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Phosphodiesterase 4 Inhibitors and the Treatment of Asthma

Where Are We Now and Where Do We Go from Here?

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Abstract

Research conducted over the last 20 years has established that inflammation of the airways is central to the airway dysfunction that characterises asthma. Typically, the airway wall is infiltrated by a variety of cells including mast cells, eosinophils and T lymphocytes, which have deviated towards a T_H2 phenotype. Together, these cells release a plethora of mediators including interleukin (IL)-4, IL-5, granulocyte/macrophage colony-stimulating factor and eotaxin which ultimately cause the histopathology and symptoms of asthma. Glucocorticosteroids are the only drugs currently available that effectively impact upon this inflammation and resolve, to a greater or lesser extent, compromised lung function. However, steroids are nonselective and generally unsuitable for paediatric use. New drugs are clearly required. One group of potential therapeutic agents for asthma are inhibitors of cyclic AMP-specific phosphodiesterase (PDE), of which theophylline may be considered a prototype. It is now known that PDE is a generic term which refers to at least 11 distinct enzyme families that hydrolyse cAMP and/or cGMP. Over the last decade, inhibitors of PDE4 (a cAMP-specific family

that negatively regulates the function of almost all pro-inflammatory and immune cells, and exerts widespread anti-inflammatory activity in animal models of asthma) have been developed with the view to reducing the adverse effects profile associated with non-selective inhibitors such as theophylline. Such is the optimism regarding PDE4 as a viable therapeutic target that more than 100 PDE4 inhibitor patent applications have been filed since 1996 by 13 major pharmaceutical companies. This article reviews the progress of PDE4 inhibitors as anti-inflammatory agents, and identifies problems that have been encountered by the pharmaceutical industry in the clinical development of these drugs and what strategies are being considered to overcome them.

Epidemiological studies indicate that the prevalence and severity of allergic asthma is increasing[1] together with the number of reported cases of fatal asthma.[2,3] These statistics are of concern given the marked increase in the prescribing of various anti-asthma therapies.[4,5] Although glucocorticosteroids are considered the most effective anti-inflammatory drugs currently available for asthma, they are nonselective and are associated with adverse effects particularly in children. Thus, new drugs with enhanced selectivity and improved adverse effect profiles clearly are required. One group of drugs that, from a theoretical perspective, may exhibit powerful anti-inflammatory and immunomodulatory activity are inhibitors of cyclic AMP phosphodiesterase (PDE) of which theophylline may be considered a weak and non-selective prototype. Currently, 11 distinct PDE families have been identified unequivocally which display a unique tissue and sub-cellular distribution, and differ in substrate specificity, inhibitor sensitivity and cofactor requirements.[6-15] With respect to asthma, 2 cAMP hydrolysing PDEs, denoted PDE3 and PDE4, have been considered as potential targets amenable to therapeutic intervention with selective inhibitors. The most important of these enzymes is PDE4, which is expressed in airways smooth muscle, pulmonary nerves, and almost all pro-inflammatory and immune cells relevant to the pathogenesis of asthma (table I). Indeed, PDE4 inhibitors suppress many processes that are believed to contribute to the inflammation associated with asthma by blocking the degradation and, thereby, increasing the level of cAMP mass in target cells and tissues (see section 3). For this reason, selective inhibitors of PDE4 have been synthesised in the hope that they will display steroid-like, anti-inflammatory activity together with a reduced adverse effect profile relative to glucocorticosteroids and nonselective PDE inhibitors such as theophylline.[16-27] Inhibitors of PDE3 also have been evaluated clinically (see section 4), but potential life-threatening cardiovascular complications preclude further development.[28-31]

This short article reviews the current status of PDE4 inhibitors for the treatment of asthmatic inflammation, identifies problems that have been en-

Table I. Human cells and tissues implicated in the pathogenesis of asthma and the distribution of phosphodiesterase (PDE) enzyme families

Cell/tissue	PDE families identified
T lymphocyte	3, 4, 7[a]
B lymphocyte	3, 4, 7
Eosinophil	4, 7[a]
Basophil	3, 4, 5
Mast cell	3, 4
Monocyte	3, 4, 7[a], 8[a]
Macrophage	1, 3, 4, 5
Neutrophil	4
Airways smooth muscle	1, 2, 3, 4, 5, 7[a], 8[a]
Epithelial cell	1, 2, 3, 4, 5, 7[a], 8[a]
Endothelial cell	3, 4
Platelet	1, 2, 3, 5
Vagus nerve	1[b], 3, 4, 5

a Identified at the mRNA level by reverse transcription-polymerase chain reaction (RT-PCR).

b Represents >90% of the total PDE activity in guinea pig desheathed vagus nerve, which contains parasympathetic and sensory fibres.

