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Electrical Responses of the Marine Ciliate *Euplotes vannus* (Hypotrichia) to Mechanical Stimulation at the Posterior Cell End

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Abstract. Electrical responses upon mechanostimulation at the posterior cell end were investigated in the marine hypotrichous ciliate *Euplotes vannus*. A new mechanostimulator was developed to mimic stimuli that are identical with those involved in cell-cell collisions. The receptor potential hyperpolarized by 18–35 mV within 12–25 msec, reached a peak value of −62 mV with a delay of 4–9 msec after membrane deformation, and was deactivated after 50–70 msec. Cirri were stimulated to beat accelerated backward. The corresponding receptor current exerted a similar time course with a peak of 2.4 nA. The shift of the reversal potential by 57.6 mV at a tenfold increase of $[K^+]_o$ identifies potassium ions as current carriers within the development of the receptor potential. An intracellular K concentration of 355 mmol/liter was calculated for cells in a medium that was composed similar to sea-water. The mechanically activated potassium current was totally inhibited by extracellular TEA and intracellular Cs^+, and partially inhibited by extracellular 4-AP. The total inhibition of the current by injected EGTA points to a Ca dependence of the posterior mechanosensitivity. It was confirmed by the increase of the peak current amplitude with rising $[Ca^{2+}]_o$. Sodium presumably repolarizes the receptor potential because the repolarization was delayed and after-depolarizations were eliminated in media without sodium. Since deciliation did not affect mechanosensitivity, the corresponding ion channels reside within the soma membrane.

Key words: *Euplotes vannus*—Posterior mechanosensitivity—Receptor potential—K current—Ca dependence

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Introduction

In ciliates, behavioral events depend on how their motor organelles, cilia or cirri, respectively, are caused to change their movements. Basically, a collective action of several ion channels is engaged therein (*reviews see* Machemer, 1989; Machemer & Sugino, 1989; Preston & Saimi, 1990). For the *vannus*-morphospecies of the hypotrichous genus *Euplotes*, the repertoire of most prominent voltage-regulated ion currents has been established (Krüppel & Lueken, 1988, 1990; Krüppel, Westermann & Lueken, 1991), together with first insights into their interactions (Westermann, Krüppel & Lueken, 1990). Here, we analyze membrane currents upon mechanical stimulation at the posterior cell end that elicits a hyperpolarizing receptor potential. Presumably, the hyperpolarization in turn triggers the activation of an inwardly rectifying sodium current (Krüppel, 1993) that quickly repolarizes the mechanically activated receptor potential back to the resting potential. The further aim of our examination is to understand the regulation of ion currents with respect to the membrane potential fluctuations and the correlated changes of single elements of the behavior of the cells, whose ethogram has been described at the phenomenological level (Ricci, Giannetti & Miceli, 1987; Ricci, 1990).

The results are compared to previous data from the freshwater species *Stylonychia mytilus* (Deitmer, 1981, 1982; Machemer & Deitmer, 1987) and to *Paramecium* (Naitoh & Eckert, 1973; Ogura & Machemer, 1980; Machemer-Röhnisch & Machemer, 1984; Machemer, 1985; Preston, Saimi & Kung, 1990). No studies of mechanoreception in marine, ciliated protozoa have been performed so far.

Materials and Methods

Stocks and Culture

Clone D_{35} has been bred from a syngen of the *Euplotes vannus* morphospecies originally collected at Naples, Italy. Its relationship to the *crassus* morphospecies within the *E. vannus/crassus/minuta* group has been recently confirmed (Valbonesi, Ortenzi & Luporini, 1992). Propagation and breeding in artificial seawater (ASW) were as described (Lueken, Gaertner & Breer, 1983). Measurements were performed at room temperature (20–22°C). Cells, from 6- to 8-day-old cultures were used, but only if they had the typical input resistance of healthy cells (mean 49 MΩ, Krüppel & Lueken, 1990) in standard recording solution.

