THE COMPENSATION OF POTENTIAL CHANGES PRODUCED BY TRIVALENT ERBIUM ION IN SQUID GIANT AXON WITH APPLIED POTENTIALS

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ABSTRACT The transmembrane potential of voltage-clamped squid giant axon is increased to compensate for a reduction in the rate of potassium channel kinetics when artificial seawater with trivalent erbium ion is substituted for artificial seawater. The additional potential required to produce an equivalent rise time is a measure of the potential shift produced by the erbium ions. When the kinetics of K^+ channels are matched in this manner, the maximal K^+ currents are larger for the larger transmembrane potential. This observation requires a functional separation of the open K^+ channel and the voltage sensor for the gating mechanism of this channel.

Small concentrations of trivalent lanthanide ions produce a decrease in the rates of channel opening for the delayed (K^+) and early (Na^+) channels of squid giant axon (Starzak et al. 1974). Frankenhaeuser and Hodgkin (1957) noted similar behavior in their studies of the effects of Ca^{2+} ion concentration on squid axon. They noted that a 30-mV depolarization was required with 112 mM Ca^{2+} artificial seawater (ASW) to reestablish K^+ channel conductance found with 4.4 mM Ca^{2+} ASW, i.e., the 30-mV depolarization was required to compensate a 30-mV hyperpolarization induced by the increased Ca^{2+} concentration. Gilbert and Ehrenstein (1969) and McLaughlin et al. (1971) have discussed changes in surface potential induced by polyvalent ions as the source of such potential shifts.

The lanthanide ions are interesting because these trivalent ions produce large potential shifts with very small ion concentrations. We have studied these potential shifts by compensating the hyperpolarizing shifts with applied depolarizing potentials. The proper compensation is established by increasing the transmembrane potential until the time required to attain maximal K^+ current (the rise time) is identical to that of an ASW control solution. This rise time is dictated primarily by the voltage-dependent rate constants for the channel kinetics. Inasmuch as the "shapes" of the two current records differ slightly because of different K^+ accumulations in the periaxonal space, different criteria for matching the kinetics of the records can yield different estimates of the potential shift. For each matching criterion, however, the total current through the K^+ channels with Er^{3+} ASW is larger than that of the control solutions and this requires an increased ion flow through the same number of open channels for the increased transmembrane potential.

The basic voltage clamp and peripheral instrumentation have been described previously (Starzak and Starzak, 1978). Although most experiments with lanthanide ions were signal averaged and stored on magnetic cartridge for detailed analysis, the kinetic changes induced by small Er^{3+} concentrations are so large that a photographic comparison is useful. The larger currents produced with larger depolarizing potentials when the kinetics are matched are conveniently observable in this format.

The series resistance compensation for this experiment was 2 ohm cm^2 (Adelman et al., 1973). The series resistance for axon 77-27 was not measured.

Axons of the squid *Loligo pealei* were obtained at the Marine Biological Laboratory, Woods Hole, Mass., and used if their action potential exceeded 100 mV. 120 nM tetrodotoxin (TTX) was added to the external bathing solutions to eliminate the Na^+ channel currents. The Er^{3+} ASW was prepared directly from the control ASW by addition of small volumes of acidic, 45 mM Er^{3+} stock solutions. The final 0.1 mM Er^{3+} ASW had a pH of 6.8 at 6.0°C, whereas the control solution pH was 7.1. Tris buffer was used.

The large potential required to reproduce the kinetic rate of the control ASW currents when Er^{3+} was present is apparent in Fig. 1. When 0.1 mM Er^{3+} ASW is present

