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Role of Calcium Ion in the Excitability and Electrogenic Pump Activity of the *Chara corallina* Membrane: I. Effects of La^{3+}, Verapamil, EGTA, W-7, and TFP on the Action Potential

Izuo Tsutsui, Taka-aki Ohkawa, Reiko Nagai, and Uichiro Kishimoto
Department of Biology, College of General Education, Osaka University, Toyonaka 560, Osaka, Japan

Summary. The cytoplasmic streaming of the normal internodal cell of giant alga *Chara* stops transiently at about the peak of action potential. Application of La^{3+} or verapamil (a calcium channel blocker) or removal of external Ca^{2+} by EGTA caused a partial depolarization of the resting potential, partial decrease of the membrane conductance and a marked decrease of the amplitude of action potential. Under these conditions, the conductance at the peak of action potential reduced markedly and the streaming of cytoplasm did not cease during action potential (excitation-cessation (EC) uncoupling). The effects of Ca^{2+} channel blockers could not be removed by addition of $CaCl_2$ to the external medium. In contrast, the effect of EGTA on the excitability could be removed to a greater extent and the cytoplasmic streaming ceased at about the peak of action potential by the addition of Ca^{2+} externally. Application of calmodulin antagonists W-7 or TFP caused similar effects on the action potential and on the cytoplasmic streaming.

Key Words action potential · Ca^{2+} channel · *Chara* · EC uncoupling · electrogenic pump

Introduction

The membrane of the freshwater alga *Chara* or *Nitella* is electrically excitable. The action potential is caused mainly by the transient increases of Cl^- channel conductance and K^+ channel conductance (Gaffey & Mullins, 1958; Kishimoto, 1961; 1964; 1965; Findlay & Hope, 1964*a*; Hope & Findlay, 1964). The marked transient increases of effluxes of K^+ and Cl^- during action potential have been well established (Gaffey & Mullins, 1958; Findlay, 1962; Mullins, 1962; Mailman & Mullins, 1965; Oda, 1976). Generation of the action potential in the *Chara* membrane can be analyzed as the open and close kinetics of Cl^- and K^+ channels (Beilby & Coster, 1979*b*; Hirono & Mitsui, 1983). Moreover, transient increase of Ca^{2+} influx or activation of the Cl^- channel by Ca^{2+} has also been suggested in *Chara* (Findlay & Hope, 1964*b*; Beilby & Coster, 1979*a*; Beilby, 1984). Hayama, Shimmen and Tazawa (1979) demonstrated a slight increase of $^{45}Ca^{2+}$ influx in the *Chara* during electrical stimulations. This amount was proportional to the number of stimuli. Recently an increase of fluorescence of aequorin during the action potential of the *Chara* was reported by Williamson and Ashley (1982) and Kikuyama and Tazawa (1983). It is very likely that Ca^{2+} influx increases to some extent at the early stage of action potential. Lunevsky et al. (1983) suggested that the Cl^- channel could be activated by Ca^{2+} influx from the external medium as well as from the vacuole.

The observed slight increase of internal free Ca^{2+} level seems to trigger the gating mechanism of Cl^-. In the *Chara*, internal free Ca^{2+} concentration is 2.2×10^{-7} M at the resting state (Williamson & Ashley, 1982). Continuous cytoplasmic streaming is maintained at this very low internal Ca^{2+} level. The slight increase in the internal Ca^{2+} level during an action potential may be closely related to the cessation of the cytoplasmic streaming. The later recovery of cytoplasmic streaming suggests the later decrease of internal free Ca^{2+} level by some active mechanism (Williamson & Ashley, 1982). The gating of Cl^- channel as well as the cessation of the cytoplasmic streaming seems to be regulated by Ca^{2+} binding protein such as calmodulin. In this paper, we report the effects of calcium and the calmodulin antagonists on the electrical characteristics and streaming in the *Chara* internode.

Materials and Methods

Internodes of *Chara corallina* were used throughout the experiments. The plant has been cultured under illumination for 12 hr a day with fluorescent lamps at an intensity of 2000 lux. Temperature was kept at 20 ± 2°C. Internodes were isolated from adjacent ones and kept in the artificial pond water (APW) at least one day before use. The average diameter of internodes used was about 0.7 mm and 4 to 6 cm in length. The APW contained (in

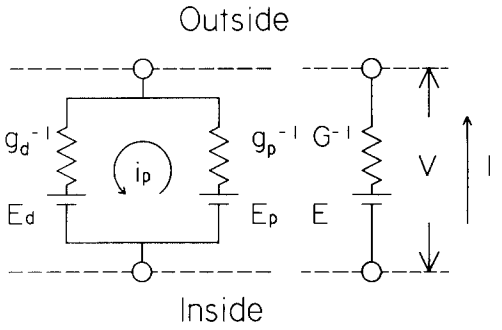

Fig. 1. A circuit model for *Chara* membrane having the electrogenic H^+ pump and the passive channel in parallel. The conductance G is the sum of the two conductances, i.e., g_p and g_d (Eq. 1), which correspond to the conductance of the pump channel and the passive channel, respectively. The E, E_p and E_d represent the electrogenic forces of the membrane, pump channel, and passive channel, respectively

mM) 0.5 KCl, 0.1 NaCl, 0.1 $Ca(NO_3)_2$ and 0.2 $Mg(NO_3)_2$, the pH of which was buffered at 7 with 2.0 mM TES.

