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Action Potentials in *Acetabularia:* Measurement and Simulation of Voltage-Gated Fluxes

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Summary. Amounts and temporal changes of the release of the tracer ions K^+ ($^{86}Rb^+$), $^{22}Na^+$, and $^{36}Cl^-$ as well as of H^+ in the course of action potentials in *Acetabularia* have been recorded. New results and model calculations confirm in quantitative terms the involvement of three major ion transport systems X in the plasmalemma: Cl^- pumps, K^+ channels, and Cl^- channels (which are marked in the following by the prefixes, *P, K* and *C*) with their equilibrium voltages ${}_XV_e$ and voltage/time-dependent conductances, which can be described by the following, first approximation. Let the maximum (ohmic) conductance of each of the three populations of transporter species be about the same (${}_PL$, ${}_KL$, ${}_CL = 1$) but voltage gating be different: the pump (${}_PV_e$ about -200 mV) being inactivated (open, $o \rightarrow$ closed, c) at positive going transmembrane voltages, V_m; the K^+ channels (${}_KV_e$ about -100 mV) are inactivated at negative going V_m; and the Cl^- channels (${}_CV_e$: around 0 mV), which are normally closed (c) at a resting V_m (near ${}_PV_e$) go through an intermediate open (o) state at more positive V_m before they enter a third "shut" state (s) in series. Model calculations, in which voltage sensitivities are expressed by the factor $f = \exp(V_mF/(2RT))$, simulate the action potential fairly well with the following parameters (${}_Pk_{co}$: $10/f\,ks^{-1}$, ${}_Pk_{oc}$: $1000 \cdot f\,ks^{-1}$, ${}_Kk_{co}$: $200 \cdot f\,ks^{-1}$, ${}_Kk_{oc}$: $2/f\,ks^{-1}$, ${}_Ck_{co}$: $500 \cdot f\,ks^{-1}$, ${}_Ck_{oc}$: $5/f\,ks^{-1}$, ${}_Ck_{so}$: $0.1/f\,ks^{-1}$, ${}_Ck_{os}$: $20 \cdot f\,ks^{-1}$). It is also shown that the charge balance for the huge transient Cl^- efflux, which frequently occurs during an action potential, can be accounted for by the observation of a corresponding release of Na^+.

Key Words *Acetabularia* · action potential · chloride channel · excitation simulation · transient fluxes · voltage gating

Introduction

The best investigated action potentials in plants are those of the cells of the fresh water Characean species. It took about three decades until the successful Hodgkin-Huxley formalism was eventually applied to these action potentials (Beilby, 1982). As a result, the previous finding (Mullins, 1962) that the depolarization of these action potentials is accomplished by a Cl^- current (after an initial influx of Ca^{2+}) was confirmed. It is not surprising that this depolarization in a Na^+-deficient environment is not due to a Na^+ influx as it is in regular animal cells with their Na^+-rich environment but due to an equivalent efflux of Cl^-. The question was now, whether the depolarization of the action potentials in marine algae is caused by a Na^+ influx as it is in animal cells according to the Na^+-rich environment, or due to a Cl^- efflux like in their green freshwater relatives.

This problem has been treated using the giant cells of the unicellular marine algae *Acetabularia* (Gradmann, 1976). The most striking result was that inactivation of the electrogenic Cl^- pump (${}_PV_e$: about -200 mV) plays an essential role in the depolarization of these "metabolic" action potentials in *Acetabularia* (Gradmann, 1976). Since these action potentials peak at transmembrane voltages, V_m, considerably more positive than the equilibrium voltage for K^+ diffusion (${}_KV_e$: about -90 mV in normal seawater containing 10 mM K^+), a contribution of transiently opening Cl^- channels (${}_CV_e$: near 0 mV in about 500 mM Cl^- of seawater) as in *Chara* could be assumed as well. Based on the data presented here in some detail, this mechanism for the action potential in *Acetabularia* has already been suggested in a previous report (Gradmann & Mummert, 1980).

As for the repolarization, action potentials in Characean cells seem to be similar to those in animal cells, namely due to a K^+ efflux through voltage- and time-dependent channels (outward rectifier). However, in *Acetabularia*, where the resting transmembrane voltage V_r (about -170 mV) is much more negative than ${}_KV_e$, the repolarization cannot be completed by an opening of K^+ channels. Complete repolarization requires activation of the electrogenic

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pump and simultaneous inactivation of the passive pathways for the translocation of Cl^- and of K^+.

These action potentials have been pointed out to play an essential role in the log-term salt balance of *Acetabularia* (Mummert & Gradmann, 1976; Wendler, Zimmerman & Bentrup, 1983): during the resting state, Cl^- (through the Cl^- pump), and of K^+ (through the K^+ channels) are accumulated considerably. It has been shown that this continuous accumulation is compensated by transient ejection of K^+ (Mummert & Gradmann, 1976) and of Cl^- (Gradmann, Wagner & Gläsel, 1973; Wendler et al., 1983) in the course of action potentials. For Na^+, the problem seems to be similar as for K^+. The Na^+ pressure is even larger (extracellular 460 mM in normal seawater, intracellular about 70 mM); on the other hand, Na^+-conducting channels have not shown up during patch-clamp investigations on the plasmalemma of *Acetabularia* (Bertl & Gradmann, 1987; Bertl, Klieber & Gradmann, 1988).

