J. Membrane Biol. 116, 79–86 (1990)

The Journal of
Membrane Biology

Calcium-Dependent Sodium Current in the Marine Ciliate *Euplotes vannus*

Thomas Krüppel and Wolfgang Lueken
Zoophysiologie, FB Biologie/Chemie, Universität Osnabrück, D-4500 Osnabrück, Federal Republic of Germany

Summary. Ca and Na inward currents were recorded upon depolarizations in *Euplotes* after the blockage of K outward currents with intracellular Cs ions. The Na current was analyzed under voltage clamp and had the following properties: it activated to a maximum within 150 msec and partly inactivated during sustained voltage steps. It had a positive equilibrium potential between 25 and 30 mV and could be carried by Na or Li ions but not by K, choline or Tris ions. The current revealed a prominent associated inward tail current which deactivated with a single-exponential time constant of 118 msec. Both the current and its tail were strongly reduced after reduction of the extracellular Na concentration. Externally applied K channel blocker tetraethylammonium chloride did not block the current. Either EGTA injection into the cell or nonlethal deciliation with ethanol eliminated the current and its tail. These results indicate the existence of a Na conductance within the membrane of *Euplotes* which is activated by the intracellular level of free Ca^{2+}.

Key Words *Euplotes* · Ca-dependent Na current · Na tail current · EGTA injection · deciliation

Introduction

In ciliates, excitability and motility result from the collective action of multiple ion channels (for review *see* Ramanathan et al., 1988). Much work has been concentrated on the Ca action potential of *Paramecium* which triggers an "avoiding reaction" (Jennings, 1904) towards several external stimuli. There is, however, in addition to the Ca inward current, a Ca-dependent Na current into the cell, when the extracellular medium contains Na^+ (Saimi & Kung, 1980). Little is known about the function of this current for cell behavior. It could help to maintain membrane depolarization after the initial fast inactivating Ca inward current (Saimi, 1986) and could be involved in the generation of "spontaneous" discharges of the cell membrane (Satow & Kung, 1974). Mixed Ca-Na action potentials have been identified in several metazoan cells (for review *see* Hagiwara & Byerly, 1981).

In the habitat of the marine ciliate *E. vannus*, Na^+ is the most abundant cation and could significantly contribute to membrane depolarizations. Recently, a Ca-dependent Na inward current upon depolarization has been identified in *E. vannus* after suppressing K currents with internally applied Cs^+ (Krüppel & Lueken, 1988). The present report characterizes this current with respect to its identity, its selectivity, its inactivation, its deactivation, its Ca dependence, and its localization within the cell membrane.

Materials and Methods

Stocks and Culture

Clone D 35 was used from a syngene of the *Euplotes vannus/crassus/minuta* group originally collected at Naples, Italy, and belonging to the *vannus* morphospecies (Génermont, Machelon & Demar, 1985; Fernandez-Leborans, 1986; Valbonesi, Ortenzi & Luporini, 1988). Cells were grown and fed as previously described (Lueken, Gaertner & Breer, 1983). Measurements were performed at room temperature (20–22°C) on cells from 8–11 day-old cultures. Investigated cells had a mean input resistance of 49 ± 6.1 MΩ (n = 23) in standard recording solution.

Solutions

Standard recording solution, termed EASW (artificial Seawater for electrophysiology), contained (in mmol/liter): NaCl 430, KCl 10, $CaCl_2$ 10, $MgCl_2$ 53, and Hepps = EPPS 10 [N-(2-hydroxyethyl)-piperazine-N′-3-propanesulfonic acid, Fluka]. The pH was adjusted according to the experimental solutions with KOH, NaOH, or HCl to 8.0–8.1. When Na^+ was replaced, either the Na analogue Li^+, or the membrane impermeant large cations choline, or Tris [Tris(hydroxymethyl)aminomethane] were used. The Na concentration was varied between 430 and "0" mmol/liter. Where no Na was added to a solution, actual Na concentrations caused by impurities of other components were not measured. The concentrations are indicated within the figures in mmol/liter. For blocking K outward currents, 10 mmol/liter TEA (tetraethyl-ammonium chloride) was added to some media. Osmolality of all solutions ranged between 950 and 1050 mOsm/kg

H_2O (Knauer Semi-micro Osmometer Type Digital). Solutions were exchanged with at least 10 times the filling volume of the chamber. At the end of an experiment some cells were transferred back to EASW to test the reversibility of the preceding treatments.

Cells were deciliated in EASW with 5% ethanol. The cirri disintegrated within 10–15 min, except those of the membranellar band, which were only removed when cells were exposed to the ethanol solution for more than 15 min. First signs of reciliation were observed 30 min after cell transfer back to EASW, and all cirri were completely reconstructed within 12 hr.

