Barium Modulates the Gating of Batrachotoxin-Treated Na^+ Channels in High Ionic Strength Solutions

Samuel Cukierman
Department of Physiology, Loyola University Medical Center, Maywood, IL 60153 USA

ABSTRACT Batrachotoxin-activated rat brain Na^+ channels were reconstituted in neutral planar phospholipid bilayers in high ionic strength solutions (3 M NaCl). Under these conditions, diffuse surface charges present on the channel protein are screened. Nevertheless, the addition of extracellular and/or intracellular Ba^{2+} caused the following alterations in the gating of Na^+ channels: (*a*) external (or internal) Ba^{2+} caused a depolarizing (or hyperpolarizing) voltage shift in the gating curve (open probability versus membrane potential curve) of the channels; (*b*) In the concentration range of 10–120 mM, extracellular Ba^{2+} caused a larger voltage shift in the gating curve of Na^+ channels than intracellular Ba^{2+}; (*c*) voltage shifts of the gating curve of Na^+ channels as a function of external or internal Ba^{2+} were fitted with a simple binding isotherm with the following parameters: for internal Ba^{2+}, $\Delta V_{0.5,max}$ (maximum voltage shift) = −11.5 mV, K_D = 64.7 mM; for external Ba^{2+}, $\Delta V_{0.5,max}$ = 13.5 mV, K_D = 25.8 mM; (*d*) the change in the open probability of the channel caused by extracellular or intracellular Ba^{2+} is a consequence of alterations in both the opening and closing rate constants. Extracellular and intracellular divalent cations can modify the gating kinetics of Na^+ channels by a specific modulatory effect that is independent of diffuse surface potentials. External or internal divalent cations probably bind to specific charges on the Na^+ channel glycoprotein that modulate channel gating.

INTRODUCTION

It has long been established that extracellular divalent cations modulate the electrical activity of excitable cells (cf. Hille, 1992). An important mechanism underlying said modulation concerns the interaction of divalent cations with Na^+ channels, which are responsible for the generation and propagation of action potentials in excitable cells. Following a suggestion made by A. E. Huxley, Frankenhaeuser and Hodgkin (1957) proposed that divalent cations might affect the activation of Na^+ currents by screening negative surface charges present on the axon membrane, thereby changing the electrostatic potential at the membrane-solution interface. *If* the gating machinery of the Na^+ channel senses this potential (Cukierman et al., 1988; Cukierman, 1991a), then the channel would behave as if a bias voltage was being added to the transmembrane potential. An essential prediction of this model is that all voltage-dependent parameters that control the gating (activation, deactivation, and inactivation) of Na^+ channels should shift along the voltage axis by the same bias voltage that is being added by the resulting screening effect of divalent cations on membrane surface charges. Overall, such a prediction has not been completely satisfied (Gilly and Armstrong, 1982a,b; Cukierman and Krueger, 1990, 1991; Clay, 1993).

By reconstituting single Na^+ channels in neutral planar lipid membranes, it has been clearly demonstrated that membrane surface charges are not really necessary for divalent cations to modify the gating of Na^+ channels (Green et al., 1987; Cukierman et al., 1988; Cukierman and Krueger, 1990, 1991; Chabala et al., 1991; Cukierman, 1991a,b; Correa et al., 1991, 1992; Moczydlowsky and Schild, 1993). The presence of negative surface charges located on both sides of the Na^+ channel glycoprotein is a sufficient condition for external (or internal) divalent cations to shift the gating curve of Na^+ channels to more positive (or negative) membrane voltages. In addition, it was also reported that the effects of divalent cations on the gating of Na^+ channels are not consistent with a change in the effective transmembrane potential: extra and intracellular divalent cations do not affect equally the opening and closing rate constants of different Na^+ channels (Gilly and Armstrong, 1982a,b; Cukierman and Krueger, 1990, 1991; Clay, 1993).