There is agreement between the reports of different groups about the transient release of large amounts of Cl^- from *Acetabularia* in a mode which does not affect the electrical potential in the cytoplasm, although it is faculatively related to action potentials (Wendler et al., 1983; Gradmann & Mummert, 1984); only some contrary opinions have been stated about the mechanism of this release. Wendler et al. (1983) and Wendler, Zimmerman and Bentrup (1984) suggested a powerful cotransport system in the plasmalemma with some unknown cosubstrate. On the other hand, Gradmann and Mummert (1984) favored a mechanism of release of NaCl-loaded vesicles from the vacuole, electrically bypassing the plasmalemma. The operation of such a vesicular shuttle has been demonstrated in the meantime (Mummert & Gradmann, 1991). In the present paper, additional evidence for this mechanism is provided by demonstrating a Na^+ release in the course of action potentials which shows great similarity with the known events of transient large Cl^- efflux.

We take advantage again of the possibility to record Cl^- efflux and transmembrane voltage, V_m, simultaneously from individual *Acetabularia* cells (Mummert & Gradmann, 1976, 1991; Mummert, Hansen & Gradmann, 1981), and from the possibility to make those recordings from cell segments with artifically altered compartmentations (Freudling & Gradmann, 1979; Goldfrab, Sanders & Gradmann, 1984; Mummert & Gradmann, 1991): vacuolar-depleted, cytoplasmic segments and cytoplasm-depleted, vacuolar segments. Whereas the voltage dependence of the Cl^- effluxes in the voltage range more negative than -100 mV was essential for the kinetic analysis of the electrogenic pump (Mummert et al., 1981), the voltage range more positive than -100 mV is now focused upon to characterize the voltage dependence of passive Cl^- permeation.

As far as the intrinsic mechanism of the action potentials themselves is concerned, some progress has been made in the past years by investigations of voltage and time dependencies of two major ion transport systems in the plasmalemma of *Acetabularia*, the electrogenic Cl^- pump (Mummert et al., 1981; Tittor, Hansen & Gradmann, 1983; Gradmann, 1989) and the principal K^+ channels (Bertl & Gradmann; 1987; Bertl et al., 1988). These previous results and the new ones on passive Cl^- permeation are used to develop a model calculation for the time course of the action potential as caused by the electrokinetic interaction between these three major ion transport systems.

Materials and Methods

All materials and methods are described in the preceding paper of this volume (Mummert & Gradmann, 1991). Briefly:

Young cells of *Acetabularia acetabulum* in artificial seawater have been equilibrated with radioactive tracer ions: K^+ ($^{86}Rb^+$), or $^{22}Na^+$, or $^{36}Cl^-$. Radioactivity of individual cells was washed out with nonradioactive seawater and sampled (most times) in 15-sec intervals. The release of the respective ion species was determined by measuring the radioactivity of each sample in a liquid scintillation counter. For the presentation of temporal changes of the fluxes in absolute units, unidirectional ion fluxes, as determined by steady-state measurement (Mummert & Gradmann, 1991) could be used as a reference. The temporal resolution of the setup was about 5 sec.

During a washout procedure, the transmembrane voltage of the plasmalemma, V_m, was routinely recorded from the individual cells, thus providing simultaneous recordings of the temporal behavior of V_m and of unidirectional fluxes from individual cells.

The kind of experiments has been carried out on normal cells and on cell segments which were either depleted of vacuoles (cytoplasmic segments) or of cytoplasm (vascuolar segments) by gentle centrifugation and appropriate ligatures. Using these different preparations enabled us to distinguish transport processes which occur at the plasmalemma itself from those which are mediated by the vesicular shuttle between vacuole and outside, bypassing the plasmalemma.

Release of protons from a cell was measured by the same washout apparatus. The pH of the washout medium was monitored continuously by a pH meter and a pen chart recorder. With a sample volume of 0.2 ml, the resolution limit was about 1 pmol H^+ (or an equivalent thereof), corresponding to a net proton efflux of 10–20 nmol $\cdot$ $m^{-2} \cdot s^{-1}$.

Temporal changes in the cell's behavior could be triggered by an optical system with a quartziodide lamp (up to 100 W $\cdot$ m^{-2}), filters, and shutter ("dark": $<10^{-3}$ W $\cdot$ m^{-2}).

