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K^+-induced Ca^{2+} Conductance Responsible for the Prolonged Backward Swimming in K^+-agitated Mutant of *Paramecium caudatum*

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Abstract. The K^+-agitated (Kag) mutant of *Paramecium caudatum* shows prolonged backward swimming in K^+-rich solution. To understand the regulation mechanisms of the ciliary motility in *P. caudatum*, we examined the membrane electrical properties of the Kag mutant. The duration of the backward swimming of the Kag in K^+-rich solution was about 10 times longer than that of the wild type. In response to an injection of the outward current, the wild type produced an initial action potential and a subsequent membrane depolarization due to *I-R* potential drop, while the Kag exhibited repetitive action potentials during the depolarization. Under voltage-clamp conditions, the depolarization-activated transient inward current exhibited by the Kag was slightly smaller than that exhibited by the wild type. In response to an application of K^+-rich solution, both the wild type and the Kag exhibited a depolarizing afterpotential representing the activation of the K^+-induced Ca^{2+} conductance. The inactivation time course of the K^+-induced Ca^{2+} conductance of Kag was about 10 times longer than that of the wild type. This difference corresponds well with the difference in behavioral responses between Kag and wild type to K^+-rich solution. We conclude that the overreaction of the Kag mutant to the K^+-rich solution is caused by slowing down of the inactivation of the K^+-induced Ca^{2+} conductance.

Key words: *Paramecium* — Mutant — Ca^{2+} conductance — Cilia — Chemoreception — Membrane excitability

Introduction

The behavior of the ciliate protozoan *Paramecium caudatum* is under the control of membrane electric events (Eckert, 1972; Naitoh, 1974). Cells show backward swimming in response to the depolarizing Ca^{2+} action potential evoked by various depolarizing stimuli (Naitoh & Eckert, 1969; Van Houten, 1992; Hennessey, Frego & Francis, 1994; Hennessey, Kim & Satir, 1995; Oami, 1996a, 1998a, b). In addition to the backward swimming associated with the action potential, it is well known that the cells of *Paramecium* show backward swimming in solutions with high K^+ concentration (Naitoh, 1968). The duration of this backward swimming is longer than that associated with the action potential. It sometimes persists for several tens of seconds. Recently, we identified the Ca^{2+} conductance responsible for the K^+-induced backward swimming in *P. caudatum* (Oami & Takahashi, 2002). The kinetics of the K^+-induced Ca^{2+} conductance were distinct from those of the voltage-gated Ca^{2+} conductance responsible for the action potential. In particular, the time course of inactivation of the K^+-induced Ca^{2+} conductance was in the order of tens of seconds. This is comparable to that of K^+-induced backward swimming in free-swimming cells.

Mutant cells of *Paramecium* defective in voltage-gated Ca^{2+} channels do not show backward swimming in response to stimulation. These mutants were originally obtained in *P. tetraurelia* by Kung (Pawn mutants; Kung, 1971a, b). In *P. caudatum*, mutants defective in voltage-gated Ca^{2+} channels are referred to as caudatum non-reversal (CNR). Presently, four CNRs with defects in different genes have been obtained (Takahashi, 1979; Takahashi et al., 1985; Takahashi, 1988). Contrary to these mutants, some mutants of *Paramecium* show prolonged backward swimming upon stimulation (Ramanathan et al.,

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1988; Takahashi, 1988). Among these mutants, K^+-agitated (Kag) mutant (Takahashi & Naitoh, 1978) has a prominent feature in responding to K^+-rich solution with unusually long backward swimming. Interestingly, Kag and one of the CNR mutants, the *cnrB*, have a defect in the same gene. Therefore, the modulation of the *cnrB* gene product can enhance, as well as suppress, the backward swimming of *P. caudatum*. To understand the regulation mechanisms of backward swimming in *Paramecium*, it is important to investigate why the Kag mutant overreacts to the K^+-rich solution.

In the present study, we have examined the membrane electric characteristics of the Kag mutant, paying special attention to the Ca^{2+} conductance responsible for the action potential as well as to that responsible for the K^+-induced backward swimming in *P. caudatum*. The results indicate that the Ca^{2+} conductance responsible for the action potential remains unaffected or slightly suppressed in the Kag mutant. On the other hand, Kag has an inactivation time course of the K^+-induced Ca^{2+} conductance that is 10 times longer than that of the wild type. This difference corresponds well with the difference in behavioral responses between Kag and wild-type cells to K^+-rich solution. We conclude that the unusual behavior of the Kag mutant to the application of K^+-rich solution is caused by slowing down of the inactivation of the K^+-induced Ca^{2+} conductance. Some of these results have been presented orally and in abstract form elsewhere (Oami & Takahashi, 1998).

