

## SERIES 'THEOPHYLLINE AND PHOSPHODIESTERASES INHIBITORS'

Edited by M. Aubier and P.J. Barnes

# Cyclic nucleotides and phosphodiesterases and airway function

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**ABSTRACT:** The cyclic nucleotides, cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) play an important role in the regulation of airway smooth muscle tone and activation of inflammatory cells. Intracellular concentrations of these nucleotides are tightly regulated by phosphodiesterases (PDEs).

Several families of PDEs are now recognized and, within each isoenzyme family, molecular cloning has revealed multiple members. PDE III and IV are important in the breakdown of cAMP; PDE III is involved in the regulation of airway smooth muscle tone, whereas PDE IV is more important in inflammatory cells, including mast cells, macrophages, eosinophils, T-lymphocytes and epithelial cells. PDE V is involved in the breakdown of cGMP in airway and vascular smooth muscle.

Regulation of PDE expression in health and disease is now under investigation. Several selective PDE inhibitors have recently been developed, and experimental studies indicate that PDE IV inhibitors may be useful as anti-inflammatory treatments in asthma. Clinical trials in asthma are now in progress.

*Eur Respir J., 1995, 8, 457-462.*

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Keywords: Anti-inflammatory drug  
cyclic adenosine monophosphate  
cyclic guanosine monophosphate  
phosphodiesterase inhibitor  
phosphodiesterase isoenzymes

Received: January 6 1995  
Accepted for publication January 6 1995

The cyclic nucleotides cyclic 3'5'-adenosine monophosphate (cAMP) and cyclic 3',5'-guanosine monophosphate (cGMP) are important second messengers and play a key role in the regulation of cell activity. In the airways, these nucleotides regulate the smooth muscle tone, mediator secretion and activation of inflammatory cells. The intracellular concentration of cyclic nucleotides is determined by stimulation of surface receptors and intracellular breakdown of cyclic nucleotides by phosphodiesterases (PDEs). Five distinct isoenzyme families have now been distinguished, based on substrate specificity and the development of selective inhibitors [1, 2], and molecular cloning studies have revealed that several additional families exist [3] (table 1).

### Theophylline as a PDE inhibitor

It is widely held that the bronchodilator effect of theophylline is due to inhibition of PDE, thereby leading to an increase in intracellular concentrations (fig. 1). Theophylline is a nonselective PDE inhibitor, but the degree of inhibition is small at concentrations of theophylline which are therapeutically relevant. Thus, total therapeutic concentrations of theophylline inhibit PDE activity in human lung extracts by only 5-10% [4]. There is no evidence that airway smooth muscle or inflammatory cells concentrate theophylline to achieve higher intracellular than circulating concentrations. Furthermore, inhibition of PDE should lead to synergistic interaction

Table 1. - Phosphodiesterase isoenzymes and inhibitors

Family	Isoenzyme features	Inhibitors
I	Ca <sup>2+</sup> /calmodulin stimulated	KS 505a, vinpocetine
II	cGMP stimulated	MEP-1
III	cGMP inhibited	Milrinone, cilostamide SK&F 94120 SK&F 94836 (siguazodan) Motipazone, Org 9935
IV	cAMP specific	Rolipram, Ro20-1742 Denbufylline
V	cGMP specific	Zaprinast, SK&F 95654
VI	Photoreceptor family	(Zaprinast)
VII	Rolipram-insensitive, cAMP specific	?
VIII	cAMP and cGMP, rolipram insensitive	?

cGMP: cyclic guanosine monophosphate; cAMP: cyclic adenosine monophosphate.

with  $\beta$ -agonists, but this has not been convincingly demonstrated. However, this might be explained by the recent observations that relaxation of airway smooth muscle by  $\beta$ -agonists may involve direct coupling of  $\beta$ -receptors *via* a stimulatory G-protein to the opening of potassium channels, without the involvement of cAMP [5, 6].