Independent of whether divalent cations alter the gating of Na^+ channels by simply modifying the effective transmembrane potential, another profound question is still lingering: Are changes in surface potential caused by screening diffuse surface charges on the channel glycoprotein the only mechanism by which divalent cations alter the gating of Na^+ channels? In this study, this question was addressed experimentally by reconstituting rat brain Na^+ channels into neutral phospholipid bilayers in high ionic strength solutions (3 M NaCl). It was previously reported that the effects of ionic strength on Na^+ channel gating are consistent with an alteration in the effective transmembrane potential via diffuse surface charge effects (Cukierman, 1991a). In that study, it was found that the voltage dependence of Na^+ channel gating was essentially unaltered in high ionic strength conditions (>0.5 M; cf. figure 6 in Cukierman, 1991a). This result was interpreted by assuming that in high ionic strength, both intracellular and extracellular surface charges in the channel glycoprotein are completely screened. Consequently, if the addition of divalent cations to high ionic strength solutions

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Address reprint requests to Dr. Samuel Cukierman, Department of Physiology, Loyola University Medical Center, 2160 South First Avenue, Maywood, IL 60153 USA.
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causes a voltage shift in the gating of Na^+ channels, this effect is unlikely to be due to alterations in surface potential generated by diffuse surface charges in the channel molecule. Here, it is demonstrated that both intracellular and extracellular divalent cations, more specifically Ba^{2+}, are still capable of altering the gating of Na^+ channels in high ionic strength solutions. These results demonstrate that, in addition to their screening effects on diffuse surface charges on the Na^+ channel glycoprotein and membrane (Cukierman et al., 1988; Cukierman, 1991a,b; Chabala et al., 1991; Correa et al., 1991), divalent cations exert a very specific modulatory effect on the gating of Na^+ channels.

MATERIALS AND METHODS

Preparation

Membrane vesicles from rat brains were prepared by conventional methods (Gray and Whittaker, 1962; Krueger et al., 1979). These vesicles were incubated with batrachotoxin (0.6 μM), an alkaloid that removes the fast and slow inactivation processes of Na^+ channels (Huang et al., 1982), before being added to the experimental chamber.

Planar bilayer set-up

Phospholipid planar bilayers had a composition of 80% 1-palmitoyl-2-oleoylphosphatidylethanolamine and 20% 1-palmitoyl-2-oleoylphosphatidylcholine in purified decane. These synthetic lipids were purchased from Avanti Lipids (Alabaster, AL). The surface charge density of these planar membranes is low ($<1e^-/2000$ Å^2) (McLaughlin et al., 1970; McLaughlin, 1977; Bell and Miller, 1984; Cukierman, 1991a), and the corresponding surface potential in high ionic strength solutions can be neglected.

Bilayers were formed by painting a 0.2-mm hole in a plastic partition separating two different compartments. The thinning of the bilayer was monitored by an increase in membrane capacitance. Membrane vesicles were added to one side of the bilayer (*cis* side). Membrane potentials were applied to the *cis* side, and membrane currents through single Na^+ channels were measured from the *trans* side using a virtual ground amplifier. The amplifier terminals were connected to both sides of the bilayer via 3 M KCl agar bridges. Junction potentials were less than 1 mV. Transmembrane potentials are expressed using the cell convention ($V_{intracellular} - V_{extracellular}$). Single channel currents were recorded with a videocassette recorder and analyzed after the experiments.

Solutions

All experiments were performed in 3 M NaCl and 0.010 M 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (pH = 7.00 with NaOH). $BaCl_2$ was added to one or both sides of the bilayer from a concentrated stock solution.

Analysis

Single channel currents were digitized and analyzed with a PC system (pClamp; Axon Instruments, Foster City, CA). Single channel recordings were digitized at a 200–300-Hz cutoff frequency using an 8-pole low-pass Bessel filter (Frequency Devices, Haverhill, MA). The open probabilities of the channel were calculated by the average current method (Cukierman, 1991a) at different membrane potentials. P_o's were fitted to membrane potentials using the Boltzmann distribution

$$\frac{1 - P_o}{P_o} = \exp\left[\frac{qF(V - V_{0.5})}{RT}\right], \quad (1)$$

where P_o is the channel open probability, q is the effective gating charge, and V and $V_{0.5}$ are the membrane potential and the membrane potential where P_o is 0.5.

For estimating the closing (k_{oc}) and opening (k_{co}) rate constants, long recordings (>60 s, >2200 events) of single Na^+ channel currents were digitized and analyzed at a frequency cut-off of 200–300 Hz. The open and closed states of the channel were delimited by a threshold located halfway between the closed and open current levels. Single exponentials were fit to histogram distributions of open and closed states (Cukierman and Krueger, 1990, 1991; Cukierman, 1991a,b).