Electrical Recording

Cells were transferred into an experimental chamber that was inserted into the slide holder of an inverted microscope (Olympus CK 2). They were penetrated from dorsal with a microelectrode that was connected to a single-electrode voltage-clamp amplifier (SEC 1L, npi, FRG). Switching frequency was 10 kHz with a duty cycle of 50%. Single electrode voltage clamp was applied to ciliates for the first time, and therefore results were thoroughly compared to former results acquired with a conventional double-electrode voltage-clamp system (Krüppel & Lueken, 1988). No qualitative differences were found. One remarkable quantitative difference concerned the maximal outward current amplitudes. With single-electrode recordings they were significantly smaller. This might either be due to a reduction of leak currents after the penetration with only one microelectrode, or to a reduced K leak from the electrode into the cell. A poor space clamp due to single-electrode recording would not be expected since ciliates have quite good cable properties (Machemer, 1988). The holding potential was usually −25 mV, exceptionally −30 mV. During a voltage step of 50 mV, the new value was stable within 2–3 msec with a 20-MΩ electrode. Voltage step duration was 160 msec and in one set of experiments 800 msec. Since the Na current was analyzed 150 msec after the onset of the step at the earliest, the clamp was appropriate to record this current.

Electrodes, either filled with 1 mol/liter KCl or 1 mol/liter CsCl, had resistances of 15–25 MΩ. Intracellular Cs^+ blocks K outward currents in *Euplotes* and thus unmasks inward currents that cannot be recorded with conventional KCl electrodes. EGTA [ethylene glycol-*bis*(2-aminomethyl)tetraacetic acid] was added to the CsCl electrodes in a concentration of 400 mmol/liter. It lowers the cytoplasmic ionized Ca level and buffers influx of Ca ions. Thus it can serve as a tool for the identification of Ca-dependent processes. EGTA and Cs ions leaked into cells by the combined action of diffusion and by iontophoresis. Up to three current pulses between 1 and 3 nA, each lasting a minute, were applied to the cells for iontophoretic EGTA injection.

Stimulation, recording, and data analysis was performed on a personal computer (PCA 40, Tandon, FRG) with pCLAMP, a suite of programs from Axon Instruments (Foster City, CA). The program was used with the 40 kHz version of the TL-1/Labmaster AD/DA board (Scientific solutions, Solon, OH). Membrane currents were on-line filtered at 1 kHz with an 8-pole Bessel filter (48 dB/oct). For later analysis and data plots, they were averaged from several cells. Leakage currents were not subtracted since they were less than 0.2 nA per 10 mV in EASW, and were significantly reduced in media with reduced Na content. They were further reduced to less than 0.1 nA per 10 mV when K channels were blocked by internal Cs^+. Capacitive transients can be seen at the beginning and the end of the current traces. The step voltage levels are indicated in mV at the zero current level of the corresponding current trace. Zero current levels are indicated in some figures by dashed lines. All experiments were performed on at least four cells, but mostly on six or more cells (*see* legends of figures). Standard error of the mean usually did not exceed 20% of the mean, except at current amplitudes below 1 nA where it could reach 40%. Several actual numbers are cited together with the results. Within the *I-V* diagrams, data point increment was 5 mV, if not indicated otherwise. It corresponds to the tick interval of the voltage axis.

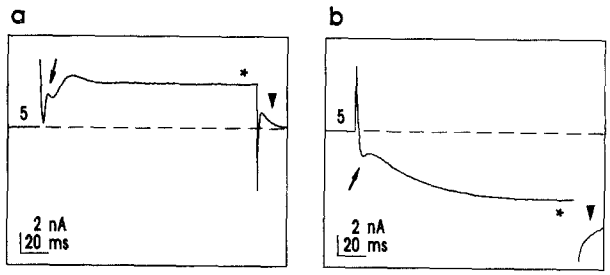

Fig. 1. Outward and inward currents in standard medium, activated by depolarizations to 5 mV. (*a*) Recording with the microelectrode filled with 1 mol/liter KCl. An early transient K outward current (arrow) develops into a second late persisting K current (asterisk) which is followed by a small outward tail current (arrowhead). Holding potential was −30 mV, averaged trace of 26 cells. (*b*) Recording with the electrode filled with 1 mol/liter CsCl. The early transient Ca inward current (arrow) is followed by a late Ca-dependent Na inward current (asterisk). A large inward tail current appears after the depolarization (arrowhead). Holding potential was −25 mV, averaged trace of 26 cells

Results

Depolarization-Induced Inward Currents Are Unmasked After K Outward Current Blockage

The standard total current pattern upon depolarization of *Euplotes* consists of two purely outward current components (Fig. 1*a*) which were formerly identified as Ca-dependent K currents. These currents mask two inward ion currents which can be seen after K channel blockage by intracellular Cs^+ (Fig. 1*b*). The early component was identified as Ca current, the late one as Na current. The latter will be called I_{Na} in this paper. I_{Na} was followed by a prominent inward tail current upon repolarization. The identity of I_{Na} was deduced from Na^+-choline$^+$ substitution experiments (Krüppel & Lueken, 1988). Further evidence for this identity will be given below. I_{Na} reached a peak between 140 and 150 msec after onset of depolarizations and slowly inactivated in the following 650 msec (Fig. 2*a*). The

