Mutation in Rod PDE6 Linked to Congenital Stationary Night Blindness Impairs the Enzyme Inhibition by Its γ-Subunit†

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Abstract

Photoreceptor cGMP phosphodiesterase (PDE6) is the effector enzyme in the vertebrate visual transduction cascade. The activity of rod PDE6 catalytic α- and β-subunits is blocked in the dark by two inhibitory Pγ-subunits. The inhibition is released upon light-stimulation of photoreceptor cells. Mutation H258N in PDE6β has been linked to congenital stationary night blindness (CSNB) in a large Danish family (Rambusch pedigree) (Gal, A., Orth, U., Baehr, W., Schwinger, E., and Rosenberg, T. (1994) *Nat. Genet.* 7, 64–67.) We have analyzed the consequences of this mutation for PDE6 function using a Pγ-sensitive PDE6α‘/PDE5 chimera, Chi16. Biochemical analysis of the H257N mutant, an equivalent of PDE6βH258N, demonstrates that this substitution does not alter the ability of chimeric PDE to dimerize or the enzyme's catalytic properties. The sensitivity of H257N to a competitive inhibitor zaprinast was also unaffected. However, the mutant displayed a significant impairment in the inhibitory interaction with Pγ, which was apparent from a ~20-fold increase in the K_i value (46 nM) and incomplete maximal inhibition. The inhibitory defect of H257N is not due to perturbation of noncatalytic cGMP binding to the PDE6α‘ GAF domains. The noncatalytic cGMP-binding characteristics of the H257N mutant were similar to those of the parent PDE6α‘/PDE5 chimera. Since rod PDE6 in the Rambusch CSNB is a catalytic heterodimer of the wild-type PDE6α and mutant PDE6β, Chi16 and H257N were coexpressed, and a heterodimeric PDE, Chi16/H257N, was isolated. It displayed two Pγ inhibitory sites with the K_i values of 5 and 57 nM. Our results support the hypothesis that mutation H258N in PDE6β causes CSNB through incomplete inhibition of PDE6 activity by Pγ, which leads to desensitization of rod photoreceptors.