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THE EFFECTS OF TETRAETHYLAMMONIUM AND OTHER AGENTS ON THE POTASSIUM MECHANORECEPTOR CURRENT IN THE CILIATE *STYLONYCHIA*

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SUMMARY

The membrane response to mechanical stimulation was investigated under voltage-clamp conditions in the hypotrich ciliate *Stylonychia mytilus* (Protozoa). The mechanoreceptor current shows high ionic selectivity, unlike that in metazoans. It is exclusively carried by potassium ions and is selectively inhibited by tetraethylammonium and 4-aminopyridine. Procaine, tetramethylammonium, caesium and divalent cations have no or little effect on this receptor current. The ionic channels mediating the currents differ from *voltage-dependent* channels in the ciliate membrane in their low sensitivity to divalent cations, and their relatively high sensitivity to tetraethylammonium and 4-aminopyridine.

INTRODUCTION

Mechanical stimulation of the posterior of ciliated Protozoa evokes a hyperpolarizing receptor potential (Naitoh & Eckert, 1969, 1973). This membrane hyperpolarization has been reported to reverse its direction near the K^+ equilibrium potential (Naitoh & Eckert, 1973; de Peyer & Machemer, 1978), suggesting that K^+ ions are the main charge carriers during the receptor current flow. Recently it has been shown in *Paramecium* that this mechanoreceptor current persists even after deciliation of the cells (Ogura & Machemer, 1980). Receptor current–voltage analysis has revealed that the amplitude and time course of this current change with the membrane potential in a non-linear fashion (Deitmer, 1981*a*). Naitoh & Eckert (1973) reported that tetraethylammonium ions (TEA) reduced the K^+ receptor current, but that this block was antagonized by the elevation of the external Ca^{2+} concentration, suggesting some interaction between Ca^{2+} and the activation of the K^+ receptor current. These authors also inferred that TEA itself may be carrying a mechanically activated inward current.

In the present study the effects on this K^+ mechanoreceptor current of TEA and other potential K^+ current-blocking agents, and of divalent cations have been investigated in the hypotrich ciliate *Stylonychia*, and compared with those on the voltage-dependent K^+ current. Some of these results have been presented in a preliminary form elsewhere (Deitmer, 1980, 1981*b*).

METHODS

Cloned cells of the hypotrich ciliate *Stylonychia mytilus syngen I* (Protozoa) were cultured in Pringsheim solution and fed with the phytomonad *Chlorogonium elongatum*. The cells were washed and equilibrated in a solution containing 1 mM-KCl and 1 mM-$CaCl_2$, buffered with 1 mM Tris-Cl or 1 mM HEPES at pH 7·3–7·4 ('normal' solution). In various test solutions the KCl concentration was lowered (to 0·1 mM) or increased (up to 4 mM), or the concentration of various divalent cations was changed, as indicated in the text. Tetraethylammonium, 4-aminopyridine and procaine (all from Merck, W. Germany), tetramethylammonium (Fluka AG, Switzerland) and CsCl were added to the normal solution, and the pH restored as necessary. The experiments were performed with the experimental chamber cooled to 17–18 °C.

The mechanical stimulation of the cell surface was performed with a fine glass stylus (2–5 μm tip diameter) connected to a ceramic phonocartridge, as described previously (de Peyer & Machemer, 1978; Deitmer, 1981*a*).

For electrical recording and voltage-clamp the cells were impaled by two microelectrodes (electrical resistance 30–80 MΩ). Experiments were continued only when the input resistance of the cell membrane was above 40 MΩ (in normal solution). Electronic circuits and recording techniques were as described previously (de Peyer & Machemer, 1978; de Peyer & Deitmer, 1980; Deitmer, 1981*a*).

