External divalent ions decrease counter-ion permeation in anion-selective glycine receptor channels without changing the minimum pore diameter of the channel

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The ligand-gated glycine receptor ion channel (GlyR) mediates fast inhibitory synaptic transmission in the central nervous system. Dilution potential measurements of P_{Cl}/P_{Na} in wild-type (WT) homomeric α1 GlyR have been found to give larger values, implying a greater anion selectivity, in the presence of external divalent ions than in their absence. In order to investigate monovalent anion-cation selectivity and particularly the mechanism underlying counter-ion permeation in ion channels (Sugiharto *et al.*, 2006), it would be ideal to make such measurements in the absence of divalent ions. However, measurements in the literature are variously made with different concentrations of divalent cations. It is therefore particularly important to know whether the presence of divalent ions affects anion-cation permeability values.

We have done a series of whole-cell patch-clamp dilution potential experiments on WT homomeric α1 GlyR channels expressed in HEK 293 cells in the presence and absence of different external concentrations of Ca^{2+} to carefully investigate the relationship between $[Ca^{2+}]$ and P_{Cl}/P_{Na}. The solutions contained 145 mM NaCl, 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 external solution contained varying concentrations of $[Ca^{2+}]$. The diluted external solutions had the diluted Na-salt concentrations reduced to about half (75 mM) and one quarter (25 mM) and in each case were replaced by an osmotically equivalent concentration of sucrose. We have shown that dilution potential experiments performed in $[Ca^{2+}]_o$ ranging from 0 to 4 mM produced the following P_{Cl}/P_{Na} values: 12.4 ± 0.4 (32) [0 mM]; 13.4 ± 0.8 (6) [0.5 mM]; 15.5 ± 0.7 (6) [1 mM]; 19.3 ± 1.2 (6) [2.0]; 24.9 ± 1.8 (6) [4 mM], where the values are given as mean ± SEM, with the number of measurements in parentheses and $[Ca^{2+}]$ in brackets. These results clearly show that the addition of at least 1.0 mM Ca^{2+} produced a significant increase in P_{Cl}/P_{Na} and at 4 mM Ca^{2+} the P_{Cl}/P_{Na} value was double the value obtained at zero calcium. It was suggested that this might be due to an effect of Ca^{2+} reducing the minimum pore diameter of the GlyR channel. To test this we measured the reversal potential in virtually symmetrical NaCl solutions (V_{rev}), the shift under bi-ionic NaCl:NaFormate conditions (ΔV_{rev}) and the relative permeability to Cl^- of a large, but not impermeant, monovalent organic anion, formate, to give the results in the table below (the data are presented as the mean ± SEM, and number of measurements in parenthesis). All potentials were corrected for liquid junction potentials.

With 0 mM $[Ca^{2+}]_o$			**With 4 mM $[Ca^{2+}]_o$**		
V_{rev} (mV) NaCl:NaCl	ΔV_{rev} (mV) NaCl:NaFormate	$P_{Formate}/P_{Cl}$	V_{rev} (mV) NaCl:NaCl	ΔV_{rev} (mV) NaCl:NaFormate	$P_{Formate}/P_{Cl}$
–1.6 ± 0.4	21.0 ± 0.7	0.38 ± 0.01 (7)	–2.4 ± 0.3	20.6 ± 0.8	0.38 ± 0.02 (7)

This clearly indicates that although formate has a much lower permeability than Cl^-, 4 mM Ca^{2+} has no effect on the $P_{Formate}/P_{Cl}$ and hence has not changed the minimum pore diameter of the channel. It hence seems likely that external Ca^{2+} ions are somehow directly reducing the permeation of the counter-ion Na^+ maybe by making it energetically more difficult for Na^+ ions to dissociate from an Na-Cl ion pair in the selectivity filter and more difficult for it to enter the external vestibule of the channel.

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