Data compiled from Giembycz,[16] Torphy[17] and author's unpublished observations.

countered by the pharmaceutical industry in their clinical development and discusses strategies that are being considered to overcome them.

1. What is Phosphodiesterase (PDE) 4?

PDE4 is a generic term used to describe a large family of enzymes that share several common characteristics. Without apparent exception, PDE4 isoenzymes are acidic proteins that exclusively hydrolyse cAMP[32,33] and are inhibited by nanomolar concentrations of rolipram, an archetypal inhibitor of this enzyme family. Molecular techniques have identified and cloned 4 mammalian cDNA homologues[34-37] of the *Drosophila melanogaster* 'dunce' cAMP PDE[38] establishing a molecular basis for the heterogeneity of PDE4 variants within this PDE family. These clones represent transcripts of 4 different genes and have been classified as RNPDE4A, RNPDE4B, RNPDE4C and RNPDE4D, where RN and the last letter refer to the species (in this case *Rattus Norvegicus*) and the gene, respectively.[6] Subsequent studies have provided evidence for at least 4 human genes that encode PDE4 isoenzymes.[33,39-45] Like their rat counterparts, these enzymes are classified similarly, i.e. HSPDE4A, B, C and D, and are encoded by distinct genes that have been localised to chromosomes 19, 1p31, 19 and 5q12, respectively.[46,47]

An astonishing finding that emerged from the molecular cloning of PDE4 isoenzymes is the presence of mRNA transcripts of different sizes for each of the 4 variants differentially expressed between tissues.[33,48,49] An example of this multiplicity is exemplified in human T lymphocytes, where at least 3 out of a possible 5 mRNA transcripts derived from the PDE4D gene have been identified.[50] PDE4 heterogeneity can be attributed to alternative mRNA splicing and PDE4 genes expressing multiple promoter regions providing several potential start codons for translation of protein.[51] Diversity of PDE4 isoenzymes presumably allows for the highly co-ordinated regulation of cAMP levels in discrete sub-cellular locations, thereby permitting fine-tuned control of specific cAMP-dependent responses.[52] Indeed, the extreme amino terminus of these enzymes features unique sequences that are believed to target spliced variants to specific intracellular organelles[53-60] expressing so-called scaffold or adapter proteins such as myomegalin[61] and receptor for activated C-kinase-1.[60] Further discussion of the structure, multiplicity and regulation of PDE4 isoenzymes is beyond the scope of this review but interested readers should consult articles by Torphy,[17] Conti et al.,[48] Houslay et al.[49] and Bolger.[62]

2. Why PDE4 Inhibitors?

The prototype PDE inhibitor that has been used in the treatment of asthma for many years is the alkylxanthine theophylline, which is widely prescribed. The main beneficial activity of theophylline was originally attributed to its weak bronchodilator action. However, evidence accumulated in the early 1990s points to an anti-inflammatory action of this compound at sub-bronchodilator doses.[63-65] This has provoked a remarkable resurgence of interest in theophylline and the so-called 'second generation' PDE inhibitors not only as smooth muscle relaxants but also as potential antiallergic and/or anti-inflammatory agents.[16-19,23,24,26,27] However, despite providing further impetus for the development of novel, isoenzyme-selective PDE inhibitors, it is unclear whether theophylline does, in fact, owe its therapeutic activity to PDE inhibition, which raises some interesting questions regarding the development of anti-inflammatory drugs in the future (see section 6.6).

Regardless of the mechanism of action of theophylline, the rationale for developing new PDE inhibitors has stemmed primarily from the realisation that these enzymes are highly heterogeneous, differentially expressed between different cell types and, presumably, regulate specific functional responses. Accordingly, it was rapidly appreciated that selective inhibition of a particular PDE isoenzyme may result in a discrete functional alteration of cells expressing that PDE variant and, theoretically, specific functional responses within the same cell. In this respect, almost every cell type that has been implicated in the pathogenesis of asthma ex-