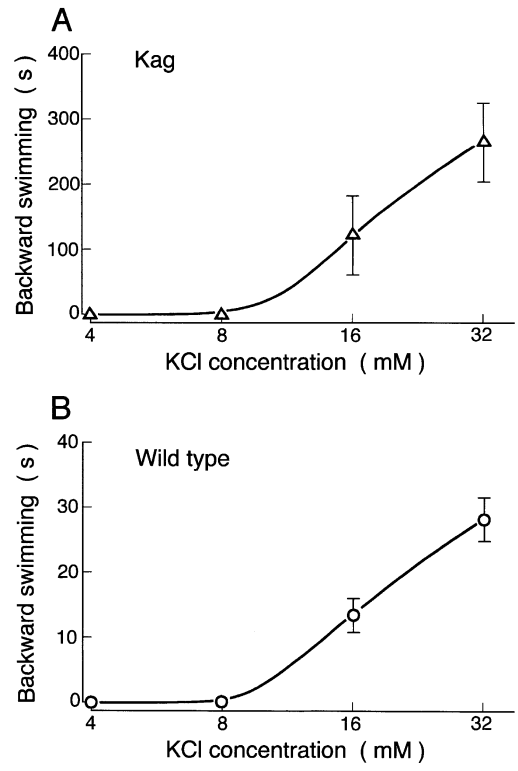

Fig. 1. Effects of the KCl concentration on the duration of the backward swimming exhibited by the K^+-agitated mutants (*A*) and wild-type cells (*B*) of *P. caudatum*. Duration of backward swimming was plotted against the K^+ concentration in the test solution. Each symbol represents the mean and its standard error of 10–20 measurements with different cells. In *B*, data are taken from a previous publication (Oami & Takahashi, 2002), replotted for comparison.

Materials and Methods

Wild-type cells of *Paramecium caudatum* (27a G3, belonging to syngen 3) were cultured in a bacterialized (*Klebsiella pneumoniae*) hay infusion medium and collected in the early stationary growth phase. The mutant used in the present study overreacts to the K^+-rich solution and is referred to as the K^+-agitated (Kag) mutant (13D401; Takahashi & Naitoh, 1978). Cells were washed three times with the reference solution, which consisted of 4 mM KCl, 1 mM $CaCl_2$ and 1 mM Tris-HCl buffer (pH 7.4) and kept in the solution for more than 30 minutes prior to the experimentation.

To examine K^+-induced backward swimming, cells were transferred from the reference solution into the test solution with varying K^+ concentration by a small pipette (ca. 500 μm inner diameter). The duration of the continuous backward swimming was then measured (Oami & Takahashi, 2002).

Conventional electrophysiological techniques were used to examine membrane-potential responses to the test solution with varying K^+ concentration and/or to an electric current pulse (Oami, 1996a). To examine the voltage-gated Ca^{2+} conductance responsible for the action potential, two-microelectrode voltage-clamp techniques were used (Oami, 1996a).

The test solution was squirted over an impaled cell through a pipette (ca. 200 μm inner diameter) by increasing the hydrostatic pressure (3–5 mm H_2O) inside the pipette, as described previously (Oami, 1996a, b).

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

Results

Concentration Effects of K^+ on the Duration of K^+-induced Backward Swimming

K^+-induced backward swimming of the cells of the Kag mutant in *P. caudatum* was measured in solutions with varying K^+ concentration. Kag showed whirling when they were transferred from a reference solution (4 mM KCl) to the test solution containing 8 mM KCl, but not continuous backward swimming. With raising the KCl concentration of the test solution to 16 mM, the cells showed backward swimming followed by whirling. The duration of the backward swimming increased in a test solution containing 32 mM KCl. As shown in Fig. 1*A*, the duration of the backward swimming of the Kag cells increased linearly with a logarithmic increase in the KCl concentration in the range of 8 mM to 32 mM. It should be noted that the duration of backward swimming

