Further analysis of counterion permeation through anion channels: liquid junction potentials and offset corrections

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To determine how positive counterions permeate through anion-selective channels it is necessary to accurately determine their anion-cation permeability ratios. These are readily obtained from whole-cell patch-clamp situations by determining the shifts in zero-current reversal potentials in salt solution dilutions, with the Goldman-Hodgkin-Katz (GHK) equation used to analyse the data. We have already shown that the anion-cation permeability ratios (P_{Cl}/P_{cation}) of wild-type (WT) and mutant (with larger pore diameter) glycine receptor (GlyR) channels in the presence of Li^+, Na^+ and Cs^+ counterions, were inversely related to counterion hydration diameter, with P_{Cl}/P_{cation} increasing as hydration diameter approached or exceeded the channel minimum pore diameter (Sugiharto *et al.*, 2008). Corrected for liquid junction potentials (LJPs; using ion activities), the P_{Cl}/P_{cation} values were 23.4±2.8 (n=6; LiCl), 10.9±0.3 (n=32; NaCl) and 5.0±0.5 (n=6; CsCl) for the smaller WT channel (note that ignoring LJPs reduced each permeability ratio to about 4). Further analysis to incorporate an initial potential offset correction, to fully allow for slight differences between internal cell composition and external control salt solution, changed the above P_{Cl}/P_{cation} values to 29.0±4.4 (LiCl), 11.8±0.4 (NaCl) and 5.0±0.5 (CsCl), adding enhanced support for the relationship between counterion permeation and the difference between pore diameter and ion hydration size. Also, new direct measurements of LJPs (*e.g.*, for NaCl salt dilutions) using a 3M KCl-agar reference salt bridge (with freshly-cut end for each solution composition change) have shown precise agreement (within 0.1 mV experimental error) with calculated LJPs (using ion activities), validating such calculated values. We suggest that counterion cations permeate with chloride ions as neutral pairs.

Sugiharto S, Lewis TM, Moorhouse AJ, Schofield PR, Barry PH. (2008) Anion-cation permeability correlates with hydrated counterion size in glycine receptor channels. *Biophysical Journal* **95**: 4698-4715.