RESULTS

Potassium-dependent mechanoreceptor current

Mechanical stimulation of the cell *posterior* evokes a hyperpolarizing receptor potential (Fig. 1A). The amplitude and rate of fall (dV/dt) of the potential varied with the stimulus intensity and had maximum values of 30 mV and 5 V/s, respectively (see also de Peyer & Machemer, 1978). The receptor current recorded under voltage-clamp conditions (Fig. 1A) reached amplitudes up to 30 nA (see also Deitmer, 1981*a*). Both receptor potential and receptor current decayed with an exponential time course (Fig. 1B). The receptor potential decay had a mean time constant of 57·3 ms (S.D. 8·3; $n = 7$). This time constant agreed with the membrane time constant, i.e. the product of membrane resistance and membrane capacitance. From small long-lasting hyperpolarizing square-wave current pulses, membrane time constants between 45 and 70 ms were determined. The decay of the receptor current occurred much faster, the mean time constant being 7·3 ms (S.D., 1·2; $n = 15$) (see also Deitmer, 1981*a*).

The amplitude of the mechanoreceptor current decreased upon membrane hyperpolarization and reversed its direction near a membrane potential of −90 mV (Fig. 2A). The receptor current–voltage relationship has been investigated more elaborately in a previous paper (Deitmer, 1981*a*). A tenfold change in external K^+ concentration produced a 57·8 mV change in reversal potential (Fig. 2B), which indicates, from the Nernst equation, that the receptor current is exclusively carried by K^+ ions, in agreement with previous studies of the unclamped *Stylonychia* (de Peyer & Machemer, 1978) and on *Paramecium* (Naitoh & Eckert, 1973; Ogura & Machemer, 1980). From this identity of the reversal potential and K^+ equilibrium potential an intracellular K^+ concentration of approximately 33 mM may be calculated for *Stylonychia*.

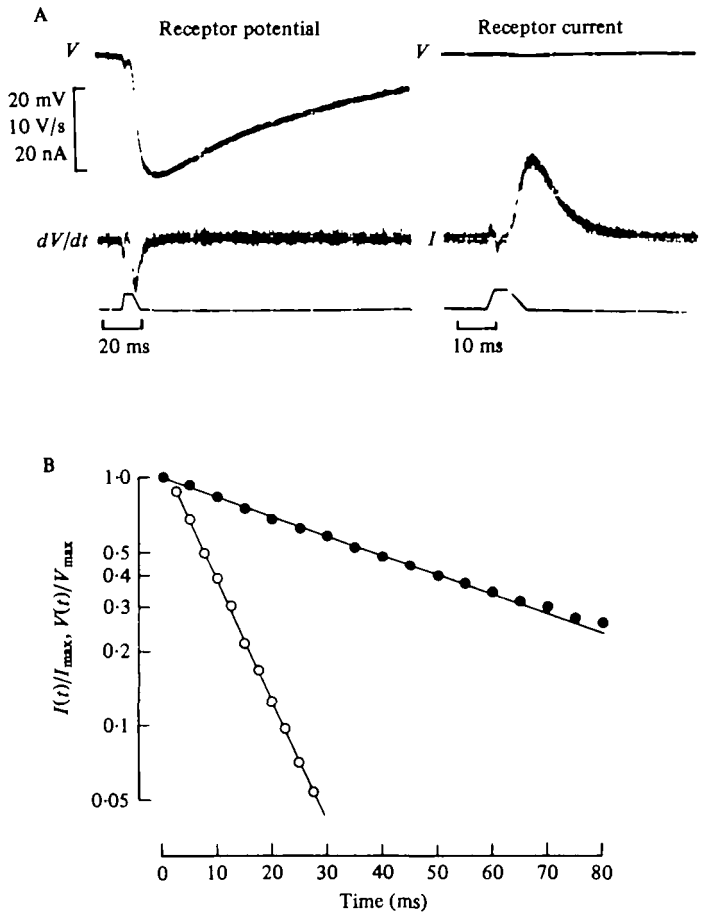

Fig. 1. (A) Membrane responses, the hyperpolarizing receptor potential and the receptor outward current, to mechanical stimulation to the cell posterior; V, the membrane potential; dV/dt, the first derivative of the potential change; I, membrane current in voltage-clamp. The lower traces show the DC pulses to the phonocartridge for mechanical stimulation; note the different time scale.

