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Inward Rectification by Hyperpolarization-Activated Na Current in the Marine Ciliate *Euplotes vannus*

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Summary. The ionic mechanisms underlying inward or anomalous rectification have been studied in the marine hypotrichous ciliate *Euplotes vannus*. Inward-current pulses of moderate amplitude elicited time-dependent rectification that started from a hyperpolarization peak and was expressed as a depolarizing sag towards rest. Voltage-clamp analysis showed that this depolarization is caused by the activation of a complex inward current that does not inactivate with time. The current is carried by a major Na and a minor K component. The Na-current component has been identified by its concentration-dependent reduction in low extracellular Na solutions and the capability of Li^+ as Na substitute to carry the current, though with a slightly reduced amplitude. The K-current component has been isolated from the total current after the replacement of Na^+ within the experimental solution. It was blocked in media that contained 10 mmol/liter TEA, a well-known blocker for K inwardly rectifying currents. TEA was only effective at membrane potentials close to or negative to the potassium equilibrium potential. The inward current was reduced after the injection of the Ca chelator EGTA into the cell. Also the elimination of the ciliary membrane, by deciliating cells with ethanol, reduced the amplitude of the inwardly rectifying currents. Both experiments indicate a regulatory function of Ca^{2+} in inward rectification.

Key Words *Euplotes vannus* · inward rectification · sodium current · potassium current

Introduction

Inward rectification is a mechanism that has been described in detail for various cells (Rudy, 1988). Usually, it is correlated to a K influx through an ion channel termed inward or anomalous rectifier that is activated at membrane potentials negative to the potassium equilibrium potential (E_K). The channel conductance is regulated by the external K concentration and increases instantaneously with hyperpolarization; it is high for voltages more negative than E_K (inward current), and low for voltages positive to E_K (outward current). In cardiac ventricular cells of the guinea pig, it has been shown by single channel recordings that the voltage dependence arises from a voltage-dependent block of the channel by internal Mg^{2+}; the single channel conductance itself is ohmic in nature (Matsuda, Saigusa & Irisawa, 1987; Vandenberg, 1987). Block of the channel also occurs when Cs^+, Ba^{2+}, or TEA are applied to the external solution (Oertel, Schein & Kung, 1978; Thompson & Aldrich, 1980; Latorre & Miller, 1983; Ballanyi & Deitmer, 1984).

A second type of inward-rectifier current, sometimes termed I_h, I_q, or I_f, is carried by both sodium and potassium ions. Its activation at potentials positive to E_K indicates the contribution of sodium as charge carrier (Angstadt & Calabrese, 1989; McCormick & Pape, 1990; Tokimasa & Akasu, 1990; Kamondi & Reiner, 1991).

In the fresh-water ciliate *Paramecium,* at least four inward currents activate upon hyperpolarization. Recently, a Ca current was characterized that is distinct from the well-known, depolarization-activated Ca current of ciliates (Preston, Saimi & Kung 1992*a,b*). Two distinct K currents were analyzed by Preston et al. (1990) and a Ca-activated Na current by Saimi (1986). The Na current presumably flows through the same type of Na channel as the one that is activated upon depolarization (Saimi & Kung, 1980). Also in the marine *E. vannus,* a Na current that activates upon depolarization has been identified (Krüppel & Lueken, 1990), and first evidence for a contribution of Na ions to inward rectification in *E. vannus* has been presented by Krüppel and Lueken (1988). In the present study, the ionic basis of the inward currents activating upon hyperpolarization, their participation in inward rectification, and their regulation by calcium have been examined with the current-clamp and voltage-clamp methods.

Materials and Methods

Stocks and Culture

Clone *D35* was derived from a syngen originally collected at Naples (Italy). It belongs to the *vannus* morphospecies of the *E. vannus/crassus/minuta* species complex (Valbonesi, Ortenzi & Luporini, 1988, 1992). Techniques for cultivation and for breeding have been reported elsewhere (Lueken, Gaertner & Breer, 1983). Measurements were performed at room temperature (20–22°C) on cells from 8- to 11-day-old cultures.

Solutions

Standard recording solution, termed EASW (Artificial Sea-Water for Electrophysiology), contained (in mmol/liter): NaCl 430, KCl 10, $CaCl_2$ 10, $MgCl_2$ 53, 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 ions were 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 (tetraethylammonium chloride) or 50 mmol/liter CsCl (*not shown*) were added to some media. 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 cilia, which are composed to cirri in *E. vannus*, 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 recirriation were observed 30 min after cell transfer back to EASW, and all cirri were completely reconstructed within 12 hr.