Results

Voltage-Dependent K^+ ($^{86}Rb^+$) Efflux

Temporal changes of the efflux of K^+ (recorded by the tracer $^{86}Rb^+$) have been reported to occur during

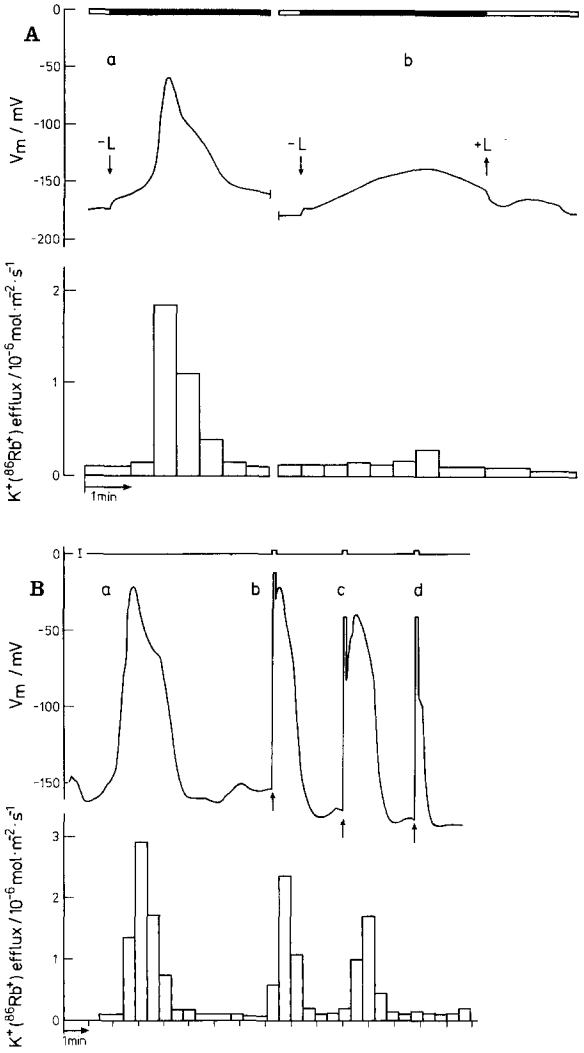

Fig. 1. Evidence for major effect of voltage and minor effect of light on K^+ ($^{86}Rb^+$) efflux. (*A*) Examples of K^+ ($^{86}Rb^+$) efflux changes during light (~80 W · m^{-2} white light) induced changes of V_m, where in *a* an action potential took place but not in *b*. (*B*) Examples of K^+ ($^{86}Rb^+$) efflux changes during changes of V_m, spontaneous action potential (*a*) or induced by injection of depolarizing current pulses (10 μA for 15 sec each, indicated by upper tracing) which triggered an action potential (depolarization and slow repolarization after current pulse in *b* and *c*) or did not (fast repolarization after local depolarization upon current pulse in *d*).

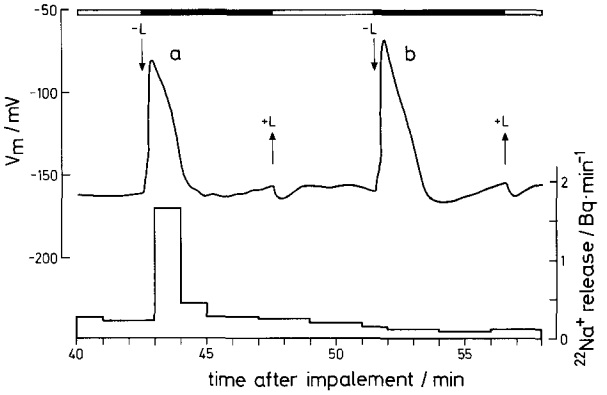

Fig. 2. Example recording for changes in $^{22}Na^+$ release during (light-off triggered) action potentials: a remarkable peak (here about sevenfold over baseline) may occur (*a*) or may be completely absent (*b*).

light-off-induced action potentials (Mummert & Gradmann, 1976). These changes have been used to record efflux-voltage relationships for K^+ ($^{86}Rb^+$) by assuming a voltage dependence of these fluxes. However, it was not clear whether the K^+ ($^{86}Rb^+$) efflux changes observed simultaneously with the change in the transmembrane voltage, V_m, are primarily the effect of the light/dark regime, or the actual result of the change in V_m. This point is examined now.

Figure 1*A* shows two events which have been triggered by light-off. The stimulus was sufficient to elicit an action potential in example *a*, but not in example *b*. Corresponding time courses of the K^+ ($^{86}Rb^+$) efflux show that it is, in fact, not the light-off signal which causes the transient increase of the K^+ ($^{86}Rb^+$) efflux, but the electrical depolarization [beyond ${}_KV_e$, as originally suggested by Mummert and Gradmann (1976)]. This is confirmed by direct electrical stimulation (Fig. 1*B*). Furthermore, these results show that the relative time integrals during $V_m > E_K$ (about -90 mV) of the four consecutive events *a*, *b*, *c*, and *d*(1 : 0.7 : 0.6 : 0) compare well with the corresponding relative amounts (1 : 0.6 : 0.5 : 0) of K^+ ($^{86}Rb^+$) release during these time periods.

