Can selectivity be functionally modulated in ion channels?

Riccardo Fesce,[1,2] Lia Forti,[1] Alice Polenghi,[1] Albina Locarno,[1] Rita Canella,[3] Oscar Sacchi,[3] and Maria Lisa Rossi[3]

[1]Neuroscience Research Center, Insubria University, 21052 Busto Arsizio, Italy
[2]Neuroscience Division, San Raffaele Scientific Institute, 20132 Milano, Italy
[3]Department of Biology and Evolution, Physiology and Biophysics Section, Ferrara University, 44121 Ferrara, Italy

Most work on ion selectivity in membrane channels has regarded K^+ channels and the structural and local field aspects that determine K^+ selectivity. Much less is known about little selective channels, such as the acetylcholine (ACh) receptor (AChR) channel. In general, multiple factors appear to underlie K^+ versus Na^+ selectivity; in addition to structural aspects, external forces derived from the surrounding environment and exerted on the ion-ligated complex play a primary role in determining selectivity (Varma et al., 2011). This points to a specific aspect that appears to have received very little attention, possibly because it is particularly elusive to experimental investigation: namely, the possibility that the potential profile along the pore and at its mouths is physiologically influenced by contingent aspects, such as posttranslational modifications (e.g., phosphorylation) or solute composition of the intra- and extracellular solutions (pH and impermeant counter-ions). Functional or experimental modulation of ion channels has been studied with regard to gating and/or inactivation mechanisms, but very little is known about the possibility of modulating ion selectivity.

In a two-electrode voltage-clamp study on the rat sympathetic neuron, the properties of the subsynaptic native neuronal AChR (nAChR) in response to the physiologically released ACh were shown to be modified within a few hours after denervation (Sacchi et al., 2008), suggesting that the nAChR ion selectivity switched from preferential permeability to potassium ions to scarce selectivity between K^+ and Na^+; the changes regarded synaptic, but not extrasynaptic, receptors and revealed an unexpected flexibility of the nicotinic channel in its permeation properties. Subsequently, a number of quite simple experimental procedures in intact ganglia were also shown to produce changes in conductance and ion selectivity properties of the nAChR; unlike denervation, such procedures (resting membrane potential shifts within a voltage range of physiological interest, ionic modifications, and the action of α-bungarotoxin) were very unlikely to acutely produce modifications in nAChR subunit composition or steric conformation (Sacchi et al., 2011).

In particular, lowering extracellular Cl^- concentration (from 154 to 18 mM) reduced synaptic conductance by ∼20%, whereas Goldman's fits to experimental I-V curves suggested a decreased channel permeability to Na^+ but not K^+ (K^+/Na^+ permeability ratio from ∼1.5 to ∼2.2) and a shift of the zero-current potential by some 9 mV toward more negative values. Similar effects were produced by α-bungarotoxin; the two treatments were occlusive rather than additive. Conversely, a prolonged sojourn at hyperpolarized potentials (−90 mV), which produces redistribution of Cl^- ions and a decrease of $[Cl^-]_i$ from ∼40 mM (at −40 mV) to ∼8 mM (Sacchi et al., 2011), decreased synaptic conductance by ∼20%, affecting K^+ rather than Na^+ permeability (K^+/Na^+ permeability ratio from ∼1.5 to ∼1) and shifting the zero-current potential by some 9 mV toward more positive values (Sacchi et al., 2011).

Posttranslational modifications of the channel protein might have occurred, but the consistency of those results with the idea that impermeant Cl^- ions might affect cation binding and/or penetration into the pore raised the aforementioned question of whether extrinsic factors might contribute, together with the structural organization of the pore, to determine the permeability and ion selectivity of the channel. Actually, any change in the potential profile along the pore (and/or at its mouths) is bound to affect both the thermodynamic aspects of ion permeation (local field profile and ion binding to sites within the pore) and the kinetic aspects (ease of ion displacement and traveling among subsequent sites). These appear to constitute two distinct factors in determining ion selectivity, but the two aspects are strictly related.

