

Calcium and Lanthanum Effects at the Nodal Membrane*

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Summary. 1. Voltage clamp experiments on single myelinated nerve fibres of *Xenopus* were done in Ringer solutions, in which the normal Ca concentration (2 mM) was substituted by 10 mM Ca²⁺, 0.1 mM La³⁺ or 0.5 mM La³⁺.

2. The activation and the inactivation curves of the Na permeability were shifted by 11 and 8 mV in positive direction on the potential axis by an e-fold increase in concentration of either Ca²⁺ or La³⁺.

3. These shifts were plotted versus the logarithm of the Ca or La concentration and could be fitted by straight lines under the assumption that 1 mM La³⁺ is equivalent to 55 mM Ca²⁺.

4. The activation curve of the K permeability was shifted 6 or 14 mV by an e-fold change in Ca or La concentration.

5. In high Ca and in the La solutions the maximum Na and K permeability and the leakage conductance were reduced.

6. Ringer solution with 0.1 mM Tb³⁺ exerted an even stronger effect than 0.1 mM La³⁺.

7. It is assumed that the observed shifts reflect a change in membrane surface potential due to electrostatic screening by the cations of the external solution. On this basis a negative fixed charge density of approximately 1/70 Å² is calculated for the vicinity of the Na channel; a lower density appears to apply near the K channel.

8. As some of the La effects cannot be interpreted in terms of screening or binding and are different from the Ca effects, La seems to be a potent Ca substituent with some specific effects in addition.

Key words: Node of Ranvier — Calcium Ions — Lanthanum Ions.

La exerts a strong Ca effect at excitable membranes as Takata, Pickard, Lettvin and Moore (1966) have shown in lobster axons. From the shift of the Na conductance curve on the potential axis they calculated that La is some 20 times as effective as Ca. Shifting effects of the relation between ionic permeability and membrane potential have first been established by Frankenhaeuser and Hodgkin (1957). These authors have suggested that an increase of the external Ca concentration induces a hyperpolarization that is "seen" by the membrane but is not measur-

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able by conventional electrodes so that the effect is perceived as a permeability shift on the voltage axis. The original notion (actually proposed by A. F. Huxley) was that Ca ions may be adsorbed at the external membrane surface. Later the idea emerged that this surface bears negative fixed charges that are the source of a surface potential. Ca ions could reduce the potential by binding or by electrostatically screening the negative fixed charges as suggested by Gilbert and Ehrenstein (1969), who applied Grahame's (1947) adaption of the Gouy-Chapman theory. In the case of a high charge density trivalent cations would be much more effective in screening than divalent ions as was confirmed by McLaughlin, Szabo and Eisenman (1971) in experiments on artificial membranes. In order to test the screening hypothesis on a natural membrane the present experiments compare the action of La to those of Ca in a voltage-clamp study on nodes of Ranvier. Screening indeed seems to explain most of the results, although some additional mechanisms, binding or other, appear to be involved.

Preliminary reports have appeared elsewhere (Vogel, 1973a, b).

Methods

From the tibial nerve of the clawed frog, *Xenopus laevis*, a single fibre was isolated and mounted in a perspex cell. The Ranvier node under investigation was permanently superfused. The nodes on either side were kept in isotonic KCl solution and cut. The membrane currents were recorded with the voltage-clamp technique of Dodge and Frankenhaeuser (1958) in a setup as described by Koppenhöfer (1967). According to Frankenhaeuser (1962) the output voltage of the clamp amplifier was multiplied by $1/(14 \Omega \text{ cm}^2)$ giving the current density in mA/cm^2 . Between the test pulses the membrane potential, E , was kept at a value, where h_{∞} the availability of the sodium system, was 0.7–0.8. Potentials are usually given as deviations, V , from the resting potential, E_r , thus $V = E - E_r$. For calculations E_r was assumed to be -70 mV (Dodge and Frankenhaeuser, 1958). Depolarization and outward current are positive.

The normal Ringer solution contained (in mM): 110 NaCl, 2.5 KCl, 2 CaCl_2 and 5 Tris-HCl buffer, pH 7.0–7.2. In the test solutions 2 CaCl_2 was replaced by 10 CaCl_2 , 0.1 LaCl_3 , 0.5 LaCl_3 or 0.1 TbCl_3 . In some experiments 5 mM tetraethylammonium chloride (TEA) was added to block the potassium currents. Junction potentials were measured to be smaller than 0.5 mV. The temperature was 19 or 8°C.

