Modification of Sodium Channel Gating by Lanthanum

Some Effects That Cannot Be Explained by Surface Charge Theory

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ABSTRACT In clonal pituitary (GH3) cells we studied the changes in sodium channel gating caused by substitution of La^{3+} for Ca^{2+} ion. Gating of sodium channels was simplified by using intracellular papain to remove inactivation. To quantify La effects, we empirically fitted closing and the late phase of opening of the channels with single exponentials, determined the opening (*a*) and closing (*b*) rate, and plotted these rates as a function of V_m (membrane voltage). The midpoint of the fraction open–V_m curve was also determined. Changing from Ca to La shifted the curves for these three measures of Na channel gating along the voltage axis and changed their shape somewhat. Surface charge theory, in the form usually presented, predicts equal shifts of all three curves, with no change in shape. We found, however, that the shift for each of the measurements was different. 2 mM La, for example, shifted opening kinetics by +52 mV (i.e., 52 mV must be added to the depolarization to make activation in 2 mM La as fast as in 2 mM Ca), the fraction open voltage curve by +42.5 mV, and the closing rate curve by +28 mV. The shift was an almost linear function of log [La] for each of the measures. The main finding is that changing from 2 mM Ca to 10 μM La causes a positive shift of the opening rate and fraction open curves, but a negative shift of the closing rate curve. The opposite signs of the two effects cannot be explained in terms of surface charge theory. We briefly discuss some alternatives to this theory.

INTRODUCTION

Divalent and trivalent cations have strong effects on the gating properties of voltage-dependent ionic channels. These effects are usually explained in terms of surface charge theory, which originated from a suggestion by A. F. Huxley (Frankenhaeuser and Hodgkin, 1957). According to this theory (see Hille, 1984, for a clear

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Volume 96 December 1990 1129–1140

exposition), di- or trivalent cations bind to specific sites at the outer surface of the membrane, attracted, in one version of the theory, by fixed negative charges distributed on the membrane surface. The adsorption of Ca, for example, causes a local change in the membrane field without (directly) altering the membrane potential measured by electrodes in the bulk solution. Since the gating apparatus of the channels is confined to the membrane, it cannot distinguish an electric field change caused by altered membrane voltage from one caused by a change of the cation composition.

The theory is attractive, and, in many respects, successful. It is worth remembering, however, that there is no independent evidence for negative fixed charges located appropriately near the gating apparatus of the channel. Although a large number of experiments involving many ionic species have been performed, it remains true that such charges are inferred from the results of a single type of experiment, variation of ionic composition and observation of the effects on gating properties.

A prediction of the theory, in its uniform surface charge version (the version usually considered) is that all aspects of channel gating should be affected equally. That is, the curves relating opening kinetics, closing kinetics, and the fraction of open channels to V_m should all be shifted along the voltage axis by equal amounts. Raising the divalent cation concentration in the medium, for example, should shift all of the curves equally to the right.

We report here on some curious effects of La on the gating properties of mammalian Na channels. La is a very potent modulator of gating activity (Takata et al., 1966; Vogel, 1974; Hille et al., 1975), and has been called a "supercalcium" (Takata et al., 1966). Its actions on channels are usually attributed to a surface charge effect (Arhem, 1980; Brismar, 1980). In the course of our experiments we found a case in which the shifts of the gating parameters are not only unequal, but even of opposite sign. This finding cannot be explained by surface charge theory, and suggests that a new theory of di- and trivalent cation action is needed. We offer some tentative suggestions.

METHODS

Na currents were recorded from GH3 cells (a clonal line of rat pituitary adenoma obtained from American Type Culture Collection, Rockville, MD) using a fast whole-cell voltage clamp with low resistance patch pipettes. The culturing and electrical recording procedures were as described previously (Cota and Armstrong, 1989). The internal recording solution (see below), contained in the patch pipette, was supplemented with papain (1 mg/ml) to remove fast inactivation of Na channels (Cota and Armstrong, 1989). Current records were taken within 20 min after the enzyme action.

The composition of the external recording solutions is given in Table I. External solutions with La concentrations ranging from 1 to 2 mM were prepared by mixing the 0 Ca (no added Ca) and 4 mM La solutions. The composition of the internal solution was (in mM): 100 NaF, 30 NaCl, 1 $CaCl_2$, 10 EGTA, and 10 HEPES. EGTA and HEPES were neutralized (to pH 7.3) with CsOH.