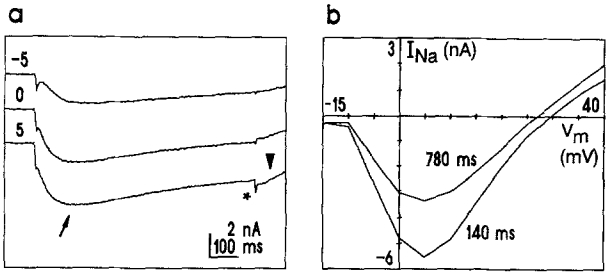

Fig. 2. I_{Na} in standard medium, average of 10 cells. (*a*) During depolarizing voltage steps of 800 msec, I_{Na} reaches a maximum between 140 and 160 msec (arrow) and then slowly decreases (asterisk). The tail current (arrowhead) is reduced compared to Fig. 1*b*. (*b*) *I-V* relationships at 140 and 780 msec. $I_{Na,140}$ reaches a peak at 5 mV and reverses direction at 29 mV. $I_{Na,780}$ is reduced to about 60% of $I_{Na,140}$

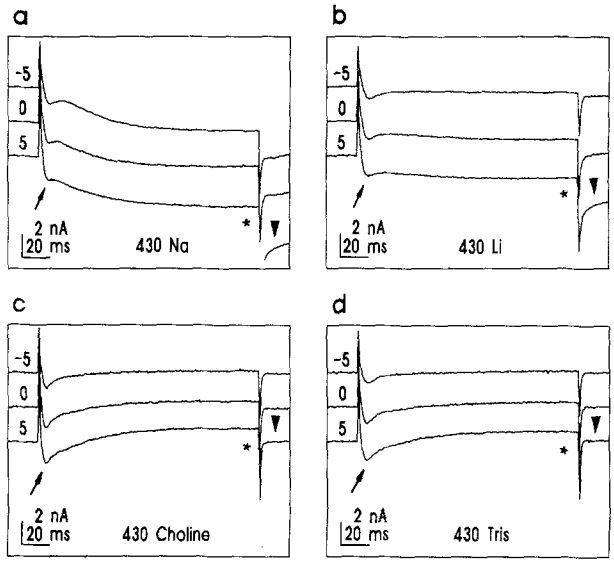

Fig. 3. The effect of replacing Na ions by Li, choline, or Tris ions on membrane currents. Typical early Ca inward current (arrow), I_{Na} at 150 msec (asterisk), and its tail current (arrowhead) are indicated. (*a*) Membrane currents in 430 mmol/liter Na medium, average of eight cells. (*b*) Membrane currents in 430 mmol/liter Li medium. I_{Na} and its tail current are reduced. I_{Ca} did not change, same cells as in *a*. (*c*) In 430 mmol/liter choline medium, I_{Na} is outward directed at 150 msec and no tail current can be seen. Again, I_{Ca} did not change, average of six cells. (*d*) As *c*, but in 430 mmol/liter Tris medium

I-V relationship (Fig. 2*b*) shows a strong voltage-dependent activation beyond −10 mV, a maximum inward current of -5.5 ± 0.3 nA, and a decline to a zero current at 29 ± 0.6 mV ($n = 10$). This potential is the reversal potential of I_{Na}, on condition that the measured ion current is only carried by Na ions. From this value an intracellular Na concentration of 130 mmol/liter can be calculated by the Nernst equation.

Identity of I_{Na}

Selectivity of the Na channel was tested by replacing Na^+ by the permeant Li^+, or the two large impermeant cations choline or Tris (Fig. 3). In the Li medium, behavior was not significantly modified. Cells survived for more than 24 hr without obvious changes in motility. I_{Na} and its tail were reduced, but both were still directed inward. The early Ca inward current was not changed (Fig. 3*a* and *b*). This points to a reduced permeability of the Na channel for Li ions, but also confirms its identity. In choline or Tris medium, no I_{Na} was measured, but in either medium, after the initial early Ca inward current, a late outward current was activated. It was not followed by a significant tail current (Fig. 3*c* and *d*). This could be expected since in Na-free media no Na is available to carry an inward tail current into the cell and the driving force for Na should be directed outward. The outward current is presumably carried by Na^+ from the intracellular Na pool, though either Cs^+ or K^+ could also carry this current.

To examine the contribution of residual K currents to either the late Na inward or Na outward current, TEA was added to the media. External TEA ions can block K channels in *Euplotes* (Krüppel & Lueken, 1988) and non-Cs-blockable K currents should be reduced by the addition of external TEA ions. In spite of these considerations, I_{Na} inward and its tail current were only slightly affected by the addition of 10 mmol/liter TEA^+ to EASW (*not shown*). Therefore it cannot contain sizable TEA-blockable components.