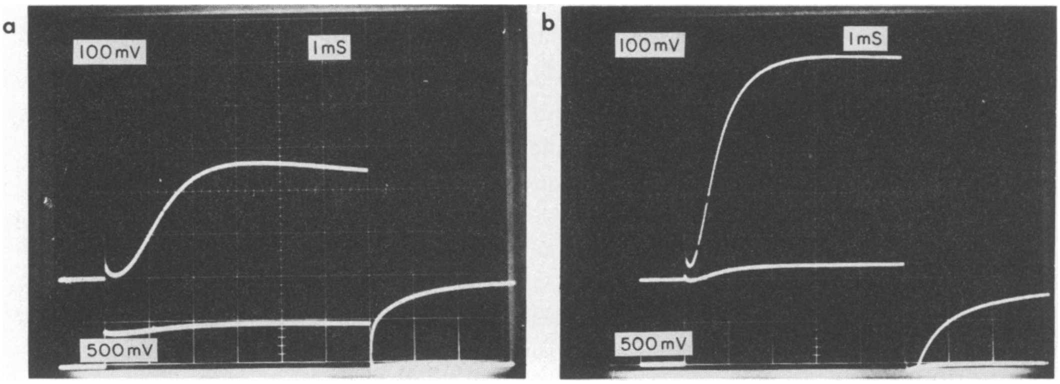

FIGURE 1 Current density and transmembrane potential vs. time records for voltage-clamped squid giant axon (77-27). All solutions contain 120 nM TTX. Current scale (lower left readout): 500 mV converts as 1 mA/cm^2. The holding potential is −60 mV and the series resistance compensation is 2 Ω cm^2. (a) Initial control ASW external solution current densities (upper trace) for an absolute potential of +20 mV. The temperature is 5.5°C; pH = 7.1. (b) ASW with 0.1 mM Er^{3+}. Current densities (upper trace) for an absolute potential of +140 mV. The temperature is 5.8°C; pH = 6.8.

(lower photograph), the transmembrane potential must be increased to +140 mV to produce a rise time comparable to the rise time in control ASW at a potential of +20 mV (upper photograph) for axon 77-27. Both current density records of Fig. 1 peak at 3.5 ms, and these data suggest that voltage sensors of the potassium channels experienced a hyperpolarizing potential shift of −120 mV when the 0.1 mM Er^{3+} ion was present. Even for this minimal concentration, the observed shifts are larger than those reported by Frankenhaeuser and Hodgkin (1957) for a Ca^{2+} concentration range of 108 mM (30 mV).

Although the magnitude of the potential shift is interesting, the observed maximal K^+ currents for the two experiments of Fig. 1 provide additional insight into the nature of the K^+ channels. The maximal current for the Er^{3+} ASW experiment (5.17 mA/cm^2) is substantially larger than the control ASW experiment (2.62 mA/cm^2) even though both have been matched to have comparable kinetics. Because the hyperpolarizing shift produced by the Er^{3+} ion is compensated by an increased transmembrane potential, the additional currents must be produced directly by the increased potential, i.e., an increase in $V - V_K$ is primarily responsible for the larger current densities. Assuming $V_K = -72$ mV, the predicted increase due to the potential change is: $I_{ss} = (212 \text{ mV}/92 \text{ mV})(2.62 \text{ mA/cm}^2) = 6.04 \text{ mA/cm}^2$.

The predicted maximal K^+ current is slightly larger than the observed current (5.17 mA/cm^2) because this approximate current prediction does not include the effects of changes in V_K and $\bar{g}_K$ when Er^{3+} is present. Because the currents are larger in the Er^{3+} ASW experiments, K^+ accumulation in the periaxonal space is also larger. The values of V_K at the termination of the 6-ms step clamp are: $V_K(\text{control}) = -40$ mV and $V_K(Er^{3+} \text{ ASW}) = 0$ mV. The corresponding values of g_K were 0.043 S/cm^2 and 0.037 S/cm^2, respectively. When these parameters are included, the predicted current is 5.24 mA/cm^2, in excellent agreement with the observed maximal current of 5.17 mA/cm^2. The major source of the increased currents is the increased transmembrane potential, and these data suggest that the ensemble of open K^+ channels functions ohmically as originally suggested by Hodgkin and Huxley (1952).

The small decrease in g_K indicates a decrease in $\bar{g}_K$ because the ratio of rate constants, $(\alpha_n/\alpha_n + \beta_n)$, is assumed identical for both experiments. Er^{3+} ion apparently blocks potassium channels although the time constant for this blockage is slow. Experiments were normally completed within 1 h to minimize such blockage.