We used La^{3+} (Lettvin et al., 1964; Takata et al., 1966) and verapamil (Lee & Tsein, 1983) as Ca^{2+} channel blockers, EGTA as a calcium chelating agent, W-7 (Hidaka & Tanaka, 1983) and TFP (Levin & Weiss, 1976) as calmodulin antagonists. One M stock solution of $LaCl_3$ was diluted to appropriate concentrations with the APW just before use (La^{3+}-APW). Calmodulin antagonists and verapamil were also dissolved in the APW just before use (verapamil-APW, W-7-APW and TFP-APW).

The resting and the action potentials were measured under the current-clamp condition ($I = 0$). Small and short square test current pulses were applied repeatedly under the current-clamp condition to measure the membrane conductance at the resting state and its change during an action potential. The details of the current-clamp and the conductance measurement were almost the same as described previously (Kishimoto Kami-ike & Takeuchi, 1980). Acquisition of data and the calculation were performed by CP/M-80 microcomputer system (Kishimoto et al., 1981; 1984). Cytoplasmic streaming was observed under a microscope (Optiphot, Nikon Optics, Tokyo, Japan).

The plasmalemma of characean internodes has at least two different, i.e., passive and electrogenic, ionic pathways. This situation can be most simply and satisfactorily illustrated with a parallel circuit model shown in Fig. 1. According to this model, the measured conductance G and the electromotive force (emf) E are as follows:

$$G = g_p + g_d \tag{1}$$

$$E = (g_pE_p + g_dE_d)/G \tag{2}$$

where g_d and g_p are conductances, E_d and E_p are emf's of the passive and electrogenic pump channels, respectively.

Abbreviations

Verapamil, α-isopropyl-α[(N-methyl-N-homoveratryl)-γ-aminopropyl]-3,4-dimethoxyphenylacetonitrile; EGTA, glycoletherdiamine-N,N,N′,N′-tetraacetic acid; W-7, N-(6,aminohexyl)-5-chloro-1-naphthalenesulfonamide; TES, N-Tris(hydroxymethyl) methyl-2-aminoethanesulfonic acid; TFP, 3-trifluoromethyl-10-(3-(4-methyldipiperazinyl)-propyl)-phenothiazine dihydrates.

Results

Effect of Calcium Channel Blockers

La^{3+}

An internode of *Chara corallina* was perfused externally with La^{3+}-APW (10 μM $LaCl_3$). In about 35 min, the resting potential depolarized from −202 to −196 mV. The peak of action potential shifted from −16 to −65 mV (curve *b* in Fig. 2*A*). The half-width of the action potential increased from 3.7 to 4.8 sec, i.e., 29% prolongation (curve *b* in Fig. 2*A*; *see* Table). The conductance at the resting potential reduced slightly from 127 to 119 μS/cm², while the peak of action potential greatly reduced from 409 to 208 μS/cm² (curve *b* in Fig. 2*B*; Table). The cytoplasmic streaming at this stage did not cease during the action potential (EC uncoupling). Generally we did not detect any change in the streaming rate, although in some cases the streaming happened to be slower transiently during the action potential. The effect of La^{3+} could not be removed even after washing the internode from outside for about 30 min or longer with APW containing 5 mM $CaCl_2$. Recovery of the shape of the action potential, the extent of conductance change and the half-width of the action potential were hardly observed (curves *c* in Figs. 2*A* and 2*B*). The cytoplasmic streaming did not cease during action potential (EC uncoupling) even after this washing. Calcium channel blocking with La^{3+} was irreversible. Longer treatment with La^{3+} blocked the generation of action potential completely, while it did not affect continuous cytoplasmic streaming.