Experimental points were fitted to different curves by nonlinear square methods based on a Marquardt-Levenberg algorithm (Sigmaplot; Jandel Scientific, Corte Madera, CA). The overall quality of fit was determined by three different parameters: standard deviation of the fit, degree of dependency between different parameters present in the equation, and by a small value for the residuals.

RESULTS

Fig. 1 shows membrane currents from a single Na^+ channel reconstituted in a neutral planar bilayer in the presence of symmetrical 3 M NaCl. In this figure, upward current deflections represent channel openings. The recordings in Fig. 1 *A* were obtained in control conditions, and those in Fig. 1 *B*, after the addition of 25 mM Ba^{2+} to the intracellular side of the channel. It is evident from the recordings that internal Ba^{2+} increased the channel's P_o. At −98 mV (middle recording of Fig. 1 *A*), the channel's P_o was 0.53 while at a slightly hyperpolarized membrane potential of −100 mV, and in the presence of internal Ba^{2+} (middle recording of Fig. 1 *B*) the P_o increased to 0.64. Similar observations can also be made in relation to the top and bottom recordings of both parts of this same figure—they show a relative increase in P_o caused by internal Ba^{2+}.

In Fig. 2, it is shown that the addition of 25 mM extracellular Ba^{2+} caused a decrease in the channel's P_o at −80 mV. In control conditions, P_o was 0.67 (upper recording), while in the presence of external divalent the channel's P_o

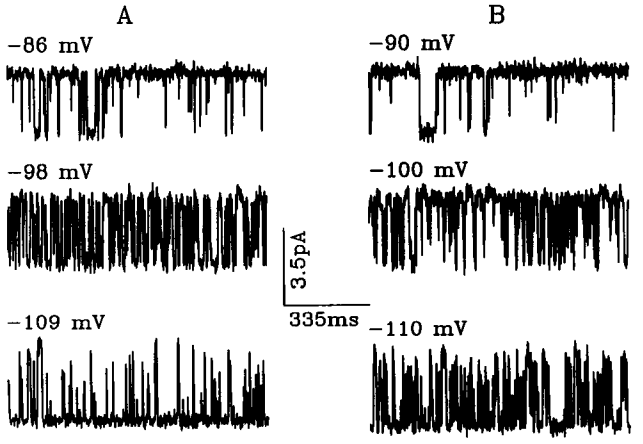

FIGURE 1 Recordings from a single sodium channel reconstituted into a planar lipid bilayer in symmetrical 3 M NaCl solutions. (*A*) control recordings; (*B*) after addition of 25 mM $BaCl_2$ to the intracellular side of the channel. Clamp potentials indicated on the upper left corner of each recording. Upward current deflections are channel openings.

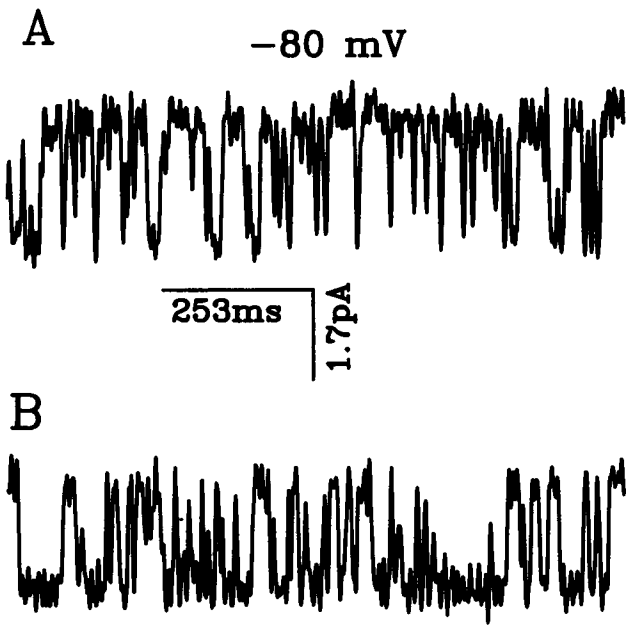

FIGURE 2 Effects of external Ba^{2+} on a single Na^+ channel in a neutral phospholipid bilayer. (*A*) control; (*B*) after 25 mM Ba^{2+} was added to the external side of the channel. Membrane potential for both recordings was −80 mV. Upward current deflections are channel openings.