Electrical Recording

Electrodes, either filled with 1 mol/liter KCl or 1 mol/liter CsCl, had resistances of 15–25 MΩ. Intracellular Cs electrodes were used to eliminate K leak out of the electrode into the cell and to block outwardly directed K-current components. K electrodes could increase the intracellular K concentration and thus artificially reduce K-inward currents. Inward-rectifier currents are usually only blocked by extracellular Cs^+, thus, the K-inward component of the total current should not be influenced by the use of these electrodes. But, K-outward currents are effectively blocked by intracellular Cs ions in *E. vannus* (Krüppel & Lueken, 1990). These currents and/or outward currents through the inward-rectifier channels that are supposed to activate above E_K, should be blocked by the use of the Cs electrodes. Otherwise, the amplitude of the inwardly directed total current would be underestimated. The Ca chelator EGTA [ethylene glycol-bis(2-aminomethyl)tetraacetic acid] was added to the microelectrodes in a concentration of 200 mmol/liter. EGTA and Cs ions leaked into cells by the combined action of diffusion and by iontophoresis. Proceeding effectiveness of the drugs became manifest in a typical change of the repolarization pattern of 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 currents (Krüppel & Lueken, 1990). The mechanical setup and general techniques were essentially as described by Krüppel and Lueken (1990). Recordings were performed by use of a single-electrode voltage-clamp system (npi SEC 1L, H.-R. Polder, D-7146 Tamm, Germany). Switching frequency was 10 kHz with a duty cycle of 50%. Data were acquired with a Labmaster analog-digital interface board (Tecmar, Scientific Solutions Division, Solon, OH) installed into a personal computer (Tandon AT). The computer generated the command signals and simultaneously recorded data by use of the pCLAMP program (Axon, Foster City, CA). Records were on-line filtered at 1 kHz with an 8-pole Bessel filter (48 dB/octave). Holding potential in the voltage-clamp experiments was usually −25 mV, or when exceptionally −30 mV, it is indicated within the legends of the figures. All experiments were performed at room temperature (19–21°C).

Voltage- or current-step duration was 160 msec and recordings were analyzed 150 msec after onset of the step. Voltages and currents were averaged from at least five cells. The current-induced hyperpolarizations are plotted within the *V-I* diagrams as absolute membrane potentials with the mean resting potential as reference data point at zero-current injection. Leakage currents were not subtracted since they were less than 0.2 nA per 10 mV in EASW and were even further reduced when Cs electrodes were used. 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 and zero-current levels by straight horizontal lines. Standard error of the mean is marked at several data points within X-Y plots; where it is not, it is smaller than the extension of the data point.

Results

Hyperpolarizations Induced by Current Injection

Small constant inward currents (−0.2 nA) elicited hyperpolarizations that were mainly determined by the ohmic and capacitive membrane properties. Inward currents of higher amplitude induced peak hyperpolarizations that emerged into steady-state voltages of reduced amplitude after 150 msec (Fig. 1*a,b*). In *Paramecium*, the regression from the peak hyperpolarization to the steady-state hyperpolarization is due to K influx and represents inward rectification or anomalous rectification (Naitoh & Eckert, 1968; Oertel et al., 1978). In *E. vannus*, the regression depends on the presence of Na^+ in the extracellular