Verapamil

An internode was perfused externally with verapamil-APW (100 μM verapamil). The resting potential was depolarized from −203 to −179 mV. The action potential prolonged (i.e., half-width increased from 3.5 to 4.9 sec) and the peak of action potential shifted from −51 to −35 mV (curve *b* in Fig. 3*A*). The conductance at the peak of action potential was reduced from 720 to 450 μS/cm² by verapamil (curve *b* in Fig. 3*B*; Table). The cytoplasmic streaming did not cease during the action potential (EC uncoupling). No recovery of the shape of the action potential, the extent of conductance

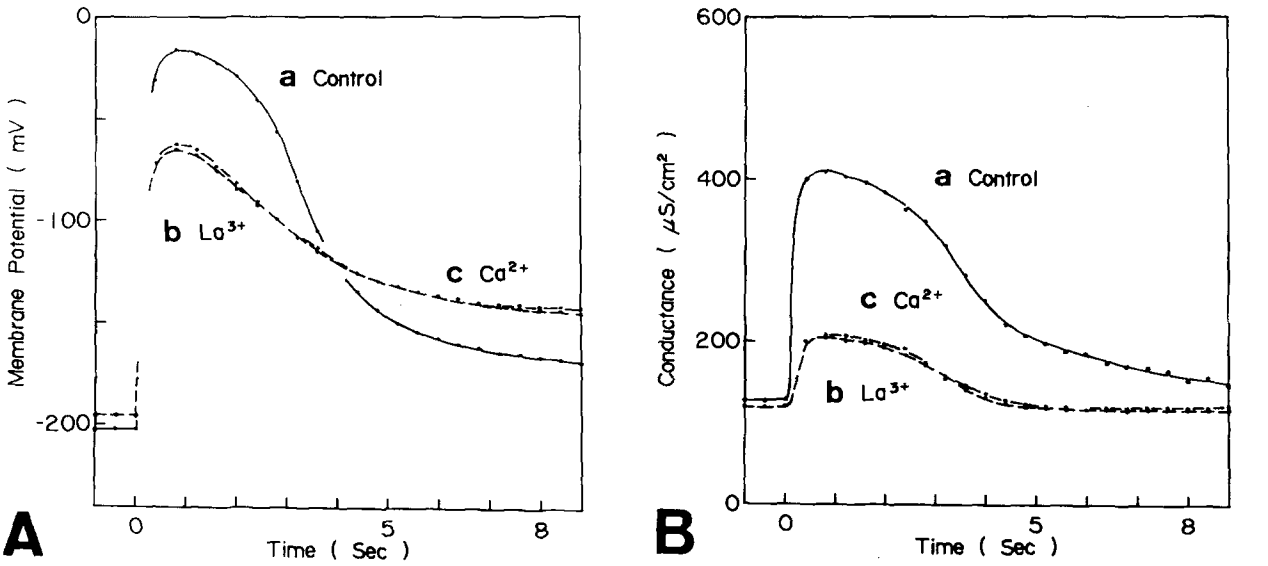

Fig. 2. (*A*) Effects of 10 μM La^{3+} on the resting potential and on the action potential of the *Chara* membrane, which were recorded under the current clamp (I = 0). (*B*) Effects of 10 μM La^{3+} on the membrane conductance at rest and during action potential. *a* (–●–●–), control. *b* (–●– –●–), 35 min after treatment with 10 μM La^{3+}. *c* (–● –●–), 30 min after washing with 5 mM $CaCl_2$ containing APW. Half-width of the action potential was about 3.7 sec in *a* and 3.9 sec in *b* and in *c*. No recovery of the extent of conductance increase during the action potential was observed even after washing with Ca^{2+}-APW

Table.[a]

	Ca^{2+} channel blockers					Ca^{2+} deficiency			Calmodulin antagonists				
	Control	La^{3+}	Ca^{2+}	Control	Verapamil	Control	EGTA	Ca^{2+}	Control	W-7	Ca^{2+}	Control	TFP
Resting potential	−202	−196	−196	−203	−178	−210	−180	−202	−201	−164	−192	−214	−121
Peak of action potential	−16	−165	−64	−51	−69	−54	−65	−64	−21	−67	−20	−36	−64
Resting conductance	127 (100)	119 (94)	120 (94)	180 (100)	168 (93)	73 (100)	41 (56)	93 (127)	102 (100)	59 (58)	75 (74)	195 (100)	191 (98)
Peak conductance	409 (100)	208 (51)	206 (50)	720 (100)	497 (69)	518 (100)	223 (43)	311 (60)	554 (100)	188 (34)	429 (77)	792 (100)	332 (43)
Half-width of action potential	3.7 (100)	4.8 (129)	4.8 (129)	3.5 (100)	4.9 (140)	2.9 (100)	3.5 (121)	3.5 (121)	3.7 (100)	5.7 (154)	4.6 (124)	3.2 (100)	6.0 (188)
EC coupling	+	−	−	+	−	+	−	+	+	−	+	+	−

[a] Units for potential and conductances are mV and μS/cm^2, respectively. Values in ( ) indicate the % value of control. + or − indicates the observation of EC coupling or EC uncoupling, respectively.

change, and the EC coupling were observed after washing the internode externally with normal APW (*data not shown*). Furthermore, calcium channel blocking by verapamil could not be removed, similar to La^{3+}-APW treatment, even by washing with APW containing 5 mM Ca^{2+} for longer than 30 min. Moreover, the continuous cytoplasmic streaming was not affected by longer treatment with verapamil.