The high internal Na^+ concentration keeps inward Na current small and therefore minimizes the voltage error associated with the access resistance. This error did not exceed 5

TABLE I

External Recording Solutions

Solution	Na*	Ca	La	Cl	HEPES‡
2 mM Ca	155	2	—	154	10
4 mM La	151	—	4	158	10
0 Ca	158	—	—	153	10

*Concentrations are in mM.

‡The pH of the solutions was adjusted to 7.3 with NaOH, yielding the total Na concentration given.

mV in most experiments. A disadvantage is that current is small near 0 mV, which in some cases is in the middle of the activation range of Na channels.

For all experiments the holding potential was −80 mV and the temperature was 15°C.

RESULTS

Even very low concentrations of La slow and depress the opening of Na channels. Fig. 1 compares opening in 2 mM Ca and in two concentrations of La. Inactivation of the channels had been previously removed by internal papain, making it possible to study activation (opening) in isolation. The upper left panel shows I_{Na} (Na current) traces recorded at −30 mV, first in 2 mM Ca and then in 10 μM La (the La solutions had no added Ca). The current activates during the 10-ms pulse to −30 mV, and is

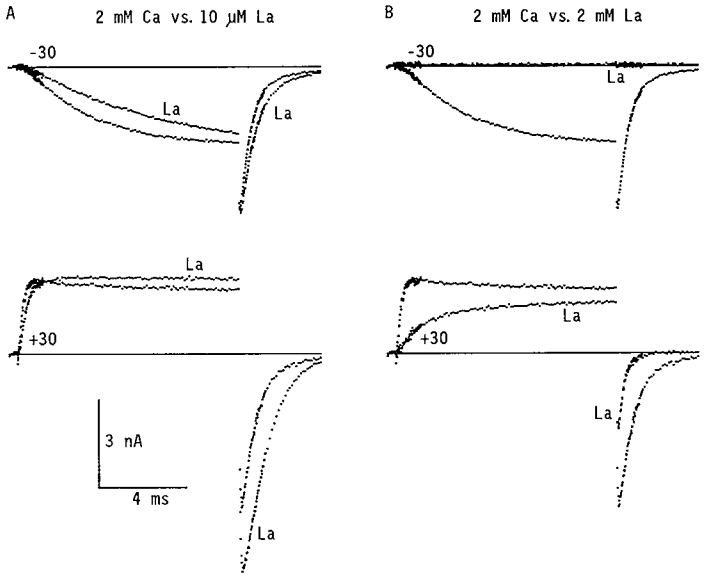

FIGURE 1. Opening and closing of Na channels in Ca and La: I_{Na} was recorded from papain-treated GH3 cells, and is shown for steps from −80 to −30 and +30 mV. E_{Na} was −10 mV. (*A*) Substituting 10 μM La for 2 mM Ca slowed opening at −30 and +30 mV, and slowed closing at −80 mV. (*B*) I_{Na} at −30 mV was completely suppressed by 2 mM La, and strongly slowed in its development at +30 mV. On stepping back to −80 mV, the current tail was smaller than expected, and fast. Experiment Se078G#2. Electrode resistance 0.6 MΩ.

inward. On stepping back to −80 mV (the holding potential) the current magnitude jumps because of the increased driving force for Na^+ ions, and then decays as the channel gates close, with a time course that can be approximated by a single exponential.

Current activation is distinctly slower in 10 μM La, and the final level of current at the end of the step is smaller. There are two significant points regarding the current tails. First, although current at pulse end is smaller in La, the initial amplitude of the tails in the two solutions is the same. As described below, this is because 10 μM La blocks the channels less than does 2 mM Ca. Second, the tail in La is a bit slower. Thus changing from 2 mM Ca to 10 μM La slows both activation and deactivation of the channels. This is the major finding in the paper.

At +30 mV current is outward (E_{Na} is near zero; see Methods). Activation of the channels is much faster than at −30 mV, as expected, but is still significantly slower in La than in Ca. Final current amplitude is slightly higher in La. On stepping back to −80 mV, the current tail in La is bigger than in Ca, and its time course is slower. The ratio of the tail amplitudes is larger than expected from the relative amplitudes at pulse end, again because there is less channel blocking in 10 μM La.

2 mM La suppresses all channel opening at −30 mV (Fig. 1 *B*). At +30 mV the channels activate very slowly, and the final level of current is smaller. On stepping to −80 mV the initial tail current is reduced, and current decays more rapidly than in 2 mM Ca. Thus 2 mM La slows opening kinetics and speeds closing. The tail in La is smaller than expected from the current at the end of the activating pulse because 2 mM La has a greater tendency to block Na channels than does 2 mM Ca (see below).