Solutions

Standard recording solution, i.e., artificial sea-water for electrophysiology (EASW) contained (in mmol/liter): NaCl 430, KCl 10, $CaCl_2$ 10, $MgCl_2$ 53, HEPPS (=EPPS, [N-(2-hydroxyethyl)piperazine-N'3-propanesulfonic acid) 10. The pH was adjusted according to experimental conditions with KOH, NaOH, or HCl to 8.0–8.1. When the potassium concentration was increased up to 150 mmol/liter by addition of KCl, osmolarity was balanced by equimolar reduction of NaCl content. Na^+ was replaced by the large membrane impermeant cation NMDG (N-Methyl-D-glucamine) and Ba^{2+} was added via equimolar substitution of Ca^{2+}. TEA (tetraethyl-ammonium chloride, 10 mmol/liter), or 4-AP (4-aminopyridine, 3 mmol/liter), or Ca^{2+} were added to the standard solution. Cells were transferred from breeding solution to EASW or solutions with increased $[K^+]_o$ by a sieving technique 1–2 hr before starting experiments. Solutions were exchanged with at least 5 times the filling volume of the experimental chamber. At the end of an experiment, cells were exposed to EASW to exclude unspecific damage by the preceding treatments. Decirriation—which means deciliation for hypotrichs (Krüppel & Lueken, 1990)—was modified by pouring EASW with 7% ethanol over cells on a sieve. This procedure disintegrated all cirri within a few sec without any visible other damage.

Electrical Recording

General techniques were as described by Krüppel and Lueken, 1990 and Krüppel et al., 1991. Electrical recordings were performed with a single-electrode voltage-clamp system (npi SEC 1L, H.-R. Polder, D-71732 Tamm, Germany), an analog-digital interface board (Tecmar, Scientific Solutions Division, Solon, OH), a personal computer (PCA 40, Tandon), and the pCLAMP software (Axon, Foster City, CA). Switching frequency was 10 kHz with a duty cycle of 50%. Records were on-line filtered at 1 kHz with an 8-pole Bessel filter (48dB/octave). Microelectrodes, either filled with 1 mol/liter KCl, or CsCl to block K channels, had resistances of 12 to 15 MΩ. To introduce EGTA [ethylene glycol-O,O'-bis(2-aminoethyl)-N',N, N',N'-tetraacetic acid] into a cell, the drug was added to the electrolyte of the microelectrode in a concentration of 200 mmol/liter. Cs ions or EGTA, respectively, leaked out of the electrodes within 5–10 min. Proceeding effectiveness of the drugs became manifest in a typical change of the current-induced action potentials. Effectiveness was evaluated as maximal when the graded action potential was transformed into an all-or-none type. This change arises either when Cs^+ blocks K-outward currents that usually repolarize the action potential, or when EGTA prevents the Ca-dependent inactivation of the depolarization-activated Ca channels in combination with the lacking activation of Ca-dependent K-outward currents (Krüppel & Lueken, 1990). In addition, we documented the increasing block of the Ca-dependent outward currents with depolarizing test pulses before we stimulated the cells mechanically. Holding potential in voltage clamp was −25 mV if not otherwise stated. Voltages and currents were averaged from at least four cells. Standard deviation is marked within figures by bars. Leakage currents were not subtracted since they are not relevant when receptor currents are mechanically activated.

The cells were fixed by a further micropipette to prevent them from turning around the recording microelectrode. Thus, the posterior cell end could exactly be exposed to the mechanostimulator rod. The behavior of the cells was observed by a video system.

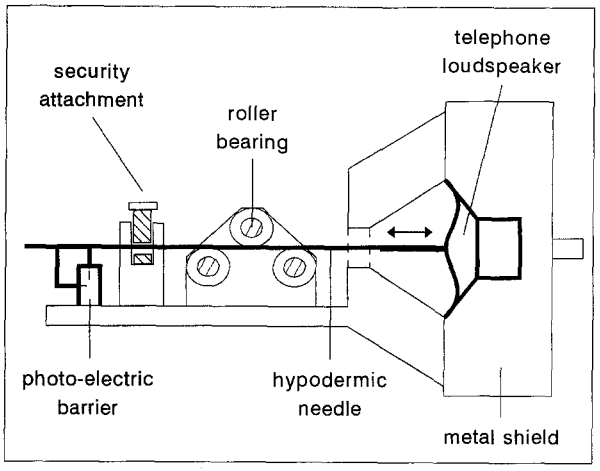

Fig. 1. Mechanostimulator moved by the electromagnetic system of a loudspeaker under feedback-control of a photoelectric barrier. The stimulator is connected to a micromanipulator at the right and a glass rod is glued to the tip of the hypodermic needle at the left. Details of the function are described within the text.