The Na permeability, P_{Na} , was obtained from the Na current, I_{Na} , by applying the constant-field equation (Goldman, 1943; Hodgkin and Katz, 1949):

$$I_{\text{Na}} = P_{\text{Na}} \frac{F^2 E}{R T} [\text{Na}]_0 \frac{\exp [(E - E_{\text{Na}}) F/RT] - 1}{\exp (EF/RT) - 1} . \quad (1)$$

$[\text{Na}]_0$ is the external Na concentration and E_{Na} is the Na equilibrium potential; F , R and T have their usual meaning. According to Hodgkin and Huxley (1952b)

$$P_{\text{Na}} = \bar{P}_{\text{Na}} m^2 h, \quad (2)$$

where \bar{P}_{Na} is a permeability constant and m and h vary between 0 and 1, respectively.

When the nodal membrane is depolarized by $V > 30$ mV, the time course of P_{Na} is given by

$$P_{\text{Na}} = P'_{\text{Na}} [1 - \exp(-t/\tau_m)]^2 \exp(-t/\tau_h), \quad (3)$$

where $P'_{\text{Na}} = \bar{P}_{\text{Na}} m_{\infty}^2 h_0$, τ_m and τ_h are the time constants of the activation and the inactivation, respectively, and the subscripts 0 and ∞ apply to the beginning and the steady state after a potential change.

The total currents were corrected for capacity and leakage current and plotted on a semi-logarithmic paper versus time after beginning of the clamp pulse. In these plots the current during the beginning of the falling phase can be fitted by a straight line. By extrapolation to $t = 0$ the maximum Na current, I'_{Na} , was obtained, which the Na current would have reached if the inactivation would stay at h_0 . A constant prepulse of about -20 mV lasting 50 msec brought h_0 to unity. P'_{Na} was calculated from I'_{Na} by means of Eqn. (1) and m_{∞} was obtained by

$$m_{\infty} = (P'_{\text{Na}}/\bar{P}_{\text{Na}})^{1/2}. \quad (4)$$

The inactivation of the Na permeability was studied with a constant test pulse to elicit a larger or smaller Na current, I_{Na} , depending on a 50 msec hyper- or depolarizing prepulse of varying amplitude. From I_{Na} the availability of the Na system in the steady state, h_{∞} , was calculated as a fraction of the maximum Na current, $I_{\text{Na max}}$, obtained after prepulses $V = -20$ to -40 mV; thus $h_{\infty} = I_{\text{Na}}/I_{\text{Na max}}$.

Results

Usually an experiment started in normal Ringer solution with constant test pulses preceded by de- or hyperpolarizing pulses to measure the inactivation curve of the Na system. Then a run was taken with depolarizing pulses of varying amplitude, V , preceded by a 50 msec conditioning pulse of about -20 mV. From these records current-voltage curves and activation curves of the Na and K system were obtained. Finally the leakage conductance was measured with hyperpolarizing pulses. Each test solution was followed by normal Ringer solution to check for reversibility.

Current-Voltage Curves. In Fig. 1A voltage-clamp records in normal Ringer solution with 2 mM Ca^{2+} are shown. A hyperpolarizing pulse of -51 mV produced a short capacity current and a small leakage current. With a depolarization of $V = 33$ mV the current following the capacity transient turned inward rather quickly and then decayed more slowly. At $V = 62$ mV the inward current reached a maximum of -20 mA/cm² within less than 0.2 msec. With an 89 mV depolarization the current was much smaller and at $V = 130$ mV the current was outward during the whole pulse. The records in Fig. 1B were taken in a test solution with 0.5 mM La^{3+} . The recording sensitivity had been increased to show the smaller currents. A much stronger depolarization was necessary to get an inward current which reached its peak value later than in normal Ringer solution. In C the node was in 10 mM Ca^{2+} . At $V = 33$ mV the inward current was still missing and at 62 mV it was smaller than in

