Eur Biophys J (1995) 24: 85–92

European Biophysics Journal

Dynamics of chloride and potassium currents during the action potential in *Chara* studied with action potential clamp

Gerhard Thiel

Pflanzenphysiologisches Institut, Universität Göttingen, Untere Karspüle 2, D-37073 Göttingen, Germany
(Tel.: 49-551-397840, Fax: 49-551-397838, e-mail: GTHIEL@GWDGV1.DENET.GWDG.DE)

Received: 1 August 1994 / Accepted in revised form: 23 January 1995

Abstract. The Cl^- and K^+ currents underlying the action potential (AP) in the giant alga *Chara* were directly recorded with the action potential clamp method. An electrically triggered action potential was recorded and repetitively replayed as command voltage to the same cell under voltage clamp. The resulting clamp current was close to zero. Only the initial rectangular current used for stimulation was approximately reproduced by the clamp circuit. Inhibition of Cl^- channels with niflumic acid or ethacrynic acid and of K^+ channels with Ba^{2+} evoked characteristic compensation currents because the amplifier had to add the selectively inhibited currents. Integration of the compensation currents revealed a mean flux through Cl^- and K^+ channels of 3.3 10^{-6} and 2.1 10^{-6} mole m^{-2} AP^{-1} respectively. The dynamics of Cl^- and K^+ channel activation/inactivation were obtained by converting the relevant clamp currents to ionic permeabilities using the Goldman-Hodgkin-Katz current equation. During the AP the Cl^- permeability reaches a peak 370 ms, on average, after termination of the stimulating pulse. The following inactivation proceeds 3.6 times slower than the activation. The increase in K^+ permeability lags behind the rise in Cl^- permeability, reaching a peak approximately 2 s after the latter.

Key words: Action potential clamp – *Chara* – Cl^- currents – K^+ currents

Introduction

Voltage clamp experiments with square pulse protocols have been used to investigate the activation and inactivation kinetics of ionic currents during the action potential (AP) in the giant alga *Chara* (Findlay and Hope 1964; Beilby and Coster 1979 a; Lunevsky et al. 1983) with the final intension of reconstructing the free running voltage from the kinetics of individual currents (Beilby and Coster 1979 b). An alternative method to directly record the dynamics of ionic currents underlying the AP is the action potential clamp technique (Fischmeister et al. 1984; Mazzanti and DeFelice 1987; de Haas and Vogel 1989; Doerr et al. 1989, 1990; Ibarra et al. 1991). The advantage of this technique, in which a cell (or a membrane patch) is clamped to the cell's own action potential, is that it allows recording of the currents as they really occur during excitation. In one version of the action potential clamp technique, currents can be investigated by allowing the cell to clamp its own action potential while simultaneously recording ion channel activity in a cell attached patch (Fischmeister et al. 1984, Mazzanti and DeFelice 1987). Using this experimental approach in *Chara* two types of transiently activating Cl^- channels and one type of K^+ channel were identified during excitation (Thiel et al. 1993; Homann and Thiel 1994). The drawback of these measurements was that the activation of Cl^- and K^+ currents could not be clearly separated (Homann and Thiel 1994). Furthermore, in order to increase resolution of small currents/single channels in membrane patches the ionic composition of the extracellular solution (pipette) contained ions in concentrations which were up to 3 orders of magnitude higher than those present in normal artificial pond water. Thus, under these conditions the voltage gradient at which these channels normally operate is greatly shifted, a factor which might affect the gating properties of these channels (Blatt 1991).

In the present investigation whole cell currents which pass the plasma membrane of *Chara* during an action potential were recorded with the more conventionel version of the action potential clamp method (de Haas and Vogel 1989; Doerr et al. 1990; Ibarra et al. 1991). In this case an action potential was recorded, stored and then replayed as command voltage of a clamp amplifier to an intact cell bathed in artificial pond water. With appropriate channel inhibitors the dynamics of the macroscopic Cl^- and K^+ currents and permeability changes as they occur during an AP could be determined.

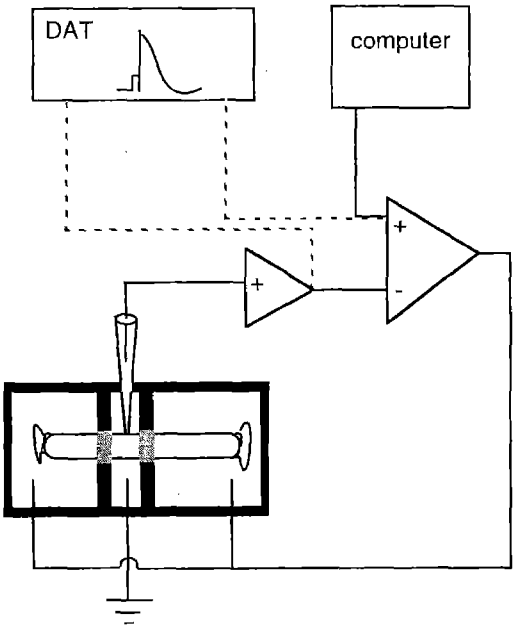

Fig. 1. Experimental set up for action potential clamp technique (not to scale). A *Chara* internodal cell placed over three electrically separated pools. The cell part in the central pool (1 mm wide) was impaled with a microelectrode for voltage recording. Current for space clamp of the central cell part was injected through Ag wires in the lateral compartments and measured to ground. The free running membrane voltage of electrically triggered action potentials was stored on a DAT recorder. Under voltage clamp, the command voltage was either determined by a computer (for excitation stimulation) or by replaying the stored AP record from the DAT recorder

Materials and methods

Plant material and solutions

Chara corallina Klein ex Wild was grown as reported previously (Thiel et al. 1993). Individual internodal cells were stored one day before experiments in standard experimental solution (APW) (in mM: 0.5 $CaCl_2$, 0.1 KCl, 1 NaCl, 5 (N-[2-Hydroxyethyl]-piperazine-N′-[2-ethanesulfonic acid])/(HEPES)/NaOH, pH 7.5. Modifications of the standard experimental solution are stated in the text.