Transient Na^+ Release

Figure 2 shows, first, that in normal cells a transient enhancement (here about 10-fold) of Na^+ release can occur during a light-off triggered action potential, and second, that the correlation between such transient Na^+ releases and action potentials is not strict. The latter observation means also, that—in sharp contrast to the observations with K^+ ($^{86}Rb^+$)—a direct voltage dependence of Na^+ efflux could not be assessed. In analog experiments with action potentials in (vacuole-depleted) cytoplasmic cell segments, a transient increase of the Na^+ efflux was never observed. This finding and the large amount of $^{22}Na^+$ released during the transient (Fig. 2*a*) strongly suggests vacuolar origin of this Na^+. Since the compartmentation of Na^+ is very complex (*see* Mummert & Gradmann, 1991), the conversion of such data of $^{22}Na^+$ release data (Fig. 2) to flux

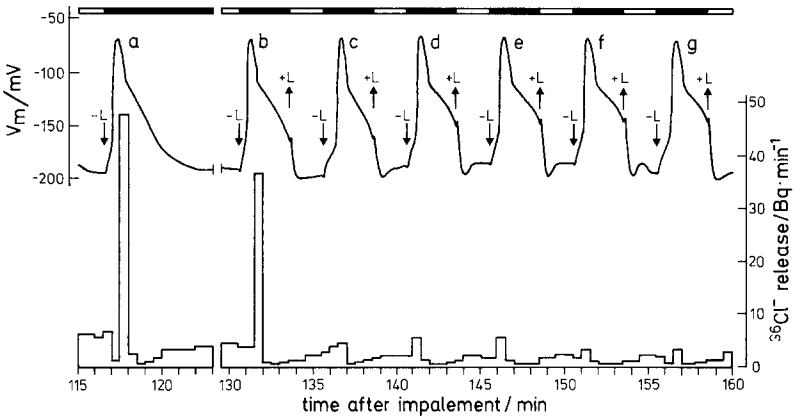

Fig. 3. Examples for changes in $^{36}Cl^-$ release in the course of light-off triggered action potentials of normal cells; events *a* and *b*: with a (faculative) efflux burst each; events *c* through *g*: without (faculative) burst but with small obligatory peak during depolarization process.

units would be problematic and has, therefore, not been carried out.

Temporal Changes of Cl^- Efflux

At least three distinct processes are involved in temporal changes of Cl^- fluxes. These processes, which have already been mentioned (Mummert & Gradmann, 1980), are supported here by additional data.

Vesicular NaCl Bursts

During the peaks of the first two action potentials of the series depicted in Fig. 3, large Cl^- bursts (as described by Gradmann et al., 1973) have been recorded again. The action potentials of this series look similar, no matter whether such a Cl^- burst is associated with them or not. This fact confirms the notion that these efflux bursts and the electrical status of the plasmalemma are primarily two independent matters.

Corresponding recordings of V_m and $^{36}Cl^-$ on cytoplasmic (vacuole-depleted) cell segments and on vacuolar (cytoplasm-depleted) ones are shown in Fig. 4*A* and *B*. The results from the cytoplasmic segments are of particular interest, because the severe problem of vacuolar compartmentation and vesicular shuttle (Mummert & Gradmann, 1991) is eliminated in these preparations. Therefore, it was only here possible to scale the events of transient Cl^- release in proper flux units.

In addition, large Cl^- bursts could not be detected in cytoplasmic cell segments which were investigated immediately (within 7 hr) after centrifugation (Fig. 4*A*). Only in preparations that have recovered for >24 hr (Fig. 35 in Mummert, 1979) and regenerated new vacuoles, Cl^- bursts have been observed in rare cases. Cl^- efflux bursts do take place frequently (Fig. 4*B*, events *b*, *c*, *e* and *f*), but not always (not during events *a* and *d*). These results, together with the equivalent ones above on Na^+ efflux (Fig. 2), confirm the hypothesis that these efflux bursts are caused by NaCl export from the vacuole *via* vesicles.

Voltage- and Time-Dependent Cl^- Channel

In a previous report (Mummert et al., 1981) the release of $^{36}Cl^-$ was focused for V_m more negative than -100 mV. On the other hand, these data (Figs. 5 and 6 in Mummert et al., 1981) show also an increase of $^{36}Cl^-$ release for V_m more positive than -100 mV, which is only observed temporarily in the course of action potentials. A closer examination of data such as in Fig. 3 (events *c* through *g*), shows that this voltage-dependent $^{36}Cl^-$ release is more pronounced during the depolarization process compared with the $^{36}Cl^-$ release during the repolarization process thereafter. These characteristics indicate that the increased release of $^{36}Cl^-$ observed during an action potential consists of two voltage-dependent gating processes which are enhanced by positive going V_m: a fast (τ some sec) activation and a slower (τ some 10-sec) inactivation. An explicit, numerical treatment of this suggested mechanism is presented in the Discussion.