Mechanostimulator

Cells were touched by the vertical flank of a tiny glass rod. The rod was connected to the tip of a hypodermic needle that was glued to the membrane of a telephone loudspeaker with its other end (Fig. 1). The needle was properly supported by three roller bearings. The loudspeaker was shielded by a metal surrounding to prevent electrical coupling to the recording electrodes. When the rod had to be replaced, the loudspeaker membrane could be protected against mechanical destruction by a security attachment of the needle. The loudspeaker membrane was precisely moved forward or backward by the electromagnetic system of the loudspeaker under feedback-control via a photoelectric barrier and a computer (SHARP MZ-3500). The stimulator was mechanically calibrated via a step-motor driven micrometer calliper that moved the needle instead of the loudspeaker membrane. At

the tip of the needle, a mechanical dial gage measured the actual position of the needle with a resolution of 1 μm. The simultaneously measured voltages of the photoelectric barrier were linear over a range of 200 μm and were attributed to an address file of the computer. Thereafter, the address file determined the command voltage for the movement of the loudspeaker membrane via an 11-bit D/A converter. The command voltage is compared to the actual voltage of the photoelectric barrier and differences are amplified and used to drive the loudspeaker membrane as long as they are zero. An optical calibration of the stimulator was performed after its installation within the electrophysiological setup. The programmed step width of the rod tip was inspected with a microscope at 400-fold magnification, an object micrometer, and an ocular micrometer with an effective resolution of 1.5 μm. No significant difference between the programmed step width and the actual position of the rod tip was measured in a range of 140 μm. The ocular micrometer was left within the microscope throughout the whole set of experiments to determine the distance between the tip of the rod and the cell surface at the beginning of each experiment. The stimulation velocity was calculated from the rise time of the actual voltage signal of the photoelectric barrier and the programmed step width, but was not determined at the tip of the rod. The mechanostimulator and the electrophysiological recording device could be mutually triggered.

Results

Physiological Calibration of the Mechanostimulator

The mechanostimulator device should mimic the mechanical irritation of a cell that is hit by a free-moving specimen. To calibrate the apparatus, responses were recorded from cells that were touched by a free-moving cell at the posterior cell end. From 60 hits, the "natural" patterns of the cell responses were established. The parameters of the mechanostimulator were adapted until the most similar receptor potentials or receptor currents were recorded with either stimulation technique (Fig. 2). The standard stimulus parameters were: position of the stylus 1–3 μm aside the cell margin, forward motion by 15 μm with a speed of 1 μm/msec.

Membrane Potential Changes upon Posterior Mechanostimulation

The total delay between activation of the stimulator stylus and onset of potential shift was 5 to 12 msec (Fig. 2*a*). From that interval, however, about 3 msec are consumed until the stylus flank reaches the cell from its parking position. The real lag-time between the first mechanical membrane deformation and potential response was therefore 2 to 9 msec. The mechanically induced hyperpolarization reached a peak

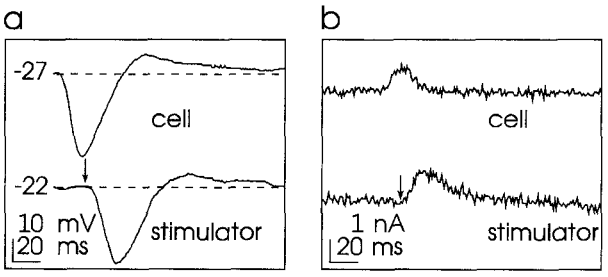

Fig. 2. Comparison of "naturally" and artificially elicited cell responses to posterior mechanostimulation. Receptor potentials (*a*) and receptor currents (*b*) upon touching the posterior cell end by a colliding cell (upper trace) or by the glass rod of a mechanostimulator (lower trace), respectively. Responses upon cell contact were at random in the time axis, those upon the mechanostimulator were induced at the arrows. Typical recordings as found in more than 40 cells. Numbers indicate membrane potentials immediately before the receptor potential.

potential of −60 to −62 mV, which is far from its reversal potential of around −90 mV (*see below*). The receptor potential rose with a speed of 1–2 mV/msec and reached its peak within 12–25 msec. The original potential was re-established with a speed of 0.3 to 0.7 mV/msec so that the total receptor potential persisted 50 to 70 msec. Approximately half of the cells showed an afterdepolarization of 5–10 mV that lasted several tens of milliseconds. When the cells were stimulated at the depolarized range of their spontaneously fluctuating membrane potential, almost no receptor potential could be elicited by posterior mechanostimulation.