decreased to 0.47. These modulatory effects of external and internal Ba^{2+} in high ionic strength conditions are manifested by voltage shifts in the gating curve of Na^+ channels. This is shown in Fig. 3. In this figure, circles are data points obtained in control conditions, squares were obtained after the addition of 30 mM Ba^{2+} to the intracellular side of the channel, and triangles were obtained after adding the same concentration of divalent cations to the extracellular side of the channel (symmetrical 3 M NaCl + 30 mM Ba^{2+}). The intracellular addition of Ba^{2+} shifted the gating curve of the channel by −5.6 mV, while extracellular Ba^{2+} shifted the gating curve by a larger voltage (8.1 mV) in the depolarizing direction. Whereas the gating curve of the channel in 3 M NaCl was shifted along the membrane potential axis by Ba^{2+}, no significant changes were observed in the effective gating charge (q) in these different experimental conditions. In different experiments in control conditions, q was 3.48 ± 0.41 (mean ± SEM, 8), while with external or internal Ba^{2+} (concentration range, 10–100 mM) the average values for q were 3.52 ± 0.20 (14), and 3.77 ± 0.26 (8), respectively. These results are in quantitative agreement with those previously obtained in low ionic strength solutions (Cukierman et al., 1988; Cukierman and Krueger, 1990, 1991).

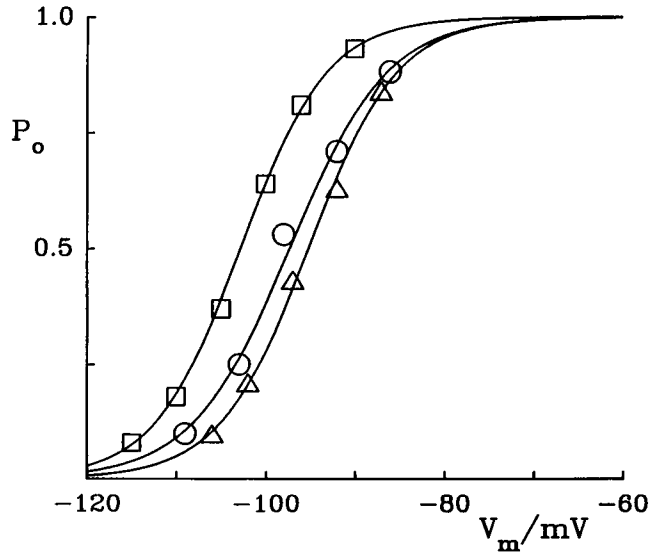

FIGURE 3 Gating curves of a sodium channel in control (○), with 30 mM Ba^{2+} added to the intracellular side of the channel (□), and in symmetrical 3 M NaCl + 30 mM $BaCl_2$ (△). Curves were drawn with Eq. 1 in text with the following values for q and $V_{0.5}$: −4.61 and −97.3 mV (○), −5.20 and −102.8 mV (□), and −5.06 and −95.2 mV (△).

In Fig. 4, the voltage shifts in the gating curve of different Na^+ channels were plotted against various concentrations of internal (*A*) or external (*B*) Ba^{2+}. Curves drawn are the best fits to the equation $\Delta V_{0.5} = \Delta V_{0.5,max}/(1 + K_D/[Ba])$, where $\Delta V_{0.5}$ and $\Delta V_{0.5,max}$ are the voltage shift and maximum voltage shift in the gating curve of Na^+ channels, respectively, and K_D is the dissociation constant of Ba^{2+} from binding site(s) in the channel. The same sets of symbols in Fig. 4 represent data from the same single channel. While the voltage shifts in the gating curve caused by different internal Ba^{2+} concentrations were usually small (<5 mV), they were consistently smaller than the shifts caused by the same concentration of external Ba^{2+} and significantly larger than those predicted for alterations in surface potential caused by screening effects.

In Fig. 5, the voltage dependence of the opening (*A*) and closing (*B*) rate constants is shown under different experimental conditions. In this experiment (same as in Fig. 3), circles were obtained in control (3 M NaCl) conditions, squares after the addition of 30 mM intracellular Ba^{2+}, and triangles in symmetrical 30 mM Ba^{2+} solutions. In this, as well as in four other experiments with single channels, both