La Shifts the Activation–Voltage Curve to the Right

Perhaps the most familiar finding when increasing divalent concentration is a shift of the channel activation, or fraction open, curve to the right along the voltage axis. In terms of surface charge, this is said to result from a change of the membrane field, caused by binding or accumulation of the di- or trivalent cation near the outer membrane surface. La exerts this effect very strongly, as shown in Fig. 2. The curves in the figure, which we will call the 'open' or fraction open curves, are plots of the initial amplitude of the tail current (at −80 mV) as a function of *V*m during the 10-ms activating pulse that preceded the tail measurement. The activating voltage is given on the abscissa. Tail current amplitude is directly proportional to the number of channels that are open at the end of the activating pulse. For example, the tail after a pulse to +40 mV, which opens almost all of the channels, is larger than the one after a pulse to −40 mV, which opens few channels. The absolute amplitude of the tail is affected by blocking of the channels by Ca or La as described below. Nonetheless, the proportionality between tail amplitude and the number of open channels holds if the voltage is always returned to the same level (−80 mV).

In almost all cases 'open' curves saturate as voltage is increased, and in some cases decline slightly above +20 or 30 mV. All curves have been normalized relative to their maximum amplitude. The tail method is the most reliable way to measure the open fraction, but results from another method, dividing the *I-V* curve by the open channel *I-V* curve (see below), gave almost identical results with regard to both the size of shifts and the steepness of the curves.

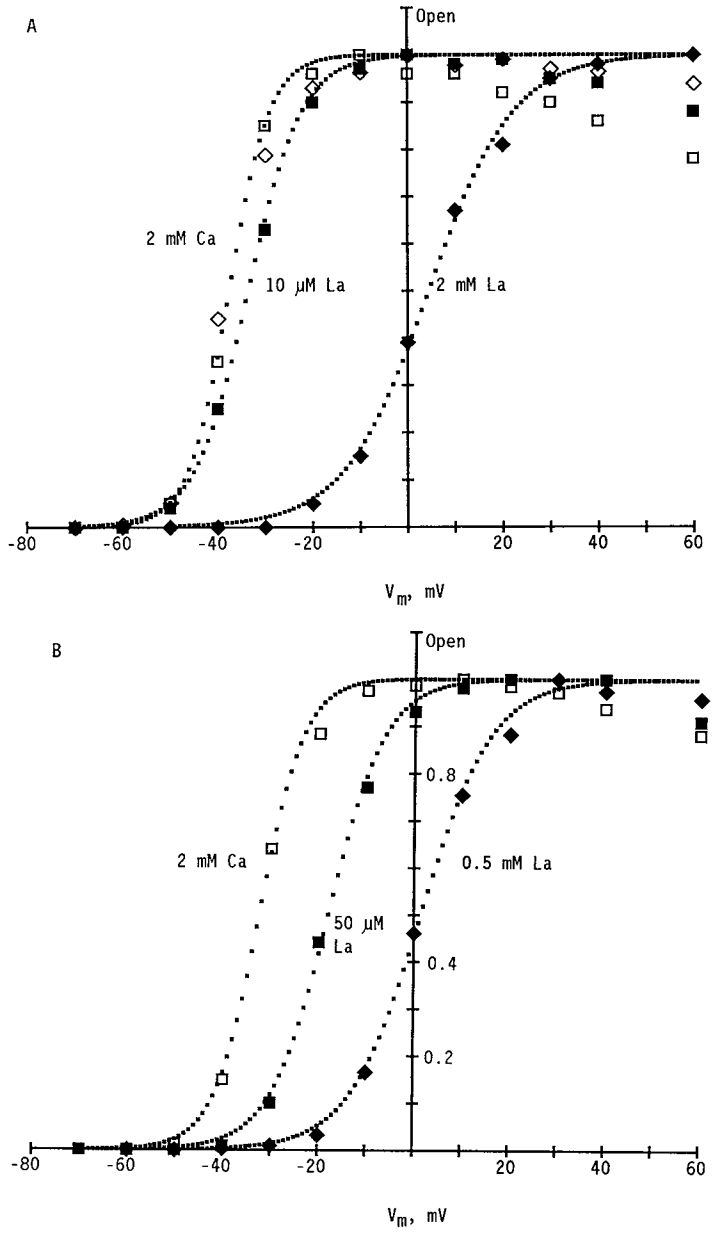

FIGURE 2. The fraction of open channels as a function of V_m. The plots give the normalized amplitude of the tail current after 10-ms pulses to the voltage on the abscissa. Tail amplitude is proportional to the number of open channels. The curves were normalized relative to the maximum amplitude in each case. The dotted lines are least-squares fits of the formula given in the text. (*A*) Curves were recorded in the order 2 mM Ca (□), 10 μM La, 2 mM La, and 2 mM Ca (◇). The fitted curve for the final 2 mM Ca determination is omitted for clarity. Experiment Se078G#2. (*B*) The 2 mM Ca points are the average of four very similar determinations, before and after 50 μM La, and before and after 0.5 mM La. Experiment Se068G#5 & #6.