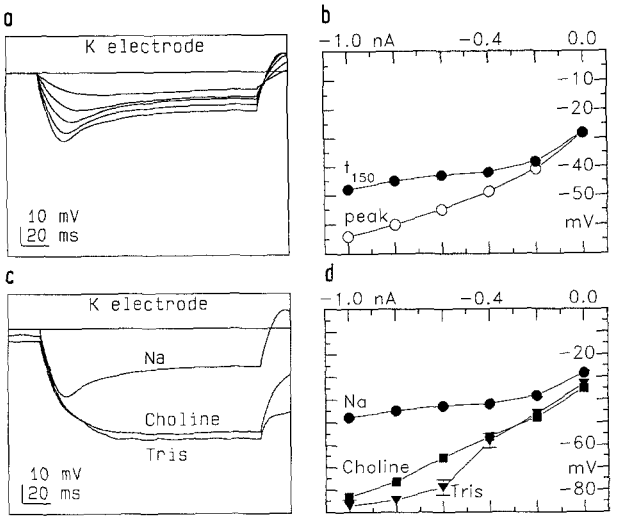

Fig. 1. Hyperpolarizations upon inward current injections in standard media and after the replacement of Na^+ by choline or Tris recorded with K electrodes. (*a*) Time-dependent inward rectification moves the membrane potential from a peak hyperpolarization back towards the resting potential. Currents of −0.2 to −1.0 nA and 160 msec were injected with an increment of 0.2 nA, 42 cells averaged. (*b*) *V-I* diagrams of the peak hyperpolarization and the steady-state hyperpolarization measured 150 msec after the onset of the current pulse. The difference between peak and t_{150} amplitude indicates inward rectification. Same cells as in *a*. (*c*) Inward rectification is lost after the replacement of Na^+ ($n = 42$) by choline ($n = 5$) or Tris ($n = 6$), and the hyperpolarization is mere ohmic-capacitive. Traces are induced by currents of −1.0 nA in either medium. The mean resting membrane potential in standard medium (straight line), slightly hyperpolarizes in Na-free media. (*d*) *V-I* diagrams of steady-state hyperpolarization measured at t_{150} are linearized in choline medium and Tris medium, same cells as in *c*.

medium. Inward rectification disappeared after totally replacing the Na ions by the large, membrane-impermeant cations choline or Tris (Fig. 1*c,d*). The exchange produced a strong increase of the steady-state hyperpolarization without considerably affecting the ohmic-capacitive response, as can be seen by the identical initial exponential time course of the voltage response in either medium (Fig. 1*c*). The time constants (τ) and input resistances (R_i) were almost identical in either medium (Table, K electrode). The loss of inward rectification linearized the voltage-current diagram especially after replacement of Na^+ by choline (Fig. 1*d*). The resting membrane potential hyperpolarized from -28.2 ± 0.5 mV ($n = 42$) in standard medium to -35 ± 1.9 mV ($n = 5$) in choline medium, and -33.1 ± 1.3 mV ($n = 6$) in Tris medium. Though the Na replacement eliminates the driving force for Na^+, only this small hyperpolarization was measured. Thus, Na ions play a minor part in the generation of the resting potential

Table. Passive membrane properties documented as time constants and input resistances after replacement of sodium by Tris or choline recorded with either K electrodes or Cs electrodes (1 mol/liter)

Medium	Sodium	Tris	Choline
K electrode			
τ [msec]	22 ± 2	20 ± 2	26 ± 5
R_i [MΩ]	74 ± 4	66 ± 5	71 ± 10
n	39	6	5
Cs electrode			
τ [msec]	22 ± 2	18 ± 3	23 ± 3
R_i [MΩ]	78 ± 9	77 ± 8	89 ± 9
n	13	9	10

τ has been calculated from a one-exponential fit of the passive hyperpolarization during the first 28 msec after an inward-current pulse of −0.2 nA. The fit was extrapolated to 150 msec. The input resistance R_i has been determined by ohmic law from the fitted hyperpolarization amplitude at 150 msec and the amplitude of the injected current. This was necessary because even during current injections of −0.2 nA inward rectification reduced the amplitude of the steady-state hyperpolarization such that the real passive voltage drop would have been underestimated. Passive membrane properties are not significantly different in either solution recorded with either type of electrode.

in *E. vannus*, but a major part in the generation of inward rectification.

Since K electrodes were supposed to artificially influence inward rectification (*see* Materials and Methods) the set of Na-exchange experiments was repeated with Cs electrodes (Fig. 2). The difference between the peak hyperpolarization and the steady-state hyperpolarization was reduced, predominantly because the peak value of the hyperpolarization is smaller. The voltage of the steady-state hyperpolarization was not significantly modified (Fig. 2*a,b*, *cf.* 1*a,b*), and the Na dependence of the inward rectification was confirmed (Fig. 2*c,d*). The resting membrane potential depolarized from -19.5 ± 0.5 mV ($n = 15$) in standard medium to -3.3 ± 1.4 mV ($n = 10$) in choline medium, and -9.4 ± 2.0 mV ($n = 10$) in Tris medium. These depolarizations might indicate time-dependent block of K conductances by the increasing accumulation of intracellular Cs ions. However, the input resistances and time constants were not significantly changed (Table, Cs electrode). In summary, the use of Cs electrodes did not modify inward rectification with respect to the recorded steady-state potentials after current injection.

