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Acetylcholine receptor channel ionic selectivity: Ions experience an aqueous environment

(channel structure/selectivity sequences/selectivity theory)

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ABSTRACT **Alkali metal and alkaline earth cations pass readily through the acetylcholine receptor channel. Monovalent cations with larger crystal radii are more permeant than ones with smaller radii. For divalent ions, this selectivity sequence is reversed: smaller ions are more permeant than larger ones. This reversal in selectivity sequence with change of valence from 1 to 2 can be naturally accounted for by electrostatic interactions between the ion and its environment in the selectivity region of the channel. For monovalent ions, ion–dipole interactions dominate, and, for divalent ions, ion-induced dipole interactions are more important. The sign of these two types of effects is opposite and produces the reversal in the selectivity sequence. The magnitude of electrostatic interactions can be estimated from experimental data and suggests that the permeating ion's environment in the selectivity region of the channel is essentially like that in free water.**

The amino acid sequences for all subunits of the acetylcholine receptor (AcChoR) channel have now been deduced from cDNA clones, and several structural models for the channel, with varying amounts of detail, have been proposed (1–5). We wish to present here information about the movement of ions through the channel and some interpretations of our data that have implications for proposed channel structures.

Alkali metal cations (Li^+ through Cs^+) and alkaline earth cations (Mg^{2+} through Ba^{2+}) all pass easily through the AcChoR channel. Of the alkali metal ions, the largest (Cs^+) is the most permeant and the smallest (Li^+) is the least permeant, as determined by reversal potential measurements. For the divalent ions, the reverse sequence is found: the smallest ion (Mg^{2+}) is the most permeant and the largest (Ba^{2+}) is the least. Our goal here is to explain how the selectivity sequence can reverse as the ionic valence is increased from 1 to 2 and to indicate what these observations tell us about the particular environment that ions find within the channel. We shall conclude that ions within the selectivity region of the channel probably interact almost exclusively with water molecules rather than with protein side chains in the pore.

EXPERIMENTAL METHODS

Biological Preparation. During the months of December through February, experiments were performed by using medium-sized *Rana pipiens*, 6.4–7.6 cm in length, which had been kept refrigerated at about 4°C. The cutaneous pectoris muscle was removed and dissected down to a monolayer by using a technique similar to that of Dreyer and Peper (6) and Dionne and Stevens (7).

Experimental Apparatus. The experimental equipment has been described (8). In summary, two microelectrodes filled with 3 M KCl were used to voltage clamp a muscle fiber. A third microelectrode, filled with ≈3 M acetylcholine chloride (AcCho) (Sigma), was used to apply AcCho to the end-plate region by iontophoresis.

Solutions. Normal Ringer solution had the following composition in mM: NaCl, 115; KCl, 2.5; $CaCl_2$, 2; and Hepes (Sigma), 4. The solution also contained 100 nM tetrodotoxin (Sigma), and the pH was adjusted to 7.4.

The test solutions were made by replacing the 115 mM NaCl with an isosmotic equivalent of the test solute. The following solutes were tested: LiCl, CsCl, RbCl, $SrCl_2$, $BaCl_2$, $CaCl_2$, and $MgCl_2$. The experiments that attempted to use RbCl and $SrCl_2$ were unsuccessful because these solutions were not well tolerated by the cells.

The activity corrections for the monovalent cations were calculated by using tables 9–11 in appendix 8.10 in Robinson and Stokes (9). The activity corrections for the divalent cations were calculated by using table 13 in the same reference along with the Guggenheim convention for relating the activity coefficient of a divalent cation to the activity coefficient of the salt (10).

Temperature. Temperature ranged between 9 and 12°C in various experiments. The temperature was measured with a thermal-sensitive probe placed in the bathing solution on top of the muscle preparation. The temperature was regulated by a peltier device in the microscope stage underneath the muscle chamber.

Procedure. The procedure for voltage clamping the end plate has been described (8). Measurements were made immediately after a solution change for a period of 15–20 min. Usually measurements in two or three different solutions were made on any one muscle preparation.

Reversal Potential Determinations. The method for determining the reversal potential is the same as described (8). Most reversal potential values were determined by interpolation of the AcCho-induced end-plate current versus voltage curve. A few reversal potential values obtained by extrapolation were included if the voltage range for extrapolation was <10 mV.

