## Relative counter-ion permeation in anion-selective glycine receptor channels did not vary between two different anions, supporting an ion pair mechanism

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The ligand-gated glycine receptor ion channel (GlyR) mediates fast inhibitory synaptic transmission in the central nervous system. The wild-type (WT) homomeric α1 GlyR, with a minimum pore diameter of 5.3 Å, is predominantly permeant to $Cl^-$ anions, while a proline deletion (P-2'Δ) creates a larger (6.9 Å) minimum pore diameter and a smaller anion-cation permeability (*e.g.*, Keramidas *et al.*, 2004).

In a series of whole cell dilution potential experiments to investigate the mechanism of counter-ion permeation in the above two channels expressed in HEK 293 cells, we have already measured relative permeabilities of counter-ion cations to anions and shown that the hydrated counter-ion size determines the anion-cation permeability (Sugiharto *et al.*, 2006). The effective hydrated diameters of $Cl^-$, $NO_3^-$, $Cs^+$, $Na^+$ and $Li^+$ ions of 5.0, 5.2, 4.9, 6.5 and 7.4 Å, respectively, were calculated *de novo,* or re-calculated, from published data (Robinson & Stokes, 1965). We used the GHK (Goldman-Hodgkin-Katz) equation with activity corrections and $P_{Cl}/P_{cation}$ values were determined in LiCl, NaCl and CsCl solutions from reversal potential shifts (corrected for liquid junction potentials) with ~50% and ~25% external salt dilutions. We showed that in the smaller WT GlyR channels, $P_{Cl}/P_{Cs} = 5.1 \pm 0.5$, $P_{Cl}/P_{Na} = 12.4 \pm 0.4$ and $P_{Cl}/P_{Li} = 32 \pm 5$. However, in the larger mutant P-2'Δ GlyR channels, $P_{Cl}/P_{Cs} = 2.0 \pm 0.1$, $P_{Cl}/P_{Na} = 3.5 \pm 0.2$ and $P_{Cl}/P_{Li} = 6.6 \pm 0.5$. For both channels, as the hydrated counter-ion cation size increased so did $P_{Cl}/P_{cation}$. In addition, the smaller channel displayed the greater range of relative permeabilities (Sugiharto *et al.*, 2006). In anion-cation permeability measurements in a neuronal chloride channel, Franciolini & Nonner (1994) had previously shown that their counter-ion/co-ion permeability ratio was approximately constant for different anions.

We used whole-cell patch-clamp measurements to explore the counter-ion/co-ion permeability ratio for different anions to see if the same relationship held true for the WT GlyR channel, and if it could elicit further information about the mechanism of counter-ion permeation in that channel. We chose the nitrate anion, $NO_3^-$, which is similar in size to $Cl^-$ but has a different bi-ionic permeability (Lee *et al.*, 2003). The solutions contained either 145 mM NaCl or $NaNO_3$, together with 10 mM HEPES titrated to a pH of 7.4 with NaOH. In addition, each internal solution contained 2 mM $CaCl_2$ and 5 mM EGTA, and the internal $NaNO_3$ solution had 5 mM of its $NaNO_3$ replaced by 5 mM NaCl to maintain a well-defined Ag/AgCl electrode potential. The diluted external solutions had the diluted Na-salt replaced by an osmotically equal concentration of sucrose. We initially measured the reversal potential in bi-ionic $NaNO_3$ : NaCl solutions and determined a relative $P_{NO3}/P_{Cl}$ of 1.6 ± 0.1. We then did dilution potential measurements in $NaNO_3$ solutions, determined their reversal potentials and, fitting the data to the GHK equation, showed that $P_{NO3}/P_{Na} = 12.8 \pm 0.6$. This was very similar to the $P_{Cl}/P_{Na}$ value of 12.4 ± 0.4 and indicates that this counter-ion/co-ion permeability is not primarily determined by the anion permeability. It is supportive of counter-ion permeation being *via* neutral anion-cation pairs with $P_{Cl}/P_{cation}$ determined by the larger hydrated size of the cation counter-ions. It can readily be explained if (1) the ions permeate as neutral ion pairs, (2) the rate at which the ion pairs are formed is proportional to the rate at which the anions permeate, and (3) the hydrated sizes of the anions are smaller than those of the cations.

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