Voltage regulation of the power stroke of the beating cirri was as stereotype as known from other ciliates. Upon high hyperpolarizations, accelerated backward beating was observed. Ciliary activity was reduced with increasing depolarization and stopped between −38 and −30 mV. Further depolarization started ciliary activity again, but with reversed (i.e., forward) beating direction. Mechanical stimulation at the posterior cell end could not reverse the direction of the forward directed power stroke (i.e., backward creeping cells) of the cirri.

Current upon Posterior Mechanostimulation

The delay between the first membrane deformation and onset of the current of 3–6 msec (Fig. 2*b*) corresponds well to the delay of the correlated hyperpolarization. Usually, the total current response was terminated within 50 msec at the latest, a response duration like that of the receptor potential without afterdepolarizations. This is different to the ciliates

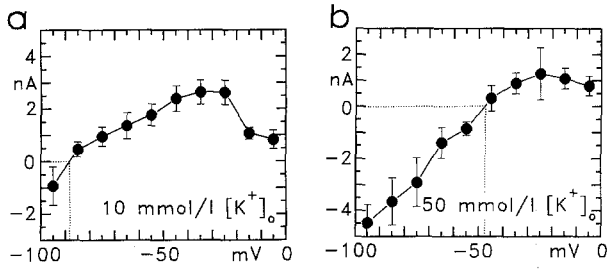

Fig. 3. Peak amplitudes of the receptor current plotted against membrane potentials in 10 mmol/liter (*a*, $n = 11$) and 50 mmol/liter (*b*, $n = 7$) external potassium concentration. Dotted lines refer to reversal potentials with zero current. Cells were polarized for 1 sec before being mechanically stimulated at the posterior cell end. *I/V* relationships are linear below membrane potentials of −25 mV, above, outward current amplitude declines unexpectedly.

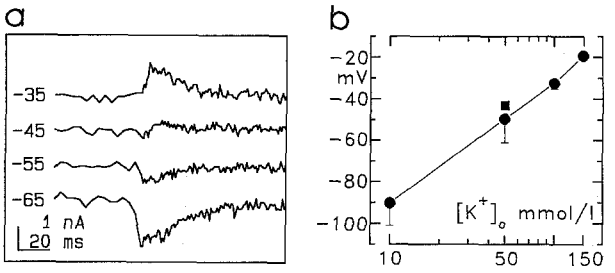

Fig. 4. K-concentration dependence of the reversal potential of the mechanoreceptor current. (*a*) Sequence of receptor currents of a cell in 50 mmol/liter $[K^+]_o$, demonstrating current reversal at a membrane potential between −45 and −55 mV. (*b*) Reversal potentials plotted against log $[K^+]_o$. The slope is 57.6 mV for tenfold $[K^+]_o$ change, $n = 2$–7. Doubling the $[Ca^{2+}]_o$ from 10 to 20 mmol/liter (■) did not significantly modify the reversal potential, $n = 7$.

Stylonychia and *Paramecium*, where the receptor potential evidently outlasts the receptor current (Ogura & Machemer, 1980; Deitmer, 1982). Times-to-peak for the receptor current was 9–12 msec independent of peak-current amplitude (*not shown*). That means, rise speed was positively correlated to current strength, e.g., it increased from 0.1 nA/msec at 0.3 nA to 0.22 nA/msec at 2.5 nA in EASW (n = 5–10 cells).

Receptor currents were determined in relation to external potassium concentration and to membrane potential (Fig. 3). The current-voltage relationships were almost linear in all media for the inward currents and for outward currents up to a threshold membrane potential of −25 mV. At more positive membrane potentials, peak-outward currents saturated or even decreased. In 100 and 150 mmol/liter, no substantial outward receptor currents were recorded, presumably due to the positive shift of the potassium equilibrium-potential and the corresponding extensive reduction of the electromotive force for potassium.

The reversal potentials of the receptor currents shifted by 57.6 mV at tenfold $[K^+]_o$ increase, thus giving strong evidence that the receptor current is entirely carried by K ions (Fig. 4). The intracellular K concentration, calculated from the potassium equilibrium potentials according to the Nernst equation, was 355 mmol/liter in EASW ($n = 11$), 360 mmol/liter in 50 mmol/liter K^+ ($n = 7$), and 324–363 mmol/liter for higher K concentrations ($n = 4$). *Euplotes* thus maintains its $[K^+]_i$ independently on $[K^+]_o$.