Electrical

Action potential clamp. Space voltage clamp experiments were carried out on 1.4 mm long sections of *Chara* internodal cells (mean surface area 3.8 ± 0.6 mm^2, n=24) according to Lunevsky et al. (1983) (Fig. 1). Voltage and current recordings were obtained with a clamp amplifier (μP, Wye Science, Wye, UK). Voltage pulse protocols, data acquisition and analysis were controlled by a micro-computer using pClamp hard- and software facilities (Axon instruments, Foster City, CA). Electrically triggered action potentials were stored on a DAT recorder (DTC-1000ES, Sony, Japan) and subsequently replayed as command voltage through the voltage clamp circuit to the same cell. Between successive clamp protocols the cells were left to repolarize to ±3 mV of the control voltage. For analysis,

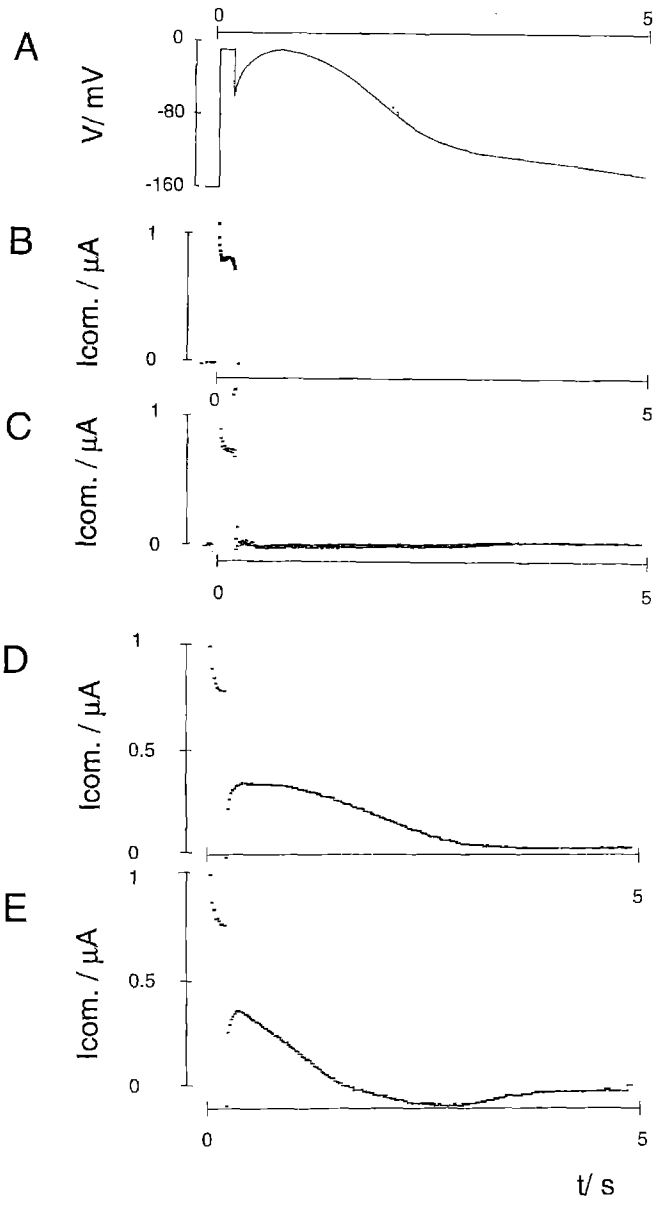

Fig. 2. Membrane voltage **A** and clamp current **B–E** recordings from a *Chara* internodal cell bathed in artificial pond water. Cell action potential **A** triggered by clamping the membrane under voltage control for 200 ms to −10 mV. The corresponding clamp current is shown in **B**. When the same action potential was used twice as voltage command for the amplifier the clamp currents in **C** were recorded. The same procedure was repeated after exposing the same cell for 5 min to the Cl^- channel blocker niflumic acid (100 μM) and for 7.5 min to niflumic acid plus the K^+ channel blocker Ba^{2+} (10 mM). The compensation currents during the action potential obtained after blocking Cl^- channels and K^+ channels are shown in **D** and **E** respectively. Cell diameter 0.8 mm; cell surface area 3.5 mm^{-2}

only those cells were considered in which all control AP-clamp protocols (n≥2) produced no significant compensation current (Fig. 2, trace C). This was best achieved using cells with resting voltages at about K^+ reversal voltage (ca. −160 mV). In cells with more negative voltages the control clamp protocols usually caused significant and variable compensation currents. As a second criterion for successful experiments, only experiments were considered

in which the inhibitor treatment affected the resting voltage by less than ±3 mV compared to the control.

Patch clamp. Ionic currents across the plasma membrane in the cell attached configuration were measured, stored and analyzed as reported previously (Thiel et al. 1993). *Chara* internodal cells were plasmolyzed in a solution containing 10 mM KCl, 1 mM $CaCl_2$, 5 mM Hepes/2.5 mM OH; pH 7.5 and 320 mM sorbitol. In a region where the protoplast had withdrawn from the cell wall, a small opening was cut into the wall, making the plasma membrane accessible for patch pipettes. For current recordings patch electrodes were filled with 50 mM $CaCl_2$. The stated membrane voltage is the sum of the negative pipette voltage, the empirically estimated liquid junction voltage of −30 mV and the mean estimated free running membrane voltage of −45 mV measured in cells kept under the same condition (Thiel et al. 1993).