Effect of External Calcium Deficiency

An internode was perfused externally with EGTA-APW (0.5 mM EGTA). The free Ca^{2+} concentration

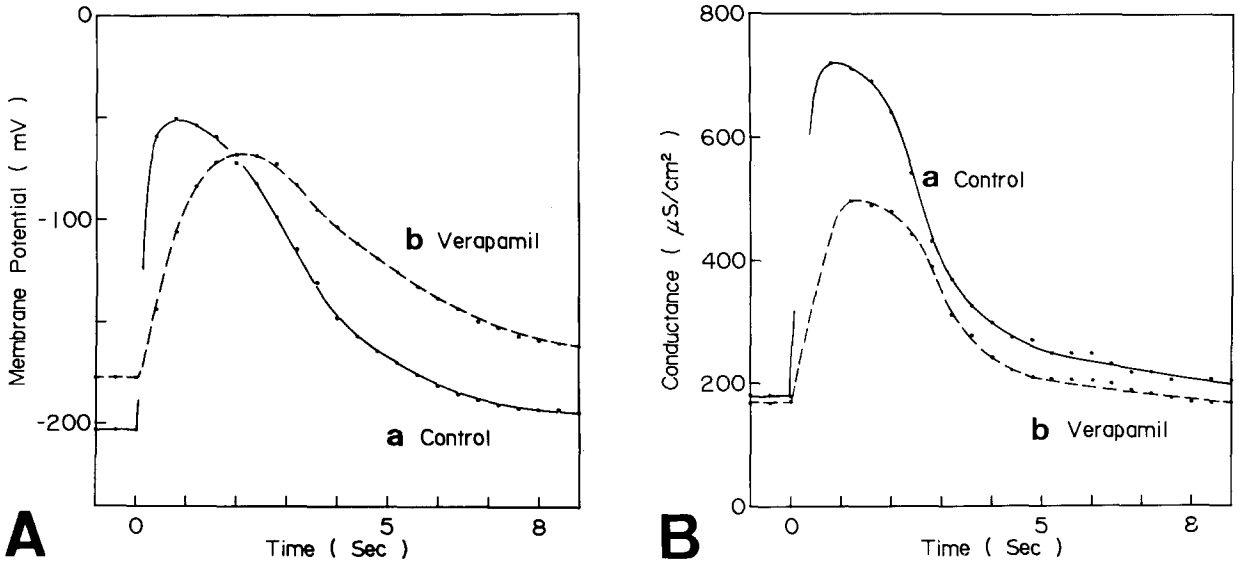

Fig. 3. (*A*) Effects of 100 μM verapamil on the resting potential and on the action potential of the *Chara* membrane (current clamped). (*B*) Effects of 100 μM verapamil on the membrane conductance at rest and during action potential. *a* (–●–●–), control. *b* (–●– –●–), 35 min after treatment with 100 μM verapamil. Half-width of the action potential was about 3.5 sec in *a* and 4.9 sec in *b*