$[Na^+]_o$ Dependence of I_{Na}

In order to test the identity of I_{Na}, it was analyzed in various reduced Na media, with Na ions replaced by Tris ions (Fig. 4). The current, upon depolarizations to 5 mV (Fig. 4*a*), decreased according to the decreasing $[Na^+]_o$. It was almost zero in 215 mmol/liter and turned to an outward direction in 108 mmol/liter. The transient Ca inward current remained unchanged, except for a slight reduction in low $[Na^+]_o$. The *I-V* diagram of I_{Na} (Fig. 4*b*) shows a small voltage shift from 5 to 0 mV for the activation of the peak Na current. In 108 mmol/liter, currents were purely outward directed. Such a Na concentration-dependent reversal confirms the identity of I_{Na}. Further evidence for this identity can be de-

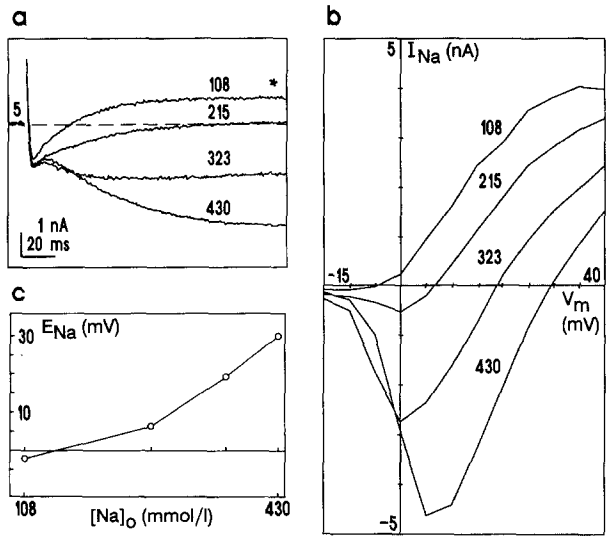

Fig. 4. Na concentration dependence of I_{Na}, average of 6–11 cells. (*a*) Membrane currents induced by voltage steps to 5 mV. I_{Na} at 150 msec (asterisk) changes from inward to outward with decreasing Na concentration in mmol/liter as marked on traces. (*b*) *I-V* relationships of I_{Na}. The peak I_{Na} shifts from 5 to 0 mV in low Na concentrations, and the current reversal shifts towards negative voltages. (*c*) Semilogarithmic plot of the reversal potential, E_{Na}, against the Na concentration. The overall slope is nonlinear, the linear part between 215 and 430 mmol/liter has a slope of more than 70 mV per decade change of the Na concentration

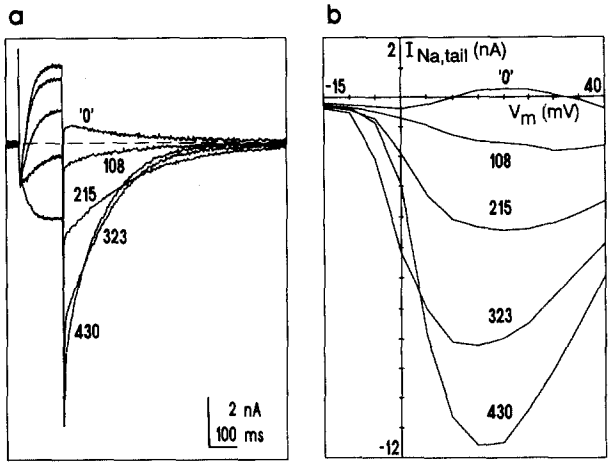

Fig. 5. Na concentration dependence of the Na tail current, average of 5–11 cells. (*a*) Tail current was recorded for 800 msec and preceded by current which was induced by a voltage step of 160 msec to 15 mV. Time constants (single-exponential) for deactivation range from 108 to 181 msec in media of 108–430 mmol/liter. (*b*) *I-V* diagram of the tail current measured 4 msec after the end of the voltage step. The tail current peaks between 15 and 30 mV. It decreases between 430 and 108 mmol/liter and reverses to outward direction in "0" mmol/liter

rived from the concentration-dependent voltage shift of the current reversal. For a pure Na current it should shift 58 mV per decade change in Na concentration. The reversal potential shifted from 29 ± 0.3 mV to −2 ± 1.1 mV (n = 6–11) between 430 and 108 mmol/liter. The semilogarithmic plot of the reversal potential of I_{Na} against the $[Na^+]_o$ (Fig. 4*c*) gave an overall nonlinear correlation. Extrapolation from the linear part between 430 and 215 mmol/liter to one $[Na^+]_o$-decade gave a slope of the reversal potential shift of more than 70 mV. These observations indicate the Na dependence of the reversal potential, but also the contribution of further ion currents.