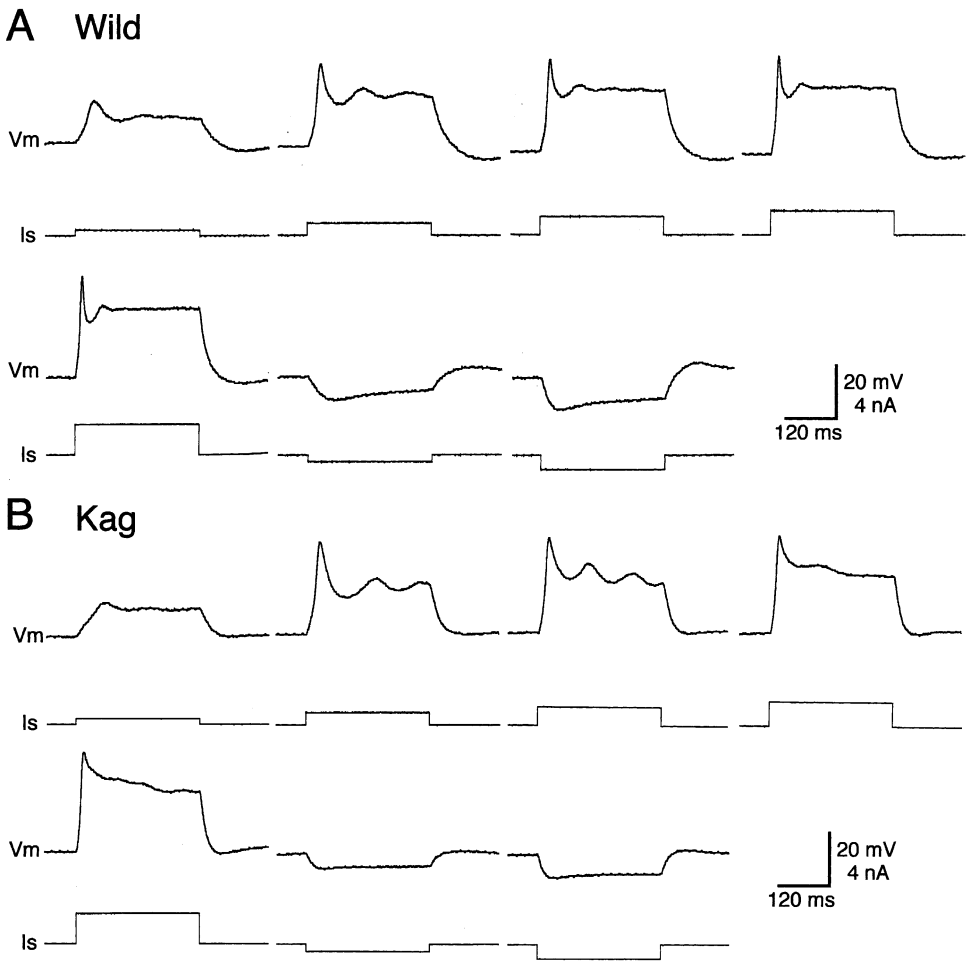

Fig. 2. Comparison of current-evoked action potentials between wild-type cell (*A*) and Kag mutant (*B*) of *Paramecium caudatum*. *Vm*, membrane potential; *Is*, injected current intensity.

was about 10 times longer than that of the wild type (Fig. 1*B*).

Membrane-potential Responses of Wild-type and Kag Cells to the Injection of Electric Current

We compared the current-evoked action potentials exhibited by wild type (Fig. 2*A*) and Kag cells (Fig. 2*B*). The wild-type cell exhibited an initial action potential and sustained depolarization due to *I-R* potential drop in response to an injection of the outward current. The amplitude of the action potential increased as the applied outward current increased. The cell exhibited sustained hyperpolarization to the inward current.

The Kag cells exhibited similar membrane potential responses to the injection of an outward current.

