The charge of the P-loop glutamate controls cation-anion selectivity in CNG channels

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Cyclic-nucleotide-gated (CNG) channels play a critical role in olfactory and visual transduction. We have used site-directed mutagenesis and inside-out patch-clamp recordings to investigate ion permeation and selectivity in two mutant homomeric rat olfactory CNGA2 channels expressed in HEK 293 cells. We showed that a single point mutation of the negatively-charged pore-loop (P-loop) glutamate to either a positively-charged lysine or arginine did produce functional channels, which consistently responded to either cGMP or cAMP, although the currents were extremely small. We found that the concentration-response curve of the lysine mutant channel was very similar to that of wild-type (WT) channels, suggesting no major structural alteration to the mutant channels. Reversal potential measurements, during cytoplasmic NaCl dilutions, showed that both the lysine and the arginine mutations switched the selectivity of the channel from cations (P_{Cl}/P_{Na} = 0.07 [WT]) to anions (P_{Na}/P_{Na} = 15 [Lys] or 10 [Arg]). In addition, we showed that these mutant channels seem to have an extremely small single-channel conductance, measured using noise analysis to be about 1 pS, compared to a WT value of about 25 pS. Our results indicated that it is predominantly the charge of the E342 residue in the P-loop, rather than the pore helix dipoles, which controls the cation-anion selectivity of this channel. However, the outward rectification displayed by both mutant channels in symmetrical NaCl solutions suggests that the negative ends of the pore helix dipoles probably play a role in reducing the outward movement of Cl^- ions through these anion-selective channels. Such a postulated mechanism is also supportive of the mutations only causing local effects within the selectivity filter region of the channel. These results may have general implications for the determinants of anion-cation selectivity in the large family of P-loop containing channels.

We also showed from measurements of reversal potentials, with different halide ion substitutions, that the relative permeability of the halide ions increases with ionic radius in these E342K and E342R mutant CNG channels. Since ionic radius is inversely related to hydrated ion size, this result indicates that it is dehydration of these ions, as they pass through the selectivity region of the mutant channel, that is the major factor determining their relative permeability, as is also observed in anion-selective $GABA_A$ and glycine receptor channels (Fatima-Shad & Barry, 1993).

Fatima-Shad, K. & Barry, P.H. (1993) *Proceedings of the Royal Society (London) B*, 253, 69-75.