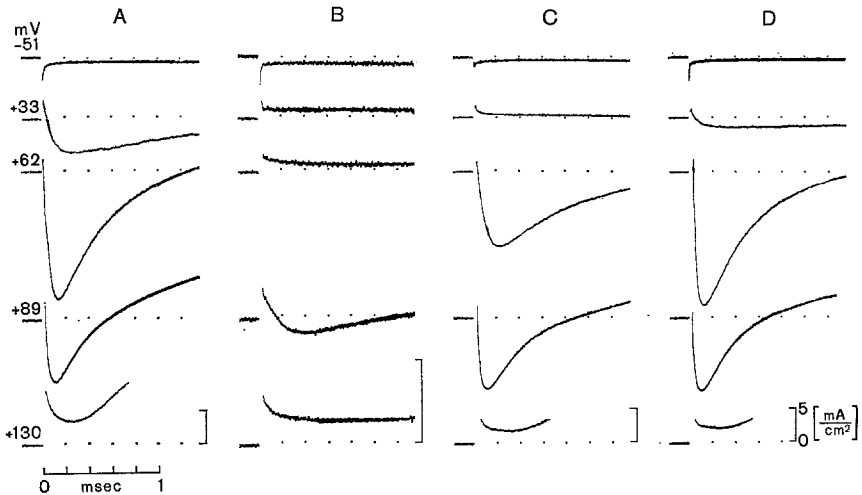


Fig. 1. Voltage-clamp records in Ringer solutions with 2 mM Ca^{2+} (A), 0.5 mM La^{3+} (B), 10 mM Ca^{2+} (C) and 2 mM Ca^{2+} (D). The numbers attached to the left records give the deviations of the rectangular pulses, V , in mV from the resting potential and apply to the four records of each row. A 50 msec conditioning pulse of -21 mV preceded the test pulses except those of the first row. The vertical bars give 5 mA/cm²; notice the different sensitivity in B. Inward currents downward. Node 21. Temperature 19°C

2 mM Ca^{2+} . At $V = 130$ mV the currents were outward in all solutions. Records *D* are taken after returning to normal Ringer solution. They demonstrate a good reversibility of the effects seen in the test solutions.

In Fig. 2 peak values of the early currents of the experiment in Fig. 1 corrected for leakage and capacity currents are plotted as a function of the depolarization, V . The measuring points in 2 mM Ca^{2+} show the well-known Na current-voltage curve beginning with a steep part of negative resistance followed by a decrease of inward current with increasing depolarization and becoming outward at the Na equilibrium potential near $V = 120$ mV. The negative resistance branch is shifted in positive direction on the potential axis by about 9 mV in 0.1 mM La^{3+} (filled circles), 20 mV in 10 mM Ca^{2+} (filled squares) and 45 mV in 0.5 mM La^{3+} (filled triangles). The reversibility of these shifts was realized in control runs following each test run. They are omitted in Fig. 1 and 2 except the last run. The maximum inward current was reduced to 92% in 0.1 mM La^{3+} , to 73% in 10 mM Ca^{2+} and to 8% in 0.5 mM La^{3+} . In most experiments the Na equilibrium potential remained practically unchanged. In summary the experiments underlying Fig. 1 and 2 suggest that rather low La concentrations cause strong Ca effects.

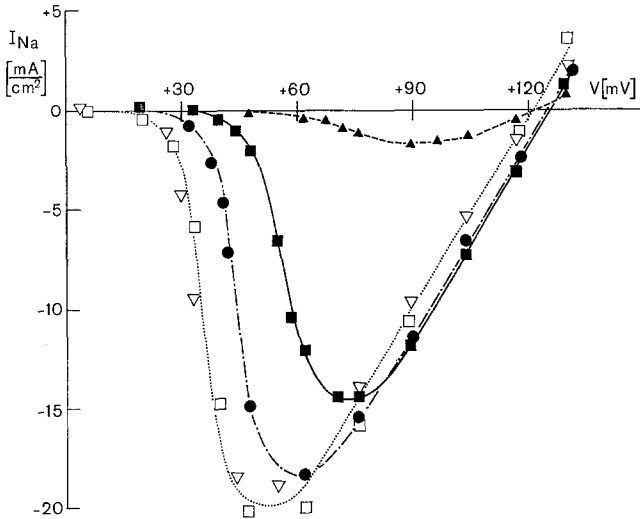


Fig. 2. Current-voltage curve of the peak sodium currents. Ordinate, sodium current density in mA/cm². Abscissa, deviation of the membrane potential, V , in mV from the resting potential. Normal Ringer solution with 2 mM Ca²⁺ at the beginning (\square) and the end (∇) of the experiment; 0.5 mM La³⁺ (\blacktriangle); 10 mM Ca²⁺ (\blacksquare); 0.1 mM La³⁺ (\bullet). Reference solutions between test solutions are omitted for clarity. The curves are drawn by eye. The same experiment as in Fig. 1

Activation and Inactivation Curves. In order to get more quantitative data the parameters of activation, m , and availability, h , of the Na permeability have been analysed.