Inward Currents Activated by Hyperpolarizations

The contribution of currents to inward rectification was tested by the voltage-clamp method with hyper-

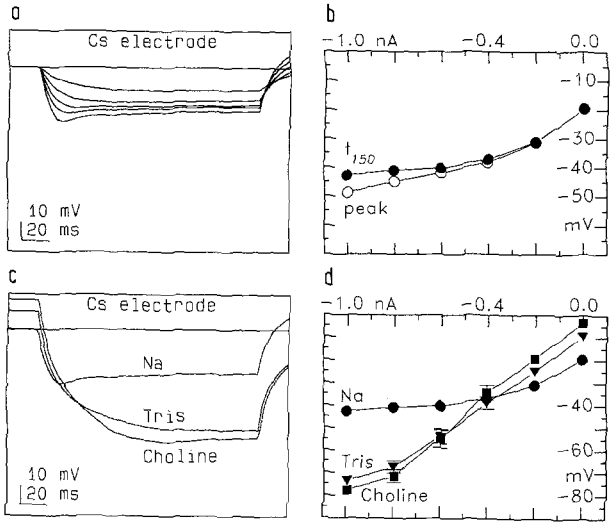

Fig. 2. Hyperpolarizations upon inward-current injections in standard media and after the replacement of Na^+ by choline or Tris recorded with Cs electrodes. (*a*) Inward rectification is still prominent ($n = 15$). (*b*) The *V-I* diagram shows a reduced difference between peak and t_{150} hyperpolarization when compared to Fig. 1, same cells as in *a*. (*c*) Inward rectification is lost after the replacement of Na^+ ($n = 15$) by choline ($n = 10$) or Tris ($n = 10$). The resting potential depolarizes after the replacement of Na^+ by Tris or choline. (*d*) *V-I* diagrams measured at t_{150} are linearized in Tris or choline medium, same cells as in *c*.

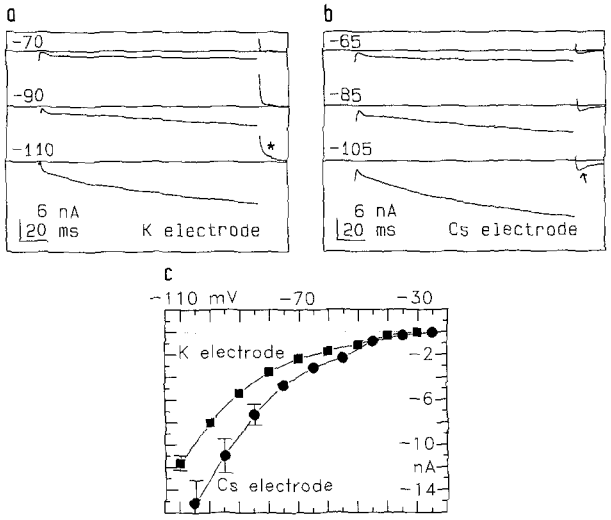

Fig. 3. Currents activated upon hyperpolarizations between −25 and −110 mV, recorded with K electrodes or Cs electrodes (note different step voltages in *a* and *b* indicated in mV). (*a*) Inwardly directed currents steadily increase within 160 msec after onset of the hyperpolarizations. Tail currents are outward directed when K electrodes are used (asterisk), holding potential −30 mV, $n = 25$. (*b*) Time course of inward current is not different when Cs electrodes are used, except that tail currents have an inward-current component (arrow), $n = 13$. (*c*) *I-V* diagrams of the current (t_{150}) recorded with either type of electrodes, same cells as in *a* and *b*. Inward currents activate at membrane potentials negative to −40 mV and reveal higher amplitudes when Cs electrodes are used.

polarizing step pulses up to −115 mV with either K or Cs electrodes (Fig. 3). A composite inward current has been recorded that steadily increased during the recording period of 160 msec after onset of the voltage pulses (Fig. 3*a,b*). The amplitude of the inward current was higher when recorded with Cs electrodes. The tail current was outwardly directed when recorded with K electrodes, and inwardly directed when recorded with Cs electrodes. This indicates a block of an outward-current component, presumably of a K current, when Cs ions diffuse out of the electrode into the cell. Therefore, and for comparison with previous results from examinations of the depolarization-activated Na current in *E. vannus* (Krüppel & Lueken, 1990), Cs electrodes were used in the subsequent experiments. The voltage-threshold for the activation of small inward currents was −40 to −50 mV (Fig. 3*c*). The threshold is positive to the potassium equilibrium potential of −90 mV that has been calculated from the reversal of the mechanosensitive K currents of *E. vannus* (T. Krüppel, V. Furchbrich and W. Lueken, *manuscript in preparation*). Because no potassium current can flow into the cell above E_K, the recorded current must be carried by sodium.

