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THE IONIC BASIS OF THE DEPOLARIZING MECHANORECEPTOR POTENTIAL OF *PARAMECIUM TETRAURELIA*

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SUMMARY

A depolarizing receptor potential produced by mechanical stimulation was studied in pawn mutants of *Paramecium tetraurelia* bathed in TEA solution. The potential was dependent upon the strength of the mechanical stimulation until a maximum response was reached. The maximum value was dependent on the concentration of external Ca^{2+}. Discounting the change in resting potential (attributable to change in surface charge), the maximal receptor potential changed by 20 mV per 10-fold change in Ca^{2+} at the concentrations tested, confirming the result of Ogura & Machemer (1980) that Ca^{2+} is the major natural cation that carries the receptor current. Mg^{2+}, Sr^{2+}, Ba^{2+} and Mn^{2+} can substitute for Ca^{2+} in the generation of the depolarizing receptor potential. Except for Mn^{2+}, this result is similar to that of de Peyer & Deitmer (1980) for *Stylonychia*. Na^+, K^+, Li^+ and TEA^+ cannot effectively substitute for Ca^{2+}.

INTRODUCTION

Mechanical stimulation of the anterior end of *Paramecium caudatum* triggers a depolarization (Eckert, Naitoh & Friedman, 1972) whereas posterior stimulation triggers a hyperpolarization (Naitoh & Eckert, 1973). The depolarizing response consists of a receptor potential and a regenerative Ca^{2+}-based action potential (Eckert *et al.* 1972). Through the use of deciliated paramecia whose voltage-sensitive Ca-channels were removed with the cilia, the receptor current has been found to be largely carried by Ca^{2+} (Ogura & Machemer, 1980). In *Stylonychia*, a hypotrichous ciliate, Mg^{2+}, Sr^{2+} and Ba^{2+} can substitute for Ca^{2+} in carrying the receptor current (de Peyer & Machemer, 1978; de Peyer & Deitmer, 1980).

Typical pawn mutants have no action potential (Kung & Eckert, 1972; Schein, Bennett & Katz, 1976; Takahashi & Naitoh, 1978; Satow & Kung, 1980*a*) and have no transient Ca-inward current upon depolarization under voltage clamp (Oertel, Schein & Kung, 1977; Satow & Kung, 1980*a*). However, a depolarizing receptor

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potential induced by the mechanical stimulation at the anterior end remai (Takahashi & Naitoh, 1978; Kung, 1979). We now use the pawn mutants to study the ionic basis of this receptor potential.

MATERIALS AND METHODS

Stocks and cultures

Three stocks of *P. tetraurelia*: 51s (wild type), d4-500 (pawn of *pwA* complementation group) and d4-95 (pawn of the *pwB* group) (Kung, 1971; Chang *et al.* 1974) were cultured by standard methods (Sonneborn, 1970). Only robust cells in the logarithmic phase of growth were used and the experiments were performed at room temperature (22 ± 1 °C).

Solutions

The Ca^{2+} solution in the experiments of Fig. 2 was 1 mM-$Ca(OH)_2$, 0·5 mM-$CaCl_2$, 1 mM-citric acid adjusted to pH 7·2 with 1·2–1·5 mM-Trisma base (Sigma). The Ca^{2+}-TEA^+ solution in the experiments in Figs 1, 2 and 3 was the Ca^{2+} solution with the addition of 4 mM freshly dissolved TEA-Cl (tetraethylammonium chloride monohydrate, Aldrich). For the rest of the experiments, chlorides were dissolved in a buffer containing 4 mM-TEA^+ (fresh), 1 mM-citric acid and 3·5 mM-Trisma base at pH 7·2. Different amounts of $CaCl_2$ were added to this buffer in other cases and the free Ca^{2+} concentrations were calculated as by Ling & Kung (1980). 1 mM-$CaCl_2$, $SrCl_2$, $BaCl_2$, $MgCl_2$, $MnCl_2$ or 8 mM-KCl, NaCl or LiCl was added to the buffer for the experiments in Figs 5 and 6. All chemicals were of reagent grade.

Electrophysiological techniques

The techniques used to record intracellularly from *Paramecium* were conventional (Naitoh & Eckert, 1972; Satow & Kung, 1976*a*). The microelectrodes were filled with 0·5 M-KCl with resistance of 80–100 MΩ. Mechanical stimulation was delivered by a glass stylus (tip diameter 5–10 μm) mounted on a phonograph cartridge (Naitoh & Eckert, 1972; Satow & Kung, 1977). Before stimulation, the stylus was micromanipulated to approach and touch the lower surface of the paramecium which was held stationary by the electrodes. The excursion of the stylus and the indentation of the cell surface could be observed under the microscope when the cartridge was piezoelectrically driven by a 2 ms d.c. pulse delivered from an isolated stimulator (Devices, type 2533). The excursion was not measured, since only maximal responses were compared, except in cases where the graded nature was to be demonstrated (Fig. 3). The paramecia were first immersed in the test solution for 5–10 min before they were captured and penetrated with microelectrodes. The combined results of mechanical prodding, electrode penetration, and the bathing of specimens in solutions containing only one, often nonphysiological, metal-cation species proved deleterious to the specimens. Thus, experiments displayed in Figs 5 and 6 were limited to durations of 5–10 min after electrode penetration.