In Fig. 3 values of m_{∞} are given as a function of V . With increasing depolarization m_{∞} increases in an s-shaped manner. In this experiment the curve was shifted by $\Delta V_m = 14$ mV when the solution was changed to 10 mM Ca²⁺ (filled squares) exhibiting the well-known stabilizing Ca effect: stronger pulses are necessary to activate the Na permeability to the same amount. In 0.5 mM La³⁺ (filled triangles) the shift of the m_{∞} curve was even 29 mV.

Inactivation curves of the Na system are shown in Fig. 4. Between the pulses the membrane was kept at a potential, where about 20% of the Na permeability are inactivated ($h_{\infty} = 0.8$, see Methods). After pulses $V \geq +40$ mV the inactivation was complete. The curve drawn through the measuring points in 2 mM Ca²⁺ was calculated from Eqn. (1) of Hodgkin and Huxley (1952a). The constant, k_h , determining the steepness of the curve was 7.5 mV in normal Ringer solution. This agrees well with the results of Frankenhaeuser (1959). But in contrast to the observations of Frankenhaeuser and Hodgkin (1957) on squid axons k_h did not change in the test solutions. In 10 mM Ca²⁺ (filled squares) the in-

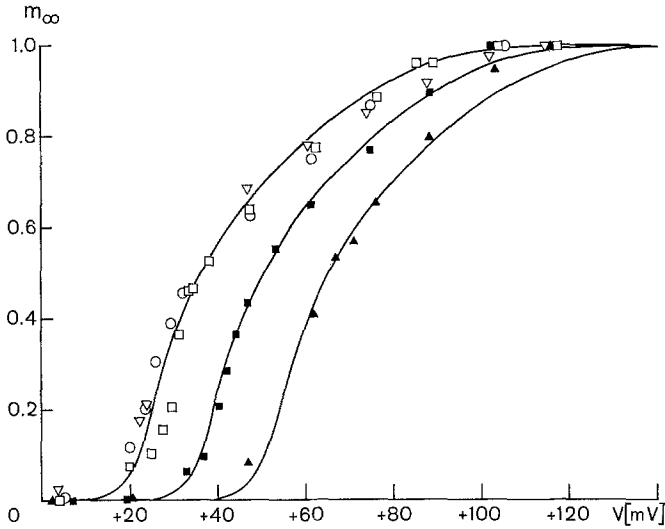


Fig.3. Activation curve of the Na system. Sequence of solutions: 2 mM Ca^{2+} (\square), 10 mM Ca^{2+} (\blacksquare), 2 mM Ca^{2+} (\circ), 0.5 mM La^{3+} (\blacktriangle) and 2 mM Ca^{2+} (∇). The left curve is drawn by eye through the measuring points in 2 mM Ca^{2+} and shifted by $\Delta V_m = 14$ mV (10 mM Ca^{2+}) and 29 mV (0.5 mM La^{3+}). Node 24. Temperature 19°C

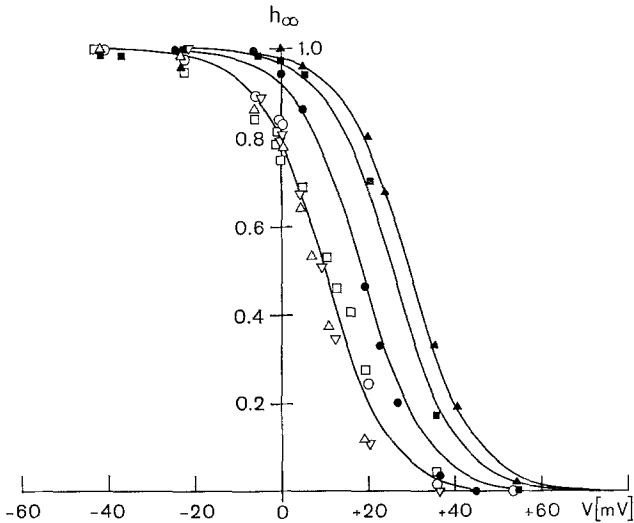


Fig.4. Inactivation curve of the Na system. Sequence of solutions: 2 mM Ca^{2+} (\square), 10 mM Ca^{2+} (\blacksquare), 2 mM Ca^{2+} (\circ), 0.1 mM La^{3+} (\bullet), 2 mM Ca^{2+} (\triangle), 0.5 mM La^{3+} (\blacktriangle), 2 mM Ca^{2+} (∇). The curve drawn through the measuring points in 2 mM Ca^{2+} was shifted by $\Delta V_h = 14$ mV (10 mM Ca^{2+}), 8.5 mV (0.1 mM La^{3+}) and 21.5 mV (0.5 mM La^{3+}). All solutions with 5 mM TEA. Node 35. Temperature 8°C