Results and discussion

Action potential clamp

Figure 2 A shows an action potential (AP) in a *Chara* internodal cell triggered by a 200 ms long voltage step to −10 mV. After recording, this AP was replayed to the same cell as command voltage of the clamp amplifier. During this procedure the clamp current produced by the amplifier closely resembled the current applied for stimulation (Fig. 2 B, C). Notably, during the originally free running period of the AP, the clamp current was approximately zero (Fig. 2 C). Comparable clamp currents were also obtained after repeating the same procedure (Fig. 2 C). The absence of significant clamp current during the AP-clamp protocol demonstrates that, on the macroscopic level, the voltage dependent activation/inactivation processes are very conservative. Hence, the randomness of voltage triggered Cl^- channel activation seen in single membrane patches (Thiel et al. 1993) is filtered out on the macroscopic level producing the impression of a simple and very predictable voltage dependent process. With these properties *Chara* cells are suitable for the study of macroscopic excitation currents with the AP-clamp method.

Compensation currents in the presence of Cl^- channel inhibitors

The dynamics of excitation currents can be studied by selectively blocking the appropriate ion channels. In this situation the amplifier has, during the AP-clamp protocol, to add the currents passing through previously non-inhibited channels (Doer et al. 1989; de Haas and Vogel 1989). For maximal inhibition of Cl^- channels in *Chara*, 100 µM niflumic acid, an inhibitor of Cl^- channels in plant cells (Marten et al. 1992) (see also below) was added to the bathing medium. This treatment evoked a transient compensation outward current during the AP clamp (Fig. 2 D). Subtracting the inhibitor induced compensation current from the control current obtained in the absence of inhibitor

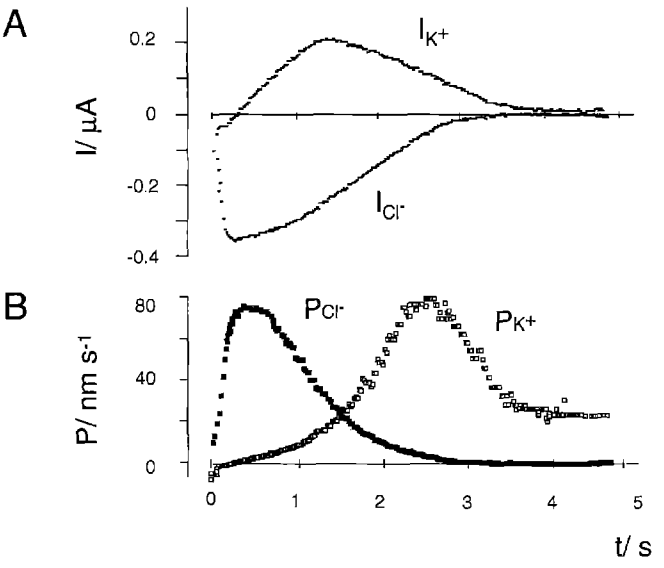

Fig. 3 A, B. Dynamics of current and ionic permeability changes during action potential in *Chara*. Compensation currents were corrected to give a real image of Cl^- and K^+ currents during excitation A by subtraction C–D and D–E in Fig. 2. The ionic permeabilities for Cl^- and K^+ were computed with the Goldman-Huxley-Katz current equation assuming cytoplasmic activities for Cl^- of 5 (Cl^-: Coleman 1986; Okihara and Kiyosawa 1988) and for K^+ the experimentally determined value of 45 mM respectively (details in text). Data same cell as in Fig. 2

(Fig. 2 C) gives a direct reading of the amount and the time course of Cl^- currents during an AP (Fig. 3 A).

The experimental protocol was, with respect to the inhibitor concentration and exposure time, such that inhibition of niflumic acid sensitive channels could be assumed to be maximal. In parallel experiments it was found that the niflumic acid sensitive compensation current developed in a time and concentration dependent manner. Half maximal effect of the inhibitor was reached after 88 ± 7 (n = 4) s with a K_D of about 25 µM (n = 3) (Fig. 5).

To confirm that niflumic acid was indeed inhibiting excitory Cl^- currents, cells were also treated with 100 µM ethacrynic acid. This inhibitor was shown to block Cl^- channels during the AP in *Nitella* (Lunevsky et al. 1983). In three *Chara* cells tested, this treatment produced compensation currents during the AP-clamp protocol qualitatively similar to those by niflumic acid (not shown). Comparable to the previous investigation (Lunevsky et al. 1983), inhibition in ethacrynic acid was very slow; significant compensation current developed only after ≥20 min incubation (not shown).

Compensation currents in the presence of K^+ channel inhibitor

Previous investigations have given evidence for transient K^+ channel activation in the course of an AP in *Chara* (Tester 1988; Homann and Thiel 1994) but without providing details on the activation kinetics. To investigate the activation/inactivation properties of these channels during an AP in more details, cells were treated with the K^+ chan-