Na Component of the Inward Current

The identity of the hyperpolarization-activated inward current was tested in various Na media, with Na ions replaced by Tris or Li^+ (Fig. 4). A dose-dependent reduction of the inward current and its associated tail current were measured along with a decrease of the extracellular Na concentration (Fig. 4*a,b*). After totally replacing Na^+ by choline instead of Tris, inward currents were slightly smaller (*not shown*). Li^+ instead of Na^+ carried 40–50% of the maximum inward current when compared to the standard currents and the currents remaining in a medium where Tris totally replaced Na^+ (Fig. 4*c,d*). This points to a reduced permeability of the inward-rectifier channel for Li^+. Reduction of the extracellular Na concentration below 108 to nominal 0 mmol/liter (Fig. 4*c,d*, Tris) did not further reduce the amplitude of the inward current (*cf.* Fig. 4*a,b*).

K Component of the Inward Current

Though the dominating component of the composite inward current has been shown to be carried by

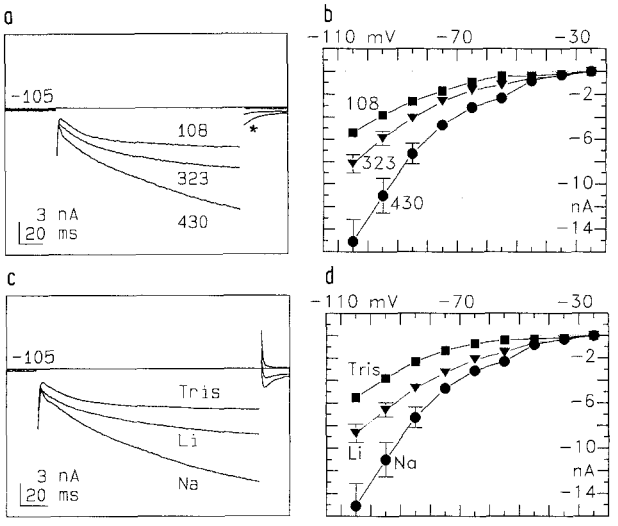

Fig. 4. Na component of the inward current. (*a*) The inward currents upon hyperpolarizations to −105 mV and their tail currents (asterisk) show a strong dose-dependent reduction in reduced Na concentrations, in mmol/liter, 430 n = 11, 323 n = 7, 108 n = 6. (*b*) *I-V* diagrams of the inward current (t_{150}) reveal the Na contribution to the inward current, same cells as in *a*. (*c*) Total replacement of Na^+ by Li^+ demonstrates a reduced permeability for Li^+ as charge carrier. After totally replacing Na^+ by Tris an inward-current component remains that steadily increases and therefore is not only carried by leak currents. Na n = 13, Li n = 9, Tris n = 18. (*d*) *I-V* diagrams (t_{150}) of the same cells as in *c*.