RESULTS

Isolation of the depolarizing receptor potential

Both the wild type and the pawn have a resting potential of about −20 mV in the Ca^{2+}-TEA solution. TEA was incorporated to suppress touch-induced hyperpolarization (see below). In the wild type, mechanical stimulation triggers a nearly all-or-none action potential (Fig. 1A) peaking some 40 mV from the resting level (Satow & Kung, 1976*b*). A similar stimulation (submaximal, see below) triggers a much smaller response in the pawn (Fig. 1B). The upstroke of the wild-type response clearly has two components: a small depolarization (the receptor potential) followed by a

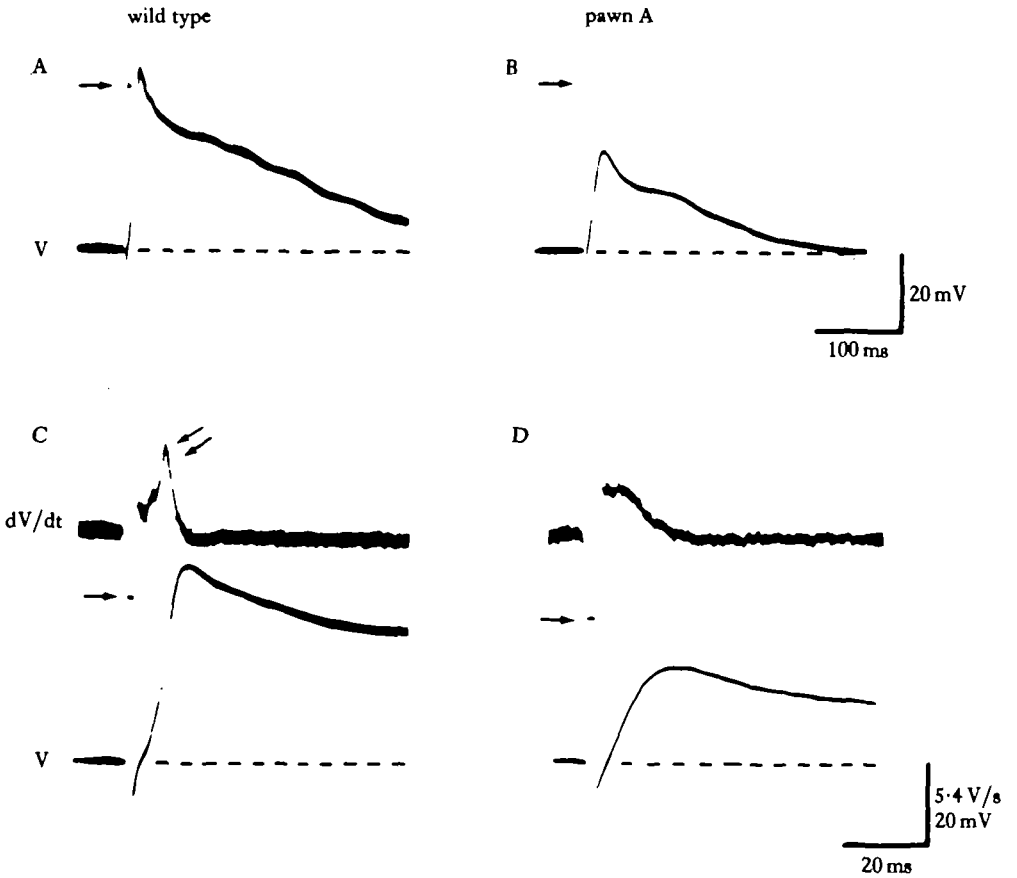

Fig. 1. Depolarizing responses (V) to mechanical stimulation of *Paramecium tetraurelia* in the Ca^{2+}-TEA^{+} solution. (A) and (C) from one wild-type cell (51s); (B) and (D) from one pawn-mutant cell (*pwA*$_{500}$). Arrows point to the marks of the 2 ms pulses which drive the glass stylus that indents the cell surface. Note that, with similar submaximal stimulations, the response of the wild type (A) is larger than that of the pawn mutant (B). At a faster sweep speed, the wild-type response (C) is seen to have two components. The regenerative cascade of the second component, the action potential, leads to a second peak (double arrow) in the first time derivative of the voltage (dV/dt). The pawn response (D) does not have this second component. The resting potential of the wild type is −20 mV; that of the pawn mutant is −25 mV. That the voltage responses appear to begin from levels more negative than the resting levels is a consistent artifact of the mechanical stimulation.

regenerative depolarization (the action potential), as evident in both the voltage a the dV/dt traces examined at fast sweep speed (Fig. 1C). The mutant response consists only of the receptor potential (Fig. 1D). The receptor potential of the pawn has a slow (hundreds of ms) biphasic fall, especially when the depolarization is large (Figs 1B, 3). The results may be caused by the several K-conductances in the *Paramecium* membrane (reviewed by Kung & Saimi, 1982), all of which are left intact by the pawn mutations (Kung & Eckert, 1972; Satow & Kung, 1980*b*). These K-conductances apparently have differential sensitivity to TEA, as is evidenced by the spontaneous action potentials of wild type bathed in TEA solutions (Satow & Kung, 1976*b*).