Table 1. Shifts of the activation and inactivation curves, reductions of maximum ionic permeabilities and of leakage conductance

	10 mM Ca ²⁺	0.1 mM La ³⁺	0.5 mM La ³⁺
ΔV_m [mV]	16.8 \pm 0.7 (8)	9.2 \pm 0.5 (5)	31.2 \pm 1.8 (5)
ΔV_h [mV]	13.1 \pm 0.5 (12)	8.1 \pm 0.5 (10)	21.2 \pm 1.3 (11)
ΔV_n [mV]	10.0 \pm 1.1 (4)	29.8 \pm 2.0 (4)	52.4 \pm 2.3 (6)
Reduction of \bar{P}_{Na} (%)	21.4 \pm 4.4 (7)	20.1 \pm 3.5 (3)	43.9 \pm 4.3 (7)
Reduction of P_{K^*} (%)	10.5 \pm 3.4 (3)	24.8 \pm 4.0 (3)	52.4 \pm 10.8 (4)
Reduction of g_L (%)	8.3 \pm 2.3 (9)	14.5 \pm 2.2 (9)	26.9 \pm 2.1 (8)

Mean values \pm S.E.M. (number of experiments). The reduction in % gives $-100 (X_{test} - X_{Ri})/X_{Ri}$, where X means \bar{P}_{Na} , P_{K^*} or g_L and the indices test and Ri denote test solution or normal Ringer solution.

activation curve was reversibly shifted by $\Delta V_h = 14$ mV, in 0.1 mM La³⁺ (filled circles) by 8.5 mV and in 0.5 mM La³⁺ (filled triangles) by 21.5 mV. After hyperpolarizations stronger than -40 mV I_{Na} was reduced, especially in the test solutions (see 10 mM Ca²⁺ in Fig. 4). A similar effect has been reported by Dubois and Bergman (1971) and can be seen in Fig. 8 of Frankenhaeuser and Hodgkin (1957).

Neither ΔV_m nor ΔV_h did change when the temperature was reduced from 19 to 8°C. In Table 1 mean values include experiments of both temperatures. La ions obviously exhibited a much stronger shifting effect than Ca ions both on the activation and on the inactivation curve. Moreover, ΔV_m was larger than ΔV_h in all test solutions.

Maximum Sodium Permeability. In addition to the voltage shifts produced by Ca and La I have studied the effects on the maximum Na permeability, \bar{P}_{Na} . If the permeabilities, P'_{Na} , are plotted against V , the potential of the test pulse, the curve saturates at larger depolarizations giving \bar{P}_{Na} . It was $4.4 \pm 0.4 \times 10^{-3}$ cm/sec (mean \pm S.E.M.) in 9 measurements which were done at the beginning of the experiments in normal Ringer solution. The agreement with Frankenhaeuser's data (1959) is good. It is assumed that in the test solution the saturation point of the $P'_{Na} - V$ curve is shifted nearly as much as the steep branch of this curve and thus \bar{P}_{Na} is reached with the applied pulses. Although the values scatter the reduction of \bar{P}_{Na} in the Ca-rich and in the La solutions was unequivocal as can be seen from the mean values in Table 1. Again

the La ions exhibited a strong Ca effect and the influence of temperature on the permeability reduction was rather small. But in contrast to the voltage shifts the reversibility of the permeability reduction was incomplete.

Potassium System. In analogy with the corresponding Eqn. (4) the steady-state activation term, n_∞ , of the K permeability, P_K , is given by

$$n_\infty = (P_K/P_K^*)^{1/3}, \quad (5)$$

where $P_K^* = k \bar{P}_K \cdot \bar{P}_K$ is the maximum K permeability. The availability of the K system, k , was close to unity and did not change in these experiments, as a 50 msec conditioning prepulse of about -20 mV preceded the test pulse which was ≤ 15 msec (Schwarz and Vogel, 1971). So P_K^* was a measure of the maximum K permeability. A shift of the inactivation curve of the K system in the test solutions as suggested for high Ca solutions by Moore (1971) has been neglected. It would cause a slight increase of P_K^* .

