

External divalent ions increase anion-cation permeability in glycine receptor channels – consideration of ion activities, surface charge and conductance measurements

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The ligand-gated glycine receptor ion channels (GlyRs) mediate fast inhibitory synaptic transmission in the central nervous system and their functional role depends on whether they are predominantly permeable to cations or anions. Measurements of relative anion-cation permeability of such channels are often made with different concentrations of external divalent ions. We have already reported (Sugiharto *et al.*, 2007) that dilution potential measurements of P_{Cl}/P_{Na} in wild-type (WT) homomeric $\alpha 1$ GlyR have been found to give larger values, implying a greater relative anion-cation selectivity, in the presence of external divalent ions rather than in their absence. We have now further evaluated the effects of divalent ions: (1) by examining the use of activities for liquid junction potential (LJP) corrections, (2) by considering surface charge effects and (3) by directly measuring the effect of divalent ions on single channel conductances.

A recent paper of ours, on the relationship between anion-cation permeability and equivalent hydrated counter-ion size (Sugiharto *et al.*, 2008), highlighted the importance of ensuring that ion activities, rather than concentrations, are used in calculating liquid junction potential (LJP) corrections in all dilution potential measurements. We used the MS Windows version of *JPCalc* for such calculations (Barry, 1994). In the case of our divalent ion experiments, using ion activities for LJP corrections and an offset correction reduced the magnitude of P_{Cl}/P_{Na} to 15 ± 1 (compared to the previous value of 25 ± 2 , using concentrations) in the presence of 4 mM external $[Ca^{2+}]$, and to 11.7 ± 0.4 (compared to the previous value of 12.4 ± 0.4 , using concentrations) in the absence of external $[Ca^{2+}]$. This means that many of the previous measurements of P_{Cl}/P_{Na} that used concentration for LJP corrections for anion-selective channels in the presence of external $[Ca^{2+}]$, in particular, have overestimated this permeability ratio.

We have also explored the effect of ionic strength and divalent ions in the presence of surface charge near ion channels on relative anion-cation permeability. We have showed in a theoretical analysis that any change in local concentration of ions due to surface charge (with a resultant surface potential) near the channel is completely compensated for by the boundary potential from that channel to within the channel vestibule, in the absence of divalent ions, and almost completely compensated for if the divalent ion concentration is only a few mM. This point and other considerations suggest that the effect of external $[Ca^{2+}]$ on P_{Cl}/P_{Na} cannot be primarily explained by surface charge effects.

The question may be asked whether the effect of external divalent ions was to increase Cl^- permeability or to decrease Na^+ permeability or both. Single channel conductance measurements on GlyR channels in NaCl solutions indicate that while external $[Ca^{2+}]$ did increase Cl^- permeability to some extent, the major effect was to decrease counter-ion Na^+ permeability.

Our results still suggest that the presence of impermeant Ca^{2+} ions in the external vestibule affects anion-cation permeation by altering the electrostatic energy profile in that region.

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