Posterior stimulation produces a hyperpolarizing response, known to be due to K^+ efflux (Deitmer, 1981), which can be completely suppressed by the addition of the K-channel blocker, TEA^+, to the external bath (Naitoh & Eckert, 1973; Deitmer, 1982). In the solution containing 4 mM-TEA, a depolarization is observed regardless of which part of the cell is stimulated (Fig. 2). Our experiments were therefore carried out in this solution. In most cases, the paramecia were prodded near their mid-sections.

The graded nature of the depolarizing receptor potential

The receptor potential was triggered by dimpling the cell surface. The magnitude of the depolarization increased with the extent of the probe excursion until further

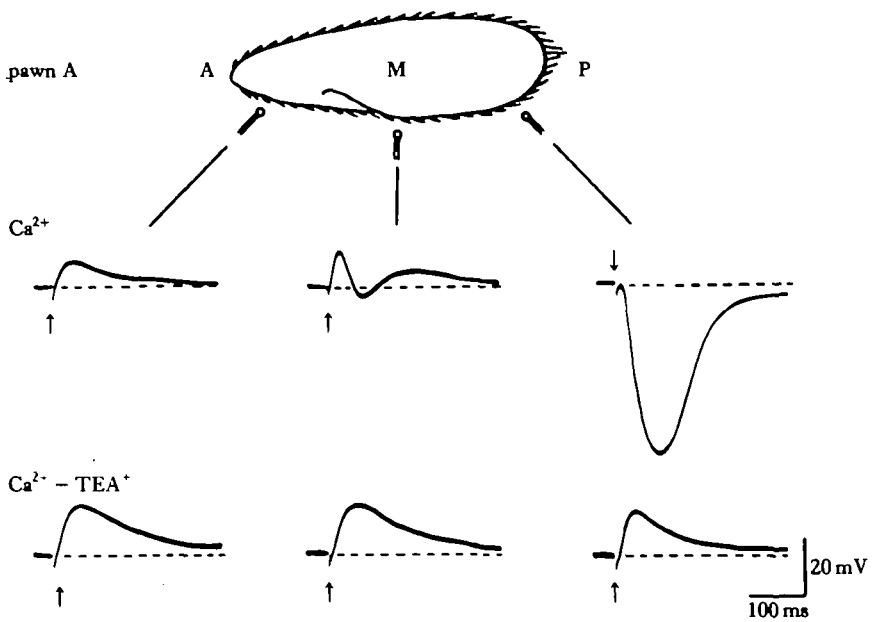

Fig. 2. Membrane potential responses to mechanical stimulation delivered at different parts of a pawn-mutant cell (*pwA*$_{500}$) bathed in the Ca^{2+} solution (upper recordings) and the Ca^{2+}-TEA^+ solution (lower recordings). Arrows indicate the time at which the glass stylus is driven to prod the cell surface. In the Ca^{2+} solution, a depolarizing response is recorded when the anterior region (A) of the cell is prodded and a large hyperpolarizing response is seen when the posterior region (P) is prodded. A biphasic response often appears when the mid-section (M) is stimulated. When 4 mM-TEA^+ is added to the Ca^{2+} solution, only depolarizations are recorded *regardless of where the cell is prodded*. All recordings are from one pawn cell in a perfusing bath.

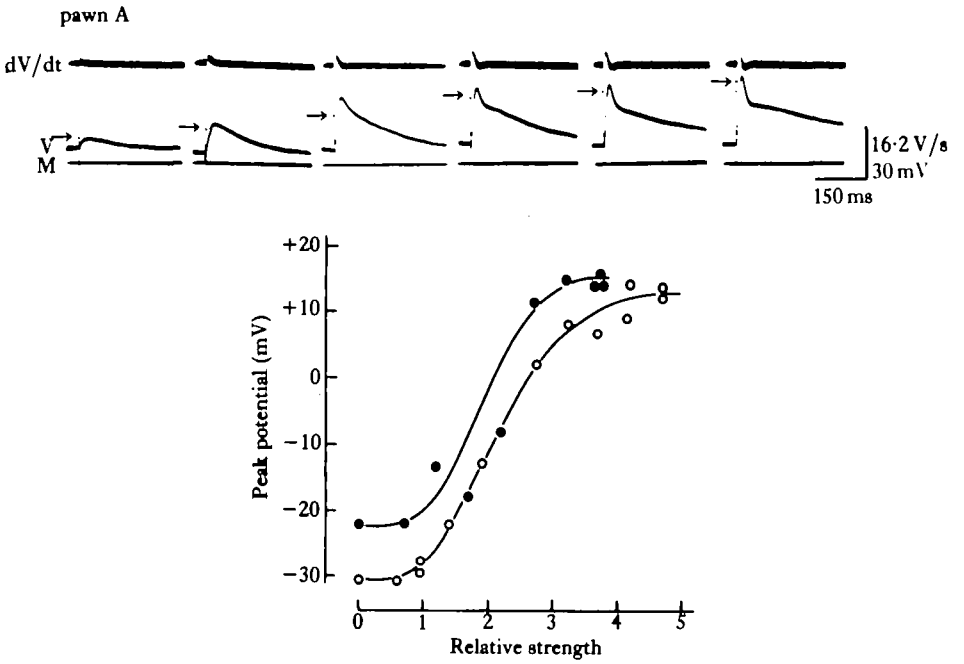

Fig. 3. Relation between the strength of mechanical stimulation and the membrane potential responses of $ptwA_{500}$ bathed in the Ca^{2+}-TEA^{+} solution. The upper six panels are recordings from one cell showing the response (V) increases with the voltage pulse (arrows on M trace) which drives the prod. The rate of rise of the response, as seen in the dV/dt trace, also increases with the strength of the stimulus. The lower graph plots the responses of two typical $ptwA_{500}$ cells (ordinate) against the voltage that drives the prod (abscissa). Although the two cells (open and filled circles) rest at different levels, they both show larger responses to stronger stimulations until their maximal responses are reached. (See text and Fig. 1 concerning the slow biphasic falls of the larger responses.)

