Mutations within the selectivity filter of the NMDA receptor channel influence voltage-dependent block by extracellular 5-hydroxytryptamine

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The NMDA receptor is a tetrameric cation channel which mediates important physiological processes such as long-term potentiation, synaptic plasticity and neurodegeneration *via* conditional Ca^{2+} signalling. The ionic influx through the open channel pore coincides with the presynaptic release of glutamate and postsynaptic membrane depolarization, which relieves voltage-dependent Mg^{2+} block. The asparagine residue on the NR2 subunit corresponding to position 596 (N+1 site) contributes to the Mg^{2+} binding site and affects channel rectification due to block by extracellular Mg^{2+}. In contrast, asparagine at position 598 (N0 site) on the adjacent NR1 subunits barely affects such events (Wollmuth *et al.*, 1998). Both residues are located near the tip of the M2 re-entrant loop and line the selectivity filter of the channel pore. Recently we reported voltage-dependent inhibition of NMDA receptor currents by 5-hydroxytryptamine (5-HT) (Kloda & Adams, 2005). The voltage sensitivity of the block indicated that 5-HT, similar to Mg^{2+}, binds within the membrane electric field.

In the present study, we assessed the effects of NR1(N0S) and NR2A(N+1Q) mutations of NMDA receptors expressed in *Xenopus* oocytes on the block by extracellular 5-HT using the two-electrode voltage clamp recording technique The mutation within the NR1 subunit of the NR1(N0S)-NR2A receptor combination, strongly reduced the magnitude of the block by 0.3 mM 5-HT and abolished the voltage dependence of block. The corresponding mutation within the NR2 subunit of the NR1-NR2A(N+1Q) receptor channels reduced the block by 5-HT to a lesser extent although the rectification of the I-V curve was similar to that observed for the wild type. This is opposite to the block produced by external Mg^{2+} where a substitution of the NR2A(N+1) site asparagine but not the NR1 N-site significantly reduces the block (Wollmuth *et al.*, 1998). Furthermore, the NR1 and NR2 mutant channels differed in their sensitivities to the 5-HT block compared to wild type. The IC_{50} values for 5-HT block at -120 mV were 59 µM for wild type but increased to 230 µM and 1.5 mM for the NR1 and NR2A mutants, respectively. These data indicate that the block by 5-HT is attenuated by corresponding asparagine mutations in the NR1 and NR2 subunits. The effect of the asparagine substitution in the NR1 and NR2 subunits on 5-HT block suggests that, in contrast to the Mg^{2+} block, 5-HT block critically depends on the NR1 asparagine residue and to a lesser extends on the NR2 residue. Thus, the binding of 5-HT to key residues in a narrow constriction of the channel pore may provide a significant barrier to ionic fluxes through the channel.

Kloda, A. & Adams, D.J. (2005) *British Journal of Pharmacology* 144, 323-330.
Wollmuth, L.P., Kuner, T., & Sakmann, B. (1998) *Journal of Physiology* 506, 13-52.