Fig. 2 *A* shows an experiment in which recordings were made successively in 2 mM Ca, 10 μM La, 2 mM La, and 2 mM Ca. Relative to the two curves in 2 mM Ca, the 10 μM La curve is slightly right shifted and appears to be slightly less steep. The 2 mM La curve is strongly right shifted, and is distinctly less steep than the 2 mM Ca curves. The reversibility in this experiment, and in many others, was excellent, as can be seen from the near identity of the two curves in 2 mM Ca. In some cases the kinetic effects of La above 0.5 mM were not completely reversible.

Fig. 2 *B* is a plot of experiments in two cells for which the control determinations (before and after La exposure in the two cells) were so nearly identical that they have been averaged and are presented as a single curve. By comparison with Fig. 2 *A*, it is clear that the shift is related to the La concentration.

Empirical Quantitation of Shifts and Steepness

The steepness and midpoint of the activation curves were quantified empirically by a least-squares fitting of each curve to the formula

$$\text{open} = 1/(1 + \exp(-Ze(V - V_{1/2})/kT),$$

TABLE II

Midpoints and Steepness of Fitted Open – V_m curves

Solution	No. of observations	$V_{1/2}$ Ca	$V_{1/2}$ La	Shift	Z_{Ca}	Z_{La}
		mV	*mV*	*mV*	*e*	*e*
2 mM Ca	14	−34.0 ± 3.7*			5.4 ± 0.6	
5 μM La	1	−32	−33.5	−1.5	5.6	4.7
10 μM La	5	−35.4 ± 3.6[2]	−33.0 ± 4.3[2]	+2.2 ± 2.3[2]	5.5 ± 0.6[2]	4.6 ± 0.4[2]
50 μM La	1	−33.5	−18	+15.5	6	4.2
0.5 mM La	1	−31.8	+1.5	+33.3	5.3	3.4
2 mM La	1	−38	+4.5	+42.5	5.3	2.8
4 mM La	1	−29	+13.5	+42.5	4.8	3.5

*Average and standard deviation.

where Z (the steepness factor), $V_{1/2}$ (the voltage where the fraction open was half maximum), and the maximum fraction open were the three parameters of the fit. The dotted lines are the calculated curves. In general the fits are adequate, but in most cases the fitted curve is not sharp enough in the region where the channels are just beginning to activate. This suggests that Z is an underestimate of the charge movement required to open a channel.

The results from the fittings are given in Table II. In 2 mM Ca the midpoint on the average is −34.3 mV and the average valence is 5.5 e (electronic charges). As noted in the Discussion, the steepness in our papain-treated cells is much greater than in cells with inactivation intact.

In 5 μM La there is a small left shift of −1.5 mV. At all higher concentrations the shift is positive, and increases from 2 mV in 10 μM La to 42.5 mV in 2 and 4 mM La.

At all concentrations La caused a measurable drop in the steepness of the curves.

The apparent valence decreased from 5.4 e to 4.6 e on changing from 2 mM Ca to 10 μM La. The largest drop was seen with 2 mM La, from 5.3 e to 2.8 e.

Quantitation of the Kinetic Effects of La

La, even at 5 μM, slows channel activation and alters closing kinetics as measured by the current tails. An empirical measure of these effects was obtained by fitting a single exponential to the activation of the current at various voltages, and another exponential to decay of the current during the tail. The rate constants from these fits are plotted in Fig. 3 for 2 mM Ca, 10 μM La, and 2 mM La.