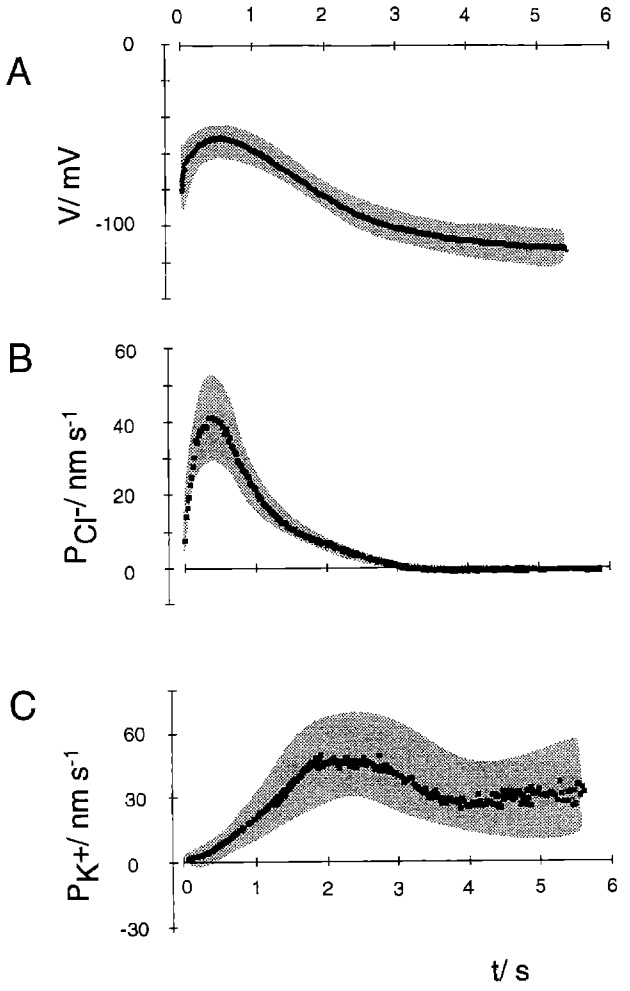

Fig. 4. Mean time course of electrically triggered action potentials **A** and the dynamics of the underlying Cl^- **B** and K^+ permeability changes **C**. Data obtained as in Figs. 2 and 3 from 9 experiments (shaded area = S.D.)

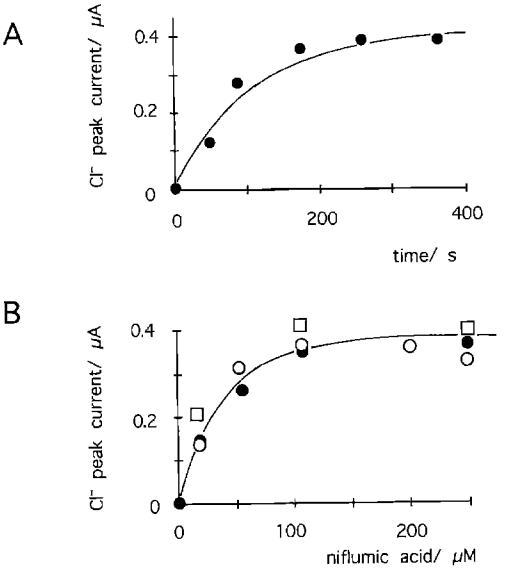

Fig. 5 A, B. Development of niflumic acid generated compensation current as a function of exposure time and inhibitor concentration. Peaks of compensation currents in presence of 100 μM niflumic acid as a function of time **A** and with different inhibitor concentrations **B**. In the latter case data were sampled 2 min after increasing the inhibitor concentration in three different cells (represented by different symbols). The solid lines represent a fit to the data with the Michaelis Menten equation

nel inhibitor Ba^{2+} (Tester 1988). Ba^{2+} was chosen as inhibitor because it effectively lengthened the action potential in *Chara* (Fig. 6), an indirect indication of the contribution of Ba^{2+} sensitive K^+ channels in the repolarization of the AP (Shimmen and Tazawa 1983; Tester 1988).

Figure 2E shows that addition of 10 mM Ba^{2+}-acetate to the bathing solution significantly reduced the niflumic acid generated compensation current during the AP-clamp protocol. Following this treatment, the clamp amplifier had now to compensate for the inhibited Cl^- and K^+ currents. Subtracting the compensation current in the presence of niflumic acid and Ba^{2+} (Fig. 2E) from that obtained in niflumic acid only (Fig. 2D), gives a direct reading of the amount and the time course of Ba^{2+} sensitive current during excitation (Fig. 3A).

In previous studies the K^+ channel inhibitor tetraethylammonium (TEA) had no effect on the course of the *Chara* action potential (Beilby and Coster 1979a; Shimmen and Tazawa 1983). In line with these observations, addition of 10 mM TEA was without perceivable effect on compensation currents during the AP-clamped protocol *Chara* cells (not shown).

To further investigate the ionic nature of the Ba^{2+} sensitive current the K^+ concentration in the bath was increased from 0.1 mM to 2 mM, which shifted the K^+ equilibrium voltage (E_K+) well positive of the resting membrane voltage. If the Ba^{2+} sensitive current was – as expected – carried by K^+, the compensation current during the AP clamp should reverse at E_K+. Figure 6 shows the Ba^{2+} sensitive current during excitation from a cell bathed in 2 mM K^+. In this case the current reversed at about –70 mV (mean –72 ± 5; n = 5 cells).