The electrical field across the membrane, determined by the membrane potential (V_m), may well be constant and produce a linear change in free energy along the pore. However, the thermodynamic profiles in ion-selective channels display nonlinear variations in the local potential seen by each ion, so that the energetic profile, $\Delta G(x) = G(x) - G(0)$ (where 0 refers to the extracellular bulk solution), is not simply a result of the presence of

Correspondence to Riccardo Fesce: riccardo.fesce@uninsubria.it

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the membrane potential. At equilibrium, the probability for an ion to be located at x, $p(x)$, is proportional to $\exp(-\Delta G(x)/RT)$; this determines the ratio between forward and backward velocities. Thus, ion flow is described by

$$J(x) = \frac{\mu_0 RT}{dx}\left[p(x) - p(x+dx)\exp\left(\frac{G(x+dx)-G(x)}{RT}\right)\right] = -\frac{\mu_0 RT \times \frac{d}{dx}\left[p(x)\exp(\Delta G(x)/RT)\right]}{\exp(\Delta G(x)/RT)}, \quad (1)$$

where μ_0 is ion mobility in the absence of field. Eq. 1 easily reduces to Planck's equation. At steady state (i.e., for $J(x) = J$ = constant), Eq. 1 integrates over the length of the pore (L) to

$$J = \mu_0 RT \frac{p(0) - p(L)\exp(\Delta G(L)/RT)}{\int_0^L \exp(\Delta G(x)/RT)dx}. \quad (2)$$

For a constant electrical field across the membrane and no other sources of local potential, the denominator reduces to $(L \times RT/zFV_m) \times (\exp[zFV_m/RT] - 1)$, as long as $p(0)$ and $p(L)$ are respectively proportional to extra- and intracellular concentrations, C_{OU} and C_{IN}, Eq. 2 reduces to Goldman's equation,

$$J_i = P_i \frac{zFV_m}{RT} \times \frac{C_{IN}\exp(zFV_m/RT) - C_{OU}}{\exp(zFV_m/RT) - 1},$$

where Pi is pore permeability for ion species i. However, any region of convexity in the potential profile ($\Delta GE(x) > zF \times V_m \times x/L$) will increase the denominator in Eq. 2 and produce a decrease in ion permeability, whereas the opposite will occur for any concavity in the profile. Thus, the local field determined by the position and charge of protein residues that line the pore will directly affect both ion binding and (kinetic) permeability.

Eq. 2 can be applied to any location along the

$$\lambda(x) = \int_0^x \exp(\Delta G(l)/RT)dl,$$

pore; for the position-independent flow is $J = \mu_0 \times RT \times (p(0) - p(x)\exp[\Delta G(x)/RT])/\lambda(x)$, so that the probability for an ion to be found at each position x can be computed as

$$p(x) = \frac{p(0) - J \times \lambda(x)/(\mu_0 RT)}{\exp(\Delta G(x)/RT)} = \frac{p(0) - \left[p(0) - p(L)\exp(\Delta G(L)/RT)\right] \times \lambda(x)/\lambda(L)}{\exp(\Delta G(x)/RT)}. \quad (3)$$

This formula displays two relevant aspects: (1) local occupancy can be considered to be comprised of an equilibrium component, $p(0) \times \exp(-\Delta G(x)/RT)$, that would produce no flow (no derivative in Eq. 1), and a flow-generating component (the rest of the right-hand side of Eq. 3), dominated by the conditions at the extremities, $p(0)$ and $p(L) \times \exp(-\Delta G(L)/RT)$; and (2) the flow (as is the case for the rate of any physical process) is determined by the ratio between a driving force (the conditions at the two sides of the membrane) and a resistance factor; here, the latter mostly arises from the exponential sensitivity of pore occupancy to the pore energetic profile (denominator in Eq. 2), which essentially acts as a resistance to the flow by reducing the availability of current carriers.