RESULTS

Among the monovalent alkali cations, the mean reversal potential becomes progressively more positive for ions with larger crystal radii. The mean reversal potentials for the monovalent alkali cations ranged from $+0.3 \pm 1.1$ mV (± SEM) ($n = 9$) for Cs to -11.1 ± 1.2 mV ($n = 11$) for Li. The mean reversal potential for Na is intermediate at -4.8 ± 0.4 mV ($n = 32$). The sequence of reversal potentials, then, is Cs > Na > Li, with Cs, the largest ion, having the most positive value.

Among the divalent alkaline earth cations, the mean reversal potential becomes progressively more positive for ions with

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Abbreviations: AcCho, acetylcholine; AcChoR, acetylcholine receptor.

smaller crystal radii. Of the divalent cations tested, Mg had the most positive mean reversal potential value of -24.0 ± 1.4 mV ($n = 7$), whereas the values for Ca and Ba were -30.1 ± 0.4 mV ($n = 5$) and -35.8 ± 1.7 mV ($n = 5$), respectively. The sequence of reversal potentials, then, is Mg > Ca > Ba, with Mg, the smallest ion, having the most positive value.

Theoretical Analysis. Selectivity of a channel, as measured by reversal potential, reflects the size of an energy barrier (B) at the channel region energetically least favorable for cations (11, 12). Therefore, the mean reversal potential values measured in the various solutions can be used to provide an estimate of the AcChoR selectivity barrier. For a single rate-limiting barrier, the reversal potential V_o is related to the barrier height by the equation:

$$V_o = \frac{RT}{F} \ln \frac{\Sigma m_{Oi} \exp(-B_i/RT) + 2\Sigma m_{Oi} \exp(-B_i/RT)\exp(-\delta F V_o/RT)}{\Sigma m_{Ii} \exp(-B_i/RT) + 2\Sigma m_{Ii} \exp(-B_i/RT)\exp((1-\delta)FV_o/RT)}, \quad [1]$$

where m_{Oi} and m_{Ii} refer to extracellular and intracellular activities, respectively, of the ith ionic species and R, T, and F are the gas constant, temperature, and faraday. B_i is the height of the rate-limiting barrier for the ith species, and δ is the fraction of the potential drop across the membrane at the location of the rate-limiting barrier. If the assumption is made that the reversal potential is dominated by the predominant external cation and by the internal cations and that the internal concentrations remain the same for the different solutions, then the change in the reversal potential compared to a reference solution (Na Ringer for monovalent ions and Ca Ringer for divalent cations) is the following:

$$\Delta B_i = -F\Delta V_i + RT \ln \frac{[i]_o}{[\text{ref}]_o}, \quad [2]$$

where $\Delta B_i = B_i - B_{\text{ref}}$, $V_i = V_o$ (ith test ion) $- V_o$ (reference ion), and the brackets indicate the activities of the ith test ion and the reference ion in the bathing solutions.

The ratio of test ion permeability P_i to reference ion permeability P_{ref} may be extracted from barrier heights from the following:

$$\frac{P_i}{P_{\text{ref}}} = \exp[-(B_i - B_{\text{ref}})/RT]. \quad [3]$$

The results of this analysis are shown in Fig. 1, where B_i is plotted versus 1/ion radius for the various monovalent and divalent cations. The straight lines fit the data points and give correlation coefficients of 0.999 and -0.998 for monovalent and divalent cations, respectively. The permeability ratios calculated from ΔB_i are Cs > Na > Li ($1.28 \pm 0.06 > 1 > 0.76 \pm 0.04$) and Mg > Ca > Ba ($1.42 \pm 0.13 > 1 > 0.73 \pm 0.08$).

Ions with different sizes and charges experience different barriers—that is, barrier height $B(z,X)$ depends on the size of the ion (measured by the reciprocal radius X) and on the valence z. The AcChoR channel is only slightly selective, which means that $B(z,X)$ is a slowly varying function of X. Consequently, if $B(z,X)$ is expressed as a power series in ion size around the size X_o of a reference ion, then only the linear term, with slope $a(z)$, needs to be retained to describe approximately the dependence of barrier height on ion size:

$$B(z,X) = B(z,X) + a(z)\,(X - X_o). \quad [4]$$

Note that this linear approximation adequately describes the data in Fig. 1 and that $a(z)$ in Eq. 4 is positive for monovalent ions and negative for divalent ions.

We now develop an approximate expression for the slope $a(z)$. Reuter and Stevens (13) have derived the following general expression for $a(z)$:

$$a(z) = \int_0^{ze} dq f(X_o, X_o, q) + \int_0^{X_o} dx \int_0^{ze} dq \left(\frac{\partial f(x,X,q)}{\partial X}\right)_{X=X_o}. \quad [5]$$

Here q is ion charge, X is ion size (i.e., reciprocal radius), X_o is the reference ion size, x is the reciprocal distance from the ion center, and f specifies the average difference between free solution and the selectivity region of the channel in the density of dipoles on the surface of each spherical shell around the ion. The second integral in this expression involves the rate at which the dipole density varies with ion size.

