Ionic Conductance Changes in Lobster Axon Membrane When Lanthanum Is Substituted for Calcium

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ABSTRACT The trivalent rare earth lanthanum was substituted for calcium in the sea water bathing the exterior of an "artificial node" of a lobster axon in a sucrose gap. It caused a progressive rise in threshold, and a decrease in the height of the action potential as well as in its rates of rise and fall. Prolonged application produced an excitation block. Voltage-clamp studies of the ionic currents showed that the time courses of the ionic conductance changes for both sodium and potassium were increased. Concurrently, the potentials at which the conductance increases occurred were shifted to more positive inside values for the La^{+++} sea water. These effects resemble changes resulting from a high external calcium concentration. Over and above this, La^{+++} also causes a marked reduction in the maximum amount of conductance increase following a depolarizing potential step. Membrane action potentials similar to those observed experimentally in the La^{+++} solution have been computed with appropriate parameter changes in the Hodgkin-Huxley equations.

On the hypothesis that Ca^{++} affects the cationic conductances through nerve membrane by virtue of its binding to the membrane it was predicted by two of us (Lettvin, Pickard, McCulloch, and Pitts, 1964) that La^{+++} should reduce, to the point of blocking, the changes in both Na and K conductances associated with changes in membrane potential. We have tested this prediction on lobster giant axons and found that blocking did occur, and that La^{+++} acted as if it were equivalent to an extraordinarily high Ca^{++} concentration. A preliminary report has been given at the 23rd International Congress of Physiological Sciences (Moore, Takata, Lettvin, and Pickard, 1965).

METHOD

The axon preparation and sucrose-gap voltage-clamp technique used to observe the sodium and potassium currents were essentially the same as described by Julian,

Moore, and Goldman (1962 *a*, *b*). The composition of the usual lobster sea water and lanthanum (La^{+++}) sea water is given in Table I. For experiments with a lower La^{+++} concentration, mixtures of the standard sea water and the La^{+++} sea water were used. The time required for a complete change from one solution to another around the "artificial node" in the axon was about 15 sec.

RESULTS

The effect of lanthanum on the shape of the action potential may be seen in Fig. 1. The rate of rise, rate of fall, and height of the peak of the action potential were all decreased in the La^{+++} sea water. The current required to

TABLE I

Standard lobster S.W.		4 × Ca^{++} S.W.	La^{+++} S.W.
	mM/liter		*mM/liter*
NaCl	465	315	457
KCl	10	10	10
$CaCl_2$	25	100	
$MgCl_2 \cdot 6H_2O$	4	4	3.9
$MgSO_4 \cdot 7H_2O$	4	4	4
$NaHCO_3$	2.5	2.5	
NaOH	0.15		*
$LaCl_3 \cdot 7H_2O$‡			11‡

* NaOH (1 M) was added to give a pH of 6 for each La^{+++} solution because lanthanum precipitates formed at higher values of pH. In control experiments, artificial sea water adjusted to a pH of 6 was found to produce only minor changes in the membrane's current-voltage relations; the maximum conductance for both sodium and potassium were a few per cent lower than for the usual unbuffered artificial sea water.

‡ We originally thought we were using La^{+++} at two-thirds that of Ca^{+++} or 16.67 mM. Subsequent consideration of the water of hydration led to the tabulated value.

elicit activity had to be increased. In this experiment the recovery of the original shape of the action potential was nearly complete when the axon was washed for several minutes in sea water. When the nerve was left in La^{+++} sea water for a longer time, the slowing of the rising phase of the action potential continued and eventually the responses appeared to be completely graded. When the La^{+++} concentration was reduced to 1.1 mM (by mixing with the standard sea water containing Ca^{++}), a nearly full sized spike was occasionally maintained, but the rates of rise and fall were still markedly decreased.