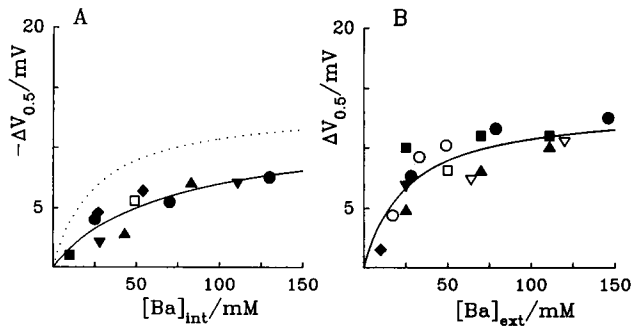

FIGURE 4 The effects of internal (*A*) and external (*B*) Ba^{2+} on the voltage shifts in the midpoint of the gating curve of Na^+ channels. The dotted line in *A* is the same curve as in *B* but with opposite polarity. Curves were drawn according to $\Delta V_{0.5} = \Delta V_{0.5,max}/(1 + K_D/[Ba])$. In *A*, K_D = 64.7 ± 31.8 (mean ± SD of the fit), $\Delta V_{0.5,max}$ = 11.5 ± 2.8. (*B*) K_D = 25.8 ± 9.1, and $\Delta V_{0.5,max}$ = 13.5 ± 1.5. Same sets of symbols are from the same channel in the bilayer.

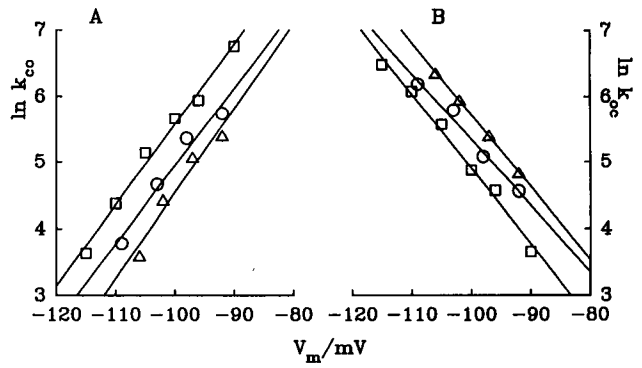

FIGURE 5 Voltage dependence of the opening (*A*) and closing (*B*) rate constants in control (○), in the presence of 30 mM internal Ba^{2+} (□) and with 30 mM Ba^{2+} on both sides of the channel (same experiment as in Fig. 3). Curves were drawn according to linear regression analyses. (*A*) $\ln k_{co} = 0.1174 \cdot V_m + 16.68$ (○), $\ln k_{co} = 0.1217 \cdot V_m + 17.74$ (□), and $\ln k_{co} = 0.1279 \cdot V_m + 17.32$ (△); (*B*) $\ln k_{oc} = -0.1 \cdot V_m - 4.66$ (○), $\ln k_{oc} = -0.1134 \cdot V_m - 6.439$ (□), and $\ln k_{oc} = -0.1095 \cdot V_m - 5.2311$ (△).

intracellular and extracellular divalent cations caused hyperpolarizing and depolarizing voltage shifts, respectively, in the opening and closing rate constants.

DISCUSSION

The implicit assumption in this study is that in 3 M NaCl solutions, there is no appreciable surface potential on the Na^+ channel. This is reasonable; assuming external and internal surface charge densities of $1e^-/533$ Å^2 and $1e^-/1231$ Å^2, respectively (Cukierman, 1991a), the external and internal potentials at the channel surface-solution interfaces in 3 M NaCl would be approximately −2 and −0.5 mV, respectively (McLaughlin, 1977; Cukierman, 1991a; Israelachvili, 1992). The calculation of these surface charge densities was based on a simple Gouy-Chapman model. Therefore, the conclusions presented in this study must take into consideration the assumptions inherent in Gouy-Chapman theory (McLaughlin, 1977; Israelachvili, 1992), which might not be entirely applicable to the surfaces of ion channels (Hille, 1992). However, it was previously found that solutions with ionic strength greater than 0.5 M do not shift the gating curve of Na^+ channels (Cukierman, 1991a). This may be considered a good indication that in 3 M NaCl, surface charges on the channel's protein were screened and the resultant surface potential under these conditions is not significant. While it is likely that external or internal Ba^{2+} binds to surface charges on the channel and shifts the channel's gating curve, this effect would occur in the absence of an appreciable surface potential (see below). The voltage shifts caused by Ba^{2+} on the gating curves observed in this study were significantly larger than expected by calculations assuming the above surface charge densities. Thus, the observed shifts cannot be easily reconciled with diffuse surface charge effects. Under the experimental conditions of this study, the novel results presented are (*a*) both extracellular and intracellular Ba^{2+} shifted the gating curve of reconstituted rat brain Na^+ channels in the depolarizing and hyperpolarizing directions, respectively; (*b*) extracellular Ba^{2+} consistently shifted the gating curve of Na^+ channels by a larger voltage than intracellular Ba^{2+}; (*c*) alterations in the P_o of the channel caused by internal or external divalent cations were a consequence of changes in both the opening and closing rate constants.

