073P A DETERMINANT OF SINGLE CHANNEL CONDUCTANCE WITHIN THE LARGE INTRACELLULAR LOOP OF A NICOTINIC ACETYLCHOLINE RECEPTOR

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Three arginine residues, particularly R436, within a putative α-helix (the HA-stretch) of the large cytoplasmic loop of the human 5-HT_{3A} receptor influence single channel conductance (γ) profoundly (Kelley *et al*., 2003). Such residues are postulated to frame narrow openings in the cytoplasmic vestibule of the channel within the ion permeation pathway (Miyazawa *et al*., 1999; Kelley *et al*., 2003). This study examines whether residues homologous to R436 within the HA-stretch of a neuronal nicotinic ACh receptor similarly affect γ.

Rat wild-type (WT), or mutant, nicotinic ACh α4 and β2 subunits were co-expressed from cDNAs introduced into HEK 293 cells by transient transfection. Mutant α4 (F588R) and β2 (Q443R) subunits were constructed by standard molecular biological techniques. Fluctuation analysis of macroscopic current responses, or single channel events recorded from outside-out membrane patches, in response to nicotine (100 nM - 100 μM) were used to estimate γ for WT and mutant receptors (Davies *et al*., 1999; Kelley *et al*., 2003).

Fluctuation analysis revealed a γ for WT α4β2 receptors of 27.3 ± 1.2 pS (n = 10). A similar value of 30.3 ± 0.8 pS (n = 7) was provided by direct recording of single channel events. The γ for receptors carrying the double mutation α4(F588R)/β2(Q443R) was significantly depressed as assessed both by fluctuation analysis (12.8 ± 0.4 pS; n = 10) and single channel recording (16.1 ± 0.5 pS; n = 7) ($p < 0.001$ t-test). Receptors assembled from the WT α4 subunit and β2(Q443R) subunit, or the α4(F588R) and WT β2 subunit, yielded single channel events with γ values of 26.8 ± 0.7 pS (n = 4) and 28.3 ± 0.9 pS (n = 4), respectively, only the former of which was significantly lower than the WT receptor ($p <$ 0.05 ANOVA and posthoc Tukey test).

The results indicate that the introduction of 2 arginine residues at a crucial location within the HA-stretch impacts significantly upon the γ of nicotinic ACh receptors assembled from α4 and β2 subunits. Cytoplasmic residues may thus prove to be important determinants of γ across additional members of the Cys-loop family.

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