In practice the permeability has been calculated from the peak value of the K current by means of the corresponding constant-field equation (Frankenhaeuser, 1963). The saturating value of the $P_K - V$ curve was P_K^* ; n_∞ was determined by means of Eqn. (5). The shape of the n_∞ curve was similar to the m_∞ curve. In 4 experiments the n_∞ curve was shifted by 10 mV in positive direction on the potential axis when the external Ca concentration was increased by a factor of 5. As the values in Table 1 indicate, the corresponding shift induced by a 5-fold increase in the La concentration was 22.6 mV.

P_K^* was $0.61 \pm 0.07 \times 10^{-3}$ cm/sec at the beginning of 6 experiments in normal Ringer solution. This is 50% of the value given by Frankenhaeuser and Huxley (1964). In 10 mM Ca^{2+} P_K^* was reduced by 10.5%. The reversibility was incomplete. As can be seen from Table 1, the reduction in the La solutions was much larger.

Leakage Conductance. Under the assumption that the leakage current, I_L , is a linear function of V , the leakage conductance, g_L , was determined as $g_L = I_L/V$. The measurements were done with hyperpolarizing pulses as shown in the first row of Fig. 1 but at higher recording sensitivity. In 10 experiments the mean leakage conductance was $12.7 \pm 0.6 \times 10^{-3} \Omega^{-1} \text{ cm}^{-2}$ in normal Ringer solution before the fibres had been in a test solution. The value of Frankenhaeuser and Huxley (1964) is 2.4 times larger. As can be seen from Table 1, the reduction in g_L was larger in both La solutions than in 10 mM Ca^{2+} . The reversibility was only partial. No effect of a temperature change was detected on the reduction of g_L .

Terbium as a Calcium Substitute. At the nodal membrane Tb^{3+} exerts an even stronger Ca effect than La^{3+} : In 0.1 mM Tb^{3+} ΔV_m was 15 and 17 mV, ΔV_h was 11.1 ± 0.7 mV ($n = 5$), \bar{P}_{Na} was reduced by 34%

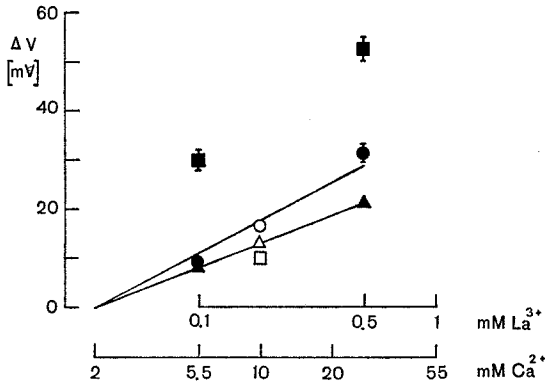


Fig. 5. Shifts of the activation and inactivation curves as dependent on the concentrations of Ca^{2+} and La^{3+} , respectively. Ordinate, mean value of shift in mV. Abscissa, ion concentration on logarithmic scale; the La scale has been displaced by a factor of 55 relative to the Ca scale. ΔV_m , circles; ΔV_n , triangles; ΔV_n , squares; open symbols, 10 mM Ca^{2+} ; filled symbols, 0.1 and 0.5 mM La^{3+} . 2 S.E.M. are given as vertical bars, when larger than corresponding symbol. The slope is 11 mV (m_∞) and 8 mV (h_∞) for an e -fold change in concentration

($n = 1$) and the leakage conductance was reduced by $13.3 \pm 3.6\%$ ($n = 4$).

Equivalence of Calcium and Lanthanum. In Fig. 5 the mean shifts of the activation and inactivation curves are plotted against the concentrations of Ca and La ions on logarithmic scales. Under the assumption that 0.1 mM La^{3+} is equivalent to 5.5 mM Ca^{2+} the shifts of the h_∞ curves (triangles) can be fitted by a straight line with a slope of 8 mV per e -fold change in concentration which corresponds to 19 mV per 10-fold change in concentration. The relative shifts of the m_∞ curves in the La solutions (filled circles) were somewhat larger than in Ca^{2+} (open circle). The common slope is 11 mV per e -fold (= 25 mV per 10-fold) change in concentration. This result suggests that the activation of the Na permeability is more strongly affected by external cations than the inactivation. With respect to ΔV_n , La^{3+} (filled squares) is disproportionately more effective than Ca^{2+} (open square) and no common function could be fitted.