movement produced no further depolarization (Fig. 3). The probe excursion was proportional to the voltage which drove it and was monitored, though not measured, through the microscope. The rate of depolarization also increased with the strength of the stimulation. In the Ca^{2+}-TEA solution, the maximal depolarization induced by mechanical stimulation was from a resting level of $-21{\cdot}6 \pm 7{\cdot}3$ mV to $-6{\cdot}6 \pm 2{\cdot}5$ mV (mean ± S.D., $N = 4$).

The ionic bases of the depolarizing receptor potential

A paramecium can survive for hours and show behavioural and electrical responses to mechanical stimulation in solutions containing Ca^{2+} as the sole metal cation. That the mechanosensory depolarization is exhibited in such solutions strongly suggests that Ca^{2+} can serve as a carrier through the ion channel opened by the mechanical stimulation. To test further the dependence of the receptor potential on Ca^{2+}, we varied the Ca^{2+} concentration and examined the maximal responses of the pawns (Fig. 4A). Increasing the external free Ca^{2+} concentration from 0·13 to 1·33 mM increased the maximal response to mechanical stimulation from $-25{\cdot}3 \pm 6{\cdot}9$ mV to $+8{\cdot}9 \pm 0{\cdot}3$ mV (mean ± S.D., $N = 4$). Increasing the external Ca^{2+} concentration also depolarized the resting membrane (Naitoh & Eckert, 1968; Satow & Kung,

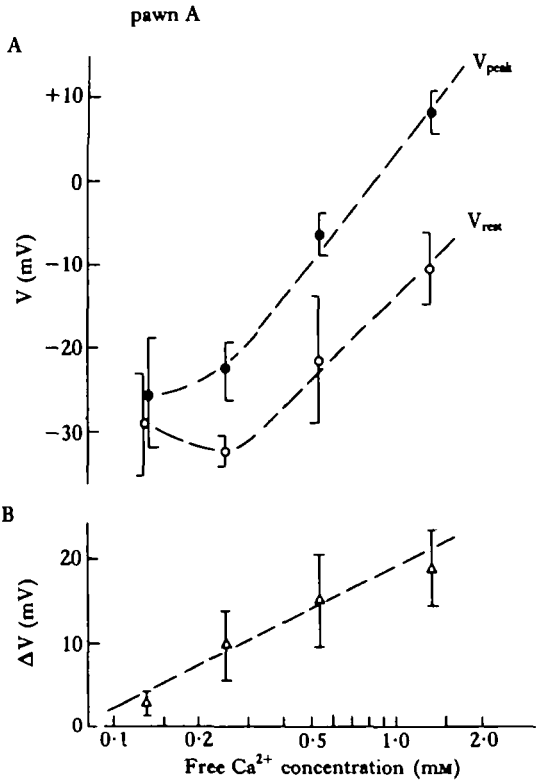

Fig. 4. Maximal depolarizing responses of $ptwA_{500}$ to mechanical stimulation in TEA^+ containing solutions of different free Ca^{2+} concentrations. The maximal depolarizations (V_{peak}) and the resting potentials (V_{rest}) are plotted in (A). The amplitudes of the maximal depolarizations (ΔV) measured from the resting level are given in (B). (See text for the rationale of the ΔV measurement.) In both plots, the response increases with increasing Ca^{2+} concentration. Mean ± S.D., $N = 4$.

1979). This apparent change in the resting level is now viewed as a response to the change in surface-charge pattern and not a genuine Nernst effect (Eckert & Brehm, 1979; Satow & Kung, 1979, 1981). Discounting the change in resting potential, the mechanically induced response increased by about 20 mV per decade increase in Ca^{2+} (Fig. 4B). These results further substantiate the view that Ca^{2+} normally carries most of the charges in the depolarizing mechanosensory response.

Ion selectivity of the mechanosensory Ca-conductance

In order to see which metal cations could carry the current for the depolarizing receptor potential, several cations were applied individually in the TEA buffer. The maximal response to mechanical stimulation recorded in the TEA buffer was small (5 mV at most, Fig. 5A) and did not follow the time course of the normal physiological response (Fig. 1). Addition of 8 mM-Na^+, Li^+ or K^+, did not significantly change the response (Fig. 5B, C, D). The usual time course was restored by the addition of 1 mM Ca^{2+}, Sr^{2+}, Ba^{2+} or Mn^{2+} to the TEA buffer (Fig. 5, E–I). Because the specimens deteriorated rapidly in these solutions, comparison of results from different cells and different solutions can only be semi-quantitative. Nonetheless, the magnitude of the maximal response increased 5- to 20-fold after the Ca^{2+}-TEA