In order to compare the reversal voltage of the Ba^{2+} sensitive current with the actual E_K+, the cytoplasmic K^+ activities where indirectly determined by current voltage difference analysis in *Chara* cells from the same batch as those used for AP-clamp experiments. Therefore, *Chara* cells were transferred to a solution containing 5 mM KCl. Under this condition the membrane conductance is dominated by a highly K^+ selective, TEA sensitive conductance (Tester 1988). Figure 7 shows the current responses to voltage clamp step from the resting voltage (–52 mV) to test voltages between –27 and –87 mV. The steady state current obtained at the end of 400 ms long clamp steps becomes reduced – over the entire voltage range tested – in the presence of 5 mM TEA (Fig. 7A). From the current voltage difference data (Fig. 7B) a reversal voltage of the TEA sensitive K^+ current at –50 mM is obtained (mean ± SD, –56.6 ± 9 mV, range, –48 to –64 mV; 6 cells). From these data a mean cytoplasmic K^+ activity of 45 mM (range, 35.1 to 89.8 mM) can be calculated using the Nernst equation. These activities are lower than those reported from direct measurements with K^+ selective microelectrodes (61 to 117 mM; Beilby and Blatt 1986). However,

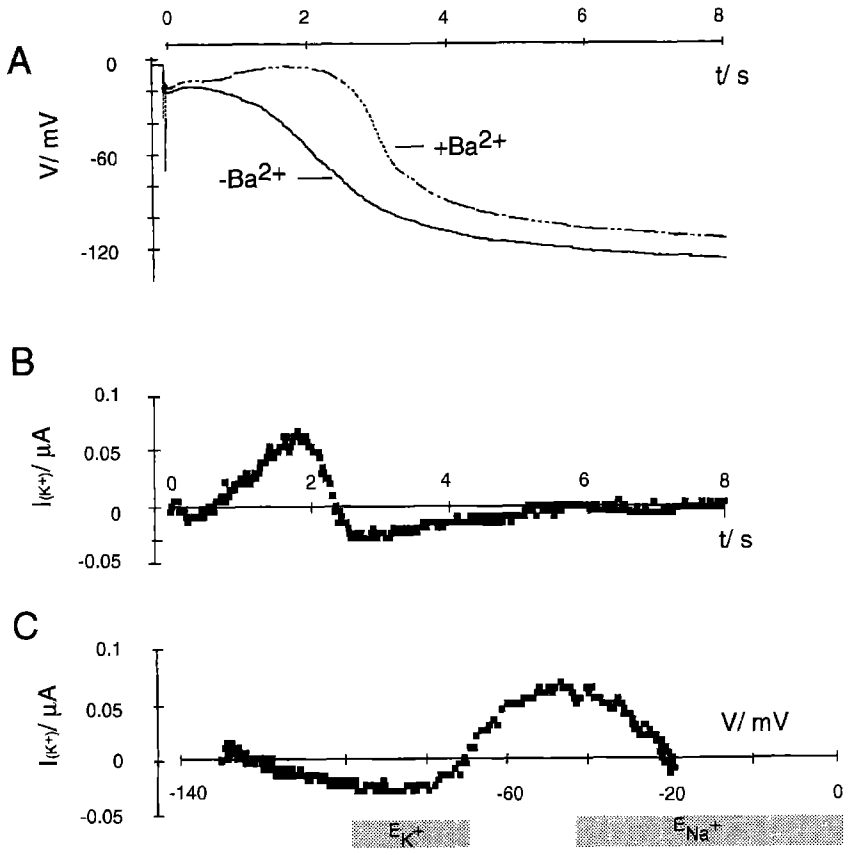

Fig. 6. Recordings of membrane voltage **A**, clamp current **B** and current voltage relation **C** from a *Chara* internodal cell bathed in artificial pond water with 2 mM K^+. Control cell action potential ($-B^{2+}$) triggered by clamping the membrane for 200 ms to –5 mV, and action potential ($+Ba^{2+}$) triggered by the some clamp step 12 min after addition of 2.5 mM Ba^{2+} to the bath. The Ba^{2+} sensitive current **B** obtained as difference between compensation current recorded during clamping cell to the control AP in absence of Ba^{2+} minus compensation current recorded under the same clamp regime in the presence of 2.5 mM Ba^{2+}. In **C** the current values from **B** are plotted against the voltage trajectory ($-Ba^{2+}$) from **A**. The shaded bars represents the expected ranges of equilibrium voltages for K^+, Na^+. Equilibrium of other relevant ions are outside of voltage range shown. Equilibrium voltages calculated considering experimentally determined range of cytoplasmic K^+ activities between 35.1 and 89.8 mM (details see text) and published data for cytoplasmic Na^+, <1 mM to 6 mM (Tazawa et al. 1974); Cl^-; 2 mM (Coleman 1986) to 9 mM (Okihara and Kiyosawa 1988) and Ca^{2+}, 2×10^{-4} (Miller and Sanders 1987) activities. Cell diameter 0.8 mm; cell surface area 3.5 mm^{-2}

considering the large differences in K^+ content generally found in *Characean* alga (Tazawa et al. 1974) also indicates that the lower concentrations found here are reasonable.

The reversal voltages of the Ba^{2+} sensitive current obtained during excitation (Fig. 6) and the calculated K^+ equilibrium voltage obtained from the TEA sensitive conductance show close resemblance. This confirms the above notion that the delayed current during an AP is predominantly carried by K^+, probably by channels with a unitary conductance of 40 pS (Homann and Thiel 1994). Nonetheless, the fact that the mean reversal voltage of the Ba^{2+} evoked currents is about 8 mV more positive than the mean value of E_{K+} may also suggest minor permeability to Na^+, Ca^{2+} and/or Ba^{2+}. The data contradict a previous hypothesis according to which the delayed current is due to a largely non-selective cation conductance (Beilby and Coster 1979 a). A reason for the discrepancy may be that in the latter study the reveral voltage of the delayed current (about –50 mV in APW) was determined from the peaks of complex excitation currents (Beilby and Coster 1979 a). Since current peaks are a function of both driving forces and kinetic parameters, their use for estimation of reversal voltages may be less reliable.