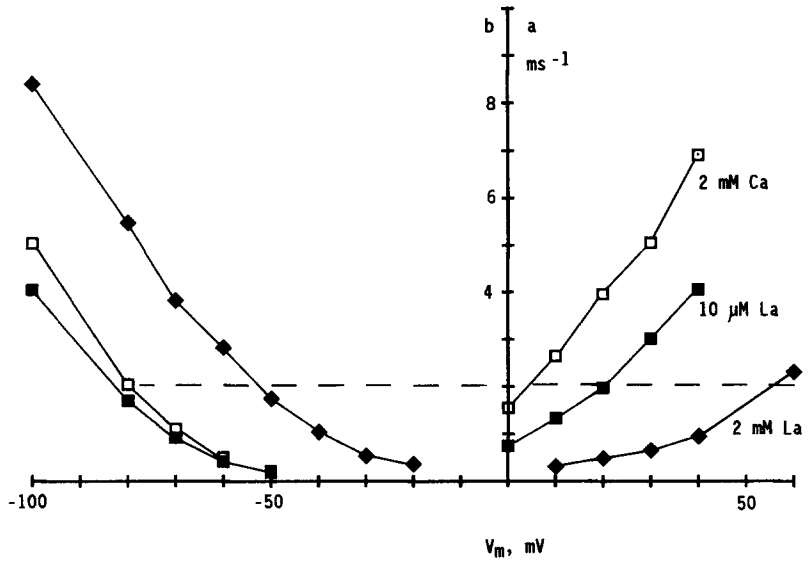

FIGURE 3. Opening (*a*) and closing (*b*) rates as a function of voltage. Opening rate at each voltage was empirically measured by fitting a single exponential, and the rate constant *a* is given for each solution. *b* is the similarly determined rate constant for closing. The shift described in the text and plotted in Fig. 4 is the horizontal displacement from the 2 mM Ca curve, measured along the dashed line. Experiment Se078G#2.

The *a* curves to the right of the origin give the rate constant of opening, which becomes larger as *V* goes in the positive direction. At any voltage, the rate is slower in 10 μM La than in 2 mM Ca, and much slower in 2 mM La. The curves cannot be made to superimpose by shifting along the voltage axis. Thus adding La does not seem to have effects that can be perfectly mimicked by changing the voltage.

The closing rates are given by the *b* curves to the left of the origin. Closing rate increases as voltage goes negative. The curve in 2 mM La is located 25–30 mV to the right of the one in 2 mM Ca. In 10 μM La closing rates were slower than in 2 mM Ca, and the La curve lies to the left of the one for Ca. The curves in La do not appear to have the same shape as the curve in 2 mM Ca.

Although the curves do not all have the same shape, it is nonetheless of interest to have a measure of their relative positions. The shifts of the La curves relative to the

2 mM Ca curve were measured along the dashed line. These shifts are plotted as a function of log [La] in Fig. 4 and are described in the next section. The shifts are clearly an empirical measure, and the line along which they are measured was selected arbitrarily. Study of Fig. 3 shows, however, that none of the conclusions of the next section would have been much altered by choosing a line at a different level.

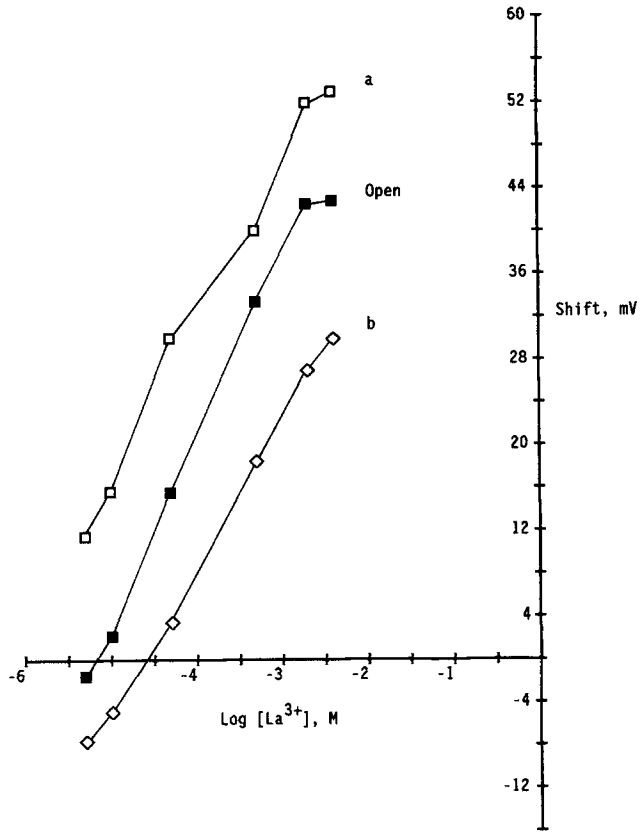

FIGURE 4. La-induced shifts of three measurable parameters of gating, opening rate (*a*), closing rate (*b*), and the fraction of open channels (*Open*), are plotted as a function of log [La]. The baseline (0 shift) is the value of each parameter in 2 mM Ca. Kinetic shifts were measured as illustrated in Fig. 3. The data points at 10^{-5} M La are the average of five determinations. The shift is different for each of the three parameters. Experiments Se068G#5, Se068H#6, Se078G#2, Mr179G#1, #5, #9.