(B) Decay of the receptor potential (●) and the receptor current (○) on a semi-log plot. The amplitude during the decaying phase, $V(t)$, $I(t)$, divided through the maximal amplitude, V_{max}, I_{max}, versus time. The potential decay to $1/e$ (37 %) as shown gives a time constant of 57 ms. The time constant of the current decay shown is 8 ms.

Effects of blocking agents

Mechanical stimulation to the cell *anterior* elicits a depolarizing receptor potential which triggers an action potential (Eckert, Naitoh & Friedman, 1972; de Peyer & Machemer, 1978). Addition of tetraethylammonium (TEA) to the external solution is known to prolong the action potential in *Paramecium* (e.g. Naitoh & Eckert, 1973; Satow & Kung, 1976). This applied also to *Stylonychia* (Fig. 3A). The action potential was significantly prolonged in the presence of 0·5 mM TEA compared to that in normal solution. The maximum rate of rise of the action potential remained unchanged by TEA.

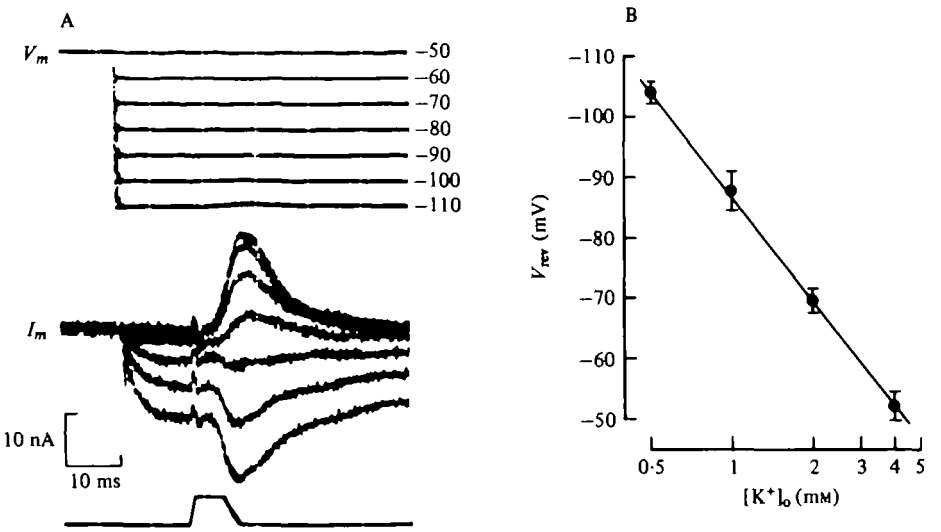

Fig. 2. (A) Superimposed recordings of the receptor current following equal mechanical stimuli at different clamped membrane potentials (V_m) between −50 mV (holding potential equal to resting potential) and −110 mV. Note the reversal of the mechanoreceptor current at the membrane potential near −90 mV. At more negative membrane potentials receptor *inward* currents are recorded.

(B) Plot of the reversal potential, V_{rev}, versus the log K^+ concentration, $[K]_o$, between 0·5 mM and 4·0 mM. The points are means, ±1 S.D., each from 4 to 18 different cells. The straight line is a least-square regression fit through all experimental points with a slope of 57·8 mV/tenfold change in the $[K]_o$ (coefficient of correlation r = 0·986).

The resting membrane depolarized by up to 3 mV after the addition of 0·5–1·0 mM TEA. The membrane input resistance increased by up to 50% to 70–100 MΩ. These effects indicated that a resting conductance to K^+ ions was decreased by TEA.

