The location of the picrotoxin binding site in the glycine receptor pore

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Picrotoxin (PTX) is an equimolar mixture of picrotoxinin (PTXININ) and picrotin (PTN). They share common structures except that PTN possesses an extra OH group. PTXININ is highly selective over PTN as a noncompetitive antagonist of the $GABA_A$ receptor, whereas PTXININ and PTN are equipotent as glycine-dependent antagonists of the glycine receptor (GlyR). Both receptors are members of the pentameric cys-loop ion channel family and mediate fast inhibitory neurotransmission in the central nervous system. This study sought to probe the location and structure of their binding sites in the GlyR pore. The pore-lining 2′ and 6′ residues are known determinants of PTX sensitivity. We employed mutant cycle analysis to compare the sensitivities of PTXININ and PTN at wild type GlyRs relative to those incorporating mutations to the G2′ and T6′ residues. Various GlyR subunit combinations were recombinantly expressed in HEK293 cells and glycine-gated currents were measured by whole-cell recording. The G2′A mutation increased the α1 GlyR PTXININ sensitivity by 10 fold, whereas PTN sensitivity was not affected. Wild type α2 and α3 GlyRs, which both contain alanines at the 2′ position, showed similar pharmacological changes as seen in the α1G2′A GlyR. On the other hand, mutation of T6′ in the α1 GlyR to F, S or A caused a loss in sensitivity that was similar in magnitude for PTXININ and PTN. These findings demonstrate that the PTXININ variable group interacts directly with the 2′ residue, whereas the structurally-similar parts of PTXININ and PTN interact with T6′. Furthermore, we investigated the sensitivities of PTXININ and PTN at heteromeric α1β and α3β GlyRs. PTXININ sensitivity was significantly enhanced in the α3β GlyR whereas PTN sensitivity was not changed. This pharmacological difference could provide a useful tool for discriminating α1β and α3β GlyRs *in vivo*.