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Structural determinants of phosphodiesterase 6 response on binding catalytic site inhibitors

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Abstract

To predict the response of retinal phosphodiesterase on binding catalytic site inhibitors, a homology model of the catalytic domain of subunit α of type 6 phosphodiesterase has been built by selecting an experimental structure of type 5 phosphodiesterase as template. Guanosine monophosphate and inhibitors (sildenafil, zaprinast) docked to the type 6 phosphodiesterase binding crevice similarly to the experimental conformations of guanosine monophosphate and sildenafil in the catalytic domain of type 5 phosphodiesterase. Inhibitors, but not guanosine monophosphate, interacted with Phe778 and Met759 (sildenafil) or Met759 (zaprinast), the key residues involved in the interaction between the catalytic binding domain and the inhibitory γ subunit of type 6 phosphodiesterase. Agreeing with predictions obtained by modelling binding, both inhibitors (1 and 10 μM) enhanced the amplitude of electric light responses of the isolated rat retina, however, the enhancement was smaller for the more efficacious inhibitor sildenafil. These paradoxical responses can be explained as a result of the enhancement of light activation of PDE6 through the competition between the catalytic site inhibitors and the γ subunit residues for catalytic domain residues Phe778 and Met759.

Keywords: Type 5 and 6 phosphodiesterases; Zaprinast; Sildenafil; Docking; Electric light response; Retina

Phosphodiesterases (PDEs) constitute a superfamily of enzymes that hydrolyse cyclic nucleotides and are recognised as important drug targets (Krier et al., 2005) in a number of pharmacological processes (Navarra et al., 2004; Prickaerts et al., 2004; Yeon et al., 2005). PDEs can be classified according to their specificity towards cyclic adenosine monophosphate (cAMP) and/or cyclic guanosine monophosphate (cGMP) (Mehats et al., 2002). A cGMP hydrolysing subtype, the heterotetrameric PDE6 is the key effector enzyme (enzyme classification number [EC]: 3.1.4.17 and 3.1.4.35) in the phototransduction cascade. PDE6 activation is responsible for the detection of light by photoreceptor cells (Norton et al., 2000).

The phototransduction cascade (Fig. 1A) starts with the capture of a single photon by the 11-cis retinal of rhodopsin, followed by photoismerisation of the chromophore and activation of rhodopsin (Choi et al., 2002). Rhodopsin then activates the photoreceptor cell-specific heterotrimeric G-protein transducin (Gt; Yeagle and Albert, 2003). Activation of Gt leads to dissociation of its α subunit (Gtα*) which subsequently binds to PDE6 (Norton et al., 2000) whereby relieves inhibition of catalytic subunits exerted by the inhibitory PDE6gamma (γ) subunits (Fig. 1B) (Granovsky et al., 1997). In its non-activated state (in darkness), PDE6γ is bound to two distinct domains of the catalytic subunits, i.e. the non-catalytic cGMP-binding GAFa and the catalytic domain (Cote, 2004). While high-affinity cGMP-binding to the GAFa domain increases subunit γ binding, cGMP-binding into the catalytic core does not seem to affect subunit γ binding (Mou and Cote, 2001). In the opposite way, subunit γ binding enhances cGMP-binding to the GAFa domain thereby stimulating its own binding in the presence of cGMP (Mou and Cote, 2001), and decreases cGMP-binding to the catalytic pocket by covering its entrance (Granovsky and Artemyev, 2001a; Zhang et al., 2005) (Fig. 1B).

During light activation, a single activated Gtα* binds to one of the γ subunits, bending it away from its contact points on the catalytic domains (Granovsky and Artemyev, 2001a) inducing a more than 300-fold increase in PDE6 activity (to about 6000–8000 cGMP hydrolysed/s/enzyme, Cote, 2004).

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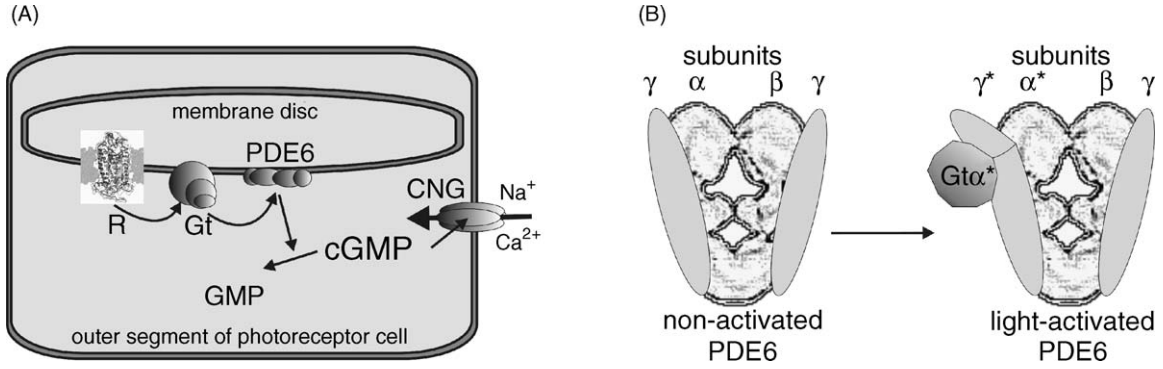

