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Oral presentation

Negative feedback control of the nitric oxide/cGMP pathway in smooth muscle

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Two major intracellular receptors for cGMP in smooth muscle are cGMP-dependent protein kinase (PKG) and phosphodiesterase-5 (PDE5), the latter of which contains both hydrolytic and allosteric sites for cGMP. We have shown that either one or both of these enzymes are involved in at least eight events leading to negative feedback control after nitric oxide-induced cGMP elevation: 1) increased hydrolysis of cGMP due to substrate elevation; 2) activation of PKG, which phosphorylates and activates PDE5; 3) enhanced phosphorylation of PDE5 by PKG due to exposure of the PDE5 phosphorylation site by elevated PDE5 hydrolytic site occupancy; 4) enhanced phosphorylation of PDE5 by PKG due to exposure of the PDE5 phosphorylation site by elevated PDE5 allosteric site occupancy; 5) increased sequestration of cGMP by PDE5 allosteric sites; 6) stimulation of PDE5 allosteric site occupancy by increased hydrolytic site occupancy; 7) stimulation of PDE5 allosteric site occupancy by increased phosphorylation of PDE5; and 8) conversion of PDE5 to a more active conformer by increased hydrolytic site occupancy. In concert, these events are predicted to result in increased cGMP breakdown and sequestration, producing negative feedback after cGMP elevation. The events could be explained by the presence of two interconvertible forms of PDE5: a less active conformer and a more active conformer that has increased cGMP hydrolytic activity, cGMP allosteric binding, and phosphorylation. By native PAGE (no SDS), cGMP or phosphorylation causes a gel shift consistent with a conformational change, and hydrolytic site-specific inhibitors such as sildenafil, vardenafil, and tadalafil cause a similar shift. Combined studies of x-ray crystal structures and site-directed mutagenesis identify three key amino acids (human Tyr-612, Gln-817, and Phe-820) in the PDE5 catalytic site that contribute importantly to high affinity of cGMP as well as potency and selectivity for PDE5 inhibitors. Binding of each of these ligands can initiate several of the cGMP feedback processes mediated by PDE5.

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