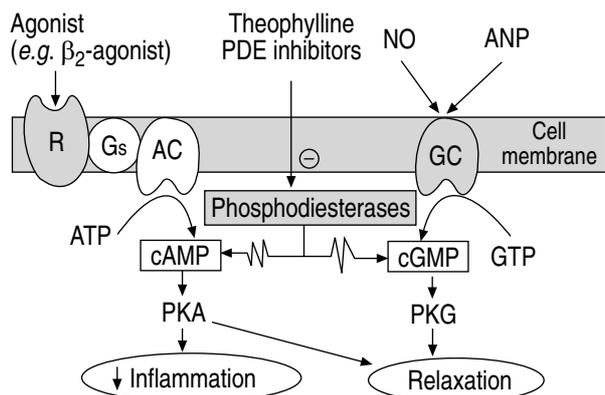


Fig. 1. — Role of phosphodiesterases (PDEs) in the breakdown of cyclic nucleotides in airway smooth muscle and inflammatory cells. R: receptor; Gs: stimulatory guanine nucleotide regulatory protein; AC: adenylyl cyclase; GC: guanylyl cyclase; NO: nitric oxide; ANP: atrial natriuretic peptide; PKA: protein kinase A; PKG: protein kinase G; ATP: adenosine triphosphate; cAMP: cyclic adenosine monophosphate; GTP: guanosine triphosphate; cGMP: cyclic guanosine monophosphate.

Some PDEs are more important in smooth muscle relaxation [7, 8], but there is no convincing evidence that theophylline has a greater inhibitory effect on the PDE isoenzymes involved in smooth muscle relaxation. It is possible, however, that PDE isoenzymes may have an increased expression in asthmatic airways, either as a result of the chronic inflammatory process, or as a result of therapy. Elevation of cAMP by  $\beta$ -agonists may result in increased PDE activity, thus limiting the effect of  $\beta$ -agonists. Indeed, there is evidence that alveolar macrophage from asthmatic patients have increased PDE activity [9]. In patients with atopic eczema, there is evidence for increased expression of PDEs in monocytes and lymphocytes in the peripheral blood, suggesting that atopic diseases may be associated with abnormal expression of PDEs in inflammatory cells [10]. This would mean that theophylline may have a greater inhibitory effect on PDE in asthmatic airways than in normal airways. Support for this hypothesis is provided by the lack of bronchodilator activity of theophylline in normal subjects, compared to the bronchodilator effect in asthmatic patients [11].

Recent studies suggest that theophylline may have immunomodulatory effects in the airways and may, therefore, have an anti-inflammatory effect in asthma. These effects of theophylline are seen at relatively low plasma concentrations ( $<10 \text{ mg}\cdot\text{l}^{-1}$ ) [12]. A recent study of placebo-controlled withdrawal of theophylline in patients who were also taking high doses of inhaled steroids has revealed that a deterioration in asthma control is accompanied by a fall in the level of activated CD4<sup>+</sup> and CD8<sup>+</sup> lymphocytes in the circulation and an increased number of these cells in the airways [13]. This suggests that theophylline may exert an inhibitory effect on the trafficking of activated T-lymphocytes into the airways of asthmatic patients. However, whether this is an effect due to inhibition of PDE or whether it is due to some, as yet undefined, cellular effect has not yet been determined. It is possible that theophylline may have a greater inhibitory effect on novel types of PDE that have not yet been

characterized. Further studies on the cellular and molecular actions of theophylline in asthmatic tissues are now required.

### Role of phosphodiesterases

Phosphodiesterases catalyse the breakdown of cAMP and cGMP within the cytoplasm of cells (fig. 1). Within each family, it is now emerging that there may be several subtypes of enzyme. Several PDEs have now been cloned, and it seems likely that different subtypes of isoenzymes may arise by alternative splicing of messenger ribonucleic acid (m-RNA) [14–16]. The recognition of multiple PDE isoenzymes provides opportunities for the development of selective inhibitors. It is now evident that the different isoenzymes are not expressed equally in all cells, so that selective actions of PDE inhibitors are theoretically possible. Four distinct genes for human PDE IV have now been identified [15, 16], and these appear to be differentially expressed in different types of cell [17]. This has raised interest in the possibility that selective PDE inhibitors may have therapeutic potential in the management of asthma [7, 8]; and has resulted in investigation of the role of different isoenzymes of PDE involved in airway smooth muscle and in inflammatory cells of the airways.