When the membrane potential was controlled by the voltage clamp, striking changes in the ionic currents in La^{++} sea water were observed. Fig. 2 shows the time course of the ionic currents through the membrane bathed in the standard (or Ca^{++}) sea water and through one which has been in La^{+++}

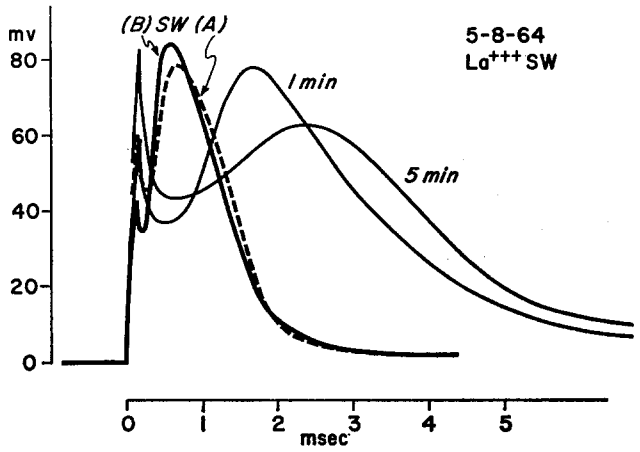

FIGURE 1. The effect of lanthanum sea water on the shape of the action potential of lobster giant axon. The delayed spikes are labeled with the time in this solution. The action potential recovers well as seen from the nearly identical shapes in normal sea water before (*B*) and after (*A*).

sea water for a few minutes. The absolute potential across the membrane during the step is indicated at the right-hand end of each curve. One first notices that the early inward sodium current is markedly delayed and greatly reduced

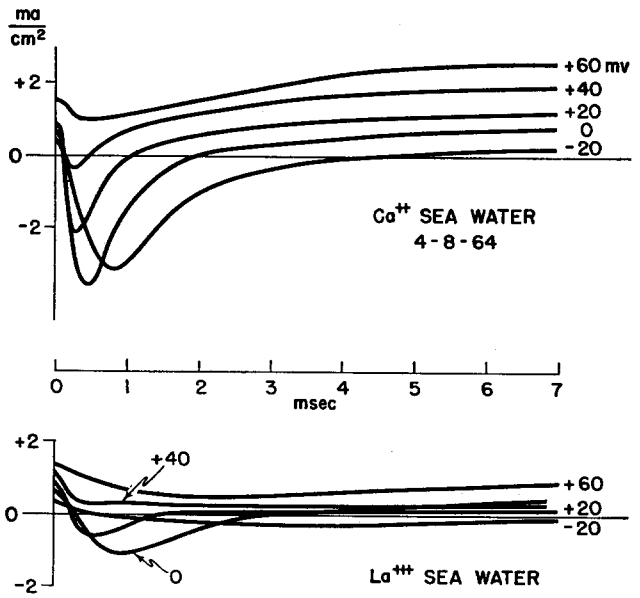

FIGURE 2. Membrane ionic currents observed in normal (Ca^{++}) sea water (upper) with a voltage clamp. The lower curve shows equivalent currents after the axon had been in La^{+++} sea water about 5 min. The membrane potential during the test pulse is shown to the right of each current curve.

in magnitude. Furthermore, the late steady-state current was reduced from large values in normal sea water to less than the initial jump of current in the lanthanum sea water. For small step changes in potential about the resting level, it was found that the leakage current was not constant but fell to a steady level of about 60% of the initial value within 1 msec. In La^{+++} sea water, the leakage current fell with the same time course, but usually to a smaller fraction of the initial current jump.

Fig. 3 shows the conventional current-voltage characteristics for normal and La^{+++} sea water. The normal (or Ca^{++}) sea water curve shown was taken