Electrogenic Cl^- Pump

Simultaneous measurements of V_m and tracer release in the course of action potentials have been used to obtain flux-voltage curves for $^{36}Cl^-$ which are consistent with the voltage-dependent reaction kinetics of the electrogenic Cl^- pump as determined by its steady-state current-voltage relationship

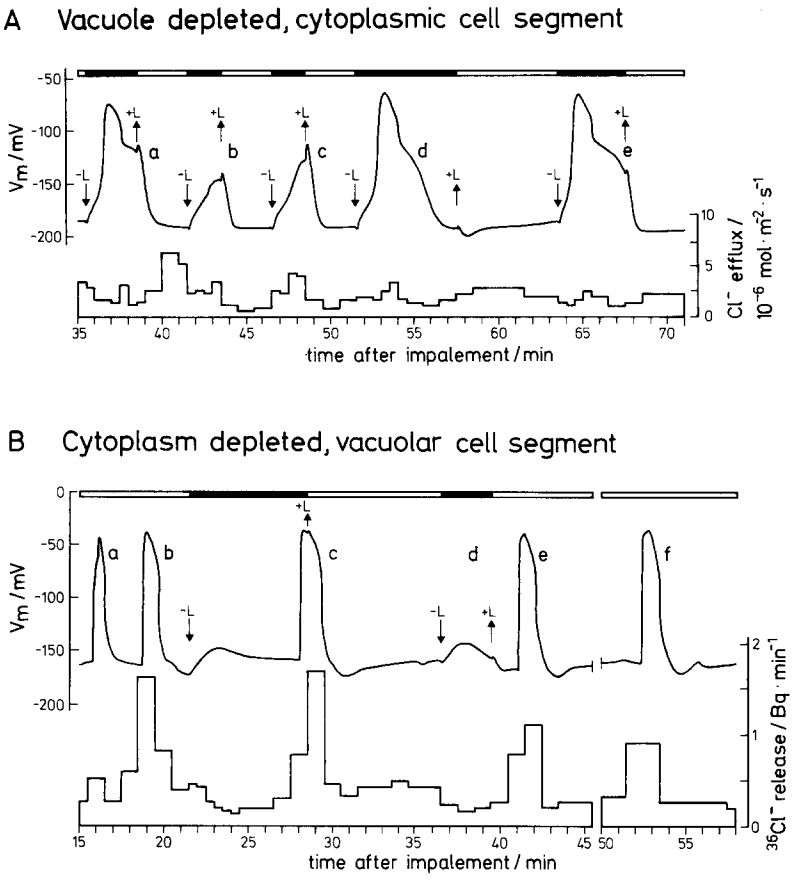

Fig. 4. Examples for changes in $^{36}Cl^-$ release in the course of light-dependent changes in V_m of cells with artificially altered compartmentation. (*A*) Cytoplasmic cell segments (vacuole depleted), show no efflux bursts. (*B*) Vacuolar cell segments (cytoplasm depleted) do show efflux bursts in a facultative mode.

(Mummert et al., 1981). In those experiments, it was already clear that the major changes of $^{36}Cl^-$ efflux are not due to changed illumination but due to changes in V_m. No additional examples are presented here.

Flux-Voltage Relationships

All the results from experiments such as in Figs. 3 and 4 have been analyzed for the flux-voltage relationship, omitting the data which were affected by the large (electroneutral) Cl^- efflux bursts. The resulting average data (Fig. 5) show two interesting features. In comparison with the data in Fig. 4*A* on normal cells, the voltage-dependent $^{36}Cl^-$ efflux of both types of centrifuged preparations is reduced in the voltage range more negative than -100 mV (about fivefold in cytoplasmic cell segments and more than 10-fold in vacuolar ones). The voltage-induced transients of Cl^- efflux at V_m more positive than -100 mV turned out to be about the same in normal cells and cytoplasmic preparations but significantly reduced in vacuolar preparations. For the presentation of the average data in Fig. 5, no correction was made to account for the finding that the stimulation of the $^{36}Cl^-$ release at V_m more positive than -100 mV is larger during the depolarization process than during repolarization (*see* Fig. 3).

Net H^+ Efflux

Since protons play a dominant role in the electrical behavior of glycophytes, their possible involvement in *Acetabularia* has been re-examined in the course of this study, although insensitivity of V_m to $[H^+]_o$ has been reported repeatedly (e.g., Gradmann, 1970, 1975). Upon light-off, an acidification of the external medium was recorded which is equivalent to a transient net efflux of 0.5–1.0 μmol $\cdot$ m^{-2} $\cdot$ s^{-1} (peak) protons. However, these H^+ effluxes also occurred when no action potential was triggered by the light-off stimulus, and they were always missing when action potentials were elicited spontaneously or by an electrical stimulus. Thus, these H^+ fluxes (*results not illustrated*) probably reflect events of the photosynthetic apparatus (maybe release of organic acids) and can apparently be ignored in the context of electrical properties of the plasmalemma. As a corollary, H^+ cannot be a candidate for the counter ion (instead of Na^+) for the large Cl^- bursts.