Receptor-current deactivation depended on the cells' membrane potential (Fig. 5) but revealed at all potentials a single-exponential decay. In standard solution (Fig. 5*a*), the time constant τ decreased from around 35 msec at hyperpolarized V_m to less

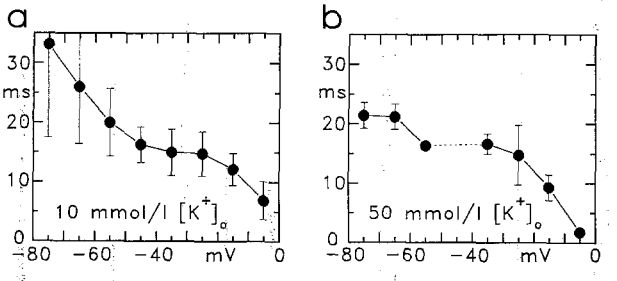

Fig. 5. Dependence of deactivation time constant, τ, of posterior mechanoreceptor current on membrane potential. (*a*) Cells (n = 11) in EASW. Within the range of most frequent values in free running V_m, −35 to −15 mV, τ was rather uniform around 15 msec. It increased in hyperpolarizing, and decreased in depolarizing direction. (*b*) Cells ($n = 7$) in 50 mmol/liter $[K^+]_o$. Notice that the inward currents (left of the dotted line that marks lack of current around the reversal potential) deactivate slowly at high current strength (*cf.* Fig. 3*b*).

than 10 msec at the most depolarized V_m. The current deactivated faster with increasing strength (*cf.* Fig. 3*a*), except at −15 and −5 mV where small currents deactivated fast. For outward currents in 50 mmol/liter $[K^+]_o$ (Fig. 5*b*, right to the dotted segment), the same results as in EASW were obtained (*cf.* Fig. 3*b*). For inward currents (Fig. 5*b*, left to the dotted segment), however, τ increased with current strength, which is the reverse relationship as for outward currents. Same results were obtained at the higher potassium concentrations (*not shown*). These kinetic data are in accordance with those formerly described for *Stylonychia* and *Paramecium* (*see* Discussion).

Block of Posterior Mechanoreception

External TEA (10 mmol/liter) totally suppressed the responses of mechanically stimulated cells (Fig. 6 *a*,*b*). Elevation of external Ca^{2+} up to 50 mmol/liter

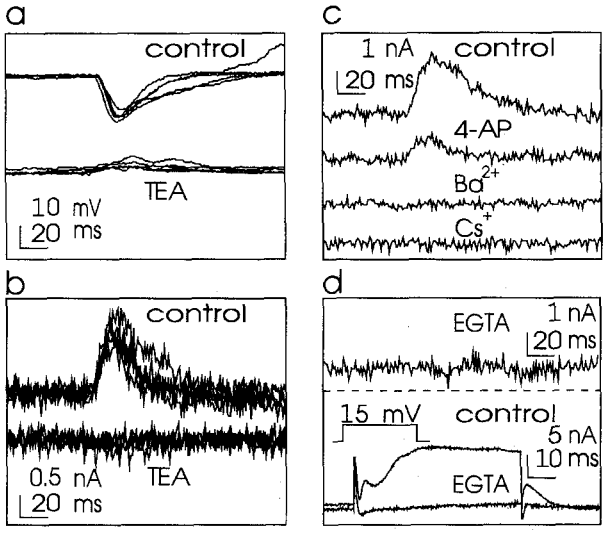

Fig. 6. Suppression of mechanoreception by blocking agents. Extracellular tetraethylammonium (TEA, 10 mmol/liter) completely blocked receptor potential (*a*) and receptor current (*b*) in all examined cells (n = 11). The figures show five superimposed reactions of one typical cell. (*c*) Effects of other agents; typical examples are shown only for outward current since the receptor potentials were always correspondingly affected. Extracellular 4-amino-pyridine (4-AP, 3 mmol/liter) inhibited 50% of the peak current (n = 4). All receptor currents were inhibited when Ba^{2+} replaced extracellular Ca^{2+} (n = 4), or when Cs ions were injected into the cell (n = 9). (*d*) No receptor current could be recorded when EGTA was injected into the cell (n = 5, upper half). The EGTA injections also reduced the depolarization-activated and Ca-dependent potassium outward current (lower half).