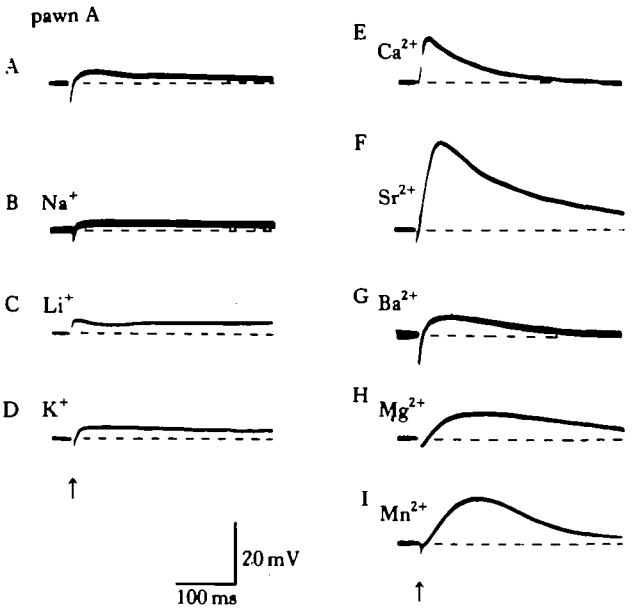

Fig. 5. Maximal depolarizing responses to mechanical stimulation (arrows) from different *pwA*$_{500}$ cells bathed in different solutions. (A) in the 4 mM-TEA^+-containing buffer solution; (B–I) in the solutions of 8 mM-monovalent cation or 1 mM-divalent cation (as labelled) added to the TEA^+-containing buffer (see Materials and Methods). Each solution was tested with at least five specimens. Traces are typical of the more robust specimens. Note that the monovalent cations have little, if any, effect upon the response, whereas the divalent cations significantly increase the response.

Sr^{2+}-TEA solution replaced the TEA buffer. The responses in Ba^{2+}, Mg^{2+} and Mn^{2+} were smaller and slower than those in Ca^{2+} or Sr^{2+}. These results indicate that all the divalent cations tested can permeate the conductance for the depolarizing mechanosensory response but the monovalent cations cannot.

The above experiments use the pawn mutant of the *pwA* complementation group (d4-500). Similar results were obtained for the key experiments with a second pawn mutant of the *pwB* group (d4-95). This pawn also gave graded mechanoreceptor potentials without action potentials in the Ca^{2+}-TEA solution. The receptor potential was also present in the Mg^{2+}-TEA solution but absent in the Na^+-TEA solution.

Ion selectivity of the voltage-sensitive Ca-conductance

To compare the ion selectivity of the mechanosensory Ca-conductance with that of the voltage-sensitive Ca-conductance, we examined the action potential of the wild-type *P. tetraurelia* bathed in these single-metal-ion solutions (Fig. 6). Judging from the potential recording or the dV/dt traces, Sr^{2+} and Ba^{2+} can substitute for Ca^{2+} in the generation of action potentials whereas Mg^{2+}, Mn^{2+} and Na^+ cannot. The Ba^{2+}-activation is small and slow, though significant. Prolonged plateau depolarizations following the action potentials are recorded from the wild type bathed in the Sr^{2+}- or the Ba^{2+}-TEA solutions. In the latter solution, the plateau lasts for several seconds.

Our results and the recent voltage-clamp results of Saimi & Kung (1982) with these solutions of single metal-ion species showed that Sr^{2+} and Ba^{2+}, as well as Ca^{2+}, can carry the current through the voltage-sensitive Ca-conductance. The results confirm

