Ligand selectivity in pentameric ligand-gated ion channels

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Members of the pentameric ligand-gated ion channel superfamily (pLGICs) select for a variety of different agonists. Recent structural and mutational analysis has highlighted the relatively conserved role of a box of aromatic residues in coordinating the agonist positive charge. From early modelling based on an acetylcholine-binding protein (AChBP), we proposed that a glutamate residue, conserved in inhibitory $GABA_A$ and glycine receptors (GlyR), would replace one aromatic box residue and form a salt-bridge with the primary amine of GABA or glycine agonists. Thus "pinning" the agonist between this Glu and a previously identified arginine from the opposing subunit that interacts with the agonist carboxyl. This proposal has been supported by mutational analysis of GlyRs and structural analysis of a bacterial homolog, ELIC. Here we show that this Glu is a key determinant of agonist selectivity in $GABA_A$Rs but, surprisingly and in contrast to the GlyR, replacement with the smaller Asp shifts selectivity towards smaller agonists. Seeking an explanation for this apparent contradiction, we show that a series of charged residues provide salt-bridges that constrain the key Glu and determine selectivity for different sized agonists. We show further that an intersubunit hydrogen-bond provides an additional determinant of agonist-size selectivity between $GABA_A$Rs and GlyRs. Thus, whilst confirming the functional role of this Glu in $GABA_A$Rs, we have identified two mechanisms that determine agonist-size selectivity between $GABA_A$Rs and GlyRs, despite using the same two charged coordinating residues. Firstly, salt-bridges constrain the side-chain of the Glu and secondly, a hydrogen-bond alters the distance between these two coordinating residues.