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The defect in a slow channel myasthenic syndrome mutant, εL221F.

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One of many mutations that cause congenital myasthenic syndromes (Vincent *et al.*, 1997) is L221F in the ε subunit of the adult form of the human nicotinic acetylcholine receptor. Steady state recordings of single ion channel currents were made from HEK293 cells transfected with wild type α, β, ε and δ subunits, or with α, β, εL221F and δ subunits. Recordings were cell-attached at 100 mV, and had a resolution of 20 – 25 μs imposed retrospectively. The mutant channels are seen (Fig. 1) to produce longer bursts of openings than wild type (and therefore produce a more slowly decaying synaptic current). In order to interpret the observations in terms of receptor mechanisms (e.g. Colquhoun, 1998), the mechanism suggested by (Milone *et al.*, 1997) was fitted by maximising the likelihood of the entire sequence of (apparent) open and shut times with exact allowance for missed brief events ((Colquhoun *et al.*, 1996); program HJCFIT). The singly-liganded openings could not always be resolved clearly (c.f. Chen *et al.*, 1999), but the rate constants for doubly-liganded openings were quite consistent despite some kinetic heterogeneity from patch to patch. Calculation of the covariance matrix for the fits showed a strong positive correlation between the estimates of the channel shutting (α) and opening (β) rate constants, so their separate values were less well defined than their ratio, $E = \beta/\alpha$, the gating equilibrium constant, or *efficacy*. The total dissociation rate from both sites of the double liganded receptor was also quite well-defined. Wild type channels gave $E = 28.4 \pm 3.4$, and a total dissociation rate of 16600 ± 2200 s^{-1} (n = 5 patches, ± SDM). Mutant channels gave $E = 64.5 \pm 8.1$, and a total dissociation rate of 4290 ± 784 s^{-1} (n = 5). The fitted rates predict that mutation will slow the endplate current decay by 6.7-fold and decrease EC_{50} by 5.7-fold, mostly (3.4-fold) because of a reduction in agonist dissociation rate, but partly (1.6-fold) because of an increase in efficacy.

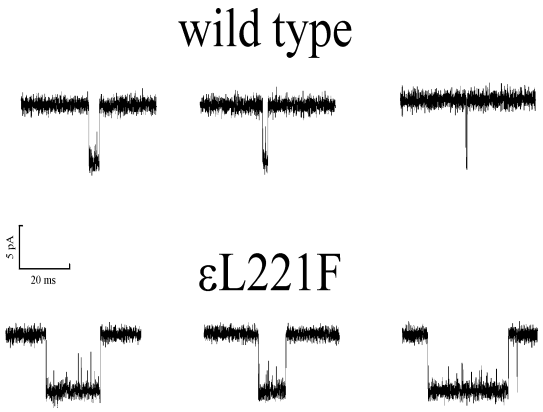

Figure 1. Wild type and mutant channel activations.

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