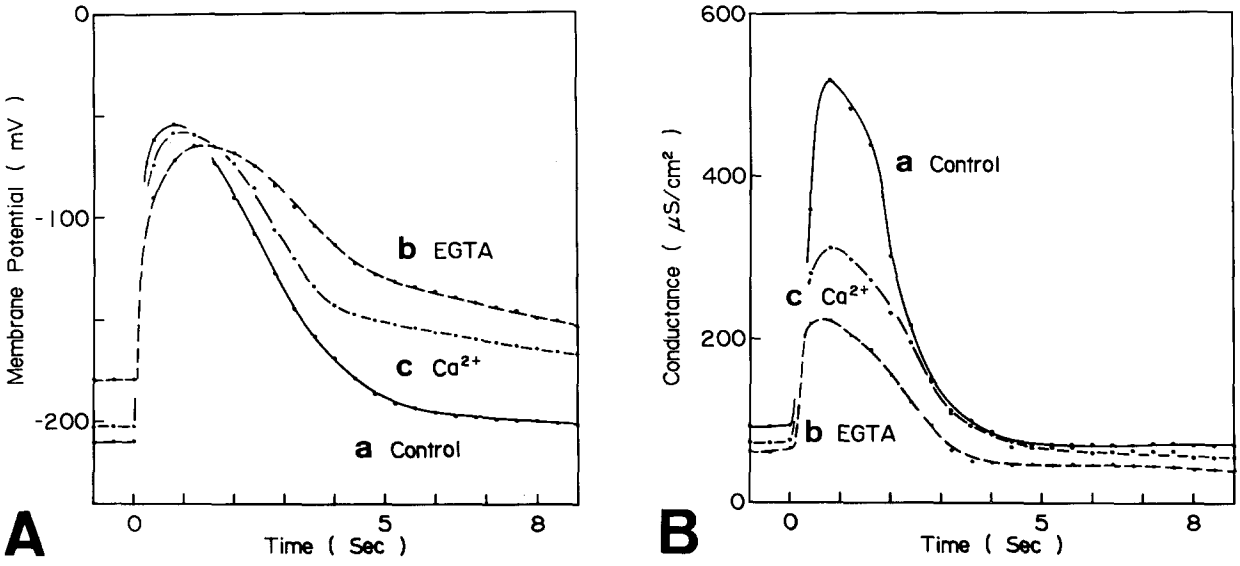

Fig. 4. (*A*) Effects of 0.5 mM EGTA on the resting potential and on the action potential of the *Chara* membrane. (*B*) Effects of 0.5 mM EGTA on the membrane conductance at rest and during action potential. *a* (–●–●–), control. *b* (–●– –●–), 30 min after treatment with 0.5 mM EGTA. *c* (–●–●–), 17 min after washing with 3 mM $CaCl_2$ containing APW. Half-width of an action potential was about 2.9 sec in *a* and 3.5 sec in *b* and in *c*. Some recovery of the extent of conductance change during the action potential was observed even after washing with Ca^{2+}-APW

in the external medium was less than 10^{-8} M. The membrane potential depolarized in about 30 min from −210 to −178 mV and the conductance at the resting potential decreased from 73 down to 41 μS/cm^2. The action potential was prolonged (half-width increased from 2.9 to 3.5 sec) and the peak of action potential shifted from −54 to −65 mV (curve *b* in Fig. 4*A*). The conductance at the peak of action potential decreased from 518 to 223 μS/cm^2 (curve *b* in Fig. 4*B*; Table). Cytoplasmic streaming did not cease during action potential (EC uncoupling). The effect of EGTA was partly removed by washing the internode for 17 min with APW (Ca^{2+}-APW) containing 3 mM $CaCl_2$. That is, the resting potential

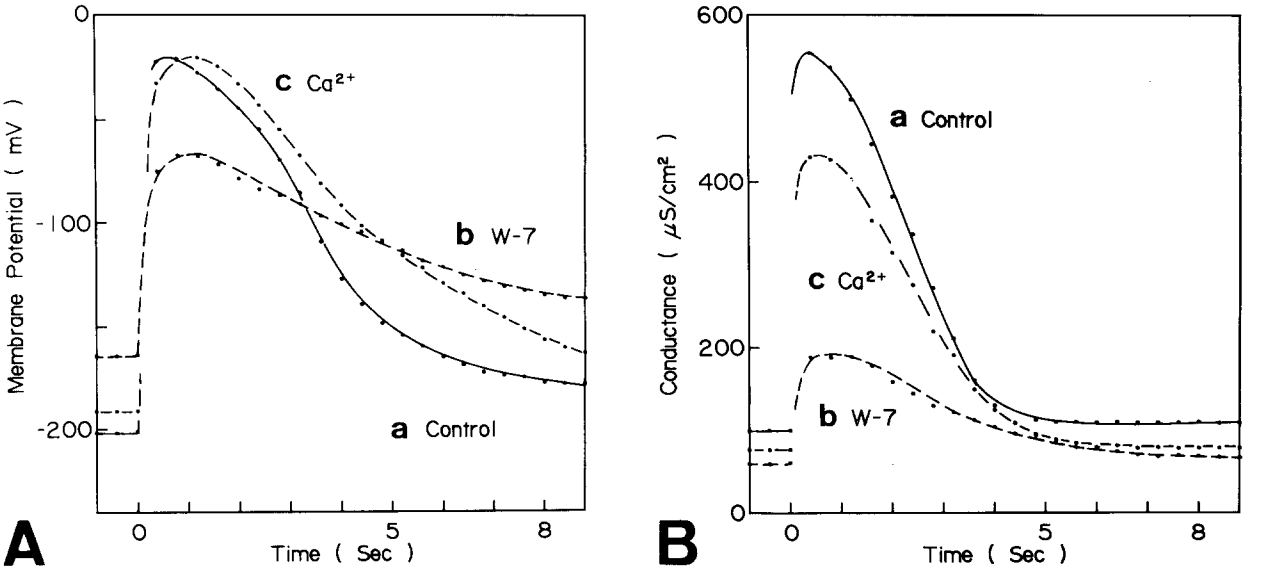

Fig. 5. (*A*) Effects of 40 μM W-7 on the resting potential and on the action potential of the *Chara* membrane. (*B*) Effects of 40 μM W-7 on the membrane conductance at rest and during action potential. *a* (–●–●–), control. *b* (–●– –●–), 30 min after treatment with 40 μM W-7. *c* (–●– ●–), 40 min after washing with 1 mM $CaCl_2$ containing APW. Half-width of an action potential was about 3.7 sec in *a*, 5.7 sec in *b* and 4.6 sec in *c*. Some recovery of the extent of conductance change during the action potential was observed even after washing with Ca^{2+}-APW

