Conformational changes associated with glycine receptor activation

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The glycine receptor Cl^- channel (GlyR) mediates inhibitory neurotransmission in the central nervous system. Like other members of the ligand-gated ion channel family, functional GlyRs comprise 5 subunits arranged symmetrically around an ion-conducting pore. Each subunit consists of a large external ligand-binding domain and 4 α-helical transmembrane domains (M1-M4). The external M2-M3 linker domain is crucial for transmitting the agonist-induced conformational change to the channel gate. Consistent with this role, a substituted cystine accessibility study on the M2-M3 linker of the α1 GlyR showed that the surface accessibility of 6 contiguous cysteine-substituted residues (R271C to K276C) was increased in the open state (Lynch *et al.,* 2001). Thus, the conformation of the M2-M3 domain depends on the degree to which the GlyR is activated by agonist. This study investigated whether the closure of the channels by picrotoxin preserves the relationship between domain conformation and fractional peak current magnitude that is seen in its absence. If this relationship is not preserved, it may be concluded that picrotoxin closes the channel by inducing a novel conformational change in this domain.

HEK293 cells were transfected with WT and mutant GlyR cDNA using the calcium phosphate precipitation method. Transfection solution was removed after 24 h and glycine-gated currents were recorded using whole-cell patch clamp techniques over the following 24–72 h. The surface accessibility of the introduced cysteines was assessed via their reaction rate with the sulfhydryl modifying agent, methanethiosulfonate ethyltrimethylammonium (MTSET) as previously described (Lynch *et al.,* 2001).

Picrotoxin significantly slowed the reaction rate of MTSET with A272C, S273C and L274C, although it had no measurable effect on R271C, P275C or K276C. Before interpreting this result as a picrotoxin-specific conformational change, it was necessary to eliminate the possibility of steric competition between picrotoxin and MTSET. One way of achieving this is to identify the location of the picrotoxin binding site. Accordingly, we showed that picrotoxin and the structurally-unrelated pore blocker, bilobalide, were both trapped in the R271C GlyR in the closed state and that a point mutation to the pore-lining T6' residue abolished inhibition by both compounds. We also demonstrated that the picrotoxin dissociation rate was linearly related to the channel open probability. These observations constitute a strong case for picrotoxin binding in the pore.

By binding in the pore, picrotoxin cannot sterically hinder MTSET from reacting with M2-M3 domain cysteines. We therefore conclude that picrotoxin changes the MTSET reaction rate by changing the intrinsic reactivity rates of the introduced cysteines. Because PTX changes the relationship between equivalent concentration and cysteine reactivity, we conclude that it alters the conformation of the GlyR M2-M3 domain in a way that cannot be achieved by simply varying the glycine concentration alone. This result implies that the M2-M3 domain integrates information from multiple categories of binding sites and sends a net signal to the activation gate. This reveals a hitherto unexpected complexity in the role of the M2-M3 domain.

Lynch, J.W., Han, N.-L. R., Haddrill, J.L., Pierce K.D. & P.R. Schofield. (2001) *Journal of Neuroscience*, 21, 2589-2599.