When the cell was mechanically stimulated at its posterior end in the presence of TEA (0·5 mM, 2 mM $[K]_o$), the evoked hyperpolarizing receptor potential was considerably reduced in amplitude and maximum rate of fall (Fig. 3B). Maximal mechanical stimulation produced receptor potentials of less than 10 mV. Decay of the potential was prolonged, presumably due to the rise in the membrane input resistance in the presence of TEA. The maximum rate of fall of the receptor potential, indicative for the maximum current flow, decreased from typically 5 V/s to less than 1 V/s.

The reversal potential of the mechanoreceptor current was around −70 mV, with an external K^+ concentration of 2 mM (Fig. 4A). In the presence of 1 mM TEA the receptor current was inhibited by about 80% without any change in reversal potential (Fig. 4B). This inhibition was readily reversible. In Fig. 4C is shown the receptor current–voltage relationship in the absence and presence of TEA. The zero current line is cut at the same potential (reversal potential) in both solutions. The slope of the lines connecting the experimental points indicates the increase in membrane conductance following a mechanical stimulus. This conductance increase, ΔG_{rec}, is given by

$$\Delta G_{rec} = I_{rec}/(V_m - V_{rev}), \quad (1)$$

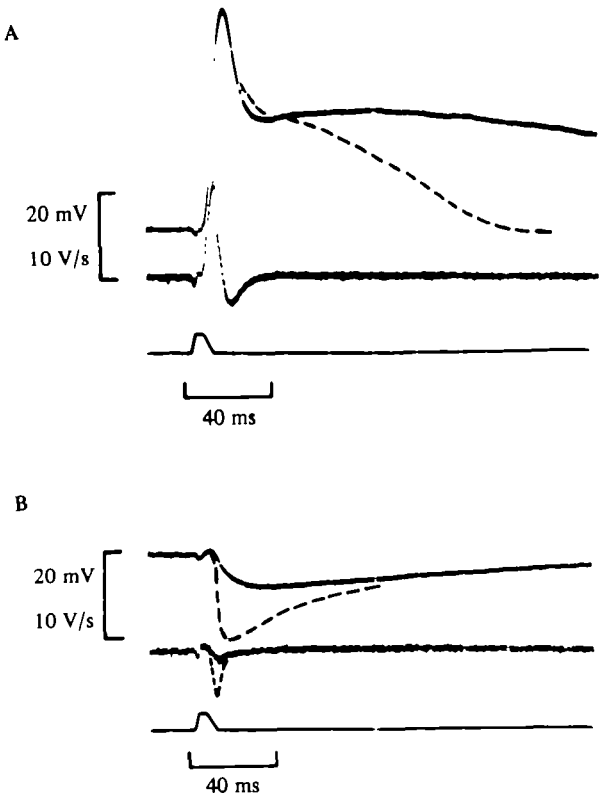

Fig. 3. The effects of 0·5 mM tetraethylammonium (TEA) on the action potential following mechanical stimulation to the cell anterior (A), and on the hyperpolarizing receptor potential following mechanical stimulation to the cell posterior (B). The dotted curves represent action potential and hyperpolarizing receptor potential, respectively, in the absence of TEA. The middle traces show the first derivative of the potential changes. The external K^+ concentration in the experiment shown was 2 mM.

where I_{rec} is the mechanoreceptor current, V_m the clamped membrane potential, and V_{rev} the reversal potential. Thus ΔG_{rec} was calculated to be 0·2 μS in the presence of TEA (1 mM) compared to 1·0 μS in normal external solution containing 2 mM $[K]_o$.

It is interesting to note that the conductance increase of the membrane following mechanical stimuli tended to increase with the $[K]_o$. Higher $[K]_o$ also antagonized the blocking effect of TEA. Thus lower concentrations of TEA were needed to inhibit the mechanoreceptor current when the $[K]_o$ was reduced. In a solution containing 1 mM-[K] a TEA concentration of 0·5 mM blocked the receptor current to approximately the same extent as 1 mM TEA in a solution containing 2 mM [K]. At 1 mM TEA and 1 mM $[K]_o$ virtually no mechanoreceptor current could be recorded.