presses representatives of the PDE4 isoenzyme family (table I).[66-108] Conceptually, PDE4 inhibitors should show a pleiotropic profile of activity on many cells types involved in the inflammation of asthma, and so differ from classical mediator antagonists whose importance in disease progression might vary between asthma patients and so have limited usefulness. Torphy[17] recently emphasised this point by reference to the eosinophil. Thus, inhibition of PDE4 can attenuate the elaboration of eosinophil chemotaxins from several cell types, the adherence of eosinophils to the post capillary microvascular endothelium, and the secretion of survival-enhancing cytokines such as interleukin (IL)-5 and granulocyte/macrophage colony-stimulating factor (GM-CSF). In addition, PDE4 inhibitors exert direct effects on the eosinophil and can suppress degranulation, activation of the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, and the generation of lipid mediators. A further prediction is that inhibiting PDE4 should potentiate the effects of endogenous anti-inflammatory agents that stimulate adenylyl cyclase through Gs-coupled receptors such as catecholamines, prostaglandin E_2 and prostacyclin.[17,109] Taken together, the pre-clinical pharmacology of these compounds provides an exciting rational basis for the development of novel anti-inflammatory pharmaceuticals for asthma.

3. What Has Been Established?

The results from extensive *in vitro* experiments and *in vivo* studies in laboratory animals have provided much optimism for the development of 'second generation' PDE inhibitors. Almost without exception, and consistent with the prediction outlined in section 2, drugs that inhibit the activity of PDE4 suppress a diverse range of functional responses across many cell types (including all of those indicated in table I) implicated in the pathogenesis of asthma.[66-108] Significantly, these agents negatively regulate the secretion not only of acute inflammatory mediators, such as histamine and cysteinyl leukotrienes, but also of other factors believed to be pivotal to disease progression and chronicity. The more important of these are the cytokines and chemokines, including GM-CSF, IL-4, IL-5 and eotaxin. Additionally, PDE4 inhibitors block the adhesion of a variety of leucocytes to vascular endothelial cells, chemotaxis, and the generation of oxygen-derived free radicals. From these data, it is clear that PDE4 inhibitors act at multiple sites and thus would be expected to have a general suppressive action on many indices of the inflammatory response. For a detailed description of the *in vitro* pharmacology of PDE4 inhibitors interested readers should consult reviews by Giembycz et al.[16] and Torphy.[17]

Generally, the *in vitro* data obtained from cell based assays are predictive of the behaviour of PDE4 inhibitors *in vivo* in a number of species including the mouse, rat, guinea-pig, dog and monkey. Given that asthmatic inflammation is believed to be eosinophil-driven, it is noteworthy that PDE4 inhibitors suppress IL-5-, platelet activating factor (PAF)-, leukotriene D_4 (LTD_4)- and, above all, antigen-induced eosinophilia in sensitised animals together with hyper-reactivity of the airways, the late phase response, microvascular leakage, and cytokine generation.[110-132] Of particular significance are the results obtained with rolipram[115] and the PDE4 inhibitor atizoram (CP-80633)[116] in cynomolgous monkeys sensitised to *Ascaris suum*. When administered subcutaneously, rolipram significantly suppressed antigen-induced pulmonary neutrophil and eosinophil accumulation, and the associated increase in IL-8 and tumour necrosis factor (TNF)-α in the bronchoalveolar lavage (BAL) fluid at a dose (10 mg/kg) that increased the cAMP content of BAL fluid leucocytes. Rolipram also reduced the pulmonary eosinophilia and airways hyper-responsiveness to methacholine chloride which occurred over a 7-day period in response to multiple antigen challenges.[115] PDE4 inhibitors are also effective after antigen challenge,[122,124] indicating that stabilisation of mast cells cannot account totally for these activities. Indeed, the finding that PDE4 inhibitors suppress pulmonary eosinophil recruitment by agents that are selective eosinophil chemotaxins, including IL-5,[126] suggests multiple sites of action including

a general suppressive effect of those mechanisms that govern the emigration of eosinophils from the circulation to the airways.[17] For a detailed description of the *in vivo* pharmacology of PDE4 inhibitors interested readers should consult reviews by Giembycz et al.[16] and Torphy.[17]