$[Na^+]_o$ Dependence of the Na Tail Current

For a further characterization of I_{Na} its tail current was analyzed in reduced $[Na^+]_o$ (Fig. 5). Deactivation of the tail current reflects the closing of Na channels, opened during the depolarization, on condition that no other ion currents contribute to the tail. As I_{Na} itself, the peak tail current showed a strong reduction in low Na media (Fig. 5*a*). It deactivated exponentially with a time constant of 118 msec in EASW. The time constant rose to 157 msec in 323 mmol/liter and to 181 msec in 215 mmol/liter Na media. It fell to 108 msec in 108 mmol/liter and was no longer a single-exponential in "0" mmol/liter. The tail current peaked, in all Na media, at 10–25 mV more positive voltages than I_{Na} (Fig. 5*b*, *compare* Fig. 4*b*). This potential difference indicates an increase of the Na conductance beyond potentials of 5 mV. The I_{Na} peaks are smaller than the tail current peaks due to the decrease of the driving force for Na^+ during the depolarizations.

Reversal Potential of the Na Tail Current

The current immediately after termination of a Na-channel activating pulse reflects the conductance of the open Na channels. The current-voltage relationship of the open channels can be measured correctly, if stepping of the membrane voltage from the activating to new levels does not take longer than closing of membrane channels. The time constants for tail current deactivation of 100 to 200 msec (*compare* Fig. 5*a*) indicate slow closing of Na channels. Therefore, the time for voltage change of 2–3 msec (*see* Materials and Methods), should allow a near accurate measurement of the "instantaneous current-voltage relationship" (Hodgkin & Huxley, 1952; Oertel, Schein & Kung, 1978). The relationship should be linear and the tail current should reverse its direction at the reversal potential of I_{Na}.

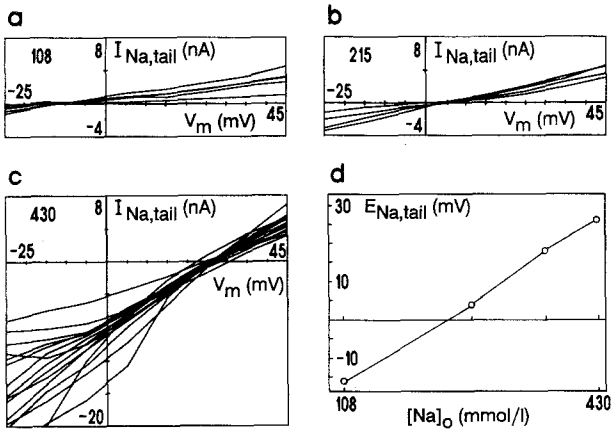

Fig. 6. Na concentration dependence of Na tail current reversal. I_{Na} was activated by step depolarizations to 5 mV. The tail current was measured 3 msec after onset of an immediately following second step to voltages between −25 and 45 mV with an increment of 10 mV. (*a–c*) *I-V* relationships of the tail current in the indicated Na concentrations. The curves are almost linear, as expected for currents through open channels (instantaneous current-voltage relationship, *see* text for interpretation), each curve represents one cell. (*d*) Semilogarithmic plot of the reversal potential of the tail current, $E_{Na,tail}$. The curve has a slope of 69 mV per decade change of the Na concentration. Data are from cells shown in *a–c*, and, in addition, from seven cells bathed in 323 mmol/liter Na medium

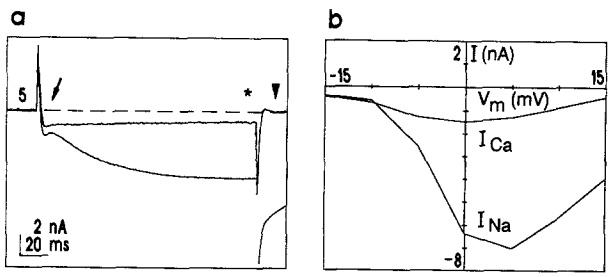

Fig. 7. Effects of intracellular EGTA on membrane currents. (*a*) Membrane currents induced by depolarizations to 5 mV, without EGTA injection (lower trace), same cells as in Fig. 1*b*, and with injected EGTA (upper trace), average of six cells. After EGTA injection the early Ca inward current persists during the voltage step, though with a slightly reduced peak amplitude (arrow). I_{Na} and its tail are lost (asterisk and arrowhead, respectively). (*b*) *I-V* diagram of the persisting I_{Ca} compared to I_{Na}. No I_{Na} activates after EGTA injection. Either current was measured 150 msec after onset of depolarizations, same cells as in *a*