Surface Charges. Gilbert and Ehrenstein (1969) have attributed shifts of potential dependent permeability parameters to screening of negative fixed charges at the membrane surface by cations of the bulk solution. McLaughlin *et al.* (1971) presented an equation according to Grahame (1947), which gives an approximation of the density of surface charges, σ , in negative fixed charge units per square Å as related to the surface

potential, ψ_0 , the concentration, c_i , and valence, z_i , of the i -th ionic species:

$$\sigma = \frac{1}{272} \left\{ \sum_i c_i \left[\exp \left(\frac{z_i F \psi_0}{R T} \right) - 1 \right] \right\}^{1/2}. \quad (8)$$

From this equation values of σ and corresponding ψ_0 have been calculated for each of the applied solutions and plotted as curves in a ψ_0 — σ diagram. Assuming that the experimentally observed ΔV reflects a change of the surface potential, $\Delta\psi_0$, and if binding is excluded, σ can be found from the corresponding curves. A mean charge density of approximately $1/70 \text{ \AA}^2$ is found for the ΔV_h and ΔV_m values of Table 1 except ΔV_m in 0.5 mM La^{3+} with a larger charge density. Allowing for binding would probably not improve the fit although the different shifting effects of the two trivalent cations La^{3+} and Tb^{3+} point to other processes than pure electrostatic screening. At any rate, experiments with more cation species, di- or trivalent, are clearly required to settle this point. ΔV_n in 10 mM Ca^{2+} leads to a lower charge density of about $1/200 \text{ \AA}^2$.

Discussion

The experiments presented in this paper have shown that an increase of the external Ca concentration and substitution of Ca by La reduced both the conductance of the resting membrane and the permeabilities \bar{P}_{Na} and P_{K}^* . The shifts of the m_∞ and h_∞ curves suggest that in the applied concentration range 0.1 mM La^{3+} is equivalent to about 5.5 mM Ca^{2+} . Under the assumption that the voltage shifts are caused by screening effects a negative fixed charge density of approximately $1/70 \text{ \AA}^2$ was found near the Na channel.

Voltage Shifts. The shifts of the m_∞ and h_∞ curves were 11 and 8 mV, respectively, per e -fold change of Ca concentration. Hille (1968) found 8.7 and 6.5 mV at the nodal membrane of *Rana pipiens*. Frankenhaeuser and Hodgkin (1957) observed the same effect at the squid giant axon: the activation is more strongly affected by external concentration changes than the inactivation of the Na system. This seems to imply that the activation gates (m) "see" more of the change in surface potential than the inactivation gates (h), possibly because they are located at different positions along the sodium channel. Recent experiments in which the inactivation was selectively impaired (Armstrong, Bezanilla and Rojas, 1973; Stämpfli, 1974) seem to confirm this notion. Changing the internal concentration of cations in perfused squid axons Chandler, Hodgkin and Meves (1965) found larger potential shifts in the inactivation than in the sodium conductance curve which is in favour of the idea mentioned above. But in similar experiments Moore, Narahashi

and Ulbricht (1964) found approximately equal shifts of the activation and inactivation curves.

The 6 mV shift of the n_{∞} curve per e -fold Ca concentration change is in agreement with the results of Brismar and Frankenhaeuser (1972) and Frankenhaeuser and Hodgkin (1957). Hille (1968) found a smaller shift of the n_{∞} curve at *Rana* nerve fibres. In the La solutions the shifts of the n_{∞} curves were much larger. This is in accordance with the finding of Mozhayeva and Naumov (1973) for the nodal membrane and of Takata *et al.* (1966) for lobster axons.

Reduction of the Permeability. An increase of Ca or La concentration reduced the maximum Na permeability. Similar results are known from the lobster axon (Takata *et al.*, 1966) and from the nodal membrane (Hille, 1968). In contrast to these observations no change of the maximum Na permeability was found in the squid axon by Frankenhaeuser and Hodgkin (1957). A reduction of the maximum K permeability as seen in the present experiments has been reported by Gilbert and Ehrenstein (1969) for squid axons. The experiments of Moore (1971) show an increase of both the maximum Na and K permeability with increasing Ca concentration. This might be due to the fact that especially in low Ca the inactivation was not fully removed (see Frankenhaeuser, 1957).

Cole (1949) was the first to report that increasing the Ca concentration decreases the leakage conductance. Similar observations have since been made (Frankenhaeuser and Hodgkin, 1957; Ulbricht, 1964; Machne and Orozco, 1970; Hartz and Ulbricht, 1973). Takata *et al.* (1966) observed a reduction of g_L in lobster axons washed in a La solution.