### Airway smooth muscle

Several PDE isoenzymes have been identified in airway smooth muscle, although the proportions of these enzymes varies between species and at different airway levels [18–20]. In human tracheal smooth muscle, we have identified the presence of PDEs I, II, III, IV and V [21], and similar results have been reported in human bronchial smooth muscle [22, 23]. Inhibition of PDE III with the selective inhibitors SK&F 94120 or SK&F 94836 (siguazodan) results in relaxation of canine and human airway smooth muscle, either on spontaneous tone or tone induced by carbachol [18, 24]. The PDE IV inhibitor rolipram does not reduce spontaneous tone but relaxes carbachol-induced tone [24]. This may be relevant in asthma, when airway tone is increased due to the release of various bronchoconstrictor mediators. A combination of PDE III and IV inhibitors is most effective in relaxing airway smooth muscle, suggesting that co-operation of the two isoenzymes PDE III and IV is necessary for optimal bronchodilator effects [23]. A combined type III/IV inhibitor, AH 21-132 (benafentrine), is very effective as a bronchodilator in bovine airway smooth muscle *in vitro* [25]. PDE V inhibitors, such as zaprinast, are also effective as bronchodilators, suggesting that cGMP is also involved in airway smooth muscle relaxation. This is consistent with the bronchodilator effect of sodium nitroprusside and other nitrovasodilators that is associated with an increase in cGMP concentration in airway smooth muscle cells [26].

Studies in guinea-pig *in vivo* have confirmed the bronchodilator effects of PDE III, IV and V inhibitors [7].

### Inflammatory cells

Several investigators have recently examined the PDE isoenzymes involved in inflammatory cells relevant to asthma (table 2). In both neutrophils and eosinophils the predominant PDE isoenzyme is type IV [27–30]. In guinea-pig peritoneal and human blood eosinophils, PDE IV inhibitors, such as rolipram and denbufylline, are highly effective in inhibiting superoxide anion release, whereas inhibitors of PDE III and V are ineffective [29, 31]. PDE IV in eosinophils is tightly membrane bound, and the kinetic analysis of cAMP hydrolysis suggests that at least two isoforms of the enzyme exist. Using reverse transcriptase polymerase chain reaction (RT-PCR), ENGEL *et al.* [17] have described the presence of PDE IV<sub>A</sub>, IV<sub>B</sub> and IV<sub>D</sub> mRNA in human eosinophils.

In cultured murine mast cells, a PDE IV has been identified and a PDE IV inhibitor inhibits histamine release [7]. In guinea-pig peritoneal macrophages, a PDE IV is also involved in the oxidative burst response [32]. However, this may not be true in human alveolar macrophages in which a selective PDE IV inhibitor, Ro 20-1724, is ineffective in inhibiting superoxide generation [33], but more recent studies suggest that PDE III and IV may be important [34]. In human peripheral blood monocytes, PDE IV inhibitors are highly effective in inhibiting the release of tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ) stimulated by exposure to lipopolysaccharide [35]. By contrast, PDE III inhibitors are ineffective. Of particular interest is the observation that PDE IV inhibitors produce significantly greater inhibition of TNF- $\alpha$  release than maximal doses of glucocorticosteroids.

T-lymphocytes appear to express multiple subtypes of PDE IV [36]. We have demonstrated the presence of PDE IV activity in CD4<sup>+</sup> and CD8<sup>+</sup> human T-lymphocytes, and there is evidence that PDE IV inhibitors inhibit the proliferative responses to anti-CD3 and phytohaemagglutinin and the synthesis of interleukin-2 (IL-2) and interferon-gamma (IFN- $\gamma$ ) in these cells [37]. Although PDE III inhibitors have no measurable effect on these cells, there is a clear synergy with PDE IV inhibitors, suggesting that combined PDE III/IV inhibitors may be useful as immunomodulators. There is also evidence that

PDE IV inhibitors impede the expression of interleukin 4 and 5 (IL-4 and IL-5) genes in Th2 cells [38]. A human T-lymphocyte-like cell line (Jurkat) has been found by RT-PCR to express PDE IV<sub>A</sub>, but not IV<sub>B</sub>, IV<sub>C</sub> or IV<sub>D</sub> [17]. Recently, a rolipram-insensitive high affinity cAMP specific PDE (which may correspond to PDE VII) has been identified in T-lymphocyte cell lines, although its functional role is not certain [39].