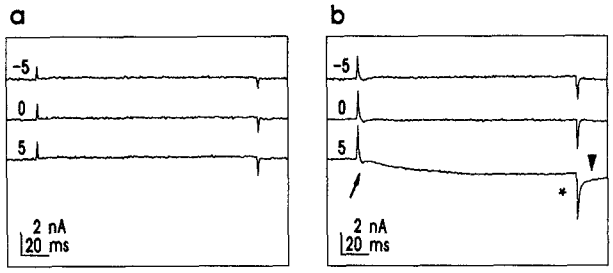

Fig. 8. Membrane currents in deciliated and partly reciliated cells. Cells were exposed to EASW with 5% ethanol for 10–15 min and then replaced into EASW. (*a*) Membrane currents recorded 15 min to 1 hr after deciliation. Only very small outward leakage currents occur, average of five cells. (*b*) Recordings 3–5 hr after deciliation. I_{Ca} (arrow), I_{Na} (asterisk), and its tail current (arrowhead) return after ciliary regeneration, average of seven cells

In reduced external Na concentrations, the reversal potential of the tail current should shift towards negative values, as described for I_{Na} itself. The relationships were linear for each cell, except for a few cells in 430 mmol/liter Na medium at low depolarizing voltages (Fig. 6*a–c*). The reversal potentials showed little variation. The semilogarithmic plot of the reversal potentials as a function of Na concentration (Fig. 6*d*) gave a slope of 69 mV. This value is higher than expected from the Nernst equation, but comes close to the slope of the reversal potential of I_{Na} that was recorded during the depolarizations (*compare* Fig. 4*c*).

Ca Dependence of I_{Na}

I_{Na} was preceded by an early Ca inward current. In order to test the Ca dependence of I_{Na}, EGTA was injected into the cells. EGTA injection has two effects: first, no Ca-dependent currents can activate, and second, the Ca inward current sustains since Ca-dependent Ca channel inactivation is lost. Figure 7 demonstrates the loss of I_{Na} and its tail when Ca^{2+} is chelated by EGTA. This strongly indicates the Ca dependence of I_{Na}.

To confirm the Ca dependence of I_{Na}, deciliation experiments were performed (Fig. 8). In *Paramecium*, deciliation only removes the voltage-activated Ca channels, because they are exclusively located on the ciliary membrane. Other ion channels, residing in the somatic membrane, are not affected (Machemer & Ogura, 1979). As a consequence of the Ca channel removal, Ca-dependent somatic ion channels cannot activate. In *Euplotes*, within 1 hr after deciliation (Fig. 8*a*) all inward currents were lost, only small outward leakage currents remained. After 3–5 hr of ciliary regeneration (Fig. 8*b*), the early Ca inward current and the late Na inward current with its tail had returned, though not yet with full amplitude when compared to the standard inward currents of Fig. 1*b*. These results indicate the Ca dependence of I_{Na}, if it is carried through the somatic membrane. But it cannot be

excluded that I_{Na} disappeared because its channels reside in the ciliary membrane, as those of the Ca current itself.

Discussion

The Identity of the Na Conductance

For the identification of one individual ion current out of the whole cell currents all other ion currents must be eliminated. In *Euplotes*, Cs injection is appropriate to block K outward currents and to unmask two separable inward currents (Fig. 1). However, the completeness of the block of Cs^+ is not known. Especially at high depolarizations, where the driving force for K^+ is very high, not fully suppressed K currents might remain. This would underestimate the amplitude of the Na inward current. Addition of extracellular TEA could block Cs-unblockable outward currents and thus increase the inward currents. But TEA was not appropriate to improve the Cs-mediated K channel blockage.

If I_{Na} is completely carried by Na^+, the ion current direction must reverse near the equilibrium potential for Na^+. Its value should be positive because cells presumably do not accumulate Na^+ beyond the extracellular Na concentration of 430 mmol/liter. The equilibrium potentials, independently measured either from the reversal current of I_{Na} or from its tail, of 29 ± 0.3 mV (n = 11, Fig. 4*c*) and 26 ± 0.5 mV (n = 15, Fig. 6*d*) confirm that this current is carried by Na ions.

Further evidence for the identity of the Na current came from the substitution experiments with Li, choline, or Tris ions. Li^+ as Na^+ analogue passes the Ca-dependent Na channel of *Paramecium* (Saimi, 1986). In *Euplotes*, the channel is less permeable for Li^+ than for Na^+ but a clear inward Li current and associated Li tail current remained (Fig. 3*b*). The strong dose-dependent reduction of the Na current and its tail (Figs. 4 and 5), after substitution of Na^+ with either the impermeant cations choline or Tris, confirms the Na dependence of these currents (shown only for Tris).

In the semilogarithmic plots of the reversal potentials of either the Na current or its tail against the Na concentration of the medium (Figs. 4*c* and 6*d*), the slopes were higher than 58 mV, and nonlinear for the Na current itself. This indicates the contribution of other ions to the Na current; they were not known until now.

Though the late inward current could be identified as Na current, the nature of the late outward current in low extracellular Na media is not evident. Beside Na ions, Cs ions which leaked out of the microelectrode into the cell could generate the outward current. This was observed in the giant axon by Meves and Vogel (1973). If K currents contribute to the late outward current, they must be unblockable by intracellular Cs^+ and extracellular TEA.