4. Clinical Experience

Despite extensive *in vitro* and *in vivo* data in laboratory animals, clinical trials with cAMP PDE inhibitors are relatively limited. However, a number of studies have been published with equivocal results. Oral administration of the selective PDE3 inhibitor cilostazol produced a bronchodilator and antispasmolytic effect in normal human volunteers,[30] and another PDE3 inhibitor enoximone, given by the intravenous route, has been shown to improve lung function in patients with chronic obstructive pulmonary disease (COPD).[29] More recently, the effect of the PDE3 inhibitor SDZ-MKS-492 at a dose of 40mg orally was examined in 18 atopic patients with asthma. In these patients, it abolished allergen-induced bronchoconstriction [as assessed by the measurement of forced expiratory volume in 1 second (FEV_1)] and significantly attenuated the late phase response.[31] However, it is likely that the diminution of the late phase response in SDZ-MKS 492-treated patients was due to functional antagonism since PDE3 inhibitors are effective airways smooth muscle relaxants[133] but have limited anti-inflammatory activity.[26,27] Unfortunately, the desirable actions of these compounds are accompanied by adverse effects which result from the inhibition of PDE3 in the cardiovascular system.[28] Tachycardia, hypotension, often with coincident headache, and arrhythmias, which reflected ventricular extrasystole in 1 patient, are the most serious deleterious effects.[29-31] However, in 1999, Myou and colleagues[134] reported that administration of the PDE3 inhibitor, olprinone (E-2010) 2mg by inhalation to 9 patients with mild asthma promoted rapid (within 15 minutes) bronchodilatation (20.5% mean increase in FEV_1) that lasted for the duration (60 minutes) of the experiment. The significance of this observation was that, in contrast to other studies, the airways effect of olprinone was evoked in the absence of tachycardia or a fall in systolic or diastolic blood pressure indicating that inhalation of this drug can reduce cardiovascular exposure. Nevertheless, the long term consequences of PDE3 inhibitors administered by this route are unexplored and ways to minimise potential adverse cardiovascular complications have been sought. One strategy has been the synthesis of compounds that inhibit both PDE3 and PDE4 in the hope that lower doses will be clinically more effective than a PDE3 inhibitor alone (see section 6.3). However, thus far, trials of this approach have yielded inconclusive results. Foster and colleagues[135] reported that, when administered by inhalation, the PDE3/PDE4 inhibitor benafentrine produced bronchodilatation in normal volunteers but when it was administered by the oral or intravenous routes it was inactive. Zardaverine, another structurally dissimilar PDE3/PDE4 inhibitor, demonstrated modest bronchodilator activity in patients with asthma when given by inhalation[136] but was inactive in a group of patients with COPD.[137] Another mixed inhibitor, tolafentrine similarly was inactive in a group of patients with mild asthma.[138] Inhalation of tolafentrine 500μg did not significantly affect airway responsiveness to histamine or AMP, which is thought to release histamine from mast cells and so promote bronchoconstriction indirectly. The level of exhaled nitric oxide, a surrogate marker of airways inflammation, was also unchanged by tolafentrine.[138]

The appreciation that many adverse effects of PDE inhibitors reflect an extension of their pharmacology has persuaded the pharmaceutical industry to concentrate on selective inhibitors of PDE4 despite their limited direct effect on airways smooth muscle tone. Perhaps the earliest clinical study of the effect of a PDE4 inhibitor in asthma was reported by Israel and co-workers with tibenelast.[139] Although this compound increased FEV_1 in a group of patients with asthma, the effect did not reach statistical significance.[139] The second generation PDE4 inhibitor piclamilast (RP-73401) is also without effect in individuals with moderate

asthma.[140] Indeed, inhalation of piclamilast 1.2mg for 15 minutes failed to attenuate allergen-induced bronchoconstriction in 11 patients[140] despite the nanomolar potency of this compound as an inhibitor of PDE4.[74] However, given that mast cell-derived mediators are primarily responsible for allergen-induced early phase responses, these data are not necessarily surprising because selective PDE4 inhibitors do not stabilise mast cells.[68] More disappointing is the finding that piclamilast 50 and 100μg inhaled twice daily for 6 weeks did not improve FEV_1, airway responsiveness to methacholine or the level of exhaled nitric oxide.[140,141]

Nevertheless, some encouragement can be derived from the results obtained with CDP-840, a potent and selective PDE4 inhibitor.[142] A double-blind, placebo-controlled study in 54 patients with asthma indicated that 9.5 days treatment with CDP 840 30 mg/day had no effect on the early response elicited by allergen but attenuated the late phase response by approximately 30%, implying that the inflammatory response *per se* was being modified.[143] Indeed, that conclusion is concordant with the inability of single doses of CDP-840 (15 and 30mg) to promote bronchodilatation in patients with asthma.[143] Unexpectedly, CDP-840 had no effect on airways responsiveness to histamine, although the authors argue that the treatment period may have been too short citing that at least 2 weeks are required before changes in reactivity are seen in response to steroids.[143]