4-AP, which has been shown to block the voltage-dependent outward K^+ current in squid axon (see, for example, Meves & Pichon, 1977) also reduced the K^+ mechanoreceptor current (Fig. 5). At a concentration of 0·5 mM (1 mM $[K]_o$) the amplitude of the receptor current was reversibly decreased by 70 to 90%. The reversal potential, again, was not altered by 4-AP.

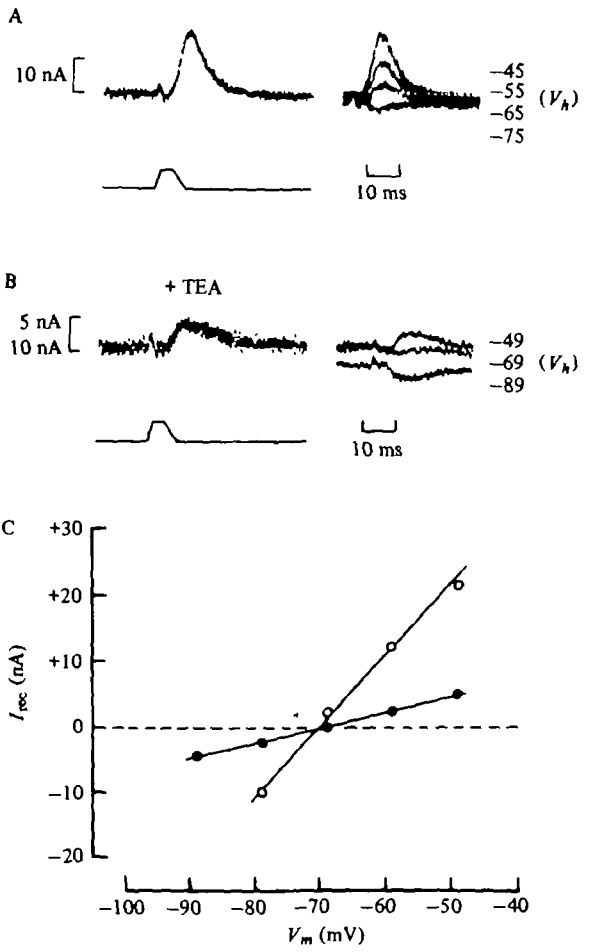

Fig. 4. (A) Mechanoreceptor currents and their reversal during membrane hyperpolarization in normal external solution (containing 2 mM-K). (B) Mechanoreceptor currents after the addition of 1·0 mM TEA. Note that the reversal of the current occurs again near −70 mV, and note the different scale for the receptor current shown on the left (5 nA). (C) Mechanoreceptor current–voltage relationships in solutions containing 2 mM-K with 1 mM TEA (●) and without TEA (○).

TMA, procaine and Cs^+, at a concentration of 1 mM, had no or very little influence on the amplitude (Fig. 5) and the reversal potential of the mechanoreceptor current.

Decay of the receptor current (see Fig. 1 and Deitmer, 1981*a*) was reversibly increased by up to 100% in the presence of 1 mM TEA (Fig. 6A) but was only occasionally affected by 0·5 mM 4-AP (Fig. 6B). This may indicate different types of action of TEA and 4-AP at this K^+-selective membrane channel. The other substances tested had no or very little effect on the time course of this mechanoreceptor current.