Relative Shifts of Activation Parameters

A basic tenet of the uniform surface charge theory is that the gating apparatus of a channel cannot distinguish between addition of a di- or trivalent cation and a suitably chosen change of the membrane potential. Thus the gating parameters in theory are shifted along the voltage axis without change of shape by changing the concentration of the multivalent cation.

The three measures of the activation process were quantified as described in the preceding sections, and the shift of each as a function of log [La] is plotted in Fig. 4. It is immediately clear that the shifts of the three parameters are not equal. Most affected are the activation kinetics: at 2 mM La the shift is ~52 mV. The shift of the open–V curve is 42.5 mV, and the 28-mV shift of b, the closing rate, is substantially smaller.

At 5 and 10 μM La, not only are the shifts different in size, but the b shift is of the opposite sign. The regular descent of the curves from right to left makes it clear that this result is not an accident of measurement, but is the end point of a steady trend.

In summary, the shifts of the gating parameters are not equal, and in some cases not even of the same sign.

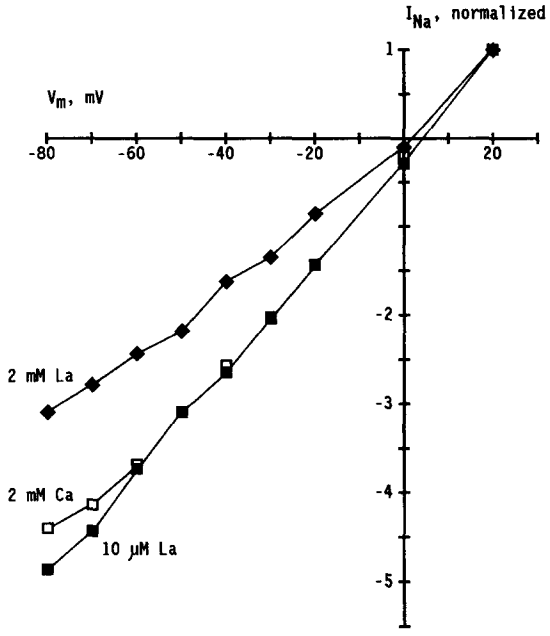

FIGURE 5. Open channel *I-V* curves in Ca and La. Channels were activated by depolarizing to +60 mV for 0.5 ms, and V_m was then stepped to a new value, given on the abscissa. The plotted current was obtained by fitting an exponential to the current trace at the new voltage and extrapolating back to the beginning of the step. The plots are normalized relative to the value of the current at +20 mV. At negative V_m, current in 2 mM La is smaller than in 2 mM Ca, because La blocks channels more efficiently. Block is slight in 10 μM La. Experiment Se078G#2.

Na Channel Blocking by Ca and La

It was noted in discussing Fig. 1 that the tail amplitudes were not always proportional to the current at the end of the pulse. An explanation for this apparent discrepancy has been in the literature for many years: the tendency of Ca ion (and presumably other ions as well) to block Na channels (Woodhull, 1973; Taylor et al., 1976; Yamamoto et al., 1984; Mozhayeva et al., 1985; Worley et al., 1986; Cukierman et al., 1988; Nilius, 1988). The degree of block is voltage dependent: block is more prominent at negative voltages, and, of course, at high concentrations of the blocking agent.

The best macroscopic measure of the blocking tendency is given by the open channel (or instantaneous) *I-V* curve. This curve is obtained by activating the channels with a large depolarization, and then, when most of them are open,

changing the voltage and measuring the current at the new voltage, ideally before any channel gates have closed. To improve time resolution, we extrapolated the current to the beginning of the step after fitting an exponential to the tail.

Open channel *I-V* curves are shown in Fig. 5 for 2 mM Ca and for La at 10 μM and 2mM. The curves were normalized relative to the current at +20 mV in each solution. The 2 mM Ca curve is slightly sublinear for voltage negative to about −30 mV. In lower [Ca] the curve is more nearly linear (not shown). The curvature in 2 mM Ca is due to Ca's blocking action. Specifically, a Ca ion enters the channel when the membrane field is negative, and, owing to binding or limited mobility in the channel, interferes with conduction until it finally emerges from the channel's inner end.