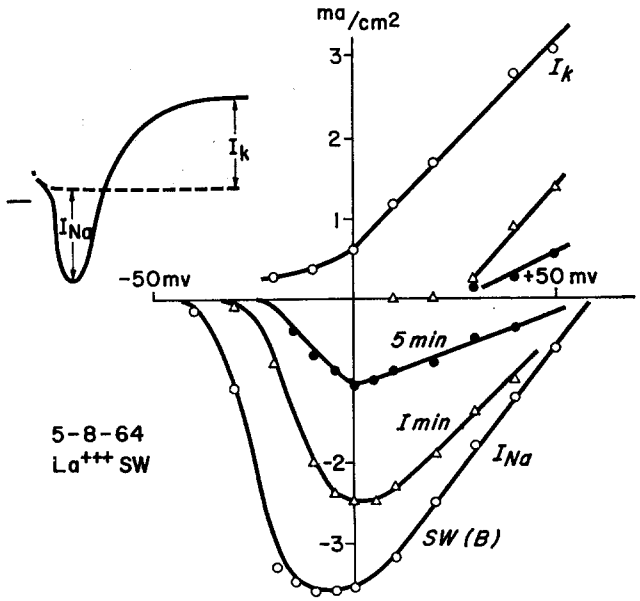

FIGURE 3. Changes in the peak sodium and steady-state potassium current-voltage characteristic curves with La^{+++} sea water.

previous to the application of La^{+++}; the curve in Ca^{++} sea water following the La^{+++} treatment was quite close to that originally obtained and is omitted in the graph for purposes of simplicity. The value of the peak sodium current was taken as the difference in the total current and the estimated leakage current. As noted above, leakage currents (observed for small positive and negative potential steps from the holding level) were not constant, but decayed exponentially to about one-half of the initial value. Therefore we estimated the leakage current by the equation $I_L = (I_0 - I_\infty)\, e^{-t/\tau} + I_\infty$ in which the subscripts represent the values of the leakage current at times $t = 0$ and $t = \infty$ (actually the steady state after a few milliseconds). In Ca^{++} sea water the ratio I_∞/I_0 was 0.6; for the La^{+++} sea water the steady-state leakage was estimated

from the individual curves. A time constant, τ, of about 0.4 ms (observed for small potential steps), was used throughout.

The potassium current I_K plotted in this figure represents the current, minus the leakage, after about 8 msec. This was sufficient time for attainment of a steady current in normal Ca^{++} sea water. In the La^{+++} sea water there appeared to be a slowly increasing current at long times for relatively strong "depolarizations" to and beyond the sodium equilibrium potential. A few observations were therefore made for very long pulses. The time course of the build-up of the steady-state current could not be determined accurately because the magnitude of change was small and somewhat obscured by 60 cycle hum. At a potential during the pulse of +50 mv, it appeared that it took at least 15 msec to reach about one-half the final steady-state level in contrast to only about 2 msec in the normal Ca^{++} sea water. In spite of these measurement difficulties, it appears from Fig. 3 that there was a shift of roughly 35 mv in the potassium conductance curve.

Not only was the conductance for sodium and potassium reduced, but also the potential at which the sodium conductance increase occurred in the La^{+++} sea water was shifted several millivolts (the inside had to be made more positive for the sodium currents to become appreciable). This latter change is similar to that observed by Frankenhaeuser and Hodgkin (1957) when they applied a high Ca^{++} sea water to the squid axon. They measured the rates and magnitudes of conductance changes and concluded that a change in the concentration of Ca^{++} bathing a squid axon was equivalent to a shift along the voltage axis of both sodium and potassium voltage-dependent conductance parameters. Fig. 4 gives one example of the changes in the sodium conductance observed in the La^{+++} and in sea water with 4 times the normal Ca^{++} concentration. The shift in the sodium conductance curve after 5 min in the La^{+++} sea water when normalized was 13 mv. This is about twice the shift of 6 mv observed when the Ca^{++} concentration in the standard artificial sea water was quadrupled. The shifts were rather variable from axon to axon (e.g. Table 3 of Frankenhaeuser and Hodgkin, 1957). Furthermore in our experiments there was variability in the shift from spot to spot along a single axon; an adjacent spot on the axon used in Fig. 4 gave a shift of 10 mv in the high Ca^{++} solution. Thus the example shown in Fig. 4 represents the lower range of shifts observed.