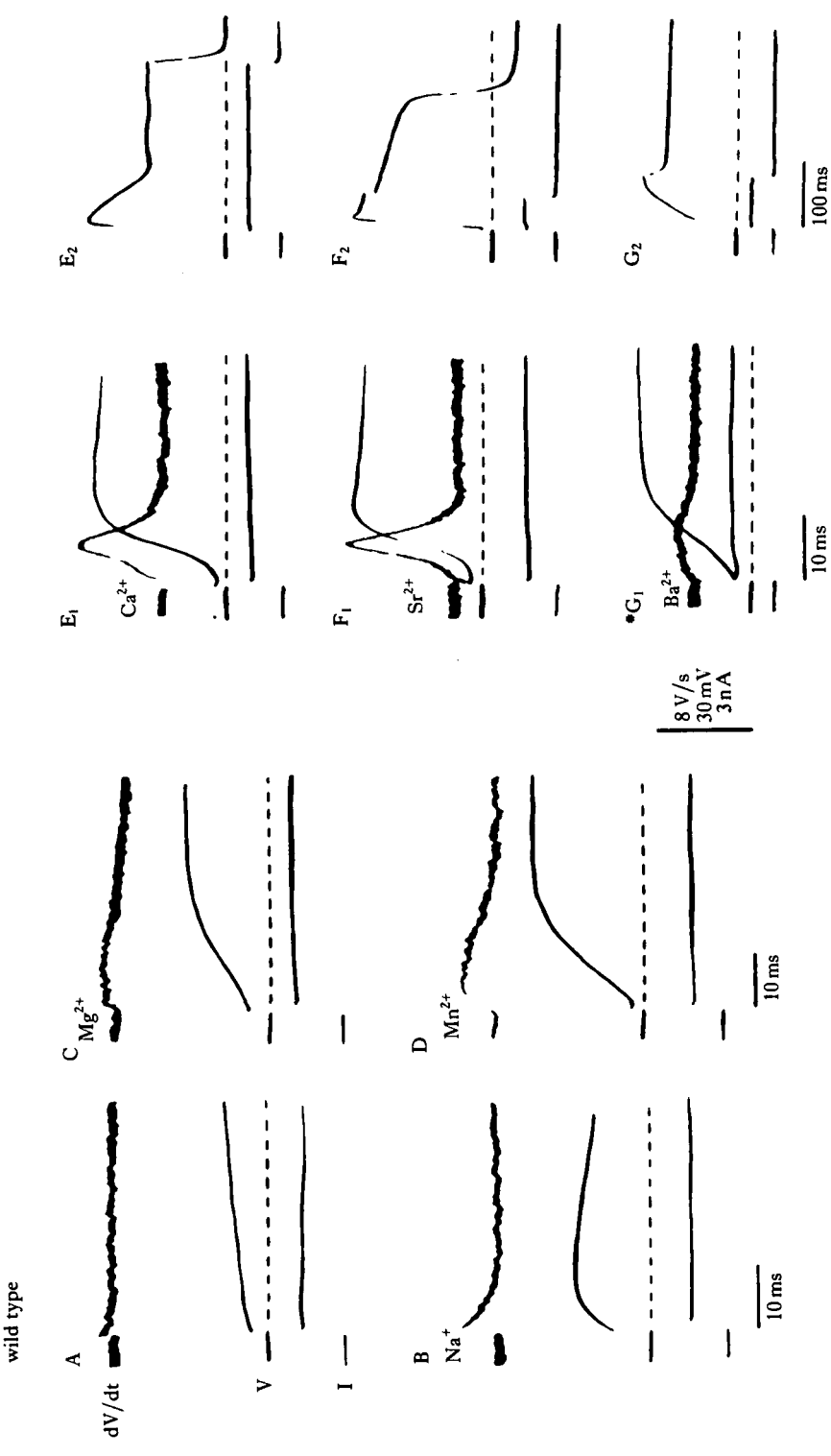

Fig. 6. Membrane potential responses (V) induced by outward current (I) injected through a second electrode in wild-type cells bathed in different solutions. (A) in TEA^+ buffer; (B–E) in different cation-TEA^+ solutions as described in the legend of Fig. 5. Note that G_1 is of a slower sweep speed. In all panels, the bottom trace is the current trace. The next is the voltage trace. The top trace, if present, is the dV/dt trace. Judging from the V and dV/dt responses, regenerative depolarization occurs in wild type bathed in Ca^{2+}-, Sr^{2+}- and Ba^{2+}-TEA^+ solutions and not in the other solutions. E_2, F_2 and G_2 are of slow sweep speed, showing the presence of prolonged depolarization after the current stimulation in Sr^{2+}- and Ba^{2+}-TEA^+ solution but not in the Ca^{2+}-TEA^+ solution.

those of other workers using solutions of mixed metal ions (Eckert & Brehm, 1979; Naitoh & Eckert, 1972).

DISCUSSION

Using a mutation to erase the action potential and thereby unmask the receptor potential, we confirmed the previous finding that Ca^{2+} is the usual ion which carries the receptor current for the depolarization response to mechanical stimulation (Ogura & Machemer, 1980). We also found that other divalent cations, but not the monovalent cations, can carry that current in *P. tetraurelia* as in *Stylonychia* (de Peyer & Deitmer, 1980).

Mg^{2+} is a candidate for being a physiological carrier of the depolarizing receptor potential. However, the internal concentration of Mg^{2+} is expected to be several orders of magnitude higher than that of Ca^{2+} (Nakaoka & Toyotama, 1979), and therefore the electrochemical gradient is less favourable for Mg^{2+} as a physiological receptor current carrier. As shown in Fig. 4, Mg^{2+} is less effective in that role than Ca^{2+}. We found that wild-type *P. caudatum* showed little electrical response to mechanical stimulation in a solution of 0·01 mM-Ca^{2+} and 0·99 mM-Mg^{2+} (data not shown), but both the receptor potential and the action potential returned when the bath was refilled with a 1 mM-Ca^{2+} solution. Our results in *P. tetraurelia* are similar to those for *Stylonychia* (de Peyer & Machemer, 1978; de Peyer & Deitmer, 1980) which showed that the depolarizing receptor potential is normally dependent on Ca^{2+}, but Mg^{2+}, Ba^{2+} and Sr^{2+} could also carry the receptor current in this hypotrichous ciliate. Unlike our finding in *Paramecium*, however, Mn^{2+} failed to carry that current in *Stylonychia*. Although systematic study of the membrane resistance in these solutions was difficult in our experiments because of the rapid deterioration of the specimens, we found that the membrane resistance was low when no metal cation was in the bath. Addition of a mono- or divalent cation increases the resistance to 20 MΩ or above. While there are differences in membrane resistance of cells bathed in solutions of different cations these differences alone cannot fully account for the presence of a receptor potential in divalent cation solutions and its absence in monovalent cation solutions (Fig. 4). It is interesting that the mechanoreceptor channels in the hair cells of the inner ear are also relatively nonselective (Corey & Hudspeth, 1979; Edwards, Ottoson, Rydqvist & Swerup, 1981) compared to voltage-sensitive channels. In the case of the hair cell, both monovalent and divalent cations permeate the receptor channel.

