In mammalian cells cGMP levels are regulated by the action of two enzymes, termed guanylate cyclase and phosphodiesterase (PDE). Guanylate cyclases catalyse the formation of cGMP from GTP, whereas the PDEs catalyse the degradation of cGMP. cGMP has re-emerged as an important second messenger in mammalian cells. Firstly, the cytosolic guanylate cyclase has been identified as an effector for NO, whereas the membrane form is part of the atriopeptin receptor and is activated by the binding of atriopeptin to the receptor (for review see [1]). Secondly, cGMP activates cell responses principally by stimulating protein kinase G (PKG).

PDEs are expressed as a family of distinct isoforms (type 1–8), with each sub-group containing multiple spliced variants [2]. The different members of the PDE family are also differentially regulated by kinases, calcium/calmodulin, cGMP and G-proteins [3–10]. The integration of these diverse cell-transduction signals enables the precise co-ordinated regulation of intracellular cyclic nucleotide levels in response to receptor stimulation. The major cGMP PDEs are the type 1, 2, 5 and 6 isoforms. The characteristics and regulation of these isoforms are discussed below.

**Regulation of PDE-1**

PDE-1 is composed of a family of proteins that are encoded by at least three genes. Each member exists as two splice variants. The 61 kDa brain, 58 kDa lung and 59 kDa heart enzymes are products of a gene known as PDE-1A. The 63 kDa brain enzyme is a product of the PDE-1B gene, whereas an additional brain isoform, PDE-1C, has a molecular mass of 74 kDa and constitutes the third distinct gene product. These isoforms hydrolyse cGMP and cAMP with different kinetics [11]. For cAMP the $K_m$ values ($\mu M$) for type 1A:1B:1C are 113:24:1.2, whereas for cGMP they are 5:2.7:1.1. The $V_{max}$ ratios (cAMP/cGMP) are 2.9:0.9:1.2. These enzymes are activated by calcium/calmodulin and are regulated by phosphorylation/dephosphorylation reactions. For instance, the PDE-1A isoform is phosphorylated by protein kinase A (PKA), whereas PDE-1B is phosphorylated by calmodulin-dependent kinase. Phosphorylation reduces affinity for calcium/calmodulin.

**Regulation of PDE-2**

PDE-2 may contain 5' splice variants. The enzyme hydrolyses both cGMP and cAMP with similar $K_m$ values, although in the presence of cGMP, cAMP PDE activity is stimulated. cGMP binds to a non-catalytic site and induces both a loss in co-operativity between cAMP catalytic sites and a reduction in the $K_m$ for cAMP.

**Regulation of PDE-5, a cGMP-specific PDE**

PDE-5 is the major cGMP-binding protein in lung [12]. It is a homodimer composed of two identical catalytic subunits, each with a molecular mass of 93 kDa. PDE-5 is a substrate for phosphorylation by both PKA and PKG. Both
kinases catalyse phosphorylation of the same site, although for PKG this occurs at a 10-fold faster rate [13]. The phosphorylation of PDE-5 is accelerated by the binding of cGMP to a non-catalytic cGMP-binding site. Corbin and colleagues have proposed that this site may act as a physiological 'sensor' for elevated cGMP levels in intact cells [13]. However, for the purified enzyme, phosphorylation does not appear to modulate activity. To the contrary, we have demonstrated that the catalytic subunit of PKA catalyses a 10-fold increase in the $V_{\text{max}}$ of the guinea-pig lung PDE-5 [14,15]. Activation of PDE-5 is likely to enhance the rate of cGMP signal termination in intact cells. Indeed, cGMP-elevating agents elicit rapid transient phosphorylation and activation of PDE-5 in vascular smooth-muscle cells [16].

We have focused upon PDE-5 in airway smooth muscle (ASM) and have investigated the mechanisms that govern its regulation. Its membrane and cytoplasmic localization in these cells may affect the dynamics of cGMP formation in response to different agonists.

**A model for the interaction of PDE-5 and PDE-3**

Based upon the ability of PDE-5 to be activated by PKA, we have proposed a model for the regulation of both intracellular cGMP and cAMP in ASM cells. Firstly, agonists that activate guanylate cyclase increase intracellular cGMP. Secondly, the cGMP formed inhibits PDE-3 [17], an enzyme which specifically catalyses the hydrolysis of cAMP and which is expressed in high levels in these cells [18]. This will result in a subsequent increase in PKA activity, which may in turn phosphorylate and activate PDE-5 [14,16]. This may serve as a feedback mechanism that ensures that agonist-stimulated cGMP formation is transient. Finally, the enhanced hydrolysis of cGMP by PDE-5 restores PDE-3 activity, thereby allowing cAMP levels to also return to basal levels.

**Comparison of PDE-5 and PDE-6**

PDE-5 from smooth muscle and PDE-6 from photoreceptors share a number of common properties. They have two tightly bound subunits containing both catalytic and non-catalytic binding sites, hydrolyse cGMP better than cAMP and are inhibited by zaprinast. The non-catalytic cGMP-binding site is formed from an N-terminal tandem repeat that is indicative of gene duplication, whereas the catalytic site is located in the C-terminal region of the protein and is conserved in a number of PDE isoforms [2,19].

