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Perchlorate Prevents Sodium Channel Gating and Sodium Protects in the Squid Giant Axon

David Landowne (University of Miami, Miami, Florida 33101)

Depolarization of nerve axon membranes induces conformational changes in sodium channel molecules which then open, allowing the flow of inward current that underlies the propagated nerve impulse. One sign of these conformational changes is the outward gating current that precedes the ionic current. The conformational changes are independent of the sodium concentration (1, 2). Therefore, the gating current—which can be obscured by the larger ionic currents present in full sodium—is best studied in low or zero sodium.

Water and small solutes in the bathing medium have access to

the working parts of sodium channel molecules, including the S4 transmembrane segments with their distinctive arrangement of positively charged amino acids (3–5). Looking for possible effects of counterions, I selected perchlorate as a chloride substitute because it is near the end of the Hofmeister series, which ranks ion effects on soluble proteins (6).

Segments of squid axons were superfused with an artificial seawater (asw) containing, in millimoles, x Na, $442-x$ tetramethylammonium (TMA), 50 Ca, y ClO₄, $540-y$ Cl and 2 HEPES, pH 7.4. That is, the sum of Na and TMA was 442 and the sum of Cl and

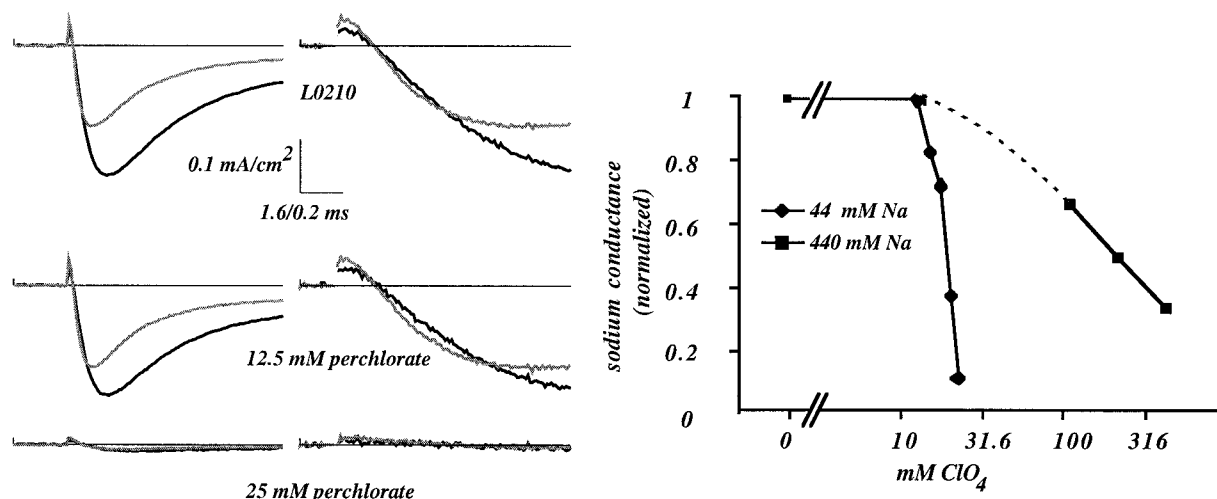


Figure 1. Perchlorate reduces gating and ionic currents. The records are for steps from a -80 mV holding potential. The gray records are to $+20$ mV; the black, to -5 mV. The records on the right are shown at the expanded timebase. The graph shows the maximum Na conductance, normalized to the value obtained in Cl artificial seawater, as a function of ClO_4 concentration for two different Na concentrations.

ClO_4 was 540. They were internally perfused with either a Cs perfusion fluid (pf) containing 240 Cs, 150 glutamate, 50 F, 750 sucrose, and 40 HEPES, pH 7.4; or a K pf containing 200 K, 80 Na, 190 glutamate, 50 F, 550 glycine, and 40 HEPES, pH 7.4. Experiments were performed at $3-4^\circ\text{C}$. The axons were voltage-clamped at a -80 mV holding potential. Currents were recorded with a p/4 protocol (7), filtered at 40 kHz and sampled at 100 kHz. The records presented are averaged over 64 cycles.

The tracings at the left of the figure were made in 44 mM Na asw, with Cs pf inside. Current traces for pulses to -5 mV (black) and $+20$ mV (gray) are shown on two different time bases. The top set of records is in Cl asw. Replacing 25 mM of Cl in the asw with ClO_4 almost eliminated both the inward ionic current and the early outward gating current, and 12.5 mM ClO_4 had hardly any effect. Intermediate concentrations of ClO_4 partially reduced both ionic and gating currents proportionally (not shown), with reduction of the sodium conductance to one half at about 16 mM. The preparation recovered partially when returned to ClO_4 -free solutions; but the recovery was less at higher ClO_4 concentrations or after longer exposure times.

The effects of ClO_4 on the maximum sodium conductance are summarized in the graph at the right of the figure. In full Na asw, with K pf internally, 25 mM ClO_4 had no effect, and reduction of the sodium conductance to one half occurred at about 200 mM. About 25% of the sodium conductance remained with 440 mM ClO_4 . In full Na asw, increasing ClO_4 altered the time course of the remaining sodium current, first reducing sodium inactivation and then replacing it with a slowly rising conductance. At 300 nM, tetrodotoxin blocked more than 90% of the current remaining in 440 mM ClO_4 .

Potassium currents were more sensitive to ClO_4 than Na currents and were also protected by external Na. In 44 mM Na asw, 15 mM ClO_4 eliminated the K currents; in 440 mM Na asw, between 220 and 440 mM ClO_4 was required.

These results in squid axons are quite different from those

reported in frog skeletal muscle, where ClO_4 shifts sodium channels excitability (8), excitation-contraction coupling, and intramembranous charge movement to more negative potentials (9). In squid axons, Na permeability was reduced 35% by 110 mM ClO_4 in the presence of 440 mM Na, compared with an 8% reduction by 115 mM ClO_4 in the presence of 115 mM Na in frog muscle (8).

In conclusion, in squid axons, anions can have large effects on ion channels. A log-log (Hill) plot of the concentration dependence of ClO_4 on sodium conductance in 44 mM Na asw has a slope of about -4 . It seems unlikely that this indicates cooperativity between the four domains of the protein (5) because gating current decreased with about the same concentration dependence, and most current models (3) involve all four domains in the gating current. There are four or more positively charged amino acids on each S4 segment that could support a cooperative effect on each domain, but this possibility does not explain the lack of cooperativity seen in 440 mM Na.

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