Estimations of relative anion-cation permeabilities deduced from reversal (dilution) potential measurements, as in glycine receptor channel studies, are essentially model independent

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In a recently completed series of structure-function studies on human recombinant glycine receptor (GlyR) channels, expressed in HEK293 cells, we have shown that a single, double (SDM) and triple (STM) point mutations in the M2 region of the glycine receptor were able to each switch the GlyR selectivity from being anion- to cation-selective (Keramidas *et al.*, 2000, 2002; Moorhouse *et al.*, 2002). In order to relate ion selectivity to changes in electrostatic effects in the channel pore and in its minimum pore diameter, we needed both anion-cation permeability ratios and minimum pore diameters. The latter were determined by measuring cation-cation (or anion-anion) permeability ratios for a series of large test cations (anions) for the different cation-selective (anion-selective) mutant GlyRs. They were determined from bionic potentials, measuring the change in reversal potential, under whole cell patch clamp conditions, when the external solution cation (anion) was substituted by various larger cations (anions) for cation selective (anion-selective) mutant GlyRs. It has been shown that for such bionic measurements, the form of the membrane potential equation is essentially independent of the mathematical model underlying it, as discussed in Barry & Gage (1984).

However, concern has been expressed about the validity of using the Goldman-Hodgkin-Katz (GHK) equation (see Barry & Gage, 1984) for dilution potential measurements, given the inherent assumptions (a constant electrical field in the membrane and independence of ion fluxes) in its derivation. Experimentally, the anion-cation permeability ratios (P_{Cl}/P_{Na}) were determined from dilution potentials by measuring the change in reversal potential, when the external solution NaCl concentration was decreased to about 50% and then to about 25% and each shift in reversal potentials plotted against external NaCl activity. Experimentally, it was noted that the data did fit the GHK equation with the predicted straight line and constant permeability ratio. The GHK equation is:

$$\Delta V_{rev} = RT/F \ln [a_{Na}^{o} + (P_{Cl}/P_{Na})a_{Cl}^{i}] / [a_{Na}^{i} + (P_{Cl}/P_{Na})a_{Cl}^{o}]$$

where ΔV_{rev} is the shift in reversal potential, R, T and F have their usual significance and a_{Na} and a_{Cl} represent the activities of Na^+ and Cl in the external (o) and internal (i) solutions respectively.

We then fitted the data to the Planck equation, derived by solving the Nernst-Planck flux equations, which has virtually opposite underlying assumptions (a non-constant electrical field and a macroscopic electroneutrality condition) to the GHK ones. The Planck equation is:

$$\Delta V_{rev} = (RT/F) \ (P_{Na} \ P_{Cl}) / (P_{Na} + P_{Cl}) \ \ln \ a_{NaCl}^{o} / a_{NaCl}^{i}$$

However, it produced very similar permeabilities to those of the GHK equation. For example, P_{Cl}/P_{Na} values using the GHK [Planck] equation for the SDM and STM cation-selective mutant GlyRs were 0.12 [0.14] and 0.27 [0.27] and P_{Cl}/P_{Na} for the anion-selective WT GlyR was 28.5 [26.2].

Hence, the anion-cation permeability ratios determined using the GHK or Planck equations are essentially independent of the limiting underlying assumptions of those equations.

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