Figure 3 shows the membrane potential responses of the wild-type (*A*) and Kag (*B*) cells in response to an injection of current for a longer

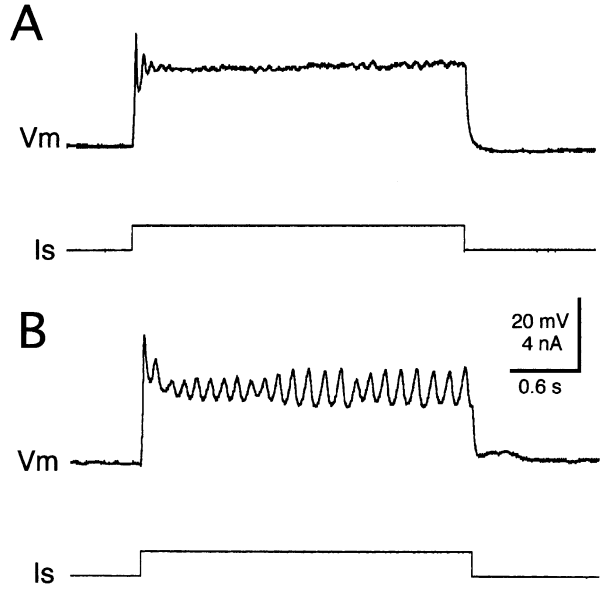

Fig. 3. Comparison of membrane potential responses exhibited by the wild-type cell (*A*) and Kag mutant (*B*) to a prolonged injection of outward current. *See* legend of Fig. 2 for details.