The voltage shift induced by Er^{3+} is also evident in the kinetics of the K^+ current tails on repolarization to −60 mV. The total rate constant, $k_n = (\alpha_n + \beta_n)$, increased from 0.118 ms^{-1} for the control ASW to 0.163 ms^{-1} for the Er^{3+} ASW when each was repolarized to −60 mV. The larger repolarization rate constant sum for Er^{3+} ASW reflects a potential shift although the direction of the shift cannot be ascertained from these data.

Analysis of the decay tail for the Er^{3+} experiment reveals a second slower decay with a rate constant of 0.056 ms^{-1}. This time constant has not been studied in detail.

Both the voltage compensation needed to restore the control kinetics and the blockage of the channels increase as the Er^{3+} concentration is increased. This phenomenon

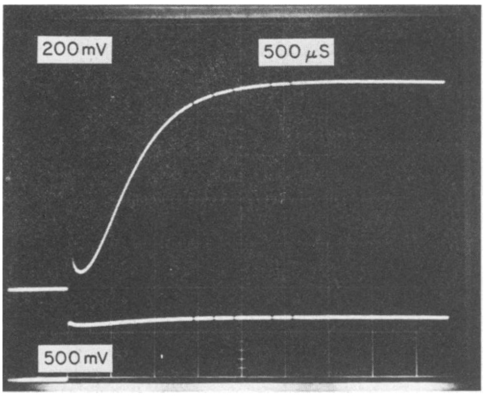

FIGURE 2 Current density and transmembrane potential vs. time for voltage-clamped squid giant axon (77-27). The ASW contains 120 mM TTX and 0.33 mM Er^{3+}. Current scale (lower left readout): 500 mV converts as 1 mA/cm^2. The holding potential is −60 mV and the series resistance compensation is 2 Ω cm^2. The absolute clamping potential is +180 mV and the temperature is 5.7°C.

is evident in Fig. 2 for an absolute potential of 180 mV with 0.33 mM Er^{3+} ASW. Despite the larger membrane potential required to compensate the potential shift, the observed current is only 4.75 mA/cm^2, i.e., the current is smaller than that observed for the 0.1 mM Er^{3+} ASW with a smaller transmembrane potential. The increased concentration of the Er^{3+} in solution and the increased potential contribute to a decrease in the number of conducting channels, i.e., $\bar{g}_K$.

Steady-state current vs. potential (I-V) curves are plotted in Fig. 3 for the control ASW and Er^{3+} ASW experiments to illustrate the combined effects of the Er^{3+} ion and the larger depolarizing potentials. The current magnitude is reduced by Er^{3+} due to the reduction of the ratio $\alpha_n/(\alpha_n + \beta_n)$. The apparent "shift" of the Er^{3+} I-V curve cannot be used as a measure of the voltage shift produced by the ion but does serve to illustrate the regular behavior of the axons at a series of potentials.

Inasmuch as the transmembrane potential must be increased to compensate for the effects of the Er^{3+} ion when the membrane is depolarized, the validity of a general hyperpolarizing shift in membrane potential was checked by applying hyperpolarizing potentials as well. These results are summarized in Fig. 4. The hyperpolarizing potential for the control ASW was increased until "punch-through" (Mauro, 1962) was observed. When the Er^{3+} ASW was introduced, punch-through at −230 mV was reduced and the absolute potential had to be hyperpolarized by an additional 20 mV (−250 mV) to induce a comparable punch-through slope. The Er^{3+} ion stabilized the membrane against punch-through. Similar effects were observed for higher concentration of Er^{3+}.

The Er^{3+} ion also decreased leakage currents. The current observed shortly after initiation of the hyperpolarizing potential with Er^{3+} ASW is roughly half that observed with the control ASW at the same potential.

