Ion selectivity of glycine receptors with mutations of charged residues in the intracellular portals

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The glycine receptor (GlyR) is a member of the nicotinic-like family of ligand gated ion channels that are comprised of five subunits, each with a similar topology of a large N-terminal extracellular domain and four transmembrane domains (TM1 to TM4). The prototypical member of this family is the muscle endplate nicotinic acetylcholine receptor (nAChR). Cryo-electron microscopy studies of nAChRs from the Torpedo electric ray provide the best structural information of this receptor (Unwin, 2005) and by homology, a good template for other members of the family, including the GlyR. The nAChR structure shows that the intracellular loop between TM3 and TM4 is, in part, an α helix (designated 'MA') and the MA helix from each subunit comes together to form an inverted 'tee-pee' underneath the intracellular mouth of the ion channel pore. Charged residues in the MA helix are hypothesized to influence the ion size and charge selectivity of the channel as a consequence of lining the 'portals' or windows that form between adjacent MA helices. In the serotonin receptor (5-HT_3R), these residues influence the channel conductance (Kelley *et al.*, 2003). We investigated homologous residues in the MA helix of the α1 GlyR to see if they influenced ion selectivity or conductance.

Homology alignment of the amino acid sequence for the α1 GlyR subunit and the α subunits of the Torpedo nAChR and 5-HT_3R was used to identify the likely charged residues in the GlyR that line the MA helix and face the portals. Four positively charged residues were identified: Arg377, Lys378, Lys 385 and Lys386. Two mutant α1 GlyR subunits were created: one where all four of these residues were substituted for neutral alanine ('4A') and another where they were substituted for the negatively charged glutamate ('4E'). The cDNA for the wild-type, 4A and 4E GlyR mutants were separately transfected into 293 cells using a polyethylenimine reagent (jetPEI™) and glycine activated currents were recorded using standard whole-cell patch-clamp techniques. Current-voltage (I-V) curves were determined from a voltage-step protocol (100 ms steps) performed during the continued application of non-desensitizing concentration of glycine. I-V curves were initially determined in an extracellular solution containing (in mM): NaCl, 145; glucose, 10; HEPES, 10 (adjusted to pH 7.4). I-V curves were subsequently determined in solutions with 50% NaCl (75 mM) and 25% NaCl (37.5 mM) that were osmotically balanced with sucrose. The internal (pipette) solution was (in mM): NaCl, 145; $CaCl_2$, 2; EGTA, 5; HEPES 10 (adjusted to pH 7.4). The reversal potentials (V_{rev}) were determined from the I-V curves for each NaCl dilution and corrected for liquid junction potentials. The shifts in V_{rev} for each dilution were fitted with the Goldman-Hodgkin-Katz equation to estimate the relative permeability for chloride ions with respect to sodium ions (P_{Cl}/P_{Na}). The mean values for the V_{rev} obtained from the wild-type, 4A and 4E (n=7 in each case) are shown in the following table:

	V_{rev} (mV)		
	wild-type	4A	4E
100% NaCl	-0.3±0.2	-0.3±0.6	-0.8±0.3
50% NaCl	11.6±0.7	10.7±0.5	11.5±0.7
25% NaCl	22.6±1.5	22.4±1.3	22.8±1.4

The corresponding P_{Cl}/P_{Na} values determined were similar in all three cases: 7.5±0.2 for the wild-type, 7.0±0.5 for 4A, and 7.6±0.3 for 4E. These results indicate that the substitution of the four charged residues in the MA helix had no significant effect upon the ion selectivity of the α1 GlyR. Preliminary data suggest that both the 4A and 4E mutations reduce the single channel conductance. Further studies are required to address the possibility that other charged residues lining the portals may be involved in ion selectivity.

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Kelley S.P., Dunlop J.I, Kirkness E.F., Lambert J.J. & Peters J.A. (2003) *Nature* **424**, 321-324.