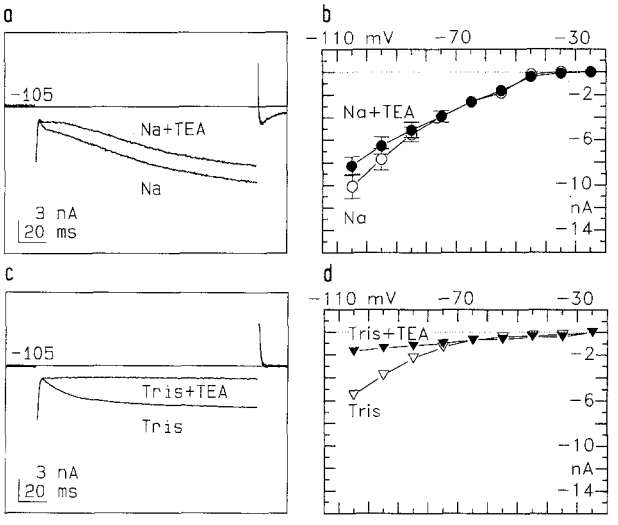

Fig. 5. K component of the inward current. (*a*) Addition of 10 mmol/liter TEA to the standard medium (Na+TEA) reduces the inward current that is activated by hyperpolarizations to −105 mV. The initial inward currents immediately after onset of voltage pulse and the tail currents are not different in either medium, Na (=EASW) n = 6, Na+TEA n = 6. (*b*) *I-V* diagrams recorded in the same media as in *a*. TEA blocks inward currents at potentials negative to −85 mV. (*c*) After replacement of Na^+ by Tris a current remains that is blockable by TEA. Addition of 10 mmol/liter TEA to the Tris medium activates a pure, time-independent leakage current during hyperpolarizations to −105 mV, Tris n = 18, Tris+TEA n = 8. (*d*) *I-V* diagram (t_{150}) of the same cells as in *c*. A linear leakage current remains in the Tris+TEA medium.

sodium, it also contains a small K current component, which has been identified by its block by extracellular TEA (Fig. 5). TEA was inefficient up to the potassium equilibrium potential of −90 mV, but slightly reduced the total inward current below E_K (Fig. 5*a,b*). A somewhat stronger blocking influence of TEA was recorded negative to −75 mV, when, in addition to the extracellular application of TEA, the Na-inward current was eliminated by the replacement of Na^+ by Tris (Fig. 5*c,d*). A linear leakage current remained (Fig. 5*c,d* Tris + TEA), that was below −2 nA even at hyperpolarizations to −105 mV. The difference between the leakage current and the current in the Tris medium presumably isolates the K-inward current component. Neither the extracellular addition of 50 mmol/liter Cs^+ nor the replacement of 50 mmol/liter Na^+ by Cs^+ changed the amplitudes of the recorded inward currents (*not shown*).

Ca Dependence of the Inward Current

To test the Ca dependence of the inwardly rectifying current, EGTA was injected into the cells through the electrodes (Fig. 6). The inward current was somewhat reduced when the Ca ions entering the cell were chelated by EGTA. An inward current remained that neither could be correlated to the Na- nor to the K-current component. The residual current is too large for an inhibition of the Na current (*cf.* Fig. 5*c*, Tris), and too small for a pure inhibition of the K-current component (*cf.* Fig. 5*b*). Presumably, a part of the Na current is not activated, and/or the Ca-inward current is enlarged after EGTA injection, or leakage currents are enlarged.

The dubious results of the EGTA experiments should be clarified by a set of decirriation experiments (Fig. 6). Decirriation removes depolarization-activated Ca channels in other ciliates and in *Euplotes*, because they are located within the ciliary membrane (Machemer & Ogura, 1979; Krüppel & Lueken, 1990). If the Ca channels which activate upon hyperpolarization also reside within the ciliary membrane, no Ca-activated ion channels should activate after decirriation. In any case, the inward current was reduced to a small component when cells were decirriated (Fig. 6). This indicates a strong, but not complete Ca dependence of the total inward current, since the remaining current is not reduced to the pure leakage current (*cf.* Fig. 5*d*, Tris + TEA). Unfortunately, it cannot be excluded that the current

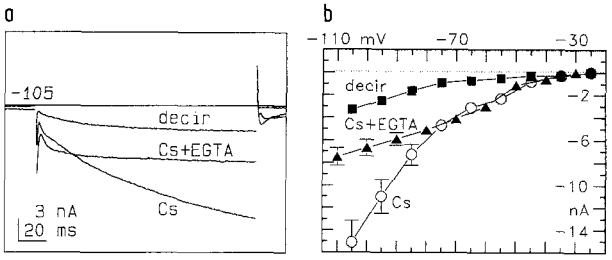

Fig. 6. Ca dependence of the inward current. (*a*) Decirriation strongly reduces inward current (decir $n = 9$). Inward currents are smaller when the Ca chelator EGTA is injected into the cells (Cs + EGTA electrode – holding potential −30 mV and $n = 6$, Cs electrode $n = 13$). (*b*) *I-V* diagram (t_{150}) of the same cells as in *a*. After decirriation, only small inward currents remain with little larger amplitudes than the pure leakage current (*cf.* Fig. 5*d*). Leakage currents might be increased after EGTA injection.

is reduced because the inward-rectifier channels reside in the ciliary membrane, as those of the depolarization-activated Ca channels.