Fig. 1. The first steps of vision – schematics of the visual transduction cascade and PDE6. (A) The key elements of the cascade are packed inside the photoreceptor cells's outer segment. A single photon is captured by rhodopsin (R), which then activates the G-protein transducin (Gt). The activated α subunit of transducin (Gtα*) then relieves the inhibition from the key effector enzyme of the cascade – phosphodiesterase 6 (PDE6). As the rate of cGMP hydrolysis is increased, the free concentration of cGMP decreases and cyclic nucleotide-gated ion channels (CNG) close, thereby shutting off the inward current of Ca^{2+} and Na^{+} ions and hyperpolarising the photoreceptor cell. (B) In its non-activated form, rod PDE6 is composed of four subunits: two catalytic subunits (α and β) with an inhibitory PDE6gamma (γ) subunit attached to each catalytic subunit. In the non-activated state, the γ subunit covers the catalytic binding pocket, inhibiting the binding of cGMP and of the cGMP-analogue inhibitors, zaprinast and sildenafil. Gtα* binding to one of the γ subunits bends it away from PDE6 catalytic subunits (α) performing catalytic activation (α*). Scheme of PDE6 αβ catalytic dimer was adapted from Kameni Tcheudji et al. (2001).

Increased PDE6 activity transiently reduces free cGMP concentration ([cGMP]) inside the photoreceptor outer segments (Ames et al., 1986). As a result, cGMP bound to the cyclic nucleotide-gated (CNG) plasma membrane channels dissociates and CNG channels close (Kaupp and Seifert, 2002). Consequently, Na^{+}/Ca^{2+} ion current through CNG channels is inhibited. This shifts the transmembrane potential from approximately −40 mV in the dark (Baylor et al., 1979) towards the K^{+} ion equilibrium potential, causing hyperpolarisation (Copenhagen and Jahr, 1989; Baylor, 1996; Witkowsky et al., 2001). Hyperpolarisation spreads to the synapses formed between photoreceptor, horizontal and bipolar cells whereby evoking the decrease of the release of the neurotransmitter glutamate (Copenhagen and Jahr, 1989; Witkowsky et al., 2001; Barabás et al., 2002).

The X-ray crystal structure of PDE6 is not available yet, mainly due to problems with its expression (Granovsky and Artemyev, 2001b; White et al., 2004). However, the catalytic domain of PDE5, another cGMP-specific PDE enzyme has been crystallised and its structure elucidated in complex with GMP and specific inhibitors (Sung et al., 2003; Zhang et al., 2004). The global structure of PDE5 closely resembles that of PDE6 on the basis of their electron microscopic images (Kameni Tcheudji et al., 2001), and is similar in many other aspects to the photoreceptor-specific enzyme PDE6, making these two enzymes the closest relatives within the PDE family (for a recent review see Cote, 2004). Moreover, PDE5-specific inhibitors like zaprinast and sildenafil can be considered rather PDE5/6-specific (Zhang et al., 2005). The most important difference between PDE5 and PDE6 is the regulation of enzymatic activity. Lacking a γ subunit homologue, functional PDE5 constitutes a catalytic dimer only. Catalytic domain residues Met759, Phe778 and Phe782 contacting γ in PDE6 (Granovsky and Artemyev, 2001b) are replaced in PDE5 by Leu804, Ala823 and Gln827, respectively. For this reason, γ subunit does not show either binding or inhibitory activity toward PDE5 (Granovsky et al., 1998).

Besides broad-spectrum PDE inhibitors (theophylline, caffeine, IBMX), a new class of high-affinity cGMP-analogue PDE5 inhibitors including sildenafil, vardenafil and tadalafil have been discovered recently. These inhibitors compete with cGMP for the catalytic binding site and have been introduced as therapeutics for the treatment of erectile disfunction (ED) (Corbin and Francis, 1999; Dinsmore, 2005; Gupta et al., 2005). Sildenafil and vardenafil are both effective inhibitors of PDE6 also, with in vitro K_i values in the order of 5–10 nM and subnanomolar, respectively (Zhang et al., 2005). PDE6 is abundant in the mammalian retina, suggesting that the effects of PDE catalytic site inhibitors (D'amours et al., 1999; Turko et al., 1999) on retinal light responses (Barabás et al., 2002, 2003, 2004) can be attributed to their interactions with the catalytic site of PDE6. By blocking the phototransduction cascade, inhibition of PDE6 is expected to block or at least inhibit the light responses of photoreceptor cells and the retina. Paradoxically, long-term (30 min) application of the PDE catalytic site inhibitor zaprinast (200 μM) enhanced the amplitude of electric light responses (ELRs) in isolated rat retina (Barabás et al., 2004).

To explore the relationship between the paradoxical long-term response of PDE6 to inhibitors and inhibitor binding to the PDE6 catalytic site, a molecular modelling-based approach has been applied in combination with measurements of the long-term effects of low concentration of zaprinast and the more efficacious inhibitor sildenafil (1 and 10 μM, both) on the amplitude of ELRs.

1. Experimental procedure

1.1. Molecular modelling and docking

Homology modelling of the PDE6 alpha subunit catalytic domain was performed by Swiss-Model (Guex and Peitsch, 1997). Rod cell-specific human PDE6A (EC 3.1.4.17) sequence corresponding to the catalytic domain of the α subunit was aligned to crystallised human PDE5 structures (Protein Data Bank (PDB) code: 1TBF and 1T9S; Zhang et al., 2004), according to the alignment