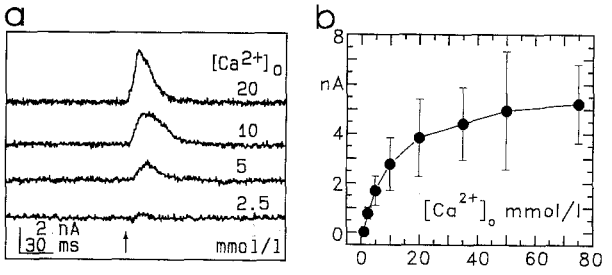

Fig. 7. Ca dependence of the mechanically activated outward current. (*a*) The peak outward current increased with increasing Ca concentration (one cell). (*b*) The dose-response relationship shows strong dependence of the peak current between 1 and 20 mmol/liter $[Ca^{2+}]_o$ (n = 8–18) and saturation at higher concentrations (n = 5–7).

did not reverse the TEA-block (*not shown*) as described for *Paramecium* (Naitoh & Eckert, 1973). No mechanical activated currents could be recorded when intracellular Cs^+ or extracellular Ba^{2+} were used as K-current blockers; the K-current blocker 4-AP (3 mmol/liter) reduced the receptor current partially (Fig. 6*c*). The intracellular-applied Ca chelator EGTA inhibited the cell responses to mechanostimulation totally (Fig. 6*d*). The effectiveness of the EGTA injection was confirmed by the block of the depolarization-activated and Ca-dependent potassium outward currents (Krüppel et al., 1991). Posterior mechanosensitive channels thus seem to be Ca regulated. This might be confirmed by the blocking effect of extracellular Ba ions that replace Ca ions, but Ba^{2+} can also act as a blocker of K channels themselves.

Decirriation did not abolish posterior mechanosensitivity (*not shown*). Therefore, mechanosensitivity must be allied to the soma membrane in *Euplotes* as in other ciliates (Machemer-Röhnisch & Machemer, 1984).

Ca Regulation of the Outward Current

Although the Ca regulation of the mechanically activated potassium current has been demonstrated by the EGTA experiments, a correlated Ca-inward current could not evidently be identified. Small Ca currents whose recording is masked by the activation of large K currents might be unmasked by the suppression of the K currents by specific blocking agents (*see above*). Small depolarizations or a mechanically activated Ca-inward current should remain, as described in other ciliates. Observations in *Euplotes*, however, were contradictory. When the outward current was blocked by intracellular EGTA neither a depolarization nor an inward current was found. When the outward current was suppressed by external TEA, a depolarization was elicited in rare cases (2 of 11), but the correlated inward current was never observed. When intracellular Cs ions were present, in some cells (3 of 9) a depolarization and a small, but distinct inward current could be recorded (*not shown*). Lag-time (3–7 msec) and time-to-peak (10 msec) were slightly shorter than those of the K current and thus this inward current partially precedes the potassium outward current.

The contribution of Ca^{2+} to the receptor current was additionally tested by the variation of the extracellular Ca concentration (Fig. 7). The peak amplitude of the mechanically activated receptor current rose with increasing Ca concentrations (Fig. 7*a*). The dose-response relationship (Fig. 7*b*) shows a strong variation of the peak current between 1 and 10 mmol/liter $[Ca^{2+}]_o$ and a saturation at concentrations between 20 and 75 mmol/liter $[Ca^{2+}]_o$. The reversal potential of the current was not modified after a Ca concentration change from 2.5 to 20 mmol/liter (*cf.* Fig. 4 for 20 mmol/liter). Therefore, the Ca ions do not directly contribute to the receptor current.

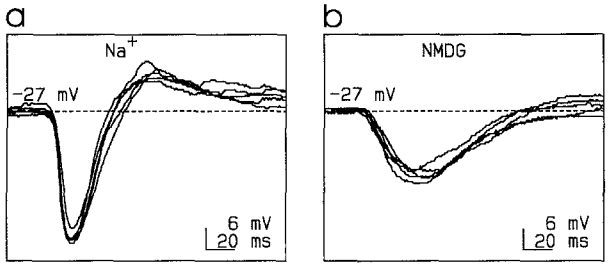

Fig. 8. Influence of Na replacement by NMDG on mechanically activated hyperpolarizations. Five responses to standard stimuli from a typical cell out of more than 10 analyzed cells are superimposed; dashed line indicates a membrane potential of −27 mV. (*a*) In standard medium with 430 mmol/liter Na^+, hyperpolarizations were followed by afterdepolarizations. (*b*) Total replacement of Na^+ by NMDG reduced the receptor potential amplitude, prolonged its duration, and eliminated the afterdepolarization.