PDE-6 is a heterotetrameric protein composed of $\alpha$ (88 kDa), $\beta$ (84 kDa) and two inhibitory $\gamma$ (8-11 kDa) subunits. It serves as an effector in the visual signal transduction cascade [20]. This involves the light bleaching of rhodopsin, the sequential receptor-dependent GDP-GTP cyclical activation of the G-protein, transducin and the subsequent stimulation of PDE-6 activity by GTP-bound transducin [21]. The GTP-bound $\alpha$ subunit of transducin binds to and displaces PDE-6$\gamma$. The PDE-6$\gamma$ has two additional functions: firstly, it increases the affinity for cGMP in the PDE-6 non-catalytic site [22,23], and secondly, it participates in the activation of transducin GTPase, thereby sequentially terminating the activation of PDE activity by the GTP-bound $\alpha$ subunit of transducin [21]. Multiple similarities in the structure and function of PDE-5 and PDE-6 point to the possibility that PDE-5 might contain its own $\gamma$ subunit that couples this enzyme to an as yet unidentified G-protein-dependent pathway. Indeed, G-proteins have been implicated in the regulation of other PDEs, such as the hepatocyte insulin-sensitive cAMP-specific PDE [9].

The following describes results that are consistent with a G-protein-dependent regulatory pathway governing the activity of PDE-5.

**Lung and airway smooth muscle membranes contain two proteins immunoreactive with anti-PDE-6$\gamma$ antibodies**

Immunoblotting lung smooth-muscle and ASM membranes with antibodies raised to bovine PDE-6$\gamma$ has allowed the identification of two major immunoreactive-staining polypeptides. These polypeptides have molecular masses of 14 and 18 kDa and migrate more slowly than recombinant PDE-6$\gamma$. They clearly share common epitopes with PDE-6$\gamma$ and form a stable complex with PDE-5 in cell membranes.

**Phosphorylation by an endogenous G-protein-regulated kinase**

In order to test the hypothesis that these small-molecular-mass proteins regulate membrane-bound PDE-5, we have shown that PDE-5 can be
activated by PKA in isolated cell membranes. The role of G-proteins in the regulation of PDE-5 is supported by the observation that GppNHp inhibits the activation of PDE-5 by PKA. We therefore dismissed the possibility that the G-protein(s) regulates PDE-5 by a completely analogous mechanism to that which regulates PDE-6. Since G-proteins can also regulate various kinases, we assessed whether the 14 and 18 kDa proteins were substrates for phosphorylation. Indeed, both proteins are phosphorylated by an endogenous kinase. GppNHp (100 µM) was shown to induce an increase in the phosphorylation state of each of these proteins. This may mediate the inhibitory effect of GppNHp on PDE-5 and is supported by the finding that recombinant PDE-6γ prevents the activation of PDE-5 by PKA.

The addition of GppNHp to membranes also reduces the immunoreactivity of these proteins. Their corresponding appearance in the subsequently obtained 'high-speed' supernatant fraction was not detected, indicating that their reduced immunoreactivity may be a consequence of a post-translational modification, i.e. phosphorylation by the endogenous kinase close to the epitope, rather than a depletion in the amount of protein in the membrane. Further evidence for a G-protein involvement was indicated by the finding that pertussis toxin (0.1 µM, 24 h pretreatment of cells) blocked the effect of GppNHp on the phosphorylation of the 14 and 18 kDa proteins.

**Effect of catalytic-site-directed PDE inhibitors on 14 and 18 kDa protein immunoreactivity**

To identify further a relationship between PDE-5 and these small-molecular-mass proteins, we have shown that the addition of PDE inhibitors (isobutylmethylxanthine and theophylline, 100 µM each) to membranes, at a concentration that inhibits PDE-5 activity, abolishes the phosphorylation of the 14 and 18 kDa proteins by endogenous kinase. Therefore, direct ligand binding to PDE-5 can affect the regulatory control of these proteins by kinase.

**Physiological significance**

It is possible that the cytosolic PDE-5 forms a contiguous signalling cassette with the NO-regulated guanylate cyclase and PDE-3. This may represent a fast signalling mechanism that is limited by both the lifetime of NO and the phosphorylation of cytosolic PDE-5. Rapid transient cGMP signalling may be adapted to regulate acute cell responses, such as the inhibition of spasmonogen-induced smooth-muscle contraction. In contrast, the PKA-catalysed activation of the membrane-bound PDE-5 is inhibited by a G-protein-dependent mechanism. This may allow the concurrent atriopeptin-induced activation of the membrane-bound guanylate cyclase to generate a prolonged cGMP signal and may be adapted to inhibit smooth-muscle proliferation. These cell proliferative responses are characteristically slow and involve the induction of immediately and late genes by mitogen-activated protein kinase, the progression between cell-cycle phases and the rearrangement of cytoskeletal proteins, e.g. spindle formation.

**Concluding remarks**

We conclude that ASM cells express two polypeptides that are immunologically related to the rod photoreceptor inhibitory PDE-6γ subunit. These may represent members of a family of proteins that contain important domains for interaction with PDEs and that prevent the regulation of these enzymes by PKA andPKG. The separate regulation of membrane and cytoplasmic PDE-5 isoforms may differentially affect the ability of guanylate cyclase activators to block cell-cycle progression. This may have some bearing on future therapeutic approaches that are designed to block entry of cells into the cell cycle.

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