Further positive clinical data have been published for another PDE4 inhibitor SB-207499 (Ariflo™).[92] At a press conference in London in 1998 it was reported that SB-207499 produced a greater effect on FEV_1 than salmeterol and that it suppressed the early and late phase responses to allergen in asthmatic subjects (see Norman[144]). Nieman and colleagues[145] also found that, in 27 patients with exercise-induced asthma, SB-207499 10mg twice daily produced significant improvements in lung function after 7 days. More recently, the results of a multicentre, placebo-controlled, double-blind, randomised, parallel group study with SB-207499 (5, 10 and 15mg twice daily for 6 weeks) involving 303 patients taking inhaled corticosteroids concurrently have been reported.[146] All patients had an FEV_1 of approximately 66% of predicted, expressed a 12% or greater responsiveness to salbutamol, and had asthma that was inadequately controlled with inhaled corticosteroids.[146] At the highest tolerated dose of 15mg twice daily, SB-207499 (n = 79) increased FEV_1 from week 1 and to a greater extent than placebo (n = 72). However, the improvement in lung function relative to placebo failed to reach statistical significance at any time except at week 2.[146] SB-207499 appeared to be well tolerated with headache and nausea accounting for the major adverse effects but affecting only 13.9 and 8.9% of patients, respectively, at the highest dose.[145]

5. Why Have PDE4 Inhibitors Not Demonstrated Clinical Efficacy?

Despite the animal and clinical results described in sections 3 and 4, experience with several structurally dissimilar 'second generation' PDE4 inhibitors in the clinic has, thus far, been disappointing. While beneficial effects on airways smooth muscle tone have been documented, little evidence is available to support an anti-inflammatory action of these compounds. A number of factors could account for the lack of efficacy reported by many investigators which are not mutually exclusive. Some of these are described in this section.

5.1 Poor Bioavailability and Short Half-Life

A major difficulty in selecting PDE4 inhibitors for asthma has been extrapolating drug metabolism and pharmacokinetic properties of promising compounds across species. This is clearly illustrated with reference to rolipram where oral administration of 50 mg/kg to the rhesus and cynomolgous monkeys, the rat, rabbit and humans results in complete absorption and an approximately equivalent half-life (1 to 3 hours) but with bioavailabilities of 0.1, 0.37, 3.6, 3.7 and 75%, respectively.[147] Reasons for the poor efficacy of CDP-840 in clinical trials, despite its potency as an inhibitor of PDE4, is provided in patent applications from Celltech

which cite low bioavailability and short half-life probably due to extensive first pass metabolism.[148,149] An assessment of biological activity in whole blood can provide invaluable information on the behaviour of PDE4 inhibitors *in vivo*. For example, CP-293121 has reduced emetic potential but is extensively protein bound and, therefore, is not bioavailable.[144,150] However, poor bioavailability is not an insurmountable problem. Using piclamilast as an example, which is only 1% bioavailable in humans as it was designed for the inhaled route, several changes to the central benzimide moiety has profound effects. Thus, replacement of the cyclopentyl and phenyl rings with tetrahydrofuran and pyridine N-oxide, respectively, and oxidation of the pyridine ring to pyridine N-oxide, produces a compound (RPR-114597) that is 77% bioavailable in humans.[151]

5.2 Species Variability in Measures of Efficacy and Toxicity

Perhaps the single most confounding approach in the selection of PDE4 inhibitors for clinical development has been the evaluation of efficacy and toxicity in different species. The emetic potential of drugs is invariably examined in dogs or ferrets whereas measures of biological activity almost always involve *in vivo* studies in rats, guinea-pigs and monkeys, and *in vitro* experiments in human blood leucocytes. From these very different test systems the efficacious and toxic effects can be separated through the calculation of therapeutic concentration/dose ratios. A tempting, although generally incorrect, assumption is that these ratios always hold in humans regardless of the test systems employed for the analyses. Thus, it is vital to select the correct models of efficacy and toxicity for the evaluation of PDE4 inhibitors.[152] Clearly, a model where indices of clinical efficacy and toxicity are measured in same species is an ideal solution to some of these limitations, although this can be technically challenging.