Effect of divalent cations

Receptor current was unaltered when Ca^{2+} was replaced by Mg^{2+}, Sr^{2+}, Ba^{2+} or Mn^{2+}. Addition of up to 1 mM Mg^{2+}, Mn^{2+} or Ba^{2+} to a Ca^{2+}-containing solution

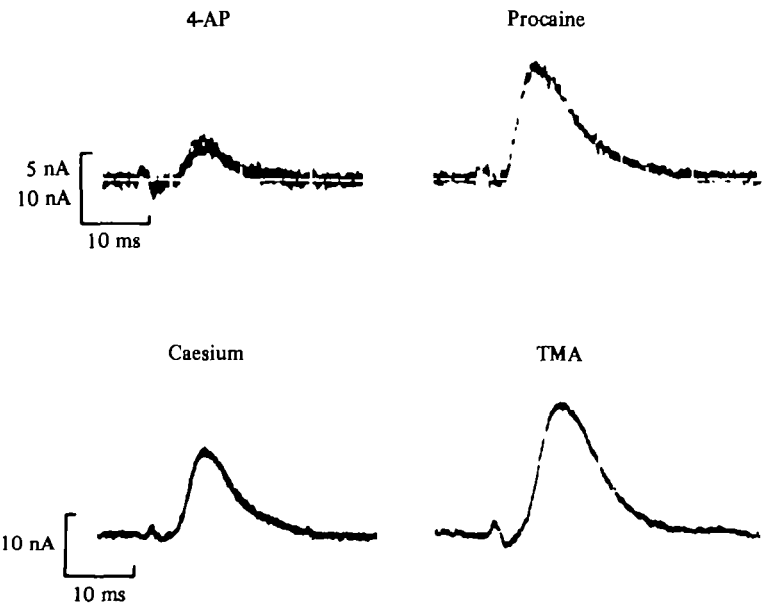

Fig. 5. Mechanoreceptor currents recorded in the presence of 0·5 mM 4-aminopyridine (4-AP), 1 mM procaine, 1 mM caesium and 1 mM tetramethylammonium (TMA). Note that the scale represents 5 nA for the upper left recording (two traces superimposed) and 10 nA for the other recordings.

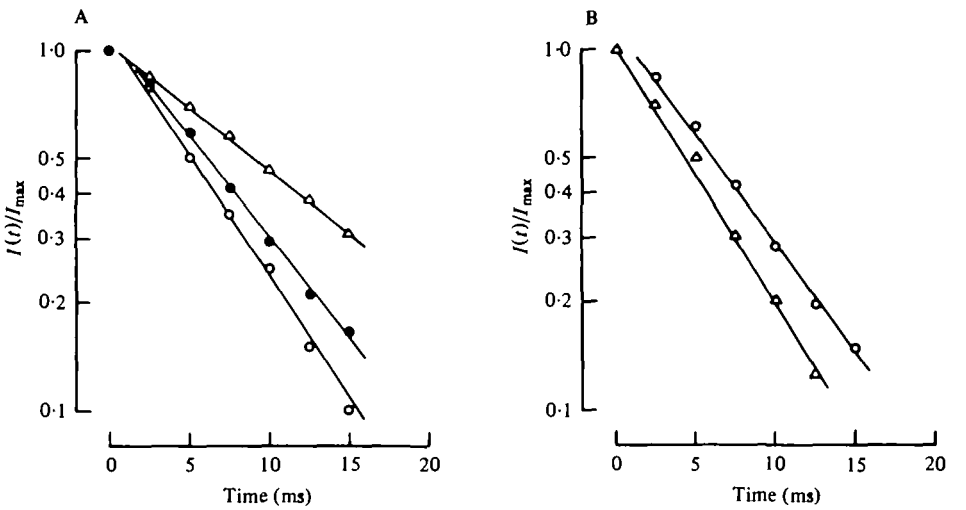

Fig. 6. Logarithmic plot of the falling phase of mechanoreceptor currents in the presence of TEA (A) and of 4-AP (B). (A) The time constant of receptor current decay was 6·8 ms in normal solution (open circles), 12·2 ms after addition of 0·5 mM TEA (triangles) and 7·8 ms after removal of TEA (filled circles). All data from one cell. (B) The time constant of receptor current decay was 7·2 ms in normal solution (circles) and 6·5 ms in the presence of 0·5 mM 4-AP (triangles). All lines were fitted by eye.