To obtain an explicit approximate expression for $a(z)$ in Eq. 4 we shall examine the limiting case in which: (A) the second integral is negligible (i.e., $\partial f/\partial X = 0$) and ($B$) $f(q)$ is adequately represented by the first two terms in its MacLaurin expansion in q with slope α and intercept f_o [$f(q) = f_o + \alpha q$]. For a relatively spacious channel such as that in the AcChoR (see ref. 14), the structure of water in the vicinity of the ion might be expected to vary with ion size approximately as free water does (assumption A). Furthermore, the difference in dielectric saturation between the ion's environment in the channel and free water should be slight (assumption B). These assumptions are plausible but difficult to verify; in any case, examination of this simple limiting case will permit insights into the mechanisms operating in selectivity.

We find, then, using this linear approximation for f in the first integral in Eq. 5, that $a(z)$ is approximately

$$a(z) = zef_o + (ze)^2\alpha/2, \quad [6]$$

where z is the valence, e the elementary charge, f_o the fixed dipole density per Å shell at the surface of the ion, and α is the induced dipole density per elementary charge per Å shell. It should be stressed that both f_o and α refer to differences be-

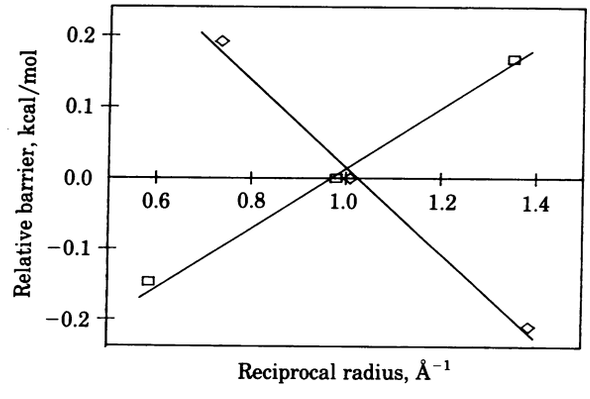

FIG. 1. Selectivity barrier energy, calculated from Eq. 2, as a function of reciprocal ion radius for monovalent (alkali metal) (□) and divalent (alkaline earth) (◇) ions. Barrier energy is relative to that of a reference ion. Na is the reference monovalent, and Ca the reference divalent ion.

tween the ionic environments in free solution and within the selectivity region of the channel.

From Fig. 1 we find that (for monovalent ions) $a(1) = 0.41 \pm 0.14$ ($\pm$ SD) kcal Å per mol and (for divalent ions) $a(2) = -0.61 \pm 0.21$ kcal Å per mol. This sign reversal from monovalent to divalent ions is an expression of the reversal of selectivity sequences. Eq. 6 for $a(z)$ together with the values for $a(1)$ and $a(2)$ permit us to find values for f_o and α—namely, $f_o = 0.016\ D/\text{A}$ and $\alpha = -0.021$ D/A elementary charge.

DISCUSSION

Comparison with Previous Results. A few studies have been reported in which the selectivity of the AcChoR channel was investigated quantitatively for a number of alkali cations. Huang *et al.* (15) measured fluxes activated by carbamoylcholine in cultured muscle cells from chicken embryos. Permeabilities were calculated from ion fluxes, and the results are Cs > Rb > K > Na (1.91 > 1.52 > 1.47 > 1.0). These numbers are somewhat higher than are reported here, perhaps because fluxes rather than reversal potentials were used to estimate permeability, but the basic sequence with Cs > Na is confirmed.

Another study was performed by Gage and Van Helden (16) on glycerol-treated sartorius muscle from the toad *Bufo marinus*. For alkali cations at a temperature of 8°C, the permeability ratios calculated from reversal potentials are Cs > Na > Li (1.14 > 1.0 > 0.88). This sequence and the magnitudes of the ratios agree quite well with the present results.

Adams *et al.* (14) examined the selectivity of AcChoR channels for both monovalent and divalent cations. A vaseline-gap voltage clamp technique was used on cut muscle fibers from the semitendinosus muscle from the frog *R. pipiens*. Permeability ratios were calculated from reversal potential measurements. For monovalent cations the result is Cs > Rb > K > Na > Li (1.4 > 1.3 > 1.1 > 1.0 > 0.9), whereas for divalent cations it is Mg > Ca > Ba > Sr (1.1 > 1.0 > 0.9 > 0.8). The magnitudes of the permeability ratios differ somewhat from the numbers reported here but the basic sequences of Cs > Na > Li and Mg > Ca > Ba are confirmed, although the position of Sr in the sequence is reversed from what would be expected on the basis of ion radii.