In contrast to the Frankenhaeuser and Hodgkin observation that high calcium shifts but does not depress the maximum sodium conductance for squid axons, we usually found some reduction of the maximum sodium conductance in lobster nerves. Fig. 4 shows about as large a reduction as we have observed. Blaustein and Goldman (1966) have made an extensive study of the changes in sodium conductance in lobster axons resulting from a fourfold increase in Ca^{++}. They found an average reduction of 8 to 10% in the maximum con-

ductance. In the La^{+++} sea water, the sodium conductance was invariably reduced to a low value; the depression shown in Fig. 4 is typical. In two experiments in which another rare earth, Pr^{+++}, was substituted for Ca^{++}, the results were qualitatively similar to those with La^{+++}.

It was noted that the time course of the ionic currents was markedly slowed after a few minutes in the La^{+++} sea water. Fig. 5 shows the relation between the change in amplitude and the time to the peak of the inward sodium current when the bathing solution is changed from Ca^{++} to La^{+++} sea water. The threefold slowing of the sodium current kinetics is equivalent to a shift of about

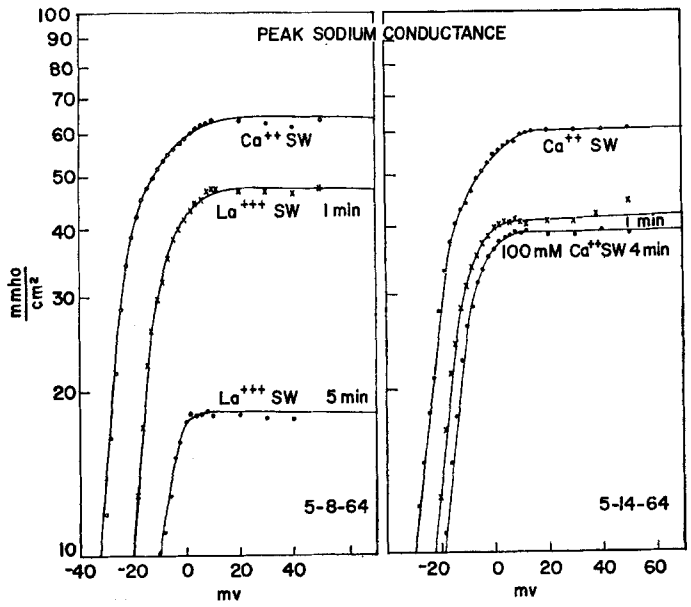

FIGURE 4. The ordinate gives the peak sodium conductance as a function of membrane potential during a pulse. The effect of La^{+++} sea water is seen on the left and of a high Ca^{++} sea water on the right The curves in the normal medium following the experiments were essentially the same as those for the controls and are not shown.

40 to 50 mv in potential. The major changes in the time to the peak of the sodium current occurred almost as rapidly as the solution change. In contrast, the change in the amplitude of the sodium current had a much slower time course.

COMPUTATIONS

It was thought useful to see whether the observed changes in the La^{+++} sea water could be simulated by changes in parameters in the Hodgkin-Huxley (1952) equations. Because the La^{+++} induced shift in the amplitude of the

sodium conductance along the voltage axis was about 13 mv while the shift of the sodium kinetic parameters was 40 to 50 mv, it was difficult to decide on appropriate parameter changes. However, we have made calculations with a compromise set of changes. The α's and β's for the factors m and h were shifted 25 mv and, for the factor n, 35 mv in a direction toward the sodium equilibrium potential (see Fig. 3). The maximum sodium conductance, $\bar{g}_{Na}$, was reduced to 30 mmhos/cm^2, one-fourth its normal value and the maximum potassium conductance $\bar{g}_K$ was reduced to 18 mmhos/cm^2, or one-