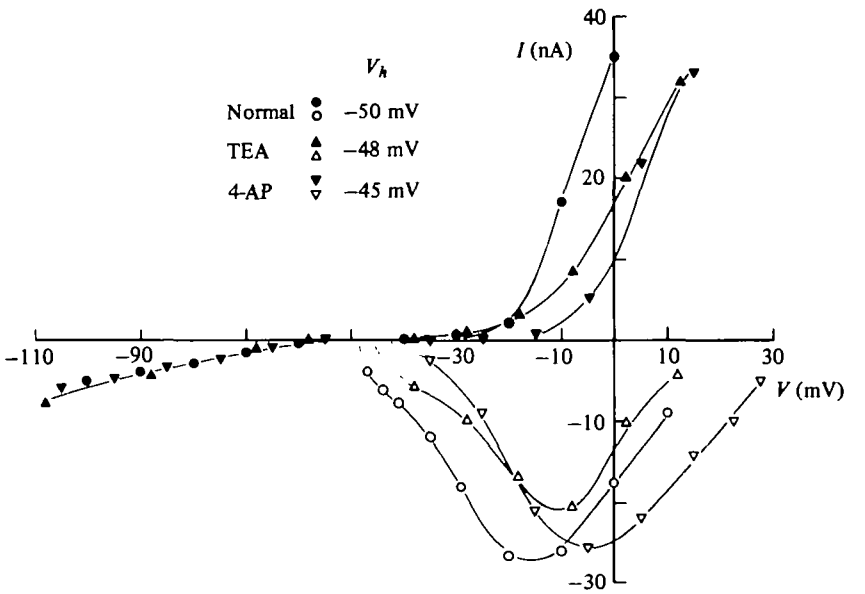

Fig. 7. Current–voltage relationships of early inward currents (open symbols) and steady-state currents (filled symbols) in normal solution (1 mM-K, circles) and in solutions containing 1 mM TEA (triangles) and 0·5 mM 4-AP (triangles upside down). Note the different holding (resting) potentials, V_h, in the different solutions.

also had little or no effect on this mechanoreceptor current. The reversal potential remained unaltered by these variations in the divalent cation species and concentration. There was no indication in all these experiments that the mechanoreceptor current in any way depended upon the presence, or upon the influx, of divalent cations.

Comparison with the voltage-dependent currents

Typical current–voltage relations in normal solution, and in solutions containing 1 mM TEA and 0·5 mM 4-AP, are shown in Fig. 7. TEA and 4-AP shifted the peak of the inward current to more positive membrane potentials by up to 5 mV, as can be seen from the different holding potentials (equal to the membrane resting potentials). The steady-state outward current was shifted by 5–10 mV to more positive potentials. In the TEA-solution the slope of outward going rectification was slightly reduced. This small shift and reduction of the outward going rectification presumably caused the prolongation of the action potential (see Fig. 3 A).

In summary, both TEA and 4-AP applied externally in concentrations up to 1 mM, which substantially blocked the K^+ mechanoreceptor current, had relatively little effect on the voltage-dependent steady-state outward current. Presumably higher concentrations or intracellular injection would have to be used to block this voltage-dependent K^+ outward current more significantly (Satow & Kung, 1976; Satow, 1978; Brehm, Dunlap & Eckert, 1978).