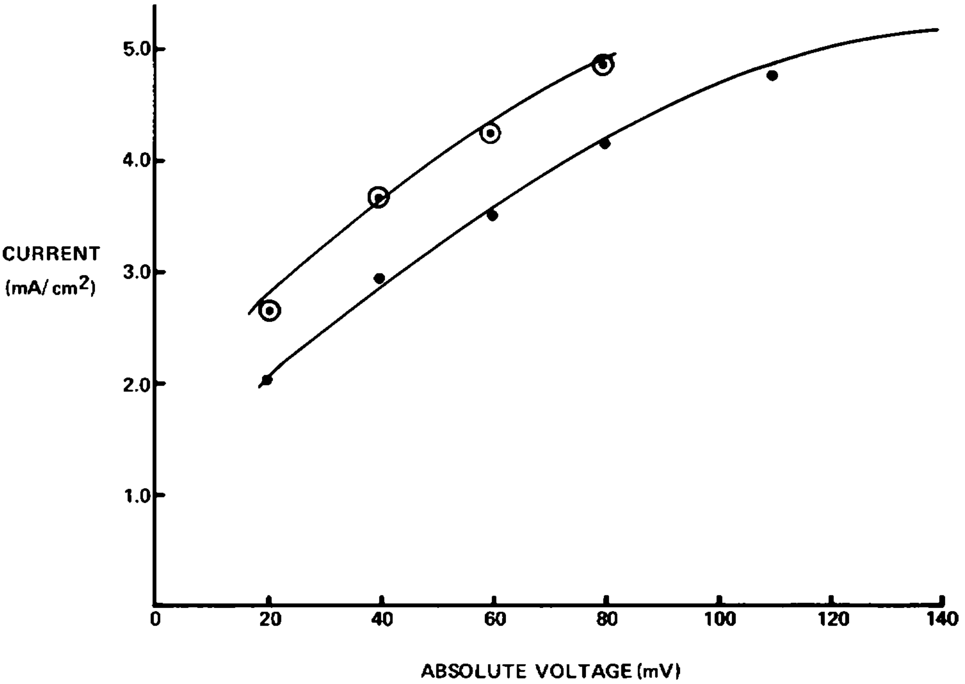

FIGURE 3 Maximal current density vs. transmembrane potential for voltage-clamped squid giant axon bathed externally with a control ASW solution (○) and an ASW solution with 0.1 mM Er^{3+} ion (●). Both solutions contain 120 nM TTX. The holding potential is −60 mV and the series resistance compensation is 2 $\Omega\,cm^2$.

The data presented here indicate that the voltage sensors of the gating machinery for the K^+ channels during a depolarization are extremely sensitive to changes in potential produced by the Er^{3+} ions. These sensors must be functionally distinct from the open K^+ channels because these channels are capable of handling increased currents when the transmembrane potential is increased to compensate the Er^{3+}-induced hyperpolarization. Begenisich (1975) has proposed such a separation for *Myxicola* axon. These observations do not preclude the possibility that the voltage sensors are associated with the membrane K^+ channels in their closed configuration.

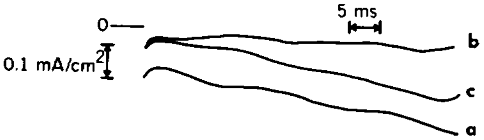

FIGURE 4 Onset of punch-through for axon 77-27. Current curves are reproduced directly from photographs. (a) Initial control ASW experiment for an absolute potential of −230 mV, 5.5°C; (b) 0.1 mM Er^{3+} ASW experiment for an absolute potential of −230 mV, 6.0°C; (c) 0.1 mM Er^{3+} ASW experiment for an absolute potential of −250 mV, 6.0°C.

However, they do preclude models (Starzak and Tranchina, 1976) where the voltage sensor and gating machinery are an integral part of the membrane channel.

Although the gating kinetics for the K^+ channels experience a hyperpolarizing shift in the presence of Er^{3+} ion, such a hyperpolarization need not be common to all membrane processes. For example, a depolarizing shift is observed for punch-through phenomena. Such differences and differences observed for the effects of trivalent lanthanide ions on the K^+ and Na^+ channels suggest that the ions alter the field locally.[1]

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