MA-stretch residues are critical for ion conduction of 5-HT_{3A} receptors

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5-Hydroxytryptamine type 3 (5-HT_3) receptors are members of the Cys-loop ligand-gated ion channel receptor superfamily. Members of this family, that include nicotinic acetylcholine (nACh), glycine and $GABA_A$ receptors, convert the chemical messages conveyed by neurotransmitters into electrical impulses through the selective conduction of ions. Electron microscopic images of the *Torpedo marmorata* nACh receptor suggest that residues within a helical stretch (the MA-stretch) of the M3-M4 cytoplasmic loop line intracellular portals that are an obligate pathway for ion flux (Unwin, 2005). It has recently been demonstrated that three arginine residues (R432, R436, R440) within the human 5-HT_{3A} MA-stretch are critical determinants of single channel conductance (γ) (Kelley *et al*., 2003). The collective replacement of these residues by their 5-HT_{3B} counterparts (producing the 5-HT_{3A}QDA receptor) increased γ 29-fold, with R436D exerting the greatest influence.

In the current study, the influence of all 5-HT_{3A} receptor MA-stretch residues (positions 426 to 442) on γ was investigated. A combination of alanine and arginine scanning and the substituted cysteine accessibility method (SCAM) was employed. Site-directed mutagenesis was used to introduce alanine, arginine and cysteine residues along the MA-stretch, one at a time. Mutations were introduced into a 5-HT_{3A}QDA receptor (Kelley *et al*., 2003) to allow detection of single channel events evoked by 5-HT in outside-out membrane patches. All mutant subunits produced functional receptors when expressed in tsA-201 cells, with the exception of the 5-HT_{3A}QDA(W442R) and 5-HT_{3A}QDA(W442C) subunits.

The introduction of alanine residues along the MA-stretch typically resulted in a decrease in γ compared to 5-HT_{3A}QDA receptors. In particular, with the exception of the R426A mutation, removal of a charged residue resulted in a significant change in γ ($p < 0.01$). The greatest change in γ was a ~2-fold decrease observed with the D436A mutation. Alanine mutant receptors were treated as controls for the remaining experiments.

The mutation of MA-stretch residues to the positively charged arginine residue typically resulted in a decrease in γ compared to alanine controls. This decrease was significant with the introduction of arginine at positions 427 and 431 to 440 ($p < 0.05$, n = 3-7). The greatest reduction in γ was observed with arginine present at the 436 and 440 positions. In contrast, γ was not reduced when arginine occupied positions 427, 428 and 431. Unexpectedly, the L429R mutation significantly increased γ ($p < 0.01$, n = 12).

Typically, the introduction of cysteine residues had little effect on receptor γ compared to control alanine mutants. Of the cysteine mutant subunits analyzed, addition of the positively charged methanethiosulfonate (MTS) reagent, MTSEA (200 μM), to the electrode solution reduced receptor γ compared to controls. The changes in γ mimicked the effects observed with the introduction of the positively charged arginine residues at all but three positions, suggesting successful reaction of MTSEA with introduced cysteine residues. This decrease in γ was significant at receptors containing the E434C to D436C and V438C to A440C mutations ($p <$ 0.001, n = 4-5), the greatest change being seen with the modification of cysteine residues at positions 435, 436 and 440.

This work establishes that a substantial portion of the 5-HT_{3A} MA-stretch influences ion conduction. In particular, residues at the 436 and 440 positions are the major critical determinants of γ.

Kelley SP, Dunlop JI, Kirkness EF, Lambert JJ & Peters JA. (2003) *Nature*, **424:** 321-4.
Unwin N. (2005) *The Journal of Molecular Biology*, **346:** 967-89.

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