DISCUSSION

The present study shows that a K^+ receptor current in *Stylonychia*, evoked by mechanical stimuli, is inhibited by TEA and 4-AP. Voltage-dependent K^+ currents were reduced to much less extent at a given low TEA or 4-AP concentration. The two membrane currents also differ in their sensitivity to external divalent cations. It has been demonstrated previously that in the absence of external Ca^{2+} (replaced by Mg^{2+}) the outward-going rectification is abolished (de Peyer & Deitmer, 1980) and that Ba^{2+} also reduced the voltage-dependent K^+ outward current (Brehm *et al.* 1978; de Peyer & Deitmer, 1980). External and internal Ba^{2+} has been shown also in other excitable cells to reduce K^+ currents specifically (Hagiwara, Miyazaki, Moody & Patlak, 1978; Hermann & Gorman, 1979; Eaton & Brodwick, 1980). The K^+ mechanoreceptor current investigated here appeared to be independent of the external concentration of divalent cations. Both in the presence and absence of the divalent cations which can act as charge carriers through the voltage-dependent membrane channels (e.g. Ca^{2+}, Sr^{2+}, Ba^{2+}), the K^+ mechanoreceptor current still persisted. It seems unlikely therefore that this K^+ mechanoreceptor current is influenced by an influx of divalent cations as shown, for example, in snail neurones (Meech & Standen, 1975; Heyer & Lux, 1976), and thus may not be due to a Ca-dependent K^+ activation reported for many excitable cells (for ref. see Meech, 1978). The latter mechanism has been proposed for the *voltage-dependent* K^+ current in ciliated Protozoa (Satow, 1978; Brehm *et al.* 1978; Satow & Kung, 1980; de Peyer & Deitmer, 1980). The different sensitivities of the K^+ mechanoreceptor current and the voltage-dependent K^+ current to TEA (and to 4-AP) support the suggestion that these K^+ activations have different modes: in a variety of other excitable cells TEA has been found to block a voltage-dependent *Ca^+-insensitive* K^+ current (e.g. Neher & Lux, 1972), but was found to be less effective on a *Ca-mediated* voltage-dependent K^+ current (Thompson, 1977; Aldrich, Gettes & Thompson, 1979).

Two results of the present investigation differ from the findings of Naitoh & Eckert (1973), for the hyperpolarizing mechanoreceptor response in *Paramecium*. First, mechanical stimulation to the cell posterior never resulted in a depolarization as reported by these authors, or in an inward current, in the presence of TEA (up to 10 mM). Secondly, the blocking effect of TEA on the K^+ mechanoreceptor current was not antagonized by external Ca^{2+}. Since TEA also did not change the reversal potential of the K^+ mechanoreceptor current I tend to conclude that (1) TEA itself does not carry current through the mechanically activated membrane channels, and (2) the posterior part of the cell membrane does not contain mechanoreceptor channels other than K^+-selective channels. The latter conclusion was also drawn by a voltage-clamp study on the distribution of mechanoreceptor channels in the *Paramecium* surface membrane (Ogura & Machemer, 1980).

It has been shown previously that the time constant of decay of the mechanoreceptor current, and thus the life time of the mechanically activated ionic channels, varies with the membrane potential (Deitmer, 1981*a*). TEA tended to increase this time constant. This contrasts with the effect of TEA to shorten the decay of a voltage-dependent K^+ current in supramedullary cells of the puffer *Spheroides maculatus* (Nakajima, 1966). 4-AP and the other substances tested had much less or no effect on the time course of

the mechanoreceptor current. This suggests that in these cells TEA may prolong the life time of these mechanically activated ionic channels or induces a two-state conformation (rapidly altering between blocked and unblocked state) of them, which might result in prolonged receptor current decay.

In conclusion, the membrane current elicited by mechanical stimuli to the cell posterior exhibits a high selectivity to K^+ ions, in contrast to mechanoreceptor responses in metazoan tissues, where these responses usually result from rather non-selective increases in membrane ionic permeability (see, for example, Edwards, Terzuolo & Washizu, 1963; Corey & Hudspeth, 1979). Furthermore, the K^+ mechanoreceptor channel is inhibited by a relatively low concentration of TEA as compared to voltage-dependent K^+ channels in ciliates, nerve and muscle cells.

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