced Ca^{2+} conductance is time-dependent (Fig. 5). As shown in Fig. 5 B, about 75% of the conductance was inactivated within 10 s from the application. The duration of the backward swimming of the cells in the Na^+ solution (Fig. 1) closely correlated with the inactivation time course of the Na^+-induced Ca^{2+} conductance.

The characteristics of the Na^+-induced Ca^{2+} conductance are similar to those of the Ca^{2+} conductance induced by the K^+-rich solution (Oami and Takahashi, 2002). Moreover, the wild-type cells did not show backward swimming in the 64 mM Na^+-containing solution when they were transferred from the 32 mM KCl-containing solution (1 mM $CaCl_2$, 32 mM KCl, 1 mM Tris-HCl; data not shown). When cells are immersed in the K^+-rich solution, the K^+-induced Ca^{2+} conductance is first activated and then inactivated (Oami and Takahashi, 2002). Therefore, it is assumed that the Na^+-induced Ca^{2+} conductance could not be activated when the K^+-induced Ca^{2+} conductance had been inactivated. These facts indicate that the K^+-induced Ca^{2+} conductance and Na^+-induced Ca^{2+} conductance described in the present study are identical or, at least, share common pathways for activation.

The effective Na^+ concentration range for the present Na^+-induced Ca^{2+} conductance was higher than the effective K^+ concentration range for the K^+-induced Ca^{2+} conductance (Oami and Takahashi, 2002). The inactivation time course of the present Ca^{2+} conductance was also much faster than that of the K^+-induced Ca^{2+} conductance (7 s for 50% inactivation of the Na^+-induced Ca^{2+} conductance; Fig. 5 and 15 s for K^+-induced Ca^{2+} conductance; Fig. 4, Oami and Takahashi, 2002). The duration of the depolarizing afterpotential was shorter than that representing the K^+-induced Ca^{2+} conductance. These facts indicate that the effectiveness for inducing the Ca^{2+} conductance differs considerably among the ion species applied.

In addition to the initial continuous backward swimming, we observed another behavioral response, namely, repeated transient backward swimming in response to the prolonged application of Na^+ ions. The membrane potential responses underlying the transient backward swimming appeared as transient depolarization overlapping the steady depolarization (Fig. 7 A). Contrary to the depolarizing afterpotential, transient depolarization could be seen in the CNR mutant cells (Fig. 7 B). Since the CNR mutant cells did not show backward swimming in the Na^+ solution, the transient depolarization does not directly control the backward swimming. In *Paramecium*, the backward swimming in response to various stimuli is mediated by voltage-dependent Ca^{2+} channels that are activated by the depolarizing receptor potentials (Naitoh, 1982). Like other receptor potential-mediated responses, the Na^+-induced transient backward swimming may be produced by an influx of Ca^{2+} ions through depolarization-sensitive Ca^{2+} channels, which are activated by the transient depolarization induced by Na^+ ions. CNR mutant cells show this depolarization without inducing backward swimming because they have defects in the voltage-gated Ca^{2+} channels.

It has been reported that the membrane of *Paramecium* depolarized in response to an application of external cations (Naitoh, 1982). Such depolarization might be attributable to various ionic channels that are permeant to cations. In *Paramecium tetraurelia,* Kung and his colleagues reported the presence of the Na^+ channel (Kung, 1971a; Satow and Kung, 1974; Satow *et al*., 1980; Saimi and Kung, 1980). Interestingly, the Na^+ channel is Ca^{2+}-dependent, and the calmodulin molecule is responsible for the Ca^{2+} dependency (Saimi and Ling, 1990; Saimi and Kung, 1994). The steady membrane depolarization during the application of the Na^+-containing solution recorded in the present study is most probably dependent on the Na^+ channels. It is assumed that the Na^+ channels are partially activated during the resting condition and that the Na^+-induced depolarization takes place due to a shift in the equilibrium potential of Na^+ ions towards the depolarizing direction.

The present results indicate that *Paramecium* has two distinct kinds of receptor systems to Na^+ ions. One shares common pathways to the K^+-receptor system, and the other seems to be specific to Na^+ ions. To understand the receptor systems of *Paramecium* to external inorganic ions, further examination of various ion species will be necessary.

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