Na Contribution to Posterior Mechanosensitivity

Recently, we have characterized inward rectification in *E. vannus* and have found that a Ca-regulated Na-inward current, and not as usual a K-inward current, causes inward rectification (Krüppel, 1993). This current activates upon hyperpolarization and might be involved in the repolarization of a mechanically activated, hyperpolarizing receptor potential. Therefore, we analyzed the contribution of Na ions to the receptor potential. When the extracellular Na^+ was totally replaced by NMDG (Fig. 8), a large molecule that presumably does not permeate the membrane, the amplitude of the mechanically induced hyperpolarization was significantly reduced, the duration of the hyperpolarization was prolonged, and the afterdepolarizations, frequently arising in EASW at the end of the receptor potential, disappeared. All responses reappeared when cells were transferred back to EASW. Similar results were recorded with Tris instead of NMDG (*not shown*). These results indicate a contribution of Na ions within the repolarization of receptor potential and will be analyzed in more detail.

Discussion

The main results of the present examination are: (i) *Euplotes* has a mechanically activated K current at its posterior cell end; (ii) this current is presumably activated by Ca^{2+}; (iii) the current shares some similarities to voltage- and Ca-activated $I_{K,slow}$ formerly examined in *Euplotes*; (iv) the receptor potential is quickly terminated by an inwardly rectifying Na current.

Distribution of Channels

The posterior part of the *Euplotes* cell membrane only contains K-selective mechanoreceptor channels (Fig. 4) as in *Stylonychia* (Deitmer, 1982). Block of these channels by extracellular TEA, by intracellular Cs ions (Fig. 6), or by clamping V_m to the equilibrium potential (*not shown*), could not unmask a further mechanically activated current, that is similar to the Ca current recorded in *Paramecium*. There, mechanosensitive K and Ca channels coexist within the larger portion of the soma membrane (Naitoh & Eckert, 1973; Machemer, 1985). Thus, with respect to the distribution of mechanosensitive ion channels, the hypotrichous ciliate *Euplotes* seems to be more closely related to the hypotrichous *Stylonychia* than the holotrichous *Paramecium*.

Neither decirriation in *Euplotes* nor deciliation in *Paramecium* (Ogura & Machemer, 1980) affects posterior mechanosensitivity. The cilia are not involved in the mechanosensory transduction, but are suited for stimulus transfer and are appropriate to defocus a local stimulus at the cilia to an extended area of the mechanosensitive soma membrane (Machemer-Röhnisch & Machemer, 1984).

Dependence of K-Receptor Current on Ca^{2+}

In *Euplotes*, intracellular EGTA totally prevented mechanically activated membrane currents (Fig. 6*d*). The loss of the receptor current after the injection of EGTA indicates the regulation of mechanosensitivity either by an influx of extracellular Ca^{2+} or the mobilization of intracellular Ca pools. The rise of the peak amplitude of the receptor current with increasing extracellular [Ca^{2+}] (Fig. 7) points to a regulatory function of an influx of Ca^{2+}. On the other hand, elevated Ca concentrations could not remove the TEA block of the K currents as has been described for *Paramecium* by Naitoh and Eckert (1973). The Ca current itself does not significantly contribute to the mechanically activated current because no shift of the reversal potential could be recorded when the Ca concentration was varied and no currents remained after the block of K channels (Fig. 6). Therefore, the postulated influx of Ca^{2+} might just be at the border of the resolution limits of the electrophysiological recording equipment. For *Stylonychia*, Deitmer (1982) found no evidence that the mechanoreceptor K current depended upon the presence, or the influx, of divalent cations. In some experiments, we recorded small depolarizations and corresponding inward currents when cells were stimulated at their posterior end. For the postulation of an inward current upon posterior mechanostimu-

lation, these results are too ambiguous, particularly because in rare cases, a parallel mechanostimulation of the anterior cell end cannot be excluded. How the Ca ions exert their regulatory function is still unknown in *Euplotes*. Recently, a hyperpolarization-activated Ca current was identified within a complex of various currents (Nakaoka & Iwatsuki, 1992; Preston, Saimi & Kung 1992 *a,b*), that should be activated together with any mechanically induced hyperpolarization in *Paramecium*. Whether the Ca current regulates the mechanosensitivity or whether the mechanically activated hyperpolarization activates the Ca current at physiological conditions is not known and should be further investigated. In *Euplotes*, a hyperpolarization-activated Ca current could not be identified, yet.