Discussion

Hyperpolarization-Activated Na and K Currents

The major conclusion of the present paper is that inward rectification in the marine ciliate *E. vannus* is predominantly due to a Na-inward current. The Na ions are exposed to a steadily increasing, inwardly directed driving force with any rise of hyperpolarization, starting from the sodium equilibrium potential of 25–30 mV (Krüppel & Lueken, 1990). Consequently, large Na-inward currents can flow into the cell. The dose-dependent reduction of the inward current in low extracellular Na media (Fig. 4*a,b*) demonstrates the Na identity of this inward current. The ionic nature is confirmed because the Na analogue Li^+ also carries the current (Fig. 4*c,d*) and extracellular Cs^+ or TEA do not block it. These features are reminiscent of those of the Na current that activates upon depolarization (Krüppel & Lueken, 1990).

Only at large hyperpolarizations negative to the K equilibrium potential of about −90 mV, as determined by the reversal of the mechanically induced K current at the posterior cell end (Krüppel et al., *manuscript in preparation*), a small TEA-blockable K current activates. This current was also identified as the remaining component after replacement of the extracellular Na^+ by Tris or choline (Fig. 5*c,d*). The K component of the inward current is not blockable by extracellular Cs ions in *E. vannus* in contrast to the typical K-inward-rectifier currents in other species (*not shown*). Recently, Preston et al. (1990) described two different K currents which activate upon hyperpolarization that can be distinguished by their kinetics, their Ca sensitivity, and their sensitivity to TEA and quinidine. The existence of these two K currents is unique for ciliates (Preston et al., 1990). Whether one or both of the correlated potassium channels are also permeable to Na^+ is an intriguing question, since Na channels were either analyzed with blocked K channels (Saimi, 1986), or K channels were analyzed without Na^+ within the extracellular medium (Preston et al., 1990).

The putative Na channels of *E. vannus* could also belong to the category of Ca-activated nonspecific cation channels (CAN), that play an important role in a wide range of cells where they provide slow depolarizing waves that regulate pacemaker activities. These channels poorly discriminate between K^+ and Na^+ (Partridge & Swandulla, 1988), as those which exist in several neural cells and are usually termed I_h, I_q, or I_f (McCormick & Pape, 1990; Kamondi & Reiner, 1991). Whether Na and/or K currents flow through the same channel in *E. vannus*, or whether different channel types are used, is not known yet and should be analyzed in further examinations.

Ca Dependence of Inward Rectification

Intracellular EGTA buffers the Ca influx and has often been used to determine Ca-dependent processes. In *Euplotes*, the inward current was reduced to 40–50% of its maximum amplitude when EGTA diffused into the cell. The remaining current could still include components of the Na-inward current, since its amplitude is larger than the current amplitudes after the replacement of Na^+ by Tris or choline. The incomplete suppression of the inward current might be explained by a remaining increase of the intracellular Ca concentration due to the limited chelating capacity of EGTA, or the nonuniform distribution of EGTA within the cell. However, the limited reduction of the inward current after Ca buffering might also reflect a residual activation by voltage instead of Ca^{2+}, or an increased Ca influx into the EGTA-loaded cells. Decirriation also indicates Ca-dependent processes, since Ca influx is carried through depolarization-activated Ca channels which are located in the ciliary membrane in ciliates. Whether these channels also activate upon hyperpolarization, or become leaky at an increase of the driving force for Ca^{2+} by hyperpolarization, is not known in *E. vannus*. Nevertheless, Ca ions can exert

their regulatory function closely related to the membrane without becoming measurable within the bulk solution of the cytoplasm, where the recording electrode is positioned. Another evidence for the Ca dependence of the inward rectification comes from former experiments (Krüppel & Lueken, 1988). Ba^{2+} instead of Ca^{2+} blocked the larger portion of inward currents activated upon hyperpolarization. This might be caused by the blocking influence of Ba^{2+} on an $I_{Ca,hyp}$ similar to the one described by Preston et al. (1992*a*) for *Paramecium*. Ba^{2+} is also known to block K channels, but the major portion of the currents identified within this paper is carried by Na^+. That Ba^{2+} acts as blocker for the Na current itself is not very likely.