Density of Cl^- and K^+ currents during AP

By integration of the niflumic acid and Ba^{2+} sensitive currents the $Cl^-$and K^+ charges which are translocated during a single AP can be obtained. In the present example 0.18 As m^{-2} of Cl^- charge and 0.12 K^+ As m^{-2} of K^+ charge were translocated during a single action potential. A summary of the analysis from 5 cells is given in Table 1. The electrical data can be directly compared with results from ion flux measurements obtained during action potentials in *Chara* (Kikuyama 1986) after converting the present data into dimensions of fluxes (Table 1). The computed data for Cl^- and K^+ fluxes are in good agreement with the range of Cl^- and K^+ fluxes ($0.7-100$ µmolm^{-2}AP^{-1}) measured with various (non-electrical) methods during excitation (Kikuyama, 1986 and references therein). These data again support the above notion that the Ba^{2+} sensitive current is predominantly a K^+ current.

Table 1. K^+ and Cl^- charge translocation/efflux during single APs in *Chara*. Cl^- and K^+ currents conducted during single APs obtained from AP-clamp protocols in presence of (a) niflumic acid (≥100 µM) and Ba^{2+} (10 mM) (as in Fig. 3) or (b) niflumic acid (≥100 µM) only. Exposure time to niflumic acid ≥3 min and to Ba^{2+} ≥5 min. Cl^- and K^+ charge translocation during AP obtained by integration of areas under respective current traces (i.e. Fig. 3) over 4 s starting with end of the triggering pulse. Charges were converted to fluxes by division of charges through the Faraday constant. Means ± s.d. of n experiments

Cl^- charge transloc. (As m^{-2} AP^{-1})	Cl^- efflux (mol m^{-2} AP^{-1})	K^+ charge transloc. (As m^{-2} AP^{-1})	K^+ efflux (mol m^{-2} AP^{-1})	n
a) 0.33 ± 0.15	$3.3\ 10^{-6}$	0.2 ± 0.07	$2.1\ 10^{-6}$	7
b) 0.39 ± 0.2	$4\ 10^{-6}$	nd	nd	11

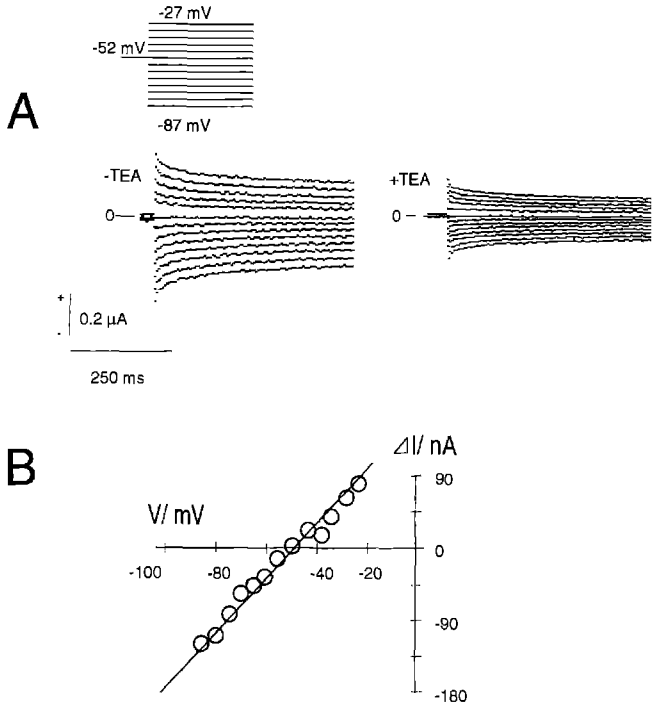

Fig. 7 A, B. Current voltage difference analysis of TEA-sensitive K^+ current in *Chara* internodal cell bathed in artificial pond water with 5 mM K^+. **A** Current response to 400 ms long clamp step protocol (*inset*) from the resting voltage (–52 mV) to test voltage between –27 and –87 in absence (–TEA) and in presence (+TEA) of K^+ channel blocker (5 mM TEA-Cl). To maintain Cl^- concentration constant the control solution contained 5 mM choline-Cl. Difference between steady state currents ($I_{-TEA} - I_{+TEA}$) at clamp voltages (mean current over final 25 ms) are plotted in **B** to give current voltage difference curve. The regression line through the data reverses at –50 mV