The Ca Dependence of the Na Conductance

The Na current was always preceded by a small early Ca inward current which can best be seen with blocked K channels in Na-free medium (Fig. 3*c* and *d*). The identity of this Ca current is well documented in freshwater ciliates (Oertel, Schein & Kung, 1977; Deitmer, 1986; Pape & Machemer, 1986) and was recently established also for the marine *Euplotes* (Krüppel & Lueken, 1988). We formerly demonstrated that Ca influx is strictly required for the activation of the Na current, because reducing external Ca^{2+} as well as replacing Ca^{2+} by Ba^{2+} or other divalent cations eliminated the Na current. Within the present examination, EGTA injection or deciliation (Figs. 7 and 8) confirmed the Ca dependence of I_{Na}. The effect of either treatment on the Ca current has been analyzed in *Paramecium* (Machemer & Ogura, 1979; Brehm, Eckert & Tillotson, 1980). I_{Na} is indirectly voltage dependent, because the Ca influx is triggered by the activation of the voltage-dependent Ca conductance. The Na current could also flow through the Ca channel, as known for the voltage-activated Na channel of the giant axon (Meves & Vogel, 1973), and as has been described for the Ca channel I in *Stylonychia* by Ivens (1986). Ivens measured a Na current through Ca channels, when the extracellular concentration of Ca^{2+} was below 10^{-3}–10^{-4} mol/liter. Since in the present examination the Ca concentration was 10^{-2} mol/liter, Na influx through Ca channels should be of minor relevance in *Euplotes*. Furthermore, the slow activation and deactivation kinetic of the Na current with deactivation time constants between 100 and 200 msec excludes this possibility, since the Ca channels inactivate almost completely in a few milliseconds (Brehm & Eckert, 1978). Recently, Martinac et al. (1988) reported upon a Ca-dependent Na channel which was identified with the patch-clamp technique in plasma membrane blisters of *Paramecium* by Y. Saimi (*unpublished*). By analogy, the observed Na current of *Euplotes* could be carried through similar Na channels, which are activated by the intracellular level of Ca^{2+}. Whether this Na channel is activated directly by binding of Ca^{2+}, or indirectly by biochemical pathways, which, for example, modify the intracellular level of cyclic nucleotides, is not yet known.

The Inactivation of the Na Conductance

Inactivation of the Na current (Fig. 2) has not been described for the Na channel of ciliates until now. Its mechanism might be correlated to its Ca dependence; reduction of the intracellular Ca^{2+} by Ca extrusion could inactivate the Na conductance. Inactivation cannot be caused by the reduction of the driving force for I_{Na}, since the intracellular Na concentration changes only by about 2.5 mmol/liter, when a current of 5 nA flows for 1 sec into a cell (estimated cytoplasmic volume: 2×10^4 μm^3).

The Physiological Function of the Na Conductance

The graded action potential of ciliates is generated by the collective action of several ion currents. Ca influx is the first step which triggers the influx of Na^+ and the efflux of K^+. Inward currents are responsible for the upstroke and maintenance of the depolarization. Outward currents limit the amount of depolarization by short-circuiting the inward currents and are responsible for the repolarization of the cell membrane. Depolarizations, and consequently backward locomotion can last tens of milliseconds up to hundreds of milliseconds (Satow & Kung, 1974; Krüppel & Lueken, 1988).

The Ca-dependent Na current could be necessary to maintain long depolarizations in ciliates as already proposed by Saimi (1986). Especially in *Euplotes*, where the Ca inward current is very small when compared to freshwater ciliates, and the early Ca current is strongly short-circuited by a fast activating K current (Fig. 1*a*; Krüppel & Lueken, 1988), a Na inward current could significantly contribute to depolarizations. A noninactivating residual Ca inward current as in *Paramecium* (Eckert & Brehm, 1979) was not yet found in *Euplotes*. Thus, the influx of great amounts of Ca^{2+}, which could sustain depolarizations, is not likely.

The inactivation of the Na conductance (Fig. 2) could be involved in the termination of sustained depolarizations. A slow diminution of Na inward current would shift an equilibrium between Na inward and K outward current during plateau depolarizations towards K outward current and thus slowly repolarize cells. Such slow repolarizations were observed within plateau depolarizations of all-or-none action potentials in *Euplotes* after K channel blockage (Krüppel & Lueken, 1988).

One further function of the Na current might concern the generation of spontaneous depolarizations. Whereas *Euplotes* is highly spontaneously active in EASW, it does not reveal spontaneous depolarizations in Na-free medium (*not shown*). In *Paramecium*, addition of Na^+ to the medium induces frequent spontaneous depolarizations (Satow & Kung, 1974) similar to those of *Euplotes*. The *paranoiac* mutant of *Paramecium* shows these depolarizations in exaggerated form and consequently, it moves spontaneously backward for tens of seconds (Saimi & Kung, 1980). This is correlated to the increased conductance of the Ca-dependent Na channel (Saimi & Kung, 1987). Potential oscillations are of general interest since they regulate important physiological processes as, e.g., heartbeat. Therefore, *Euplotes* might serve as a model organism for the analysis of the collective action of multiple ion channels in the generation of pacemaker-like potential oscillations.