That extracellular or intracellular Ba^{2+} is still capable of modulating the gating of Na^+ channels in conditions where the potentials generated by diffuse surface charges are negligible suggests that divalent cations do not really need the presence of a significant potential generated by diffuse surface charges in the channel glycoprotein to shift the gating curve of the channel. This is an important concept because historically the effects of divalent cations on the gating of Na^+ as well as other channels have been attributed, at least in part, to alterations in surface potentials. Under the experimental conditions of this study, it was noticed that relatively higher concentrations of divalent cations in relation to physiological conditions are necessary to cause a measurable voltage shift in the gating curve of Na^+ channels. It is important to ask whether these modulatory (independent of surface charge screening effects) effects have some significance under more physiological conditions. In relation to the intracellular side of the Na^+ channel, the modulatory as well as the screening/binding effects of diffuse surface charges by divalent cations cannot be considered physiologically relevant; the intracellular surface charge density is relatively low ($1e^-/1$, 231 Å^2; Cukierman, 1991a), and as such, the concentration of intracellular divalent cations necessary to shift the gating curve of Na^+ channels by a significant voltage would not be compatible with cell integrity (Cukierman et al., 1988; Cukierman and Krueger, 1990, 1991). However, extracellular divalent cations have an important physiological role in determining the relative position of the gating curve of Na^+ channels and thus the threshold voltage for action potential firing. Let us assume the following conditions: (*a*) an extracellular surface charge density on the channel protein of $1e^-/533$ Å^2 (Cukierman, 1991a); (*b*) an extracellular ionic strength of 0.15 M with 2 mM bulk concentration of divalent cations; (*c*) no specific binding of divalent cations to these surface charges. These conditions will generate a surface potential of approximately −25 mV. This potential would have the effect of increasing the concentration of divalent cations in relation to their bulk concentration by approximately exp(25/12.5)-fold to 15 mM at the surface of the channel protein. This can induce a specific modulation of the channel's P_o. Because changes in channel gating caused by alterations in the concentration of extracellular divalent cations have been used as a method for determining (or correcting for alterations in) surface potentials, the present results raise a word of caution. Different Na^+ channels possess different degrees of glycosilation, and consequently, different extracellular surface charge densities (Recio-Pinto et al., 1990). Depending on the specific experimental conditions, it is conceivable that alterations in

the gating of Na^+ channels caused by external divalent cations could be attributed to a modulatory effect (independent of diffuse surface charge effects).

Another finding in this study was that in the concentration range tested, external Ba^{2+} induced a larger shift in the gating curve of Na^+ channels than internal Ba^{2+}. Even though this difference is small (2–3 mV), it was consistently observed and could in principle be attributed to a larger surface charge density on the extracellular side of the channel (Cukierman, 1991a). Even in 3 M NaCl, there still is an approximately −2 mV potential difference between the extra and intracellular surfaces of the channel. It is important to stress, however, that for each side of the channel, the voltage shifts caused by Ba^{2+} are significantly larger than those predicted by simple surface potential calculations (see above).

Fig. 6 summarizes the basic mechanisms by which divalent cations might affect the gating of Na^+-channels. On the membrane, divalent cations screen/bind negatively charged lipid headgroups, thereby reducing the potential at the membrane-solution interface (cf. McLaughlin, 1977) (Fig. 6 *A*). The gating machinery of the channel seems to respond to this potential, but at distances of approximately 48 and 28 Å away from the plane of the membrane on the extra- and intracellular sides of the channel, respectively (Cukierman, 1991a). Also, it has been previously demonstrated that divalent cations shift the gating curve of the channel by a larger voltage when Na^+ channels are reconstituted in a negatively charged membrane than when they are present in a neutral bilayer (Cukierman et al., 1988; Cukierman and Krueger, 1990, 1991). On the channel itself, divalent cations screen/bind negative surface charges (Fig 6 *B*). This decreases the diffuse surface potential, and shifts the gating curve of the channel. The present results demonstrate that divalent cations may also bind to surface charges in the absence of a significant surface potential (gating modulation) (Fig. 6 *C*). Our previous results suggest that these binding sites are specific and have different chemical affinities for group IIA divalent cations and that intracellular and extracellular sites