From the curves it seems that 2 mM La is more effective at blocking the channels than is 2 mM Ca, since the current is smaller at all negative voltages. This reflects either a superior ability of La to enter the channel, or a lower mobility/greater tendency to bind once it does enter, or both. For both ions at 2 mM, it seems likely that the blocking process reaches equilibrium in a time too short for us to resolve (see Discussion).

When [La] is 5 or 10 μM, the points lie on an almost straight line. Since there is a pronounced tendency for La to block (as in the 2 mM La curve), this can only be the result of the lower rate of entry of La into the channels at the lower concentration.

DISCUSSION

Several of the actions of La that are reported here cannot be explained by surface charge theory, particularly if the charges are assumed to be uniformly distributed.

1. The three measurable parameters of gating, activation rate, deactivation rate, and the fraction open–voltage relation, are not shifted equally along the V_m axis (Fig. 4), as the uniform surface charge theory predicts. This discrepancy may be resolvable by invoking a nonuniform charge distribution.
2. At low [La] the shifts of opening and closing kinetics are of opposite sign. This seems impossible to explain by any modification of the surface charge theory.
3. La causes a marked decrease in the steepness of the open–voltage curve, which is not explained by surface charge theory.
4. La, like Ca, blocks Na channels at negative V_m, an action that lies outside of the surface charge theory.
5. The shifts of the fraction open–V_m curve on changing to various La concentrations are of sizes that cannot be explained simply by screening of surface charge. We first calculated the surface charge density necessary to account for experimentally observed shifts of the fraction open–V_m curve when [Ca] was altered (data from two cells, with [Ca] from 0.2 to 50 mM), using the Grahame equation. Using this charge density (−1.5 elementary charges/nm^2, which is slightly higher than the largest density cited by Hille, 1984), the predicted shifts on changing from 2 mM Ca to 10 μM and 4 mM La were −6 mV (+2.4 mV observed) and +28.5 mV (+42.5 mV observed). Even larger discrepancies were found for the opening rate–V_m curve. These discrepancies could probably be partially resolved by postulating that the charged sites specifically bind La in preference to Ca. Hille

et al. (1975) invoked specific binding. Their extensive data necessitated a model with three types of surface charge, each with different binding affinities.

Of these points, one (2) is incompatible with surface charge theory in any form, two (3 and 4) are phenomena that lie outside of surface charge theory, and the other two (1 and 5) are quantitative discrepancies that may be within reach of amended surface charge theory.

A first question is the relevance of these findings to divalent cations, e.g., Ca. Do the La results suggest a general problem with surface charge theory? This question is, of course, hard to answer, but the following points can be made. First, previous authors (Vogel, 1974; Arhem, 1980; Brismar, 1980) have considered La to act by modifying surface charge. Brismar did note, however, that La reduced the steepness of the g-V curve, and pointed out that surface charge theory provides no explanation.

Second, and most important, most if not all of the actions of La are qualitatively similar to actions of Ca. Thus increasing the concentration of either ion slows opening, speeds closing, shifts the fraction open-V_m curve to the right, and causes voltage-dependent block of the channels.

Finally, it is worth remembering that uniform surface charge theory cannot explain some of the actions previously reported for divalent cations (see Hille, 1984). Zn^{2+}, an agent that is usually listed among surface charge modifiers, affects the opening kinetics of Na channels much more than the closing kinetics (Gilly and Armstrong, 1982). Ca (in the absence of extracellular K) causes a substantial slowing of opening kinetics and has almost no effect on closing kinetics (Armstrong and Matteson, 1986). These findings are similar to the unequal shifts seen with La. Similar discrepancies have been noted for the actions of protons on Na channel gating (Shrager, 1974; Schauf, 1983; Campbell and Hahin, 1984).

To our minds, a theory that explained all of the effects of multivalent cations, including blocking, steepness changes, and the oppositely directed shifts in low La, would clearly be preferable. No theory with these capabilities exists at present, but the following suggestions will be briefly noted. (*a*) During the opening and closing of Na and K channels, there are large transmembrane movements of gating charge. Multivalent ions may bind to gating charge (Gilly and Armstrong, 1982) rather than to sites on, for example, membrane phospholipids. (*b*) Ca binds in the lumen of closed K channels, and serves as a gating cofactor for these channels, which cannot close stably in low Ca (Armstrong and Lopez-Barneo, 1987). (*c*) Our major dissatisfaction with surface charge theory is that it does not account for the blocking effects of di- and trivalent ions. All of the multivalent cations we have tested on Na channels block the channels as well as shifting their gating behavior along the voltage axis. In this regard it is interesting to note that there is at least a qualitative correlation between blocking by La and the speed of closing (Fig. 1). Similarly, there is a qualitative correlation between blocking and the shift of the conductance–voltage curve to the right along the voltage axis. We are attempting to develop from such observations a theory that relates the blocking and gating effects of multivalent cations.