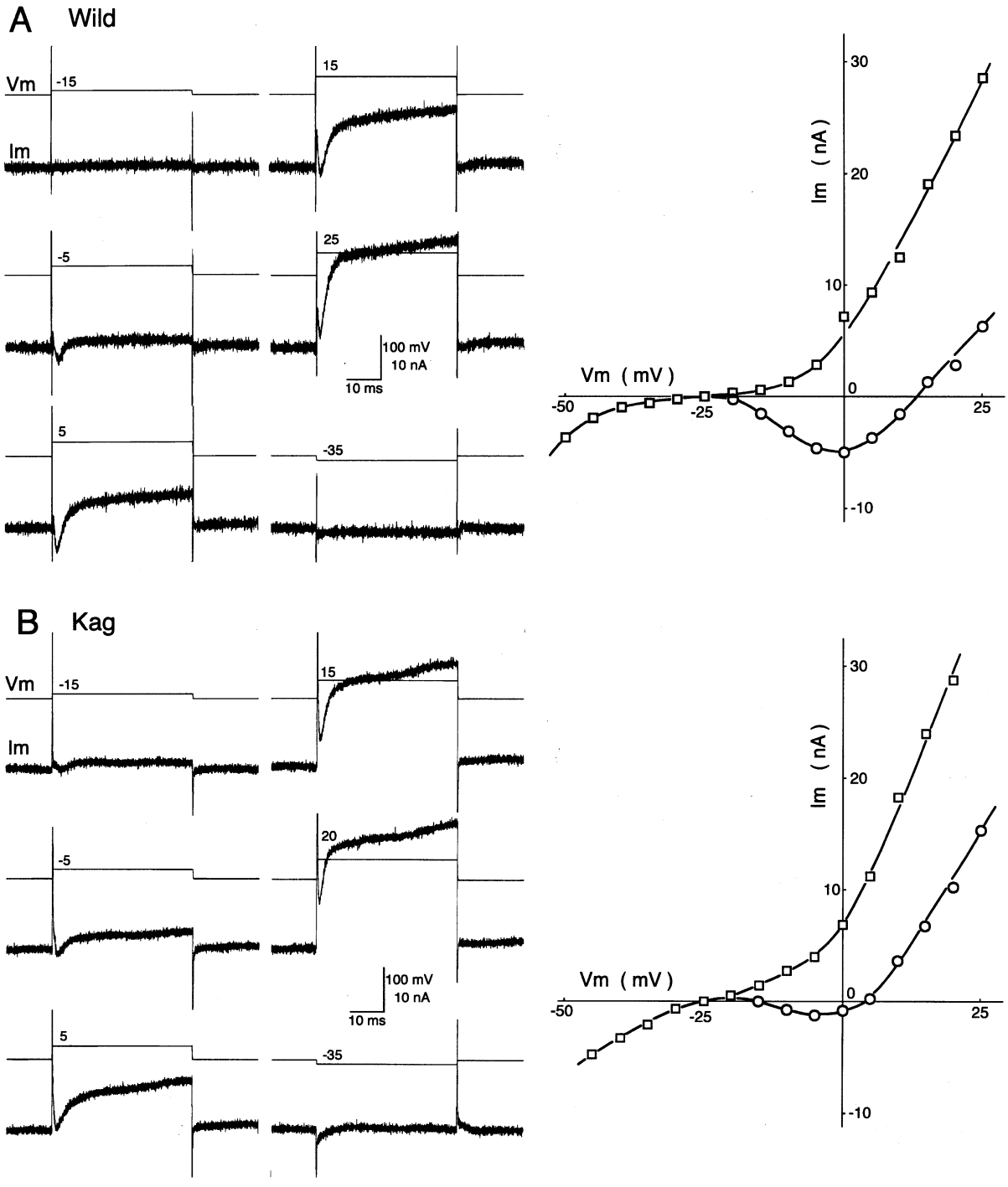

Fig. 4. Membrane-current responses exhibited by the wild-type cell (*A*) and Kag mutant (*B*) of *Paramecium caudatum* to step changes in membrane potential under voltage-clamp conditions. Upper trace in each pair of recording shows the membrane potential (*Vm*), and the lower trace, membrane currents (*Im*). The holding potential was set at the resting level (−25 mV in this case). Stepped potential level is indicated on the *Vm* trace. Right-hand figure shows the current-voltage relationship of the membrane plotted from the traces shown on the left. *Circles*: peak inward current. *Squares*: current levels 50 ms after onset of the step changes in membrane potential.

duration. Following the initial action potential(s), the wild type showed sustained steady depolarization in response to outward current (Fig. 3*A*), while Kag showed repetitive action potentials during the plateau phase (Fig. 3*B*).

Membrane-Current Responses of *Paramecium* under Voltage-clamp Conditions

To examine the Ca^{2+} conductance responsible for the action potential quantitatively, the depolarization-

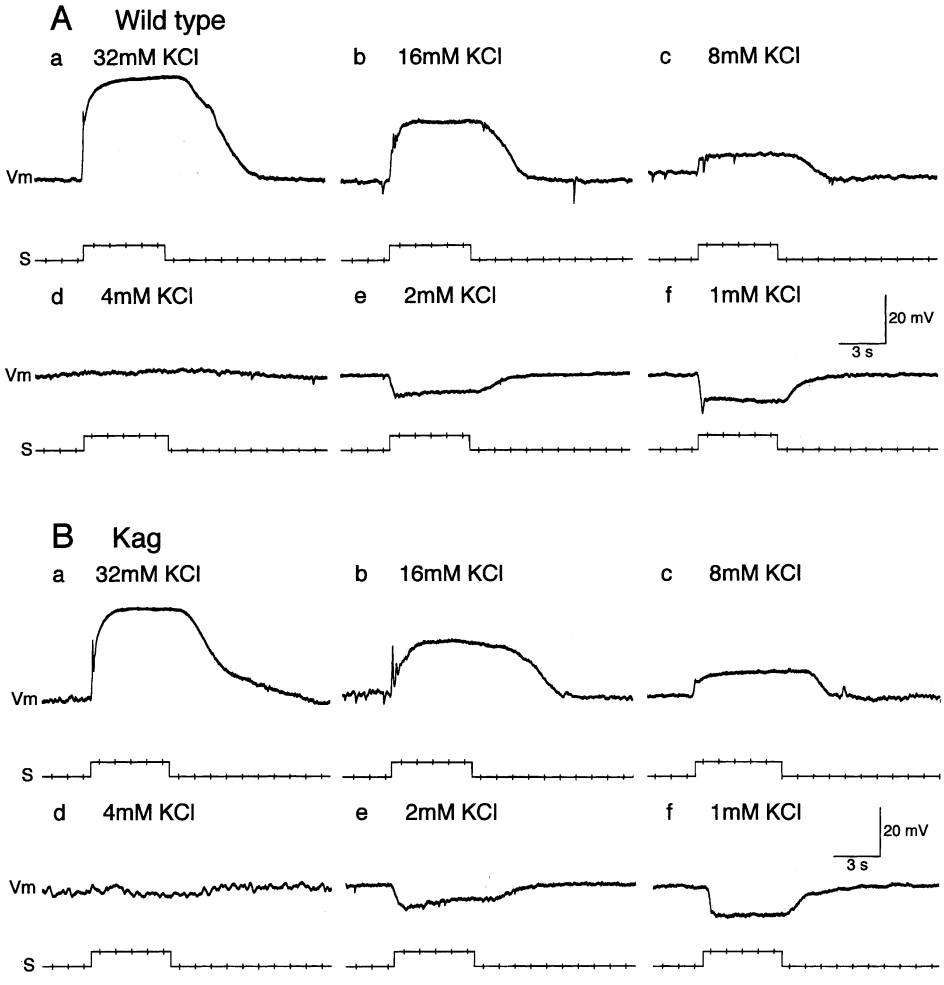

Fig. 5. Membrane potential responses of a wild-type cell (*A*) and a Kag mutant (*B*) of *P. caudatum* to an application of test solution with different K^+ concentrations. The upper trace in each pair of recordings shows the membrane potential (*Vm*), and the lower trace, timing and duration of the application of test solution (*S*).