As a purely qualitative, numerical example, an arbitrary pore energy profile has been simulated and is illustrated in Fig. 1 A for the Na^+ and K^+ ions. The profiles for the ions are arbitrarily designed, based on the suggested differential coordination of Na^+ and K^+ with the charges lining the pore (Nimigean and Allen, 2011). The same energy profile in the presence of a constant electrical field ($V_m = -3RT/zF \approx -75$ mV) is illustrated in Fig. 1 C. This gives rise to the integral of the energy factor, $\exp(\Delta G(x)/RT)$, which is illustrated in Fig. 1 D. In principle, the contribution of other ions to the local field can be taken into account, the resulting modified energy profile computed, and a similar though quite more complex computation can be performed to estimate the relative occupancy along the pore for two or more ions.

If reducing Cl^- ion concentration were to differentially elevate the energy barrier for the two ion species to enter the pore, as illustrated in Fig. 1 C (left and right panels), reducing $[Cl^-]_i$ or $[Cl^-]_o$ would respectively produce the energy integral profiles illustrated in Fig. 1 D (left and right panels) and determine the changes in I-V curves illustrated in Fig. 1 B. These curves well reproduce the experimental observations reported by Sacchi et al. (2011). Analysis of these curves according to Goldman's equation would lead to an estimate of P_K/P_{Na} ratios of 1.68 in control conditions and of 1.02 or 2.62 in decreased $[Cl^-]_i$ or $[Cl^-]_o$, respectively; the nAChR zero potential would shift from −16.1 mV (control) to −25.5 mV (low $[Cl^-]_o$), accompanied by an ∼18% decrease in synaptic conductance, and would shift to −5.5 mV in low $[Cl^-]_i$, with a decrease in conductance by ∼26%. These values are consistent with the experimental measurements reported by Sacchi et al. (2011).

Of course, none of this helps to explain selectivity; here, we simply assumed that the energy profile seen by distinct ion species at the channel access and along the pore would be different, examined the kinetic consequences of this, and observed that local alterations of ion–protein interactions would produce differential effects on the permeability of distinct ion species, giving rise to changes in permeability ratio and experimental estimates of ion selectivity. The fine mechanisms by

