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ACETYLCHOLINE-ACTIVATED CHANNEL CURRENT-VOLTAGE RELATIONS IN SYMMETRICAL Na^+ SOLUTIONS

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A primary function of the acetylcholine receptor-channel complex (AChR) is transporting ions. The channel is impermeable to anions but passes divalent cations and a wide variety of monovalent cations with diameters less than ~7–8 Å (1, 2). A simple two-barrier, one-site (2B1S) model has been used to describe the transport of the simplest and biologically most important monovalent cations (3–6). In each case certain restrictions were applied to the model, such as arbitrarily setting the position or affinity of the site. In addition, because of experimental limitations the model was applied to data obtained at one symmetrical concentration or by changing only the external concentration.

Certainly permeation through ion channels is very complex. A simple barrier model may be viewed as a crude approximation of reality most useful as a phenomenological description of transport. The model can serve a functional role in the analysis of new data; any extensions or revisions necessary to describe transport of other permeants can give clues regarding the different interactions between the ions and channel. The model is also useful when considering the effect of electrolyte composition on other properties of the channel such as gating (4). Exciting hypotheses have recently been proposed relating the primary amino acid sequences to the structure of the AChR subunits, and relating subunit organization to the formation of the ion channel (7, 8). A simple barrier model can provide the first step toward integrating the details of permeation to the overall structure and function.

Here we test if a 2B1S model, applied without restricting the location or energies of the barriers and site, can describe current-voltage (*I-V*) relations obtained over a wide concentration range of symmetrical Na^+ solutions. The shortcomings of such an absolute rate theory model, like those that arise when the energy barriers are small (9), are not addressed. Although we shall conclude that a general 2B1S model without additional provisions is not completely adequate to describe the data, it does provide a surprisingly good first approximation and gives information about the energy profile that may be useful in further investigation of AChR permeation, structure, and function.

RESULTS

Single AChR events from rat myotubes were studied by voltage clamping membrane patches in symmetrical Na^+ concentrations (10). To obtain *I-V* relations, voltage ramps were applied to the membrane (11). The legend of Fig. 1 describes the solutions, methods, and procedure for obtaining *I-V* relations from single-channel events. Fig. 1 *C* shows a leak-corrected single-channel response to a voltage ramp. Fig. 2 is a plot of the zero-voltage conductance (G_0) vs. activity. A Michaelis-Menten curve having a dissociation constant of 74 mM (activity) is drawn through the high concentration data. The insert is an Eadie-Hofstee plot (G_0 vs. G_0/activity) that shows the deviation of the low concentration data from an expected straight line. Fig. 3 shows the *I-V* relations obtained in symmetrical Na^+ solutions of 45 mM, 150 mM, and 450 mM. The smooth curves result from the best theoretical fit of the 2B1S model. Although the fit to the *I-V* relations is quite good,

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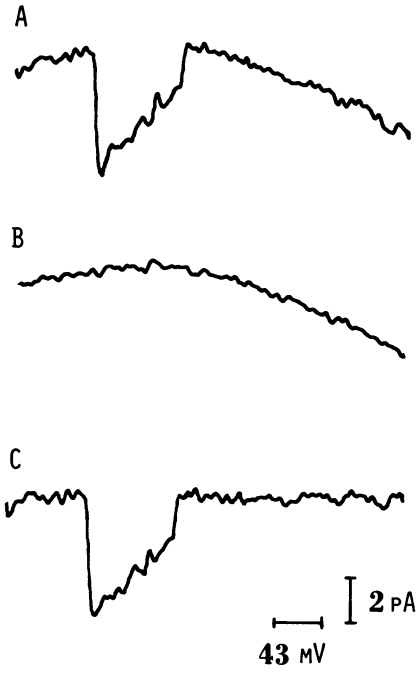

FIGURE 1 Standard patch-clamp techniques were used. Our rate of obtaining gigaohm seals increased following James Rae's suggestion to use Corning's Kovar 7052 pipettes (Corning Glass Works, Corning, NY). The Pt wire used in fire polishing should be frequently coated with Kovar glass. We believe the glass vaporizes from the hot wire, depositing a thin film on the patch pipette. The sealing rate of electrically superior pipettes can be improved by this procedure (19). The pipette was usually filled with 45, 150, or 450 mM NaCl, osmolarity unadjusted; 5 mM HEPES, pH 7.4; 0.1 to 0.6 μM ACh and 0.5 mM $BaCl_2$ was added to the 45-mM and occasionally the 150-mM solution. After a patch was obtained it was placed deeply into an inflow port containing an equivalent Cl^- or F^- solution. *A*, raw record of a single channel response to a 51 ms, -170 to $+175$ mV ramp voltage clamp, filtered at 600 Hz with 8-pole Bessel, 21°C, in 150 mM Na^+. *B*, average of two selected nearby blank records that accurately overlap the baseline of *A*, leak correction was made by subtracting *B* from *A*. Rarely, a second order polynomial was used to correct short events if blank records were unavailable or did not fit the event record baseline. *C*, leak-corrected event record. Many digitized current points were averaged by computer at their corresponding voltage point to obtain the *I-V* (11).

the model does not accurately predict G_0. The dashed line in Fig. 2 shows the 2B1S model poorly predicts the G_0 data, especially at low concentrations.