activated ion currents were recorded under voltage-clamp conditions. Figure 4*A* shows representative membrane-current responses produced by the wild-type cell. Upon step changes in the membrane potential toward the depolarizing direction, the cell produced a transient inward current. With increasing the degree of depolarization, the amplitude of the current rose to reach its maximum at about 0 mV and then declined with further depolarization. The inward current was followed by a delayed outward current at potentials more positive than 0 mV. The right-hand figure shows the amplitude of the early inward and delayed outward currents plotted against the membrane potential (the *I-V* relationships).

Figure 4*B* shows similar recordings obtained with a Kag cell. The characteristics of the early inward current and the delayed outward current were similar to those of the wild-type cells. The right-hand figure shows the *I-V* relationships of the early inward current and the delayed outward current exhibited by the Kag cell. The characteristics of the *I-V* relationships were similar to those obtained in the wild-type cells. The amplitudes of the early inward current measured at 0 mV were -3.42 ± 1.17 nA (mean and its standard error of 8 measurements with different cells) in Kag and -6.77 ± 2.76 nA (mean and its standard error of 7 measurements with different cells) in wild type.

Membrane-potential Responses of *Paramecium* to an Application of the Solution with Varying K^+ Concentration

We next examined the membrane-potential responses of *P. caudatum* in response to an application of the test solution with varying KCl concentration. Figure 5*A* shows representative membrane-potential responses exhibited by the wild-type cells. In response to a 5-s application of 32 mM KCl-containing solution, the wild-type cell showed depolarizing membrane-potential responses consisting of initial action potential(s) and subsequent sustained depolarization. After termination of the application, a prolongation of the depolarizing potential responses (the depolarizing afterpotential) took place (Fig. 5 *Aa*). This depolarizing afterpotential represents the K^+-induced Ca^{2+} conductance (Oami & Takahashi, 2002). The amplitude of the sustained depolarization

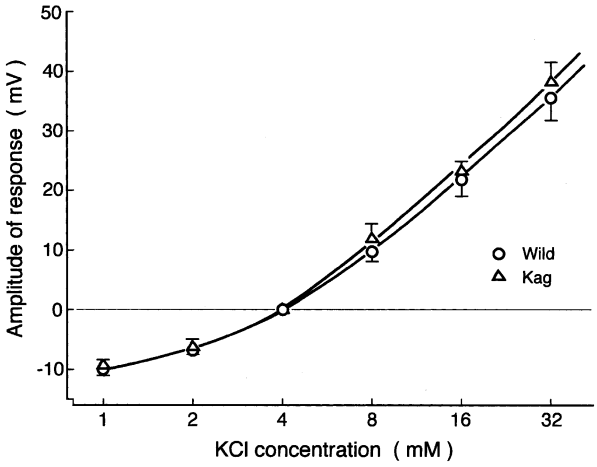

Fig. 6. Plots of the amplitudes of K^+-induced membrane potential responses of wild type (*circles*) and Kag (*triangles*) as a function of the K^+ concentration applied. Mean and standard error of 5–7 measurements with different specimens are indicated.

decreased as the applied KCl concentration was reduced (Fig. 5 *Ab*, *c*). An application of the reference solution did not affect the membrane potential (Fig. 5 *Ad*). The cells exhibited sustained membrane hyperpolarization to the test solution with a reduced KCl concentration (Fig. 5 *Ae*, *f*).

Similar to the wild-type cells, the Kag cells showed sustained membrane depolarization and a depolarizing afterpotential in response to an application of 32 mM KCl-containing test solution (Fig. 5 *Ba*). The characteristics of the membrane potential responses induced by an application of the test solution with varying K^+ concentration for a duration of 5 s were almost identical to those exhibited by the wild type.