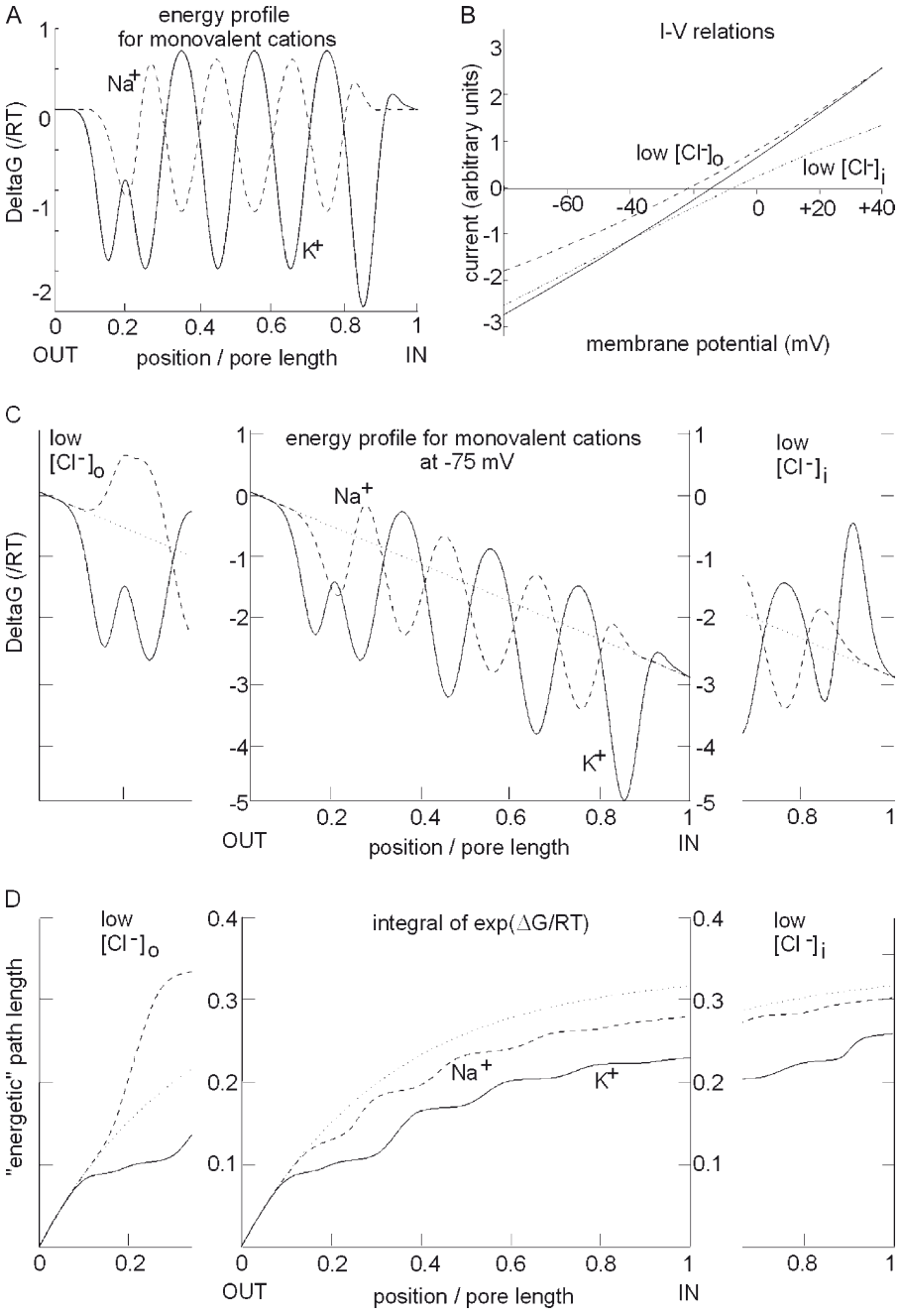

Figure 1. Simulation of the influence of the energy profile along the pore on Na^+ and K^+ permeation through an ion channel. (A) Simulated energy profile along a pore for Na^+ and K^+ ions. (B) I-V curves for such a channel, in control (solid line) or decreased $[Cl^-]_o$ (left dashed line) or $[Cl^-]_i$ (right dashed line). (C and D) The energy profile (C) and the integral of the energetic barrier (D) in the presence of −75 mV membrane potential (middle) and possible effects of decreased Cl^- concentration on the outer (left) or inner side (right) of the membrane.

which the binding of a toxin, or simple changes in Cl^- concentrations, may induce such effects remain elusive. Cl^- ions and toxin binding might mask fixed charges, interfere with the location and movement of water molecules at the pore mouth, or generate a Donnan equilibrium in possible channel vestibules, thereby affecting the energetic profile of the pore and its access regions. A Donnan equilibrium, in particular, might act either by altering local ionic strength or by interfering with the knock-on mechanism that favors K^+ flow through the channel (Nimigean and Allen, 2011); at the outer mouth of the channel, a decreased cation concentration would reduce interference with knock-on by the abundant Na^+, favoring K^+ flow over Na^+, whereas at the inner mouth, the knock-on mechanism may be impaired by the decreased K^+ concentration.

Posttranslational modifications may ensue as well during prolonged sojourns at altered membrane potentials. For example, phosphorylation, a small ubiquitin-like modifier protein (SUMO), and free polyunsaturated fatty acids have been shown to modulate gating and conductance properties of ion channels (Park et al., 2006; Börjesson and Elinder, 2011; Plant et al., 2011). The question of whether any of these mechanisms might interfere with ion selectivity as well, by changing the energetic profile of the path through the channel, has not been faced

experimentally so far. However, as clearly pointed out by Varma et al. (2011), structural analysis indicates that the preferred coordination patterns of K^+ and Na^+ ions do differ, and protein composition determines the steric organization of binding sites, but the availability of water molecules to a binding site and further interactions between the ion–ligand complex and the remainder of the protein, membrane surfaces, other ions, or the solution may alter the properties of the environment surrounding channel binding sites and modulate their selectivity.

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