Mechanically Activated and Voltage-Dependent K Currents

Nonlinearity in mechanoreceptor current-voltage relationships above a certain membrane potential (Fig. 3), together with voltage dependence of the deactivation time constant (Fig. 5), seem to be a special feature of mechanosensitive K channels. In *Stylonychia*, the lifetime of the mechanically activated channels decreased with depolarization and increased with hyperpolarization. The phenomenon remains unresolved as understanding of channel function is concerned, although several attempts have been made to explain these results for ciliates (Deitmer, 1981; Machemer, 1985), and also for mechanically activated channels of other cells (Morris, 1990). In *Stylonychia*, the mechanosensitive K conductances can be separated from the voltage-dependent K conductance, because the latter was blocked to a much lesser extent by TEA and by 4-AP (Deitmer, 1982). Another difference was its (most probable) independence on influx of divalent cations, which seems regular for voltage-dependent K currents in ciliates (Deitmer, 1982). All evidences have been summarized by Machemer (1985) into the statement that the posterior mechanoreceptor current of *Stylonychia* differs from voltage-dependent and leakage conductances for K^+ and is unrelated to the K conductance of both the resting and electrically stimulated membrane. Unfortunately, single channel recordings have identified only a K channel, which is activated by Ca^{2+} and negative voltage (Saimi & Martinac, 1989). This current belongs neither to the BK channels nor to the SAK channels (Kubalski, Martinac & Saimi, 1989; Morris, 1990).

In *Euplotes*, on the other hand, the mechanically sensitive channels share several common features with the voltage-dependent $I_{K,slow}$ (Krüppel & Lueken, 1988; Krüppel et al., 1991): it is highly susceptible to external TEA and to internal EGTA, and it is only partially suppressed by external 4-AP. Therefore, it is not possible until now to decide whether these channels are different. A last insight into the identity of the multiple ion channels of ciliates and their mutual regulation by voltage, calcium, or mechanical stimuli is presumably only possible when the patch clamp technique is more successfully adapted to ciliates than it has been until today.

Na Contribution to the Repolarization of the Receptor Potential

When Na^+ was replaced by NMDG, the receptor-potential duration was prolonged and repolarization was delayed. The change of the kinetics was accompanied by the reduction of the hyperpolarization amplitude (Fig. 8) and the outward current amplitude (*not shown*). These changes do not reflect voltage dependence of the outward-current deactivation because at minor hyperpolarizations the receptor current deactivates even faster (Fig. 5*a*). Therefore, the change of the amplitude and the kinetics must be due to the lack of Na^+ or the presence of NMDG. The delayed repolarization and the lack of the after-depolarization is presumably correlated to the elimination of the inwardly rectifying Na^+ current (Krüppel, 1993), that quickly terminates mechanically activated hyperpolarizations. Therefore, the duration of the hyperpolarization does not outlast the duration of the outward current as known for other ciliates (Ogura & Machemer, 1980; Deitmer, 1982). But the lack of Na^+ does not explain why the receptor potential amplitude is reduced. If a Na inward current antagonizes the K outward current and thus contributes to the peak amplitude of the receptor potential, one would rather expect an increase of the hyperpolarization when Na^+ is replaced by NMDG. Thus, some additional effect of NMDG must be assumed. The amplitude of the receptor potential might be reduced because the NMDG ions themselves block the K outward current partially, or the large NMDG ions compete with the Ca ions at the membrane surface. A reduced receptor potential then reflects the reduction of the Ca activation of the K-outward current similar to the one seen in low $[Ca^{2+}]_o$ (Fig. 7*a*).

To summarize, Na ions participate in the repolarization of the receptor potential that is mechanically activated at the posterior cell end of *Euplotes*. Their influence on the amplitude of the receptor potential and of the receptor current is not clear and is currently analyzed in more detail.

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