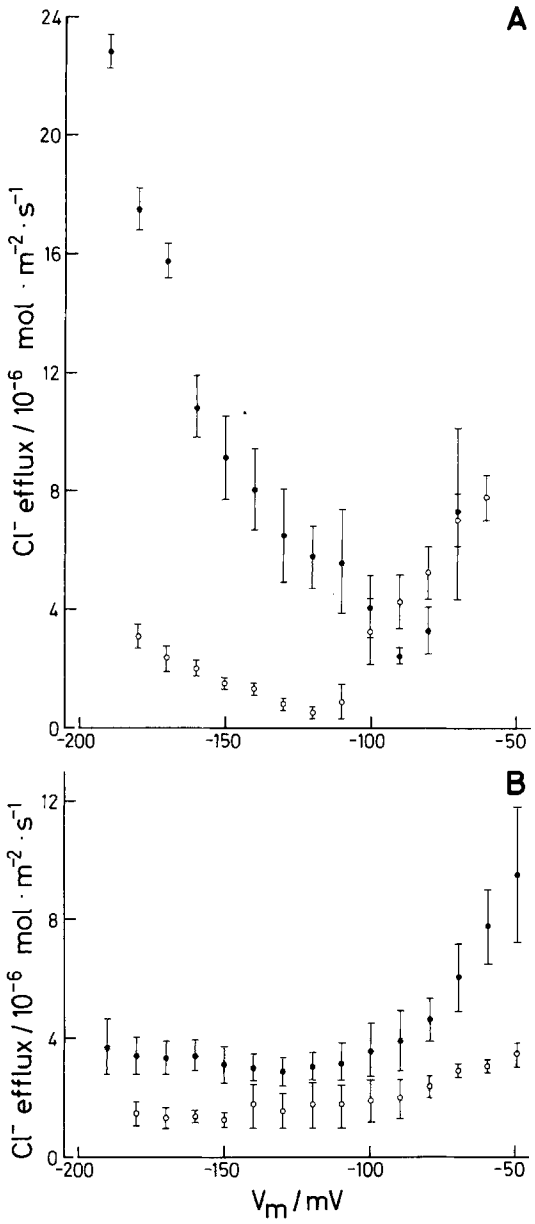

Fig. 5. Mean ±SE data of Cl^- efflux/V_m-relationships of (*A*) normal cells from experiments such as in Fig. 3, ●: total, ○: slow component, and (*B*) of cell segments with artificially altered compartmentation showing one component only, from experiments such as in Fig. 4, ●: cytoplasmic cell segments (vacuole depleted), ○: vacuolar cell segments (cytoplasm depleted).

Discussion

The aim of this study is to help in understanding the mechanisms involved in the action potential of *Acetabularia*. The role of voltage- and time-dependent K^+ channels has already been demonstrated by flux measurements (Mummert & Gradmann, 1976) and by patch-clamp studies on single K^+ channels (Bertl & Gradmann, 1987; Bertl et al., 1988). Also, the involvement of the electrogenic Cl^- pump has already been pointed out in some detail (Gradmann, 1976). On the other hand, the essential role of passive Cl^- diffusion has only been mentioned briefly at a meeting (Gradmann & Mummert, 1980). In the following, we can complete this picture by the additional efflux recordings and by a model calculation.

Passive Ion Fluxes in the Course of the Action Potential

From the data in Fig. 1 we can read peak effluxes of about 2 μmol · m^{-2} · s^{-1} K^+ ($^{86}Rb^+$) and total releases of about 300 μmol · m^{-2} within about 100 sec during the course of an action potential. These numbers are compared with the enhanced Cl^- efflux during an action potential. A direct example is given by Fig. 4*A* (event *d*) showing a Cl^- efflux peak of about 3 μmol · m^{-2} · s^{-1}. From the average data in Fig. 5 on normal cells and cytoplasmic cell segments, peak effluxes of about 6 μmol · m^{-2} · s^{-1} Cl^- can be recalled. During an action potential, total release of Cl^- is about 1 mmol · m^{-2}. These numbers exceed the above numbers from the K^+ ($^{86}Rb^+$) experiments by a factor of about 3, which may correspond to the permeability ratio between K^+ and its tracer Rb^+ (Mummert & Gradmann, 1991). Thus, the data are consistent with the idea that a transient release of Cl^- and an equimolar release (for final electroneutrality) of K^+ occur in the course of an action potential. The temporal lag of the K^+ release (repolarization toward E_K) behind the Cl^- release (depolarization to $V_m > E_K$) can be seen by comparing Figs. 1 and 3: the location of peak fluxes of K^+ ($^{86}Rb^+$) relative to the peak depolarization (Fig. 1) is delayed, while the peaks of Cl^- release in Fig. 3 (events *c* through *g*) are essentially over *after* the peak depolarization. The actual amounts of translocated ions (about 1 mmol · m^{-2}) of either sign, exceed the amounts which are required for the observed changes in V_m by a factor of about 100 ($c_m \cdot \Delta V_m/F < 10$ nmol · m^{-2}, with a membrane capacitance $c_m = 10$ mF · m^{-2}, a voltage change $|\Delta V_m| < 100$ mV, and the Faraday constant $F \cong 10^5$ A · sec · mol^{-1}). This means that the main ion transport during the action potential takes place in a simultaneous and eventually electroneutral release of Cl^- with the counterion K^+.