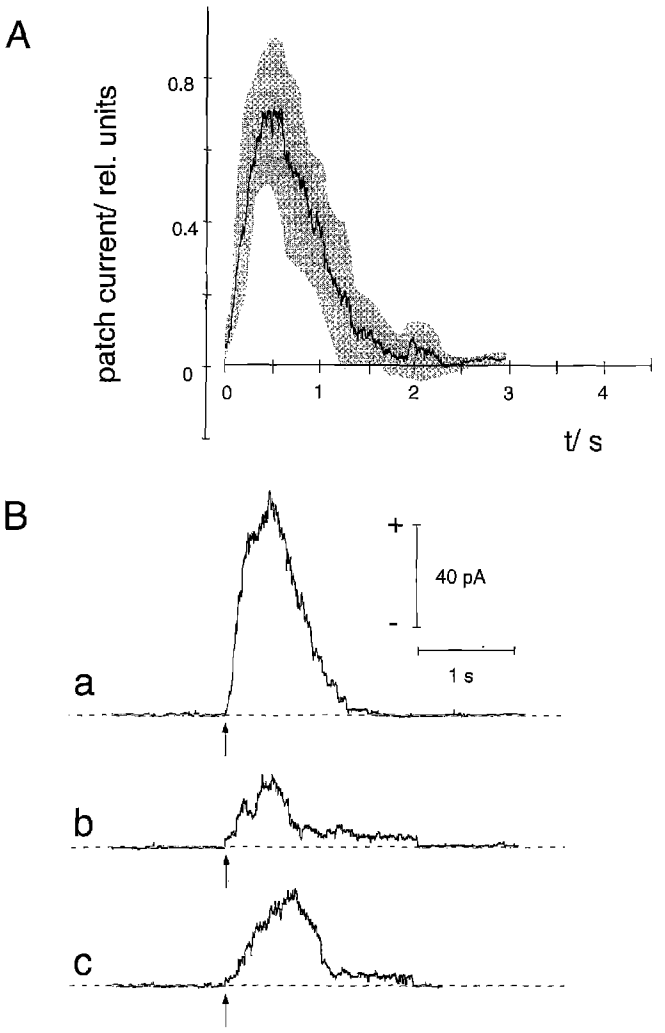

Fig. 8 A, B. Transient activity of multiple channels in cell-attached patch. **B** Individual transient Cl^- currents (a–c) were obtained in a plasma membrane patch clamped continuously to 0 mV with 50 mM $CaCl_2$ as pipette electrolyte. Sample rate 500 Hz, filter rate 100 Hz. 28 of these current transients were scaled to unity and summed beginning with the time of current activation (*arrows*). The mean value of the scaled currents (*line*) and the standard deviation (*shaded area*) is shown in **A**

Kinetics of Cl^- and K^+ permeability changes during AP

To quantify the dynamics of changes in Cl^- and K^+ permeability during the AP, the currents were converted to the corresponding permeabilities (P) using the Goldman-Hodgkin-Katz current equation (de Haas and Vogel 1989) (Fig. 3 B). Typically, the peak of the Cl^- permeability was reached at about 370 ms after terminating the stimulation pulse (Fig. 4). The following decay in permeability (between 10 and 90% from baseline to maximum) was, on average, 3.6 times slower than the rise (Fig. 4 B, C). The present data directly confirm previous predictions for the time course of the Cl^- conductance during excitation which were obtained from the Hodgkin-Huxley parameters (Beilby and Coster 1979 b): The computed Cl^- conductance reached a maximum about 370 ms after stimulation and decayed about 3.5 times slower than it activated.

The present results provide the first quantitative account for the dynamics of K^+ channel activation during an AP: the permeability changes reveal that the rise in Ba^{2+} sensitive K^+ permeability lags behind the fast increasing Cl^- permeability; it reached a peak only about 2 s after the Cl^- permeability. The experimental data confirm early estimates of the time course of Cl^- and K^+ current/conductance development during an AP predicted from Teorell's model (Hope and Walker 1975). On the other hand, the calculated activation of the Ba^{2+} sensitive K^+ permeability is much slower than the conductance rise of a proposed nonselective cation conductance predicted from the Hodgkin-Huxley parameters. This conductance already reaches a peak within the first 300 ms of excitation (Beilby and Coster 1979 b). It must hence be either different from the latter – or more likely – the discrepancy arises from the different reversal voltages (see above) considered in the permeability calculations (Figs. 3, 4) and the Hodgkin-Huxley fitting procedure (Beilby and Coster 1979) respectively.

In terms of activation/inactivation kinetics the situation in *Chara* derived from the AP-clamp procedure closely resembles – albeit on a much slower time scale – the scenario of channel activation/inactivation during excitation

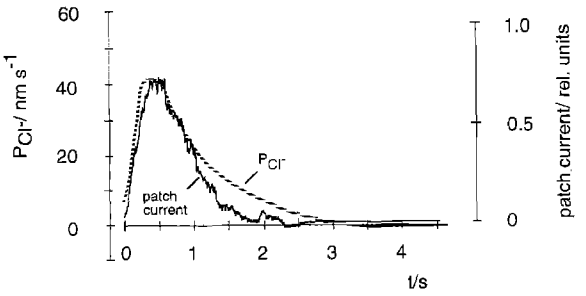

Fig. 9. Comparison between mean change in Cl^- permeability (*dashed line*, same as Fig. 4B) and mean time course of patch current activation/inactivation (*solid line*, same as Fig. 8A). Both curves were scaled to a common ordinate

in animal cells (Frankenhaeuser and Huxley 1964; de Haas and Vogel 1989). While the Cl^- permeability in *Chara* develops with kinetics similar to the Na^+ permeability in animal cells, the pattern of K^+ permeability changes in Chara resembles that of the delayed outward K^+ rectifier (Frankenhaeuser and Huxley 1964; de Haas and Vogel 1989).

Comparison between changes in Cl^- permeability and transient activation of multiple Cl^- channels in plasma membrane patches

The dynamics of permeability changes during the AP reflects – on the macroscopic level – the activation/inactivation kinetics of the elementary Cl^- ion channels activated in membrane patches. Transient activation of multiple Cl^- channels, responsible for conducting the Cl^- excitation current (Homann and Thiel 1994), can be elicited by clamping plasma membrane patches to sufficiently depolarized voltages (Thiel et al. 1993). Figure 8B shows three examples of such a transient activation of multiple Cl^- channels. The illustrated Cl^- currents were recorded in patch clamp measurements in the cell attached configuration with 50 mM $CaCl_2$ as pipette electrolyte after holding the patch constantly at 0 mV. Typically, the duration and the kinetics of the transient currents are similar between individual transients. The mean rising time between 10% to 90% of the maximum is about two times faster than the trailing time in the reverse direction (Table 2). Similar times were obtained from comparable transient patch currents obtained in the same patches but at other membrane voltages (Table 2). In this respect the present data compare well with those obtained previously for transiently activating Cl^- currents (Thiel et al. 1993; Homann and Thiel 1994).