The PDE isoenzymes in endothelial cells have not been investigated in detail, and the role of cyclic nucleotides in the expression of cell adhesion molecules is not known. There is evidence that both PDE III and IV inhibitors abolish the increase in permeability of cultured porcine endothelial cells induced by hydrogen peroxide [40], suggesting that PDE inhibition may result in reduced microvascular permeability. PDE IV has also been identified in airway epithelial cells [41]. Since these cells may be an important source of inflammatory mediators in asthma, PDE IV inhibitors may have an important regulatory effect on them.

The role of PDEs in the regulation of neurotransmission has not yet been studied in detail. There is evidence that PDE IV inhibitors modulate the release of neuropeptides from airway sensory nerves and may, therefore, modulate neurogenic inflammation in the airways [42].

Several studies have investigated the potential anti-inflammatory effects of selective PDE inhibitors on airway responses *in vivo*. Combined PDE III/IV inhibitors impede inflammatory cell infiltration into the lungs of guinea-pigs after allergen and platelet-activating factor (PAF) inhalation [43–45]. In addition, similar inhibitory effect of rolipram on allergen-induced eosinophilic inflammation and airway hyperresponsiveness has recently been reported in monkeys [46]. A PDE IV inhibitor is also effective in inhibiting airway microvascular leakage induced by PAF and allergen in guinea-pigs [47].

### Regulation of PDEs

There is relatively little information about the regulation of PDE expression in different cells relevant to asthma. PDE III is inhibited by cGMP and, therefore, stimulation of cells with agents that stimulate guanylyl cyclase may result in an increase in cAMP *via* inhibition of cAMP hydrolysis by PDE III. Thus, in guinea-pig trachea sodium nitroprusside, which increases cGMP, enhances the increase in cAMP induced by rolipram [48].

PDE IV expression is regulated by intracellular concentrations of cAMP. A long-term increase in cAMP in response to  $\beta$ -agonists or rolipram results in increased PDE IV gene expression in a human monocyte cell line (U937) [49]. This may be due to the activation of the transcription factor, cAMP response element binding (CREB) protein (fig. 2). Elevation of cAMP induces an increase in PDE IV<sub>A</sub> and IV<sub>B</sub>, but not IV<sub>D</sub> in these cells, suggesting that there may be differential regulation of PDE IV isogenes by cAMP [17]. There may be important therapeutic implications from these observations, as this suggests that chronic treatment with PDE IV inhibitors may result in increased PDE IV expression, thus overcoming the therapeutic effect of the

Table 2. – Distribution of phosphodiesterases (PDEs)

	Predominant PDE isoenzyme
<b>Smooth muscle</b>	
Airway smooth muscle	III, IV, V
Vascular smooth muscle	III, V
<b>Inflammatory Cells</b>	
Mast cell	IV
Macrophage	IV (III)
Monocyte	IV
Eosinophil	IV
Neutrophil	IV
Platelet	III, V
T-lymphocyte	IV
Endothelial cell	III, IV
Sensory nerves	IV
Airway epithelial cells	IV

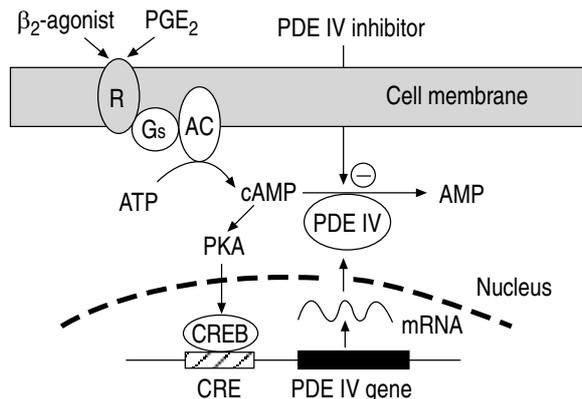


Fig. 2. – Effect of cAMP on PDE IV gene transcription. CREB: cAMP response element binding protein which binds to a putative CRE in the promoter sequence of a PDE IV gene. mRNA: messenger ribonucleic acid; PGE<sub>2</sub>: prostaglandin E<sub>2</sub>. For further abbreviations see legend to figure 1.

inhibitor. Whether this means that tolerance will develop with long-term clinical use has not yet been determined.