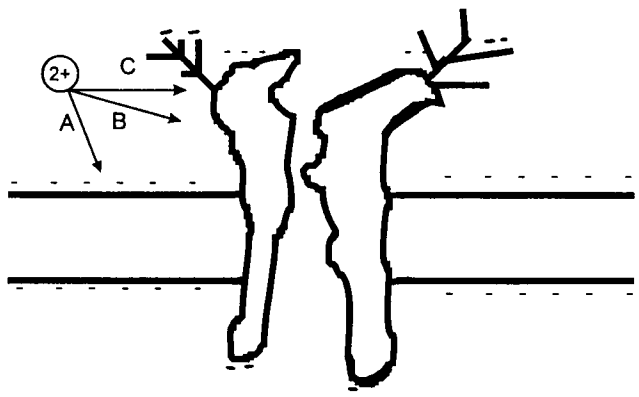

FIGURE 6 Representation of the different effects of divalent cations on the gating of reconstituted batrachotoxin-activated Na^+ channels. The effects of divalent cations are indicated on the extracellular side of the channel. While not indicated in this figure, qualitatively similar effects are also present on the intracellular side. See text for discussion of this figure.

possess different chemical properties (Cukierman and Krueger, 1990, 1991). Because the order of efficacy, among different group IIA divalent cations, in blocking Na^+ currents is different from the order of efficacy in shifting the Na^+ channel gating curve (Cukierman and Krueger, 1990, 1991), these sites are probably located on the surface of the channel and not inside the pore. Gilly and Armstrong (1982a,b) proposed that the binding sites for divalent cations on the Na^+ channel surfaces might well be part of the gating machinery of the channel. However, Armstrong and Cota (1991) have recently raised the possibility that the gating modulation of Na^+ channels by Ca^{2+} might be linked to the blocking effect of this cation on Na^+ currents inside the pore.

Gilly and Armstrong (1982a,b) showed that external divalent cations slowed the activation of Na^+ and K^+ currents in squid axons without affecting considerably the deactivation of these currents. Using internal Mg^{2+} on K^+ currents in squid axons, Clay (1993) showed that the deactivation kinetics of this current is markedly slowed while the activation is not (a "mirror" image of the effects of external divalents). We have found that external (or internal) divalent cations added to low ionic strength solutions (0.15 M) caused a more marked effect on the opening (or closing) than on the closing (or opening) rate constant of rat brain Na^+ channels reconstituted in bilayers. These results, which are in qualitative agreement with those in squid axons (Clay, 1993; Gilly and Armstrong, 1982a,b), argue against a model in which divalent cations affect the channel gating by simply changing the electric field across the membrane. We have proposed (Cukierman and Krueger, 1990, 1991) that divalent cations interact with the Na^+ channel and cause changes in the gating behavior of the channel by two different mechanisms: (*a*) by screening/binding negative surface charges on the channel protein, thus changing the effective transmembrane potential (Cukierman, 1991a) (this mechanism would lead to the same proportional change in both the opening and closing rate constants; effect B in Fig. 6), and (*b*) by a specific modulatory effect (effect C in Fig. 6) that would cause a more marked effect in one of the rate constants. A prediction of this model is that in high ionic strength solutions, where surface potentials are negligible, one should demonstrate that divalent cations induce only the modulatory effect, i.e., external (or internal) Ba^{2+} should modify only the opening (or closing) rate constant. In this study, it was found that external or internal Ba^{2+} is still capable of shifting the voltage dependence of both rate constants.

The analyses of the effects of divalent cations on Na^+ channel gating in very high ionic strength conditions could be very useful in association with molecular biological experiments aimed at defining the residues in the channel that interact with divalents. Because these sites seem to modulate channel gating very effectively, resolving their locations will be of considerable importance for understanding the functional relationships between different parts of the Na^+ channel molecule during the process of opening-closing. This information should also prove useful in understanding the organization of the channel in the membrane.

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REFERENCES

Armstrong, C. M., and G. Cota. 1991. Calcium ion as a cofactor in Na channel gating. *Proc. Natl. Acad. Sci. USA.* 88:6528–6531.