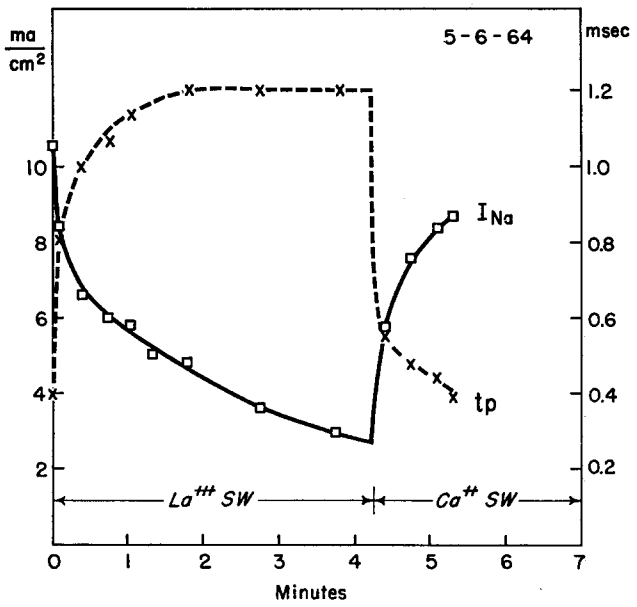

FIGURE 5. Changes in the amplitude and time to the peak of the inward sodium current at a fixed membrane potential (zero during the test pulse) for solution changes from normal to La^{+++} sea water and back.

half its normal value. The membrane action potential computed from the Hodgkin-Huxley equations with these changes may be seen in Fig. 6 B. For purposes of comparison, an action potential membrane conductance calculated from normal parameter values is shown in Fig. 6 A. The difference in the shapes of the action potential in Figs. 6 A and B resembles the experimentally observed difference between the spike in normal sea water and La^{+++} sea water shown in Fig. 1. The computed spike with altered parameters shows somewhat less delay and a somewhat faster recovery than that experimentally observed in La^{+++}. However, in view of the difficulties in parameter choices, the similarities of the general pattern of change were thought to be sufficient for practical purposes and other sets of parameter changes were not investigated.

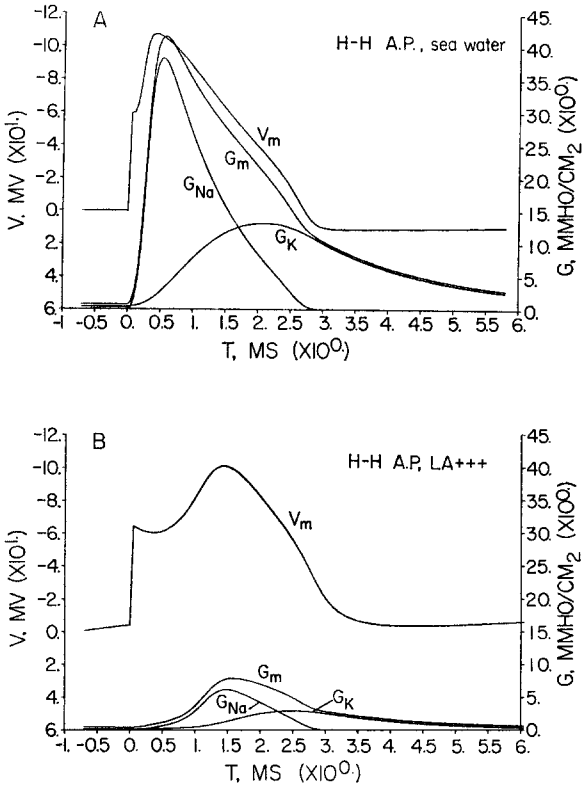

FIGURE 6. Membrane action potentials and associated conductance changes computed from the Hodgkin-Huxley equations. A, normal parameter values. B, Parameters altered to simulated voltage-clamp results with La^{+++}; α_m, β_m, α_h, β_h shifted 25 mv, α_n, β_n shifted 35 mv toward V_{Na}, $\bar{g}_{Na}$ = 30 mmho/cm², $\bar{g}_K$ = 18 mmho/cm².

DISCUSSION

The action of La^{+++} on lobster axon membrane appears to simulate a high calcium concentration in both shifting the characteristic conductance curves along the potential axis, and, at the same time, greatly reducing the maximum conductances. Computations with the Hodgkin-Huxley equations, whose parameters have been changed to approximate experimentally observed changes in the voltage-clamp data, give action potentials which resemble those in La^{+++} sea water.