The conductance for the depolarizing mechanosensory receptor of *Paramecium tetraurelia* is apparently not very permeable to Na^+, Li^+ or K^+, and is not blocked by TEA^+ (Fig. 4). Since the equilibrium potential of K^+ is usually tens of millivolts more negative than the resting level (Oertel, Schein & Kung, 1978; Satow & Kung, 1980*a*; Eckert, Naitoh & Machemer, 1976) membrane hyperpolarization is to be expected upon mechanical stimulation, if K^+ permeates the receptor conductance in question. Our results show that no such hyperpolarization occurs even when the bath is completely devoid of K^+. This result cannot be attributed to the presence of TEA^+ since TEA^+ does not block the depolarizing receptor conductance as shown by the receptor potentials when permeant ions are provided. Under natural conditions,

without TEA^+, stimulation at the midsection activates both the Ca^{2+}-based depolarizing and the K^+-based hyperpolarizing conductance as seen in the biphasic response of Fig. 2. From their elegant experiments with deciliated, voltage-clamped *P. caudatum*, Ogura & Machemer (1980) concluded that the receptor current induced by mechanical stimulation of the cell's anterior is the sum of a K^+ and a Ca^{2+} current. Stimulation of the cell's posterior can induce depolarization as revealed when TEA^+ is included in the bath (Fig. 2). These results mean that the receptors with the Ca^{2+} channels and those with the K^+ channels can both be activated by stimulations delivered to any part of the paramecium. There are at least two possible explanations of the natural responses: depolarization dominates the response to anterior stimulation and hyperpolarization dominates the response to posterior stimulation. The two populations of receptors may be mingled and distributed along two overlapping gradients over the entire surface of the paramecium (Ogura & Machemer, 1980). Alternatively, the two kinds of receptors, with unspecified distributions, may have different sensitivity. It is known that a smaller mechanical stimulation is needed to elicit a response from the 'posterior receptor' (considered to be the same as the K^+-based hyperpolarizing mechanoreceptor here) than the 'anterior receptor' (the Ca^{2+}-based depolarizing mechanoreceptor) (see Material and Methods of this paper and Naitoh & Eckert, 1973; Naitoh, 1974). Because the depolarizing response is nearly the same regardless of where the stimulation is delivered, when TEA^+ is included in bath (Fig. 2, bottom), it is possible that the Ca-based depolarizing receptors are not direction specific but detect impact from any direction. To account for the different responses in the normal, TEA^+-free, solution (Fig. 2, top), one may speculate that the K^+-based hyperpolarizing receptors may be much more easily activated and prefer posterior impact to anterior impact. This alternative hypothesis allows us to rationalize Ogura & Machemer's observation (1980) that anterior stimulation induces both Ca^{2+} and K^+ currents, and the previously unexplained result of Naitoh & Eckert (1973) that TEA^+ converts the hyperpolarizing response to a depolarizing one.

While the voltage-sensitive conductance for the action potential and the mechanosensory conductance for the depolarizing receptor potential both use Ca^{2+} as their major natural current carrier the two conductances are quite different. First, they differ in their triggering mechanisms (Eckert *et al.* 1972). Second, the voltage-sensitive Ca-conductance is present exclusively on the ciliary membrane while the mechanosensory Ca-conductance is on the soma membrane (Ogura & Takahashi, 1976; Machemer & Ogura, 1979). Third, the two conductances clearly differ in their ion specificities. Finally, pawn and CNR mutations eliminate the function of the voltage-sensitive Ca-conductance but not the mechanosensory Ca-conductance. These results, taken together, indicate that these two conductances not only serve different functions but most likely also have different molecular components.

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REFERENCES

CHANG, S. Y., VAN HOUTEN, J., ROBLES, L. G., LUI, S. S. & KUNG, C. (1974). An extensive behavioural and genetic analysis of the pawn mutants of *Paramecium aurelia*. *Genet. Res.* **23**, 165–173.

HUDSPETH, A. J. (1979). Ionic basis of the receptor potential in a vertebrate hair cell. *Nature, Lond.* **281**, 675–677.

DEITMER, J. W. (1981). Voltage and time characteristics of potassium mechanoreceptor current in the ciliate *Stylonychia*. *J. comp. Physiol.* **141**, 173–182.

DEITMER, J. W. (1982). The effects of tetraethylammonium and other agents on the potassium mechanoreceptor current in the ciliate *Stylonychia*. *J. exp. Biol.* **102**, 239–249.

DE PEYER, J. E. & DEITMER, J. W. (1980). Divalent cations as charge carriers during two functionally different membrane currents in the ciliate *Stylonychia*. *J. exp. Biol.* **88**, 73–89.

DE PEYER, J. E. & MACHEMER, H. (1978). Hyperpolarizing and depolarizing mechanoreceptor potentials in *Stylonychia*. *J. comp. Physiol.* **127**, 255–266.

ECKERT, R. & BREHM, P. (1979). Ionic mechanisms of excitation in *Paramecium*. In *Annual Review of Biophysics and Bioengineering*, Vol. 8, (eds L. J. Mullins, W. A. Hagins & C. M. Newton), pp. 353–383. Palo Alto: Annual Reviews, Inc.

ECKERT, R., NAITOH, Y. & FRIEDMAN, K. (1972). Sensory mechanisms in *Paramecium*. I. Two components of the electric response to mechanical stimulation of the anterior surface. *J. exp. Biol.* **56**, 683–694.