repolarized by about 22 mV (curve *c* in Fig. 4*A*) and the half-width of the action potential became shorter (curve *c* in Fig. 4*A*). The conductance at the peak of action potential recovered up to 60% of the control by washing the internode with Ca^{2+}-APW externally (curve *c* in Fig. 4*B*; Table). In such a partially recovered internode the cytoplasmic streaming ceased during the action potential (i.e., recovery of EC coupling). The speed of cytoplasmic streaming stayed at the original level and was not affected by longer treatment with EGTA, if not stimulated.

Effect of Calmodulin Antagonists

Calmodulin has been identified in animal as well as in plant cells as a typical intracellular Ca^{2+} receptor. It plays an important role in many physiological processes which is regulated by Ca^{2+}. We tested the effect of two different calmodulin antagonists. One was amino-naphthalene type W-7 and another was phenothiazine type TFP.

W-7

An internode was perfused externally with W-7-APW (40 μM W-7). In about 30 min, the resting potential was depolarized from −201 to −164 mV and the peak level of action potential shifted from −21 to −67 mV (curve *b* in Fig. 5*A*). The conductance at the resting potential reduced from 102 to 59 $\mu S/cm^2$, while that at the peak of action potential reduced greatly from 554 to 188 $\mu S/cm^2$ (curve *b* in Fig. 5*B*; Table). Half-width of action potential was prolonged from 3.7 to 5.7 sec by W-7 treatment. The cytoplasmic streaming at this stage did not cease during the action potential (EC uncoupling). The effect of W-7 was removed largely by washing the internode for 40 min with APW (Ca^{2+}-APW) containing 1 mM $CaCl_2$. The size of action potential recovered to a great extent. The conductance at the peak of action potential also recovered to 75% of the control level (curve *c* in Fig. 5*B*, Table). The resting potential and the peak of action potential were almost the same as the ones before W-7 treatment (curve *c* in Fig. 5*A*). Half-width of the action potential decreased to 4.6 sec (124% of control level, Table; curve *c* in Fig. 5*A*). Furthermore, in such a partly recovered internode the cytoplasmic streaming ceased again during the action potential (i.e., recovery of EC coupling). The continuous cytoplasmic streaming stayed at the original level and was not affected by longer treatment with W-7, if not stimulated. Further treatment (2 to 3 hr) with W-7 caused an irreversible depolarization of the *Chara* internode with a marked increase of the leakage and electrical collapse of the plasmalemma.

TFP

An internode was perfused externally with TFP-APW (100 μM TFP). In about 30 min, the resting potential depolarized from −214 to −121 mV and the peak of action potential shifted from −36 to −64 mV (curve *b* in Fig. 6*A*). The conductance at the resting potential reduced slightly from 195 to 191 $\mu S/cm^2$ (curve *b* in Fig. 6*B*, Table). Marked increase of the half-width (i.e., from 3.2 to 6 sec) of the action potential was observed. Like the case of W-7 treatment, the extent of conductance increase at the peak of action potential reduced from 792 to 322 $\mu S/cm^2$ by treatment with 100 μM TFP (curve *b* in Fig. 6*B*, Table). Cytoplasmic streaming did not cease during the action potential (EC uncoupling). The effect of 100 μM TFP was removed partially by an external perfusion with Ca^{2+}-APW (3 mM $CaCl_2$). The cytoplasmic streaming in such a partially recovered internode ceased again during the action potential (i.e., recovery of EC coupling). The continuous cytoplasmic streaming stayed at the original level and was not affected by longer treatment with TFP, if not stimulated. Further treatment (2 to 3 hr) with TFP caused irreversible depolarization of the *Chara* internode similar to W-7 with a marked increase of the leakage and electrical collapse of the plasmalemma.