Thus, the mechanism of these action potentials would be a bad device for economic voltage changes but a good device for osmotic relations, especially when the vesicular release of large amounts (about 4% of the entire content) of NaCl can be triggered thereby as well.

Model Calculation

For the simulation of the action potential (Fig. 6), the analog circuit used consists of three parallel batteries X: Cl^- pumps (prefix P), K^+ channels (prefix K), and Cl^- channels (prefix C), with the equilibrium voltages ${}_XV_e$ and the conductances ${}_XG = {}_XL \cdot {}_XP_o$, where ${}_XP_o$ ($0 < {}_XP_o < 1$) are the mean open probabilities and ${}_XL$ the maximum conductances; Cl^- pumps and the K^+ channels can be in an *o*pen state (${}_PO$ and ${}_KO$ with the mean probabilities ${}_PP_o$ and ${}_KP_o$) or in a *c*losed state (${}_PC$ and ${}_KC$ with the mean probabilities ${}_PP_c$ and ${}_KP_c$) which add up to respective unity (${}_PP_o + {}_PP_c = 1$ and ${}_KP_o + {}_KP_c = 1$); the Cl^- channels have an open state as well (${}_CO$, mean probability ${}_CP_o$), but two inactive states (named ${}_CC$ for closed and ${}_CS$ for shut) in the serial arrangement ${}_CC \rightleftharpoons {}_CO \rightleftharpoons {}_CS$. The three mean probabilities add up to unity as well: ${}_CP_c + {}_CP_o + {}_CP_s = 1$. Transitions from one state (i) to another state (j) of the channel X occur with the probability ${}_Xk_{ij}$ in a given period of time. These transitions are voltage dependent in the form of a symmetric Eyring barrier: $k = k^0 \cdot f$ or $k = k^0/f$ depending on the direction, with k^0 being k at $V_m = 0$ and $f = \exp(V_m \cdot zF/(2 \cdot R \cdot T))$, $z(=1)$ F, R and T having their usual thermodynamic meanings. A detailed treatment of voltage- and time-dependent channel gating in *Acetabularia*, has been presented previously (Bertl et al., 1988).

The time course of the voltage changes as shown in Fig. 6 was calculated as follows: starting condition was a steady voltage clamp at V_0, where the probabilities ${}_XP_0$ and thus the conductances ${}_XG(V_0) = {}_XL \cdot {}_XP_o$ are constant in time; upon clamp-off, the voltage immediately jumps to $V_1 = V_{t=0}$ as given by ohmic circuitry with ${}_XV_e$ and ${}_XG(V_0)$ of the three limbs. The probabilities P of the states now relax from $P(V_0)$ towards $P(V_1)$ with the velocities dP/dt, as given by the three differential equations (${}_X1$) for linear temporal changes of the mean open probabilities of the pumps, for the K^+ channels and for the Cl^- channels:

$$\frac{d({}_PP_o)}{dt} = -{}_Pk_{oc}(V_1) \cdot {}_PP_o(V_0) + {}_Pk_{co}(V_1) \cdot {}_PP_c(V_0) \qquad ({}_P1)$$

$$\frac{d({}_KP_o)}{dt} = -{}_Kk_{oc}(V_1) \cdot {}_KP_o(V_0) + {}_Kk_{co}(V_1) \cdot {}_KP_c(V_0) \qquad ({}_K1)$$

$$\frac{d({}_CP_o)}{dt} = -\{{}_Ck_{oc}(V_1) + {}_Ck_{os}(V_1)\} \cdot {}_CP_o(V0) + {}_Ck_{co}(V_1) \cdot {}_CP_c(V_0) + {}_Ck_{so}(V_1) \cdot {}_CP_s(V_0). \qquad ({}_C1)$$

Thus, after a small time increment Δt, the probabilities will have changed by Δ_XP_1 to ${}_XP_{t=1} = {}_XP_0 + \Delta_XP_1$ yielding $V_{t=1}$ as calculated by ${}_XV_e$ and the new values of ${}_XG_{(t=1)} = {}_XL \cdot {}_XP_{t=1}$. This procedure is now repeated with $V_0 = V_{t=0}$, and $V_1 = V_{t=1}$ to yield $V_{t=2}$ and so on, e.g., 200 times as done for the time course of V_m in Fig. 6*A*. The time courses of the specific ion conductances ${}_XG(t)$ and of the specific currents ${}_XI = {}_XG \cdot (V_m - {}_XV_e)$ in Fig. 6*B* and *C* can easily be extracted from the calculations.