The amplitudes of the sustained membrane depolarization and hyperpolarization are plotted against the applied KCl concentration in Fig. 6. As shown in the figure, the amplitudes of the membrane depolarization are almost the same in both cell types and they increased linearly with a logarithmic increase in KCl concentration applied.

Comparison of the Time Course of Inactivation of the K^+-induced Ca^{2+} Conductance in the Wild-type and Kag Cells

Since the Kag has a prominent feature in producing the prolonged backward swimming in the K^+-rich solution, we examined the time course of the inactivation of the K^+-induced Ca^{2+} conductance of the Kag mutant. Figure 7*A* shows overlapped recordings of the relaxation process of the depolarizing membrane-potential responses following the application of 32 mM K^+-containing solution for varied duration. When the duration of the application was shorter than 5 s, the Kag cell produced a depolarizing

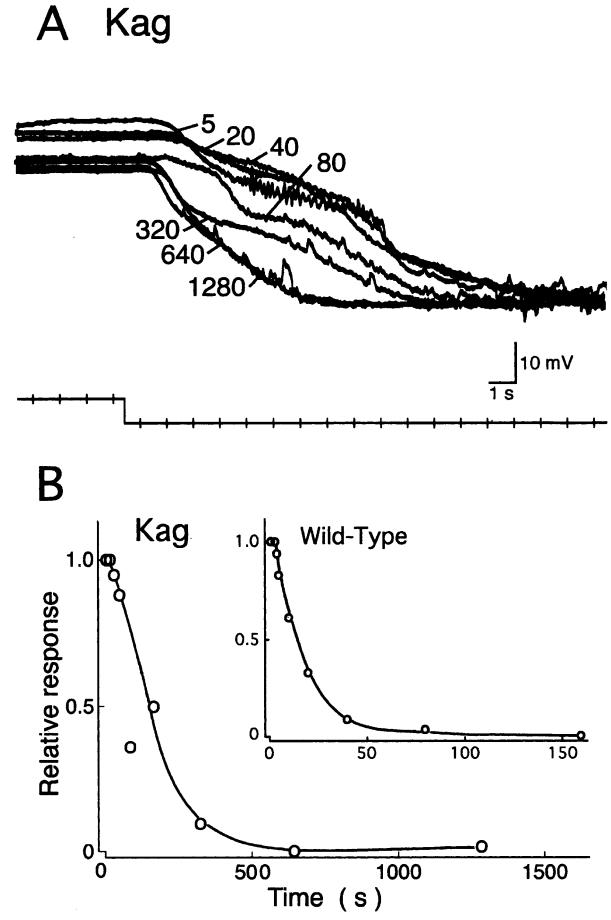

Fig. 7. (*A*) Effects of the duration of application of K^+-rich solution on the recovery phase of the K^+-induced membrane potential responses of the Kag mutant of *P. caudatum*. The duration of the application was changed from 1 s to 1280 s, while the KCl concentration was kept constant at 32 mM. Records were overlapped so that the timing of the termination of the application became identical. (*B*) Measurements of the duration of the prolongation of the depolarization (depolarizing afterpotential) following the application plotted against the duration of application of the test solution. The duration of the prolongation was measured at the half-decay point and is expressed as a relative value normalizing the maximum response as 1 and the minimum value as 0. Inset shows the results obtained with a wild-type cell, taken from a previous publication (Oami & Takahashi, 2002 J. Membrane Biol. 190:159–165; reproduced with permission from Journal of Membrane Biology) for comparison. Note that the abscissa is 10 times larger in the figure for the Kag than that for the wild type.

after-potential that continued for about 10 s after the termination of the application. With increasing duration of application, the duration of the depolarizing after-potential became shorter. The duration of the prolongation decreased to a steady level of about 3 s when the application was longer than 640 s.

To express the degree of decrease in prolongation quantitatively, we measured the duration of the depolarizing afterpotential. The duration was measured from the time when the application was terminated to the time when the depolarizing response decayed to

its half level. To obtain the net duration of the prolongation, the minimum stable value obtained when the application longer than 160 s was subtracted from the measured value and the result expressed as a value relative to the maximum value. As shown in Fig. 7*B*, the duration of the prolongation declined more or less exponentially with increasing duration of the application of the K^+-rich solution. It should be noted that the time course of the inactivation is about 10 times slower in Kag than in wild type (compare plots for Kag with those for wild type in the inset of Fig. 7*B*).