Although there is an extensive literature on the pharmacology of the lanthanons (Steidle, 1935; Trombe et al., 1959), we are not aware of any La^{+++} experiments with which our data could be compared. Our present results on lobster nerve with changes in Ca^{++} are similar to those of Frankenhaeuser and

Hodgkin (1957) on squid nerve in that they show shifts of the sodium and potassium conductance curves along the potential axis. However, we were unable in our experiments to extend the comparison to the rate of shut off of sodium conductance upon repolarization since our voltage-clamp control system was not sufficiently fast to follow these shifts reliably when La^{+++} sea water was used.

It appears that Ca^{++} does not produce as large a shift in the sodium conductance curve in lobster nerves as the 9 mv per e-fold observed by Frankenhaeuser and Hodgkin (1957). Julian, Moore, and Goldman (1962 *b*) reported a 10 mv shift for a fivefold reduction in Ca^{++} (6 mv for an e-fold change). Blaustein and Goldman (1966) quote an 8 mv shift for a 3.7–fold increase in Ca^{++} (also 6 mv for an e-fold change). Our results on axon 5–14–64 (shown in Fig. 4) range from 4.5 to 7 mv for an e-fold change. However, Frankenhaeuser and Hodgkin thought that their estimate of 9 mv was on the high side. They cited Weidmann's (1955) observations on Purkinje fibers and Frankenhaeuser's (1957) experiments on myelinated nerves, both giving an equivalent shift of about 6 mv per e-fold change in Ca^{++}. Unpublished measurements of Hodgkin and Keynes on *Sepia* axons were reported as showing an e-fold shift of 7 mv at low Ca^{++} concentrations and 12 mv at high concentrations. Taking the Ca^{++} e-fold shift as 6 mv, the La^{+++} sea water, producing a shift of 13 mv, was equivalent to an eight- to nine-fold increase in 25 mM Ca^{++} (210 mM). Thus 11 mM of La^{+++} gives a sodium conductance location equivalent to 210 mM of Ca^{++}. In other words, La^{+++} is some 20 times as effective as Ca^{++} in its effect on the sodium conductance curve and might be called "supercalcium."

If we double the Blaustein and Goldman (1966) observation of an 8 to 10% depression of the maximum sodium conductance for a fourfold Ca^{++} increase to simulate the eight- to ninefold effect of La^{+++} noted above, we can account for a depression of only 20%. The depression of the maximum sodium conductance seen in Fig. 4 is some 70% and indicates that there is a blocking effect over and above the Ca^{++} simulation.

Shorn of detail, the prediction of Pickard and Lettvin (1964) was that La^{+++} ought to be bound more strongly than Ca^{++} to whatever in the membrane takes up Ca^{++}, and that Ca^{++} ought to be bound more strongly than K^{+} or Na^{+} to whatever in the membrane accepts these cations. If Ca^{++} has to be released somehow from sites at which it is bound in order for Na^{++} current and K^{+} current to flow, and this release depends upon changes in the electric field within the membrane, then La^{+++} ought to be more difficult to dislodge electrically than Ca^{++}. The net result of substituting La^{+++} for Ca^{++} ought to be to increase the voltage necessary to produce a particular ionic flux and to lower the upper bound of such a flux. Clearly such an action will not distinguish between the reaction sequence of Goldman (1964) and

the notion of independent potassium and sodium channels. But our results, while not confirming any particular view of the means by which ions pass through a membrane, certainly tend to support the notion of calcium ions (and Ca^{++}-like substances) acting as gates, occluders, or competitors for sodium and potassium channels whatever their character.

An alternative hypothesis for the mechanism of the calcium effect was originally suggested by Huxley and noted in the paper of Frankenhaeuser and Hodgkin (1957). It was postulated that calcium ions may be adsorbed at the outer edge of the membrane and thereby create an electric field inside the membrane which adds to that provided by the resting potential. In this manner, adsorbed lanthanum ions might also alter the distribution of the charged particles within the membrane, without changing the over-all potential difference between inside and outside. While this idea may satisfactorily account for the shift of the conductance with potential, it does not explain the reduction of the maximum conductance nor the other problems noted by Frankenhaeuser and Hodgkin, namely, that of the large effect of calcium on the rate at which sodium conductance is shut off under an anode nor for the increased calcium entry associated with the conduction of impulses.

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