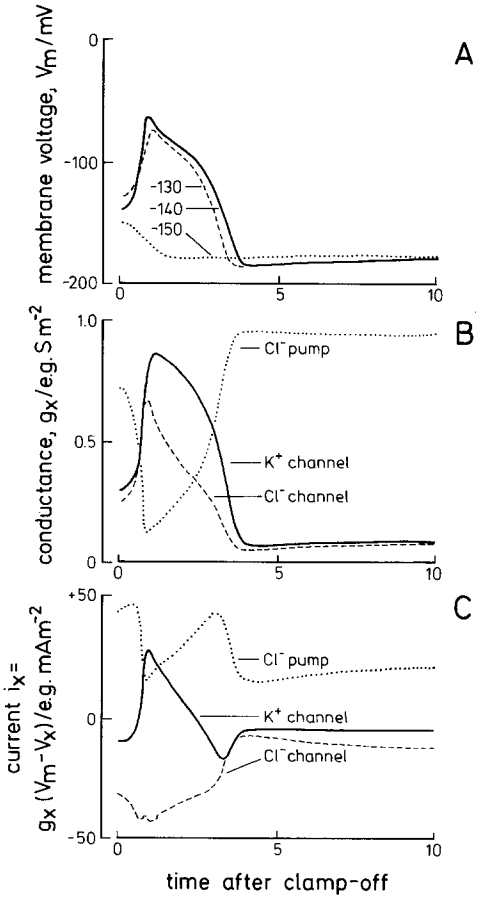

Fig. 6. Simulation of action potentials of *Acetabularia* by V_m-mediated interaction of voltage-gated Cl^- pumps (p), K^+ channels (K), and Cl^- channels (C) in parallel, with the following parameters: ${}_PV_e = -200$ mV, ${}_KV_e = -100$ mV, ${}_CV_e = 0$ mV; ${}_PL_1 = {}_KL = {}_CL = 1$ (e.g., $S \cdot m^{-2}$); ${}_Pk_{co} = 10/f\,ks^{-1}$, ${}_Pk_{oc}$: $1000 \cdot f\,ks^{-1}$, ${}_Kk_{co}$: $200 \cdot f\,ks^{-1}$, ${}_Kk_{oc}$: $2/f\,ks^{-1}$, ${}_Ck_{co}$: $500 \cdot f\,ks^{-1}$, ${}_Ck_{oc}$: $5/f\,ks^{-1}$, ${}_Ck_{so}$: $0.1/f\,ks^{-1}$, ${}_Ck_{os}$: $20 \cdot f\,ks^{-1}$; time units on graph: 100 sec.

Values of ${}_XV_e$ for the calculations for Fig. 6 are rounded according to previous work (Gradmann, 1975). The voltage dependencies as given in the legend to Fig. 6 reflect the following physical meanings: activation of K^+ channels with positive going V_m, yielding steady-state current-voltage relationships of the K^+ channel with negative slope conductance (Bertl & Gradmann, 1987) due to vanishing negative currents for V_m considerably more negative than

$_KV_e$; inactivation of pump with positive-going V_m, yielding steady-state current-voltage relationships of the pump with negative slope conductance (Gradmann, 1975, 1989) due to vanishing positive currents for $V_m \gg {}_PV_e$; fast activation and slow inactivation of Cl^- channels for positive-going V_m, yielding transient Cl^- releases as described above.

Calculated time courses of the free running V_m after a steady-state voltage-clamp situation are given in Fig. 6*A*. Voltages were clamped at a subthreshold value of −150 mV and at two suprathreshold depolarizations of −140 and −130 mV, respectively. These tracings show the essential features of an action potential in *Acetabularia* with a peak between $_KV_e$ and zero voltage, a shoulder around $_KV_e$ and an "after potential," slightly more negative than the resting voltage. The tracings of the conductances (Fig. 6*B*) and of the currents (Fig. 6*C*) through the three types of transporters illustrate the role of the electrogenic pump in the action potential (Gradmann, 1976). Figure 6*B* shows transient and overlapping increases of the conductances for Cl^- and for K^+, where the Cl^- transient precedes the K^+ transient. This protocol is paralleled by the corresponding experimental results (in Figs. 1 and 3).

Comparative Notes

As for plant cells, the action potentials of the freshwater cells of *Chara* are the only ones which have been treated and simulated hitherto by a Hodgkin-Huxley type of model (Beilby, 1982), with the main result that voltage- and time-dependent activation/inactivation of Cl^- channels play the corresponding role of Na^+ channels in animal action potentials. Although the formalism of the model calculation presented here is more closely related to enzyme kinetics, the essential device for action potentials in animals and plants seems to be the same, namely ion channels (for Na^+ in animals and for Cl^- in plant cells) which are rapidly activated and slowly inactivated at V_m more positive than the resting voltage.

It is remarkable, that the seawater alga *Acetabularia* does not use the high extracellular [Na^+] to perform the depolarization during an action potential by a Na^+ influx as in animal cells. Instead, *Acetabularia* employs a corresponding Cl^- efflux, just like their freshwater relatives *Chara* do. Of course, these cells which live without appreciable concentrations of Na^+ and other cations in their environment, must employ an efflux of anions to accomplish an electrical inward current. An increased K^+ efflux during the repolarization of the action potential seems to be ubiquitous in plants and animals. This means in osmotic terms that the action potentials in (maybe all) plants are accompanied by an intrinsic loss of salt, whereas the action potentials in animal cells (with Na^+ influx and K^+ efflux) are osmotically neutral.

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