To compare the temporal changes in Cl^- permeability under AP-clamp conditions (Figs. 3, 4) with the kinetics of the transient currents in membrane patches (Fig. 8), the mean time course for activation/inactivation of 28 transient Cl^- currents (from two different patches) was obtained from superposition of individual current traces with the maximum current scaled to unity (Fig. 8A). At a constant voltage the time course for activation/inactivation of multiple channels is proportional to the temporal changes in channel open probability. It can therefore directly be compared to the dynamics of change in Cl^- permeability

Table 2. Rising and trailing times of transient Cl^- currents recorded in cell attached patch clamp measurements with 50 mM $CaCl_2$ as pipette electrolyte (compare Fig. 8B). Values obtained as time for current rising between 10% to 90% of the maximum and time for trailing in reverse direction. n transient currents from two patches

Membrane voltage/mV	Rising time/s	Trailing time/s	n
–20	0.38 ± 0.08	0.72 ± 0.4	6
0	0.47 ± 0.08	0.93 ± 0.3	28
+20	0.45 ± 0.1	0.9 ± 0.4	7

obtained during the AP clamp. Figure 9 shows a plot with the mean changes in Cl^- permeability and the time course of multiple channel activation/inactivation. Both parameters follow a nearly identical time course. Thus, it must be concluded that once activated by depolarizing voltages (Thiel et al. 1993) the endogenous kinetics of multiple Cl^- channel activation/inactivation seen in membrane patches determines the dynamics of the whole cell Cl^- excitation current. Furthermore, while the activation of these transient currents is voltage dependent (Thiel et al. 1993), the factors which control this conservative activation/inactivation kinetics of Cl^- channels in individual patches must be voltage independent.

Acknowledgements. I am grateful to A. Bertl and D. Gradmann for helpful comments. The work was supported by the Deutsche Forschungsgemeinschaft.

References

Beilby MJ, Coster HGL (1979a) The action potential in *Chara corallina* II. Two activation-inactivation transients in voltage clamps of the plasmalemma. Aust J Biol Sci 6:323–335

Beilby MJ, Coster HGL (1979b) The action potential in *Chara corallina* III. The Hodgkin-Huxley parameters for the plasmalemma. Aust J Plant Physiol 6:337–353

Beilby MJ, Blatt MR (1986) Simultaneous measurements of cytoplasmic K^+ concentration and plasma membrane electrical parameters in single membrane samples of *Chara corallina*. Plant Physiol 82:417–422

Blatt MR (1991) Ion channel gating in plants: physiological implications and integration for stomatal function. J Membrane Biol 124:95–112

Coleman HA (1986) Chloride currents in *Chara* – a patch clamp study. J Membrane Biol 93:55–61

De Haas V, Vogel W (1989) Sodium and potassium currents recorded during an action potential. Eur Biophys J 17:49–51

Doerr T, Denger R, Trautwein W (1989) Calcium currents in single SA nodal cells of the rabbit heart studied with action potential clamp. Pflügers Arch 413:599–603

Doerr T, Denger R, Doerr A, Trautwein W (1990) Ionic currents contributing to the action potential in single ventricular myocytes of the guinea pig studied with action potential clamp. Pflügers Arch 416:230–237

Findley G, Hope A (1964) Ionic relations of *Chara australis*. IX. Analysis of transient membrane currents. Aust J Biol Sci 17:400–411

Fischmeister R, DeFelice LJ, Ayer RK, Levi R, DeHaan RL (1984) Channel currents during spontaneous action potentials in embryonic chick heart cells. Biophys J 46:267–272

Frankenhaeuser B, Huxley AF (1964) The action potential in the myelinated nerve fibre of *Xenopus laevis* as computed on the basis of voltage clamp data. J Physiol (London) 171:302–315

Homann U, Thiel G (1994) Cl^- and K^+ channel currents during the action potential in *Chara*; simultaneous recording of membrane voltage and patch currents. J Membrane Biol 141:297–309

Hope AB, Walker NA (1975) The physiology of giant algal cells. Cambridge University Press, London

Ibarra J, Morley GE, Delmar M (1991) Dynamics of the inward rectifier K^+ current during the action potential of guinea pig ventricular myocytes. Biophys J 60:1534–1539

Kikuyama M (1986) Ion effluxes during excitation of *Characeae*. Plant Cell Physiol 27:1213–1216

Lunevsky VZ, Zherelova OM, Vostrikov IY, Berestovsky GN (1983) Excitation of *Characeae* cell membranes as a result of activation of calcium and chloride channels. J Membrane Biol 72:43–58

Marten I, Zeilinger C, Redhead C, Landry DW, Al-Awqati Q, Hedrich R (1992) Identification and modulation of a voltage-dependent anion channel in the plasma membrane of guard cells by high-affinity ligands. EMBO J 11:3569–3575

Mazzanti M, DeFelice LJ (1987) Na channel kinetics during the spontaneous heart beat in embryonic chick ventricle cells. Biophys J 52:95–100

Miller AJ, Sanders D (1987) Depletion of cytosolic free calcium induced by photosynthesis. Nature 326:397–400

Okihara K, Kiyosawa K (1988) Ion composition of the *Chara* internode. Plant Cell Physiol 34:75–82

Shimmen T, Tazawa M (1983) Activation of K^+-channel in membrane excitation of *Nitella auxilliformis*. Plant Cell Physiol 24:1511–1524

Tazawa M, Kishimoto U, Kikuyama M (1974) Potassium, sodium and chloride in the protoplasm of characeae. Plant Cell Physiol 15:103–110

Tester M (1988) Blockade of potassium channels in the plasmalemma of *Chara* corallina by tetraethylammonium, Ba^{2+}, Na^+ and Cs^+. J Membrane Biol 105:77–85

Thiel G, Homann U, Gradmann D (1993) Microscopic elements of electrical excitation in *Chara*. Transient activity of Cl^- channels in the plasma membrane. J Membrane Biol 134:53–66