Discussion

Pump Activity

Keifer and Spanswick (1978) reported on the depolarization of the resting potential by the addition of 0.5 mM $LaCl_3$ in *Chara*. Beilby (1984) applied 0.1 mM $LaCl_3$ in the external solution for the purpose of blocking the passive channel in their analysis of the pump activity. Shimmen and Tazawa (1983) found a depolarization of resting potential by external application of 3 mM $CoCl_2$ in *Nitella*. Our results on the effects of La^{3+} (Figs. 2*A* and 2*B*) and verapamil (Figs. 3*A* and 3*B*) confirmed some of these data. However, in our results the conductance at the resting potential decreased to some extent by La^{3+} and by verapamil (curve *b* in Figs. 2*A* and 3*A*, Table). This is especially true at the late stage of these treatments. Two hours after La^{3+} treatment both the excitability and the H^+-pump activity were lost almost completely (Tsutsui et al., 1987). Dual effects of La^{3+} or verapamil, i.e., depolarization and decrease of membrane conductance cannot be attributed simply to the blocking of the passive channel alone. If the passive channel alone were blocked and the activity of the pump remained unaffected, as suggested by Keifer and Spanswick (1978) and Beilby (1984), then, not a depolarization, but a hyperpolarization of the membrane emf (E) would occur, since E_p is more negative than E_d (Eq. 2). On the contrary, E was depolarized by La^{3+} or verapamil treatment (Fig. 2*A*, Table). Accordingly the main reason for the decrease of conductance G and the depolarization of membrane emf (E) (Table) should be in the decrease of pump channel conductance. In other words, the decrease of the H^+-pump conductance (g_p) is the main reason for the depolarization of the resting potential and the decrease of conductance.

The chelation of external Ca^{2+} by EGTA also caused depolarization and decrease in conductance of the resting membrane (curve *b* in Figs. 4*A* and 4*B*). The internal Ca^{2+} concentration decreased in the *Vallisneria* mesophyll cell, when the cell was exposed to EGTA (Takagi & Nagai, 1983). The internal free Ca^{2+} level maintained at 2.2×10^{-7} M in the intact internode of *Chara* as described above and this level should be balanced between the leak influx of Ca^{2+} (Hayama et al., 1979) and the active Ca^{2+} extrusion in the plasmalemma. Such a balance may be lost by the decrease of Ca^{2+} influx either by chelating external Ca^{2+} with EGTA or blocking the Ca^{2+} channel with La^{3+} or verapamil. What is expected there is a decrease of internal Ca^{2+} concentration below a certain level. Our data show a general decrease of the membrane conductance by these treatments (Table). This is caused partly by the decrease of the passive channel conductance and mainly by the decrease of electrogenic pump conductance as described above. We found with the aid of the voltage-clamp experiments that all these reagents not only reduced the transient inward current markedly, but also markedly decreased the conductance of the pump channel (Tsutsui et al., 1987). The internally perfused internode with EGTA showed a marked suppression of the pump activity and also the excitability (*in preparation*). On the other hand, ATP-dependent H^+ extrusion was markedly reduced in the internally perfused *Chara* membrane when the internal free Ca^{2+} level was higher than 10^{-4} M (Lühring & Tazawa, 1985). Therefore, the normal pump activity seems to be maintained in a comparatively narrow range (i.e., around 10^{-7} M) of internal free Ca^{2+} level.

Excitability

The action potential of *Chara* membrane is caused mainly by the increases of Cl^- conductance and K^+ conductance as described above. The marked de-

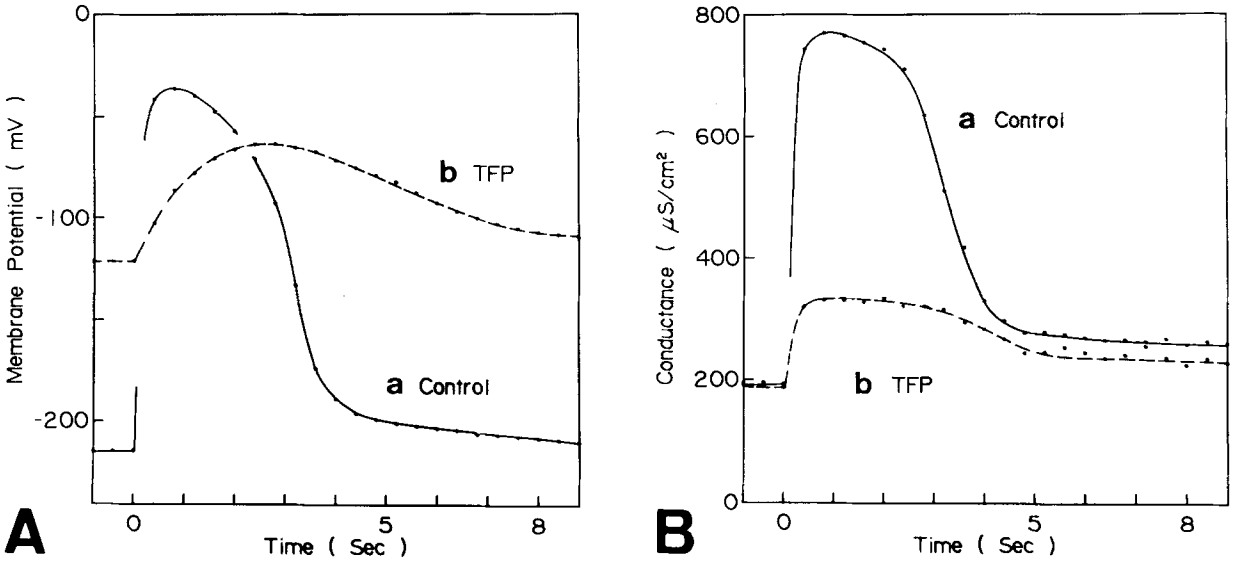

Fig. 6. (*A*) Effects of 100 μM TFP on the resting potential and on the action potential of the Chara membrane. (*B*) Effects of 100 μM TFP on the membrane conductance at rest and during action potential. *a* (–●–●–), control. *b* (–●– –●–), 30 min after treatment with 100 μM TFP. Half-width of an action potential was about 3.2 sec in *a* and 6.0 sec in *b*