This work was supported by NIH grant NS-12547.

Original version received 25 July 1989 and accepted version received 25 June 1990.

REFERENCES

Arhem, P. 1980. Effects of rubidium, caesium, strontium, barium and lanthanum on ionic current in myelinated nerve fibres from *Xenopus laevis*. *Acta Physiologica Scandinavia*. 108:7–16.

Armstrong, C. M., and J. Lopez-Barneo. 1977. External calcium ions are required for potassium channel gating in squid neurons. *Science*. 236:712–714.

Armstrong, C. M., and D. R. Matteson. 1986. The role of calcium ions in the closing of potassium channels. *Journal of General Physiology*. 87:817–832.

Brismar, T. 1980. The effect of divalent and trivalent cations on the sodium permeability of myelinated nerve fibres of *Xenopus laevis*. *Acta Physiologica Scandinavia*. 108:23–29.

Campbell, D. T., and R. Hahin. 1984. Altered sodium gating current kinetics in frog skeletal muscle caused by low external pH. *Journal of General Physiology*. 84:771–788.

Cota, G., and C. M. Armstrong. 1989. Sodium channel gating in clonal pituitary cells. The inactivation step is not voltage dependent. *Journal of General Physiology*. 94:213–232.

Cukierman, S., W. C. Zinkand, R. J. French, and B. K. Krueger. 1988. Effects of membrane surface charge and calcium on the gating of rat brain sodium channels in planar bilayers. *Journal of General Physiology*. 92:431–447.

Frankenhaeuser, B., and A. L. Hodgkin. 1957. The action of calcium on the electrical properties of squid axons. *Journal of Physiology*. 137:218–244.

Gilly, W. F., and C. M. Armstrong. 1982. Slowing of sodium channel opening kinetics in squid axon by extracellular zinc. *Journal of General Physiology*. 79:935–964.

Hille, B. 1984. The Ionic Channels of Excitable Membranes. Sinauer Associates, Sunderland, MA. 426 pp.

Hille, B., A. M. Woodhull, and B. I. Shapiro. 1975. Negative surface charge near sodium channels of nerve: divalent ions, monovalent ions, and pH. *Philosophical Transactions of the Royal Society of London B*. 270:301–318.

Mozhayeva, G. N., A. P. Naumov, and E. D. Nosyreva. 1985. Potential-dependent calcium blockade of normal and aconitine-modified sodium channels in frog node of Ranvier. *General Physiology and Biophysics*. 4:425–427.

Nilius, B. 1988. Calcium block of guinea-pig heart sodium channels with and without modifications by piperazinylindole DPI 201-106. *Journal of Physiology*. 399:537–558.

Schauf, C. L. 1983. Evidence for negative gating charges in *Myxicola* axons. *Biophysical Journal*. 42:225–231.

Shrager, P. 1974. Ionic conductance changes in voltage clamped crayfish axons at low pH. *Journal of General Physiology*. 64:666–690.

Takata, M., W. F. Pickard, J. Y. Lettvin, and J. W. Moore. 1966. Ionic conductance changes in lobster axon membrane when lanthanum is substituted for calcium. *Journal of General Physiology*. 50:461–471.

Taylor, R. E., C. M. Armstrong, and F. Benzanilla. 1976. Block of sodium channels by external calcium ions. *Biophysical Journal*. 16:27*a*. (Abstr.)

Vogel, W. 1974. Calcium and lanthanum effects at the nodal membrane. *Pflügers Archiv*. 350:25–39.

Woodhull, A. M. 1973. Ionic blockage of sodium channels in nerve. *Journal of General Physiology*. 61:687–708.

Worley, J. F., III, R. J. French, and B. K. Kreuger. 1986. Trimethyoxonium modification of single batrachotoxin-activated sodium channels in planar bilaryers. Changes in unit conductance and in block by saxitoxin and calcium. Journal of General Physiology. 87:327–349.

Yamamoto, D., J. Z. Yeh, and T. Narahashi. 1984. Voltage-dependent block of normal and tetramethrin-modified single sodium channels. *Biophysical Journal*. 45:337–344.