Physiological Function of Na and K Current Activated by Hyperpolarization

The physiological significance of inwardly rectifying K currents is poorly understood. Mostly, the regulation of pacemaker potentials (DiFrancesco & Torotora, 1991), is assigned to these currents. They may also be involved in the regulation of stimulus-secretion-coupling processes (Mukai, Kyogoku & Kuno, 1992) or may be used as negative feedback currents for the restoration of the resting potential after hyperpolarizations as the afterhyperpolarizations of action potentials (Spain, Schwindt & Crill, 1987). The vertebrate photoreceptor hyperpolarizations and electrogenic transport processes, as produced by the Na/K-exchange pump which extrudes Na^+, causing hyperpolarizations by a net-outward current, may be compensated by these currents (Phillips, Bacigalupo & O'Day, 1992).

In *E. vannus*, the largest physiologically relevant hyperpolarizations ever recorded (-60 to -65 mV) occur after mechanical stimulation at the posterior cell end and are caused by an efflux of K ions (Krüppel et al., *manuscript in preparation*). Hyperpolarizations exclusively evolve after these stimuli, spontaneous hyperpolarizations are never observed. Since the free running or mechanical modified membrane potential does not exceed the potassium equilibrium potential of -90 mV at physiological conditions, the K-inward current that activates at more negative potentials, presumably plays no part in the day-to-day life of the marine *E. vannus*. Also in fresh-water ciliates, where the activation of a potassium inward-rectifier current is supposed to activate upon hyperpolarization, it is questionable whether these currents activate within the physiologically relevant range of membrane potentials. The mechanically induced receptor potential, reported in *Paramecium caudatum* by Naitoh and Eckert (1973), reached a peak value of -60 mV in 1 mmol/liter KCl solution. In the same species, an E_K of about -85 mV has been determined by Ogura and Machemer (1980). Thus, even at the peak of the largest hyperpolarizations, the driving force for potassium is still outwardly directed and as a consequence, no inwardly rectifying K currents can activate. In *Paramecium tetraurelia,* the situation is not different since E_K is about -60 mV and the mechanically induced peak hyperpolarization just reaches this value (Oertel et al., 1978). In *Stylonychia,* an E_K of -90 mV has been determined by the reversal of the mechanosensitive K current in a solution that contains 1 mmol/liter K^+ (De Peyer & Machemer, 1978). With a mean resting potential of -51 mV (Deitmer, 1981) and a maximum amplitude of the hyperpolarization of 30 mV (Deitmer, 1982), *Stylonychia* hyperpolarizes to a peak potential of -81 mV where K currents are still outwardly directed.

Above E_K the inward-rectifier channels can only pass K-outward currents which might increase hyperpolarizations in a positive feedback-loop. In *Paramecium,* a regenerative hyperpolarization has been recorded (K-spike), but only when the driving force for potassium outward current was artificially increased either by reduction of the potassium concentration in the experimental solution or when the intracellular K concentration was increased (Satow & Kung, 1977). The K-spike was also triggered by mechanical stimulation at the posterior cell end. Mechanically and hyperpolarization-activated K currents thus might be additive mechanisms to induce fast accelerations of the cells' swimming speed which are correlated to hyperpolarizations. But, the regenerative hyperpolarizations do not occur either in the everyday life of *Paramecium* or of *Euplotes*. Therefore, the underlying K-outward currents might only be relevant in a somewhat reduced form that do not allow regenerative hyperpolarizations. The increased inward currents, recorded with Cs electrodes (Fig. 3), are presumably due to the block of such a K-outward-current component. This K current antagonizes the Na inward current and together they might regulate the peak value of any hyperpolarization.

Na-inward currents can activate upon any hyperpolarization and could repolarize cells back to the resting membrane potential. Physiologically relevant hyperpolarizations have been recorded in *E. vannus* as afterhyperpolarizations that follow depolarizations elicited by mechanical stimulation at the anterior cell end, or as mechanically induced hyperpolarizations at the posterior cell end. Indeed, the latter are often prolonged in Na-free media (Krüppel et al., *manuscript in preparation*). Na-inward currents might also contribute to spontaneous mem-

brane potential fluctuations in *E. vannus*. Influx of Na^+ at small hyperpolarizations would in turn depolarize cells and perhaps initiate spontaneous depolarizations. The afterdepolarizations, which were recorded after the end of the current pulses (Fig. 1*a*), are presumably caused by such an increased Na conductance. In *Paramecium*, spontaneous membrane potential depolarizations only arose when Na^+ was added to the experimental solution (Satow & Kung, 1974). Therefore, Na^+ might play an important role in the regulation of the spontaneous membrane potential fluctuations of ciliates and thus influence the locomotive behavior.

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