crease of conductance at the peak of action potential (curve *b* in Figs. 2*B*, 3*B*, 4*B*, 5*B* and 6*B*, Table) indicates a suppression of the Cl^- channel activation. The general prolongation of the action potential by La^{3+}, verapamil, EGTA or TFP (Table) can be interpreted as a slowing down of the opening and closing kinetics of Cl^- and/or K^+ channels. The suppression of excitation by La^{3+} was reported in the *Chara* (Keifer & Spanswick, 1978; Beilby, 1984), *Mimosa* (Campbell & Thomson, 1977) and many animal cells [*see* reviews by Kostyuk (1982) and Schwarz and Passow (1983)]. Similar prolongation of action potential and the decrease of its size was reported already in the lobster axon membrane which was treated with 11 mM La^{3+} (Takata et al., 1966), and in *Nitella* which was treated with 3 mM of $CoCl_2$ (Shimmen & Tazawa, 1983). According to Takata et al. (1966) and Hagiwara and Takahashi (1967), the effect of La^{3+} was often irreversible. Contrary to the effect of La^{3+} or verapamil, the effect of Co^{2+} was removed by the external application of 5 mM $CaCl_2$ (Shimmen & Tazawa, 1983). The fact that La^{3+} or verapamil suppresses the activation of the excitable channels (curve *b* in Figs. 2*A* and 3*A*) indicates that the normal excitatory mechanism is closely related to the Ca^{2+} influx. Removal of the external Ca^{2+} by the external perfusion with EGTA caused almost a similar effect on the action potential (curve *b* in Fig. 3*A*). Recovery of the action potential in EGTA-treated *Chara* by the external perfusion with Ca^{2+}-APW (curve *c* in Figs. 4*A* and 4*B*) emphasizes the initial triggering role of Ca^{2+} for the action potential. Lunevsky et al. (1983) mentioned the possibility that Ca^{2+} might enter into the cytoplasm, during excitation of *Chara* membrane, both from the external surface and from the vacuole. However, vacuolar perfusion in the *Chara* internode, with the artificial cell sap containing $LaCl_3$ caused almost no effect on the action potential (*unpublished*). Moreover, the internal perfusion of the tonoplast-depleted *Chara* cell with EGTA ($[Ca]_i < 10^{-8}$ M) causes a marked decrease of the resting membrane conductance and a deformation of the action potential into a flip-flop type with a much reduced conductance increase (*unpublished*). This further supports the notion that a proper range of internal Ca^{2+} level is necessary to activate the passive channels as well as the pump in *Chara*.

Calmodulin

Calmodulin antagonists caused depolarization of the resting potential, shift of the peak of action potential to a more negative level and the reduction of conductance at the peak of action potential. These are similar to the effects of La^{3+}, verapamil and EGTA. It is very likely that the opening and closing kinetics of the passive channels (Ca^{2+}, Cl^- and K^+) and also activation of the pump channels are controlled by calmodulin. Beilby and MacRobbie (1984) reported on the spontaneous action potential in the *Chara* treated with 5 μM TFP. We found that this occurred only at the early stage of TFP treatment.

In our study TFP caused a depolarization, decrease in conductance and a marked suppression of the action potential in about 30 min. The scheme of generation of action potential may be as follows: Ca^{2+} is introduced first into the cell through Ca^{2+} channel, then binds to calmodulin or calmodulin-like protein and finally activates the excitable Cl^- channel.

Cytoplasmic Streaming

Both EGTA and calmodulin antagonists caused EC uncoupling (Table). Beilby and MacRobbie (1984) reported on the cessation of streaming during the spontaneous action potential which was caused by 5 μM of TFP. However, concentration of TFP in their case was not high enough to block the excitability. We found at the early stage of concentrated TFP (20 to 30 μM) application that the cytoplasmic streaming did not cease during action potential. Whether the amount of Ca^{2+} influx itself is sufficient enough to cease cytoplasmic streaming or the internal Ca^{2+} level is further multiplied in some cascade fashion with an unknown mechanism remains to be solved. It also remains unsolved whether cessation of the cytoplasmic streaming is mediated by calmodulin or not.

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