Discussion

The inactivation time course of the K^+-induced Ca^{2+} conductance of the Kag cells was about 10 times slower than that of the wild-type cells. The K^+-induced Ca^{2+} conductance has been found to be responsible for the K^+-induced backward swimming in free swimming cells (Oami & Takahashi, 2002). This difference of the K^+-induced Ca^{2+} conductance corresponds well to the difference in the duration of the K^+-induced backward swimming between the wild-type and the Kag cells. Therefore, it is strongly suggested that the prolonged backward swimming of the Kag in the K^+-rich solution is attributable to changes in the inactivation kinetics of the Ca^{2+} conductance induced by the K^+-rich solution.

We could not detect the differences in the characteristics of the early inward current corresponding to the action potential between the Kag and wild-type cells. As a matter of fact, the amplitude of the inward current exhibited by the Kag was slightly smaller than that exhibited by the wild type. Therefore, it is difficult to explain the prolonged backward swimming of Kag cells in terms of the differences in the amplitude of the Ca^{2+} current corresponding to the action potentials. This indicates that the mechanisms underlying the K^+-induced backward swimming and the action potential-mediated backward swimming are different. Thus, the present results strongly support the idea that the Ca^{2+} conductance responsible for K^+-induced backward swimming is distinct from that responsible for the action potential (Oami & Takahashi, 2002).

There were some differences regarding the plateau phase of the current-evoked membrane potential responses exhibited by the wild type and those exhibited by Kag. The wild-type cells produced an initial conspicuous action potential followed by a more or less steady depolarization, while Kag cells showed repetitive firing following the initial action potential (Fig. 3). Though the mechanisms underlying the repetitive firing of this mutant are not clear presently, it should be noted that the depolarization-activated Ca^{2+} conductance in *P. tetraurelia* consisted of at least two distinct components with different inactivation kinetics. One inactivates rapidly, depending on Ca^{2+} ions introduced into the cell during excitation (Brehm & Eckert, 1978) and the other inactivates slowly, independently of Ca^{2+} ions (Hennessey & Kung, 1985). Because the time course of the repetitive firing exhibited by the Kag cells in the present study is comparable to that of the long-term inactivation of Ca^{2+} conductance (order of seconds), the repetitive firing exhibited by Kag might be attributable to the change in the characteristics of the long-term Ca^{2+} conductance. Thus, there is a possibility that Kag mutation might somehow strengthen the long-term Ca^{2+} conductance that caused the Kag cells to fire repetitively. If that is the case, the Ca^{2+} conductance characterized by its long-term inactivation might play an important role in activation of the K^+-induced backward swimming.

Preston and Hammond (1998) reported another example of the Ca^{2+} conductance that is responsible for the backward swimming in *Paramecium*. After a long-time exposure to the K^+-rich solution, the Pawn mutant (*pawnB*) of *P. tetraurelia* became capable of exhibiting the backward swimming in a K^+-rich solution. They measured the Ca^{2+} current controlling the backward swimming and termed it I_{queen}. This Ca^{2+} current seems to be different from that responsible for the action potential because Pawn mutants have a defect in the voltage-gated Ca^{2+} channels. Currently, the relation between I_{queen} and the present K^+-induced Ca^{2+} conductance is not clear.

As mentioned above, the Kag mutant of *P. caudatum* shows unusually long backward swimming in the K^+-rich solution due to delayed inactivation of the K^+-induced Ca^{2+} conductance. Therefore it is one of the up mutants. In *P. tetraurelia*, several up mutants have been described, such as dancer (voltage-gated Ca^{2+}current; Hinrichsen & Saimi, 1984) or paranoiac (Ca^{2+}-dependent Na^+ current; Saimi & Kung, 1980). The calmodulin mutants show both prolongation and shortening of the backward swimming upon stimulation, depending on the site of the mutation on the calmodulin molecule (Kink et al., 1990; Saimi & Kung, 1994). K-shy has a defect in the regulation of intracellular Ca^{2+} concentration and shows prolonged backward swimming (Hennessey and Kung, 1987). Among these mutants, it is interesting to note here that the Kag, an up mutant, is an allele of a down mutant, *cnrB*. The identification of the *cnrB* gene product will contribute greatly to understanding the regulation mechanisms of the ciliary motility in *Paramecium*.

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