ECKERT, R., NAITOH, Y. & MACHEMER, H. (1976). Calcium in the bioelectric and motor functions of *Paramecium*. In *Calcium in Biological Systems*, (ed. C. J. Duncan), pp. 233–255. New York: Cambridge University Press.

EDWARDS, C., OTTOSON, D., RYDQVIST, B. & SWERUP, C. (1981). The permeability of the transducer membrane of the crayfish stretch receptor to calcium and other divalent cations. *Neuroscience* **6**, 1455–1460.

KUNG, C. (1971). Genic mutants with altered system of excitation in *Paramecium aurelia*. II. Mutagenesis, screening and genetic analysis of the mutants. *Genetics* **69**, 29–45.

KUNG, C. (1979). The biology and genetics of paramecium behavior. In *Topics in Neurogenetics*, (ed. X. O. Breakfield), pp. 1–26. New York: Elsevier North-Holland.

KUNG, C. & ECKERT, R. (1972). Genetic modification of electric properties in an excitable membrane. *Proc. natn. Acad. Sci. U.S.A.* **69**, 93–97.

KUNG, C. & SAIMI, Y. (1982). The physiological basis of taxes in *Paramecium*. *Ann. Rev. Physiol.* **44**, 519–534.

LING, K. Y. & KUNG, C. (1980). Ba^{2+} influx measures the duration of membrane excitation in *Paramecium*. *J. exp. Biol.* **84**, 73–87.

MACHEMER, H. & OGURA, A. (1979). Ionic conductances of membranes in ciliated and deciliated *Paramecium*. *J. Physiol., Lond.* **296**, 49–60.

NAITOH, Y. (1974). Bioelectric basis of behavior in protozoa. *American Zool.* **14**, 883–893.

NAITOH, Y. & ECKERT, R. (1968). Electrical properties of *Paramecium caudatum*: Modification by bound and free cations. *Z. vergl. Physiol.* **61**, 427–452.

NAITOH, Y. & ECKERT, R. (1972). Electrophysiology of ciliate protozoa. In *Experiments in Physiology and Biochemistry*, Vol. V, (ed. G. A. Kerkut), pp. 17–38. New York: Academic Press.

NAITOH, Y. & ECKERT, R. (1973). Sensory mechanisms in *Paramecium*. II. Ionic basis of the hyperpolarizing mechanoreceptor potential. *J. exp. Biol.* **59**, 53–65.

NAKAOKA, Y. & TOYOTAMA, H. (1979). Directional change of ciliary beat effected with Mg^{2+} in *Paramecium*. *J. Cell Sci.* **40**, 207–214.

OERTEL, D., SCHEIN, S. J. & KUNG, C. (1977). Separation of membrane currents using a *Paramecium* mutant. *Nature, Lond.* **268**, 120–124.

OERTEL, D., SCHEIN, S. J. & KUNG, C. (1978). A potassium conductance activated by hyperpolarization in *Paramecium*. *J. Membrane Biol.* **43**, 169–185.

OGURA, A. & MACHEMER, H. (1980). Distribution of mechano-receptor channels in the *Paramecium* surface membrane. *J. comp. Physiol.* **135**, 233–242.

OGURA, A. & TAKAHASHI, K. (1976). Artificial deciliation causes loss of calcium-dependent responses in *Paramecium*. *Nature, Lond.* **264**, 170–172.

SAIMI, Y. & KUNG, C. (1982). Are ions involved in the gating of calcium channels? *Science, N.Y.* **218**, 153–156.

SATOW, Y. & KUNG, C. (1976*a*). A mutant of *Paramecium* with increased relative resting potassium permeability. *J. Neurobiol.* **7**, 325–338.

SATOW, Y. & KUNG, C. (1976*b*). A 'TEA-insensitive' mutant with increased potassium conductance in *Paramecium aurelia*. *J. exp. Biol.* **65**, 51–63.

SATOW, Y. & KUNG, C. (1977). A regenerative hyperpolarization in *Paramecium*. *J. comp. Physiol.* **118**, 99–110.

SATOW, Y. & KUNG, C. (1979). Voltage-sensitive Ca-channels and the transient inward current in *Paramecium tetraurelia*. *J. exp. Biol.* **78**, 149–161.

SATOW, Y. & KUNG, C. (1980*a*). Membrane currents of pawn mutants of the *pwA* group in *Paramecium tetraurelia*. *J. exp. Biol.* **84**, 57–71.

SATOW, Y. & KUNG, C. (1980*b*). Ca-induced K^+-outward current in *Paramecium tetraurelia*. *J. exp. Biol.* **88**, 293–303.

SATOW, Y. & KUNG, C. (1981). Possible reduction of surface charge by a mutation in *Paramecium tetraurelia*. *J. Membrane Biol.* **59**, 179–190.

SCHEIN, S. J., BENNETT, M. V. L. & KATZ, G. M. (1976). Altered calcium conductance in pawns, behavio mutants of *Paramecium aurelia*. *J. exp. Biol.* **65**, 699–724.

SONNEBORN, T. M. (1970). Methods in *Paramecium* research. In *Methods in Cell Physiology*, Vol. 4, (ed. D. Prescott), pp. 241–339. New York: Academic Press.

TAKAHASHI, M. & NAITOH, Y. (1978). Behavioural mutants of *Paramecium caudatum* with defective membrane electrogenesis. *Nature, Lond.* **271**, 656–658.