An interpretation of the mechanism underlying these reductions is difficult. Following the screening hypothesis McLaughlin *et al.* (1971) pointed out that divalent cations should "reduce the concentration of permeant monovalent ions at the outer membrane-solution interface, and therefore decrease the magnitude of the conductance". Bergman and Dubois (1972) have reported that when increasing the external Ca concentration the K inward currents were diminished but the K outward currents were not. On the other hand Meves and Vogel (1973) observed that the Ca conductance of the TTX-sensitive Ca channel was reduced at perfused squid giant axons, when the external Ca concentration was increased. We also have to keep in mind that at the potential range where \bar{P}_{Na} and P_K^* are measured the currents are directed outward and can hardly be diminished by a shortage of the corresponding current carriers in the external solution. For the time being the idea cannot be excluded that the long-lasting but electrically invisible hyperpolarization which is induced in high Ca or La solutions reduces the maximum perme-

ability by the same mechanism as strong hyperpolarizing prepulses do (see Fig. 4).

Ca-like stabilizing effects can be induced by a change of pH (Hille, 1968; Drouin and The, 1969; Mozhayeva and Naumov, 1970; Woodhull, 1973). Protonation of negative fixed charges of the membrane and the concomitant change in surface potential seem to be responsible for the curve shifts. Decrease of the maximum Na permeability is thought to be due to voltage-dependent binding of H ions to a site in the Na channel (Woodhull, 1973). Possibly Ca and La ions bind to these sites thereby reducing the fraction of permeable channels.

Negative Surface Charges. The Gouy-Chapman theory has often been used to calculate fixed charge densities for biological and artificial membranes (Chandler *et al.*, 1965; Gilbert and Ehrenstein, 1969; Mozhayeva and Naumov, 1970; McLaughlin *et al.*, 1971; Brismar, 1973; d'Arrigo, 1973). Originally, the Grahame equation is based on the assumptions of a constant value of the dielectric constant, point charges of the ions and a plane membrane surface. For a biological membrane in contact with concentrated salt solutions these conditions do not hold (Stern, 1924; Brodowsky and Strehlow, 1959; Haydon, 1964), but possibly, the errors may cancel one another out as can be seen from the results of McLaughlin *et al.* (1971).

Additionally, two errors of the voltage measurement have to be considered: 1. Voltage attenuation (Dodge and Frankenhaeuser, 1958) has been determined using the method of Hille (1971) and amounted to 5% ($n = 6$). 2. As the current amplitude is reduced in the test solutions the potential drop at the resistance of the external solution in series with the membrane (Dodge, 1963) will change. On the basis of the data given by Dodge the shifts in Fig. 3 and Fig. 4 are measured too large by maximally 1.5 mV. Thus the charge densities calculated from the voltage shifts by means of the Grahame equation have to be regarded as an approximation which may lie at the high side.

In the vicinity of the sodium channel a mean charge density of 1 electronic charge per 70 \AA^2 is found. For crayfish axons d'Arrigo (1973) calculated $1/43 \text{ \AA}^2$. For the K channel my measurements in the Ca solutions lead to $1/200 \text{ \AA}^2$ and other results from the nodal membrane of frog nerve are near $1/300$ (Brismar, 1973) and $1/600 \text{ \AA}^2$ (Mozhayeva and Naumov, 1970); Gilbert and Ehrenstein (1969) have found $1/120 \text{ \AA}^2$ for squid axons. These numbers suggest that the charge density near the Na channel is higher than near the K channel.

As the charge densities deduced from ΔV_m and ΔV_n in the 0.5 mM La^{3+} solution are higher than those estimated from the other test solutions, electrostatic screening does not seem to be the only process involved in the effect of La ions. The possibility of additional binding has already

been pointed out on p. 34 and differences in binding may explain why, of the tested divalent cations, Mg has a weaker and Ni, Co and Cd have a stronger effect than Ca (Frankenhaeuser and Meves, 1958; Blaustein and Goldman, 1968; Brismar, 1973). On the other hand the strong effect of the trivalent La ion as compared to the divalent Ca ion or monovalent ions (see Brismar, 1973) is more easily explained by screening.

La has been termed "supercalcium" by Takata *et al.* (1966) implying only quantitative differences between the two cations. This is true for the effects on the Na permeability but neither the effects on the leakage conductance nor those on the K permeability can easily be fitted into this picture; additional "pharmacological" actions of La^{3+} in concentrations above 0.1 mM must hence be postulated. In spite of these corrections the disproportionate potency of trivalent cations relative to divalent ones, as borne out by the present results, is in favour of a screening mechanism of stabilization.

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