Bell, J., and C. Miller. 1984. Effects of phospholipid surface charges on ion conductance in the K channel of sarcoplasmic reticulum. *Biophys. J.* 45:279–287.

Chabala, L. D., B. W. Urban, L. B. Weiss, W. N. Green, and O.S. Andersen. 1991. Steady-state gating of batrachotoxin-modified sodium channels. Variability and electrolyte-dependent modulation. *J. Gen. Physiol.* 98: 197–224.

Clay, J. R. 1993. Blockade of the delayed rectifier by calcium ions. Relationship to the effects of divalent cations on gating (Abstract). *Biophys. J.* 64:A312.

Correa, A. M., R. Latorre, and F. Bezanilla. 1991. Ion permeation in normal and batrachotoxin-modified Na^+ channels in the squid giant axon. *J. Gen. Physiol.* 97:605–625.

Correa, A. M., F. Bezanilla, and R. Latorre. 1992. Gating kinetics of batrachotoxin-modified Na^+ channels in the squid giant axon. *Biophys. J.* 61:1332–1352.

Cukierman, S. 1991a. Asymmetric electrostatic effects on the gating of rat brain sodium channels in planar lipid membranes. *Biophys. J.* 61: 845–856.

Cukierman, S. 1991b. Inactivation modifiers of Na currents and the gating of rat brain sodium channels in planar lipid membranes. *Pfluegers Arch. Eur. J. Physiol.* 419:514–521.

Cukierman, S., and B. K. Krueger. 1990. Modulation of sodium channel gating by external divalent cations studied in planar lipid bilayers. Differential effects on opening and closing rates. *Pfluegers Arch. Eur. J. Physiol.* 416:360–367.

Cukierman, S., and B. K. Krueger. 1991. Effects of internal divalent cations on the gating of rat brain sodium channels reconstituted in planar lipid bilayers. *Pfluegers Arch. Eur. J. Physiol.* 419:559–566.

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. *J. Gen. Physiol.* 92:431–447.

Frankenhaeuser, B., and A. L. Hodgkin. 1957. The action of calcium on the electrical properties of squid axons. *J. Physiol. (Lond.).* 137:218–244.

Gilly, W. F., and C. M. Armstrong. 1982a. Divalent cations and the activation kinetics of potassium channels in squid giant axons. *J. Gen. Physiol.* 79:965–996.

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

Gray, E. G., and V. P. Whittaker. 1962. The isolation of nerve endings from brain. An EM study of cell fragments derived by homogenization and centrifugation. *J. Anat.* 96:79–87.

Green, W. N., L. B. Weiss, and O. S. Andersen. 1987. Batrachotoxin-modified sodium channels in planar lipid bilayers. Ion permeation and block. *J. Gen. Physiol.* 89:841–872.

Hille, B. 1992. Ionic channels of excitable membranes. 2nd Ed. Sinauer Associates, Sunderland, MA.

Huang, L. M., N. Moran, and G. Ehrenstein. 1982. Batrachotoxin modifies the gating kinetics of sodium channels in internally perfused neuroblastoma cells. *Proc. Natl. Acad. Sci. USA.* 79:2082–2085.

Israelachvili, J. 1992. Intermolecular and Surface Forces. 2nd Ed. Academic Press, San Diego, CA.

Krueger, B. K., R. W. Ratzlaff, G. R. Strichartz, and M. P. Blaustein. 1979. Saxitoxin binding to synaptosomes, membranes, and solubilized binding sites from rat brain. *J. Membr. Biol.* 50:287–310.

McLaughlin, S. 1977. Electrostatic potentials at membrane solution interfaces. *Curr. Top. Membr. Transp.* 9:71–144.

McLaughlin, S. G. A., G. Szabo, G. Eisenman, and S. M. Ciani. 1970. Surface charge and the conductance of phospholipid membranes. *Proc. Natl. Acad. Sci. USA.* 67:1268–1275.

Moczydlowski, E., and L. Schild. 1993. Unitary properties of the batrachotoxin-trapped state of voltage-sensitive sodium channels. *In* Handbook of Membrane Channels: Molecular and Cellular Physiology. C. Peracchia, editor. CRC Press, Chicago. In press.

Recio-Pinto, E., W. B. Thornhill, D. S. Duch, S. R. Levinson, and B. W. Urban. 1990. Neuroaminidase treatment modifies the function of electroplax sodium channels in planar lipid bilayers. *Neuron.* 5:675–684.