5.3 Adverse Effects are Dose-Limiting

Another explanation for the lack of efficacy of PDE4 inhibitors in asthma is that the level of drug ingested is too low to inhibit PDE4 in target cells and tissues. Invariably this occurs because of dose-limiting adverse effects, nausea and vomiting, which are believed to represent an extension of the pharmacology of these compounds.[153] It would seem to be important in clinical evaluations of PDE4 inhibitors to establish that the amount of drug given inhibits PDE4 *in vivo*. While this is difficult to accomplish directly, several surrogate markers of PDE activity can be used such as the *ex vivo* measurement of cAMP in bronchoalveolar lavage leucocytes, akin to the experiments performed in nonhuman primates,[115] or more indirect measurements such as *ex vivo* cytokine production from peripheral blood leucocytes.[154]

6. How Can Adverse Effects be Minimised?

If it is accepted that dose-limiting adverse effects account, in part, for the poor clinical activity of PDE4 inhibitors in human asthma, then what strategies could be adopted to increase the therapeutic ratio? Several possibilities have been considered.

6.1 Exploitation of the PDE4 Isogene Family

Molecular genetics has established that PDE4 is a universal term that refers to a family of closely related proteins (see section 1). Thus, one potential approach to minimise adverse effects while retaining beneficial activity might be the development of 'third generation' inhibitors selective for a particular PDE4 gene product.[155,156] Currently, there is little information in the literature addressing this issue, and the functional significance of a particular PDE4 gene product in pro-inflammatory and immune cells is unknown. However, evidence is available that subtype-selective compounds can be synthesised. Indeed, SB-222618, SB-254375 and SB-254376 are up to 17-fold selective for human recombinant PDE4A/B over PDE4D. Conversely, SB-207039 and SB-250583 are PDE4D-selective

compounds with respect to PDE4A/B. Moreover, using a range of compounds with varying degrees of subtype selectivity, Manning and colleagues[157] have reported that inhibition of lipopolysaccharide-induced TNFα production from human monocytes and antigen-stimulated T lymphocyte proliferation correlates more closely with suppression of PDE4A/B than of PDE4D. These data, thus, represent the first evidence for distinct functional roles of PDE4 isoenzymes in human cells. With regard to PDE4 inhibitors in clinical development, it is of interest that SB-207499 and V-11294A are, respectively, 10- and 30-fold more selective for PDE4D than for other enzyme families.[151,158] In this respect it is intriguing that V-11294A is nonemetic in ferrets at 30 mg/kg despite being >70% bioavailable and achieving a plasma concentration (> 1 μmol/L) that is sufficient to significantly inhibit PDE4 *in vitro*.[151,159,160] The lack of emesis has also been observed in human male volunteers in Phase I clinical trials at oral doses of up to 300mg. These findings are significant, as V-11294A is similarly bioavailable (approximately 50%) with a half-life of approximately 7 hours,[151,159,160] and achieves a plasma concentration that suppresses the activation of inflammatory cells [e.g. lipopolysaccharide (LPS)-induced TNFα generation from monocytes, phytohaemagglutinin (PHA)-induced T cell proliferation] *ex vivo*.[154]

However, a note of caution is merited here. While, conceptually, this approach seems logical, it pre-supposes that all dose-limiting adverse effects are, indeed, attributable to PDE4 inhibition (see Robichaud et al.[161]). Moreover, the deliberate targeting of a PDE4 subtype also assumes that deleterious actions of PDE4 inhibitors are associated with a specific gene product(s) that is distinct from those which regulate cAMP levels in pro-inflammatory and immune cells. This latter assumption seems unlikely. Even if supporting evidence is ultimately provided, it is difficult to envisage how this strategy could be exploited given that almost all peripheral cells and tissues contain representatives of the HSPDE4A, B and D gene families (table II). Indeed, human T lymphocytes express at least 5 proteins that are derived from these 3 genes.[50] The same is true for other cells including human eosinophils, neutrophils, monocytes and macrophages.[160,163,165] It is also possible that there is significant PDE4 redundancy such that inhibition of a specific subtype will have little long-lasting impact because of the induction of an alternative isoenzyme. However, this proposal may not be correct. In mice, in which the PDE4D gene has been disrupted, a marked decrease in rolipram-sensitive PDE activity was reported in the pituitary gland, cerebellum and ovary when compared with wild type animals.[168] Thus, it would appear that other PDE4 isoenzymes cannot compensate for the loss of PDE4D variants in these murine tissues. The results suggest that the functional roles of PDE