There is evidence for increased PDE IV activity in peripheral blood monocytes from patients with atopic dermatitis [10]. It is possible that PDE IV genes have been induced by inflammatory signals, such as cytokines. Indeed, stimulation of rat thymic lymphocytes with concanavalin A results in a marked increase in PDE IV activity [50]. If PDE IV induction occurred in inflammatory cells in asthma, possibly in response to proinflammatory cytokines, this would result in reduced intracellular cAMP levels, leading to increased release of inflammatory mediators and to reduced  $\beta$ -adrenergic responsiveness (fig. 3). The effect of glucocorticosteroids on PDE IV gene expression is not yet known.

### Clinical implications

Whether selective PDE inhibitors will prove to be useful new anti-asthma compounds, and whether they will improve on theophylline is not yet certain. Current evidence suggests that PDE VI inhibitors will have anti-inflammatory potential, whereas a combination of type III and IV inhibitors will have bronchodilator effects. Several combined III/IV inhibitors, such as zardaverine and benafentrine, have been developed, and preliminary evidence suggests that they have some bronchodilator effect and inhibit methacholine-induced bronchoconstriction [51, 52], although more extensive investigations are required. A selective PDE III inhibitor, enoxamine, acutely reduced airway resistance in patients with decompensated chronic obstructive pulmonary disease (COPD) [53]. A PDE V inhibitor, zaprinast, was found to inhibit exercise- but not histamine-induced bronchoconstriction after oral administration [54].

The most promising type of drug for future production is a PDE IV inhibitor, and several are now under development for asthma. It is predicted that such drugs will have anti-inflammatory effects, since *in vitro* and animal studies predict inhibition of mast cells, macrophages, eosinophils and CD4<sup>+</sup> lymphocytes. In addition, there

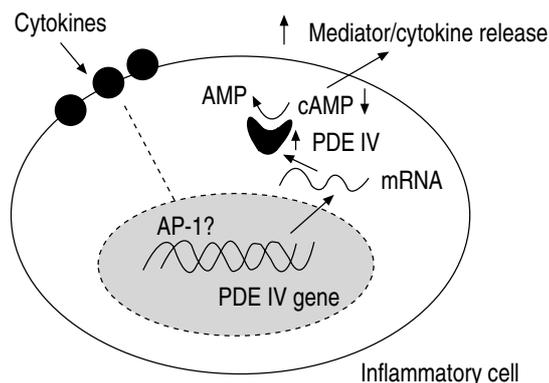


Fig. 3. – Possible increase in PDE IV activity in asthmatic inflammatory cells. AP-1: activator protein-1, a transcription factor that may mediate the effects of cytokines on PDE IV gene expression. For further abbreviations see legend to figures 1 and 2.

may be some bronchodilator activity, which would improve compliance. If side-effects are a problem, it may be possible to develop an inhaled formulation. These clinical studies are at a very early stage and more information is required, particularly after long-term administration, to assess potential anti-inflammatory effects.

### Side-effects

One of the most important considerations is whether selective PDE inhibitors will have side-effect. Because of their cardiotonic action, selective PDE III inhibition may be associated with cardiac arrhythmias, tachycardia and vasodilatation. A major side-effect of PDE IV inhibitors may be nausea and vomiting [2]. These side-effects prevented the clinical development of rolipram as an antidepressant. PDE V inhibitors may cause systemic vasodilatation, since cGMP mediates the effects of exogenous and endogenous vasodilators. It may be possible to overcome these problems with the inhaled route of delivery. It may also prove possible to identify subtypes of isoenzymes which are less likely to have these side-effects. Some recently developed PDE IV inhibitors appear not to give rise to vomiting, but whether they will be effective in asthma remains to be determined.

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