Implications. Eq. 6 provides a natural explanation for the sequence reversal for mono- and divalent ions. For a weakly selective channel, the sign of the slope $a(z)$ for the linear relationship in Eq. 4 between barrier height and reciprocal ion radius determines the sequence. If $a(z)$ is positive, then larger ions are more permeant; conversely, if $a(z)$ is negative, then smaller ions are more permeant. The slope $a(z)$ is, in the limiting case considered here, composed of two terms: the first term in Eq. 6 reflects ion–dipole interactions and the second, ion-induced dipole interactions. As the first term varies linearly with z and is positive, whereas the second term varies as the square of z and is negative, the quantity $a(z)$ is positive when $z = 1$ and negative when $z = 2$. Thus, ion–dipole contributions dominate for monovalent ions, and ion-induced contributions are greatest for divalent ions.

The idealizations made in developing the results presented here have been discussed by Reuter and Stevens (13). Because we do not know the extent to which the limiting case treated here is a realistic representation of the actual situation, the quantitative accuracy of our conclusions is similarly uncertain. Our explanation for the sequence reversal certainly is a plausible and natural one, and the theory seems as if it should be accurate within an order of magnitude.

The values of the quantities f_o and α are also informative, even if they are only accurate to an order of magnitude. Extremely small differences in the fixed and induced dipole moments of the environments within the channel, as compared to free solution, are adequate to account for the selectivities we have observed. For example, an average difference (again, as compared to free water) in the fixed dipole moment within the channel of about 0.016 D for a distance of 1 Å and a difference in polarizability of about 4.4×10^{-27} cm^3 for one elementary charge and a distance of 1 Å account for our values of $a(z)$. These quantities may be compared, for example, to the dipole moment of water (1.8 D) and the average polarizability of the water molecule, 1.4×10^{-24} cm^3. In other words, the ionic environment within the selectivity region of the channel is almost identical to free solution: a difference between the selectivity region and free water in dipole moment and polarizability of <1% are sufficient to account for the selectivity properties of the AcChoR channel.

Can side chains of hydrophilic amino acids offer a sufficiently water-like environment to be consistent with our observations? Both atomic dipole moments and polarizabilities depend fairly strongly on details of chemical structure (17). For example, an OH bond has a moment of about 1.5 D, whereas an OC bond has a moment about half that value (table 1.1 in ref. 17). Furthermore, protein side chains are constrained in their movements so that orientations of protein groups interacting electrostatically with the permeating ion would, in general, present a different average structure to the ion than would free water of hydration. If the permeating ion were selected by interacting with hydrophilic groups on walls of the pore, then one would expect the ion to experience a less water-like environment than we infer to exist in the selectivity region. Therefore, selectivity in the AcChoR channel probably does not occur through ion–protein interactions.

Claudio *et al.* (1), Noda *et al.* (3), and Devillers-Thiery *et al.* (2) have all proposed, with varying degrees of detail, structures for AcChoR subunits that have four transmembrane helices (designated 1–4, with 1 closest to the amino terminus of the polypeptide). Noda *et al.* (3), using sequence information from all four subunits, have suggested that the wall of the transmembrane pore is formed by helix 1 (contributed by five protomers); Devillers-Thiery *et al.* (2), considering only information about the α subunit sequence, have proposed that the transmembrane pore is formed by helix 3 from each of the two α subunits and an unspecified helix from each of the other three subunits. In both proposed structures, the transmembrane pore would be uncharged but would be lined with hydrophilic side chains. It is not clear that such a structure could provide a sufficiently water-like environment to give the selectivity results reported here.

Kristofferson *et al.* (4) and Guy (5) have suggested an alternative structure with a fifth transmembrane helix that would have charged side chains along one face of the helix. Five such helices, one contributed by each protomer, would then provide a very hydrophilic charged pore that would have a ring of alternating positively and negatively charged groups located roughly in the middle of the membrane. This ring of positive and negative charges would form an energy barrier, the selectivity of which would depend on ion–water interactions in that region. Again, it is not clear that water in this region would be sufficiently like free water.

Although more detailed studies of possible pore structures are required, any proposal should, according to our present conclusions, provide for a selectivity region with an environment for the ion that is electrically very much like water.

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