The authors thank Mrs. B. Oelgemöller for skillful technical assistance and for preparing the figures. This work was supported by Deutsche Forschungsgemeinschaft, SFB 171, C7.

References

Brehm, P., Eckert, R. 1978. Calcium entry leads to inactivation of the calcium channel in *Paramecium*. *Science* **202:**1203–1206

Brehm, P., Eckert, R., Tillotson, D. 1980. Calcium-mediated inactivation of the calcium current in *Paramecium*. *J. Physiol. (London)* **306:**193–203

Deitmer, J.W. 1986. Voltage dependence of two inward currents carried by calcium and barium in the ciliate *Stylonychia mytilus*. *J. Physiol. (London)* **380:**551–574

Eckert, R., Brehm, P. 1979. Ionic mechanisms of excitation in *Paramecium*. *Annu. Rev. Biophys. Bioeng.* **8:**353–383

Fernandez-Leborans, G. 1986. Morphology of a marine ciliate of genus *Euplotes*. Considerations about the taxonomic determination of *Euplotes* with single *vannus* dargyrome. *Arch. Protistenkd.* **131:**215–224

Génermont, J., Machelon, V., Demar, C. 1985. The *vannus* group of genus *Euplotes*. Sibling species and related forms; evolutionary significance and taxonomical implications. *Atti Soc. Toscana Sci. Nat. Ser. B.* **92:**53–65

Hagiwara, S., Byerly, L. 1981. Calcium channel. *Annu. Rev. Neurosci.* **4:**69–125

Hodgkin, A.L., Huxley, A.F. 1952. The components of membrane conductance in the giant axon of *Loligo*. *J. Physiol. (London)* **116:**473–496

Ivens, I. 1986. Different properties of two voltage-dependent inward currents of the ciliate *Stylonychia mytilus*. *J. Physiol. (London)* **381:**1–15

Jennings, H. 1904. The behavior of *Paramecium*. Additional features and general relations. *J. Comp. Neurol. Psychol.* **6:**441–510

Krüppel, T., Lueken, W. 1988. Membrane excitability and membrane currents in the marine ciliate *Euplotes vannus*. *Eur. J. Protistol.* **24:**11–21

Lueken, W., Gaertner, M., Breer, H. 1983. Mating-type-specific loss of conjugation competence by irritation in *Euplotes vannus*. *J. Exp. Zool.* **226:**11–17

Machemer, H. 1988. Motor control of cilia. *In:* Paramecium. H.-D. Görtz, editor. pp. 216–235. Springer-Verlag, Berlin—Heidelberg—New York—London—Paris—Tokyo

Machemer, H., Ogura, A. 1979. Ionic conductances of membranes in ciliated and deciliated *Paramecium*. *J. Physiol. (London)* **296:**49–60

Martinac, B., Saimi, Y., Gustin, M.C., Culbertson, M.R., Adler, J., Kung, C. 1988. Ion channels in microbes. *Period. Biol.* **90:**375–384

Meves, H., Vogel, W. 1973. Calcium inward currents in internally perfused giant axons. *J. Physiol. (London)* **235:**225–265

Oertel, D., Schein, S.J., Kung, C. 1977. Separation of membrane currents using a *Paramecium* mutant. *Nature (London)* **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

Pape, H.-C., Machemer, H. 1986. Electrical properties and membrane currents in the ciliate *Didinium*. *J. Comp. Physiol. A* **158:**111–124

Ramanathan, R., Saimi, Y., Hinrichsen, R., Burgess-Cassler, A., Kung, C. 1988. A genetic dissection of ion-channel functions. *In:* Paramecium. H.-D. Görtz, editor. pp. 236–253. Springer-Verlag, Berlin—Heidelberg—New York—London—Paris—Tokyo

Saimi, Y. 1986. Calcium-dependent sodium currents in *Paramecium:* Mutational manipulations and effects of hyper- and depolarization. *J. Membrane Biol.* **92:**227–236

Saimi, Y., Kung, C. 1980. A Ca-induced Na-current in *Paramecium*. *J. Exp. Biol.* **88:**305–325

Saimi, Y., Kung, C. 1987. Behavioral genetics of *Paramecium*. *Annu. Rev. Genet.* **21:**47–65

Satow, Y., Kung, C. 1974. Genetic dissection of active electrogenesis in *Paramecium aurelia*. *Nature (London)* **247:**69–71

Valbonesi, A., Ortenzi, C., Luporini, P. 1988. An integrated study of the species problem in the *Euplotes crassus-minuta-vannus* group. *J. Protozool.* **35:**38–45

Received 21 September 1989; revised 20 December 1989