The dissociation constant is in reasonable agreement with values obtained at 100 mV from myotubes (12), and from *Torpedo* AChR reconstituted into liposomes (13). These values are about four times smaller than those estimated in frog or toad endplates (14, 15).

The deviation of the low concentration data from a single site binding curve in Fig. 2 suggests possible shortcomings of the 2B1S model. Such a deviation would result if the channel had more than one nonequivalent site or if negative dipoles or charges increased the concentration of Na^+ near the channel entrance. Lewis and Stevens (5) extended the 2B1S model to include an external surface

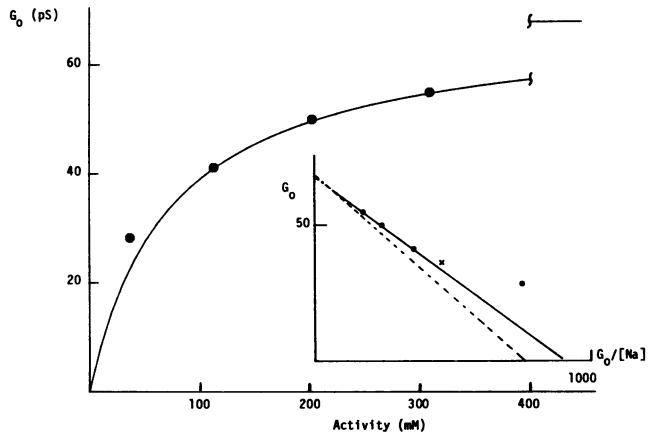

FIGURE 2 Zero-voltage conductance vs. activity fitted by $G_0 = G_{max}$ [Na]/([Na] + K_D) with G_{max} = 68 pS and K_D = 74 mM, where [Na] is activity. *Insert*, an Eadie-Hofstee plot of G_0 vs. G_0/[Na]. The solid line is drawn through the high concentration data using the same G_{max} and K_D as above. The dashed line is the prediction of the 2B1S model with G_{max} = 68 pS and K_D = 90 mM. The *x* is taken from reference 12.

potential to fit their data. The surface potentials they used are larger than suggested by our results. Given that the channel extends 55 Å from the outer surface of the membrane and that the channel entrance is ~25 Å in diameter (16, 17), it seems likely that the effective field of

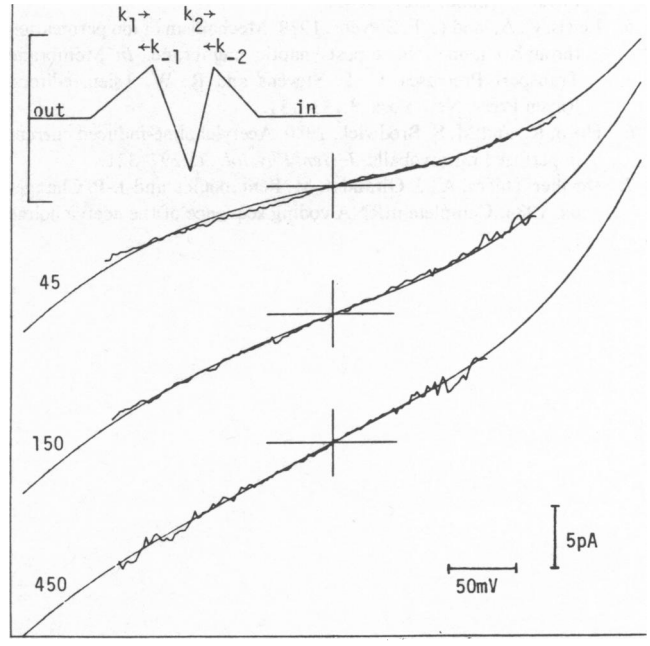

FIGURE 3 Shows the *I-V* relations at 45, 150, and 450 mM Na^+. The vertical and horizontal lines mark $V = 0$ and $I = 0$ positions. The smooth curves are the result of a nonlinear least-squares Levenberg-Marquardt fit (algorithm developed by C. Clausen) to all the data using the 2B1S model. The net flux of ions is given by J = [Na]$(k_{-1}k_{-2} - k_1k_2)$/[Na] $(k_1 + k_{-2}) + k_{-1} + k_2$. The upper insert shows the free energy profile of the best fit labeled with the rate constant used in Eq. 1. The marks on the *Y*-axis represent + or − 10 kT. Unlike previous findings, this fit predicts the outer barrier to be larger than the inner.

dipoles and charges within this large vestibule will vary with ionic strength and will influence ion loading. The introduction of "surface potentials" into the barrier model using the Grahame equation (18) is an approximation that does not deal with these intricacies, but extends the model to handle the overall effect. Additional data may allow us to determine whether "surface potentials" can adequately diminish the shortcomings of a 2B1S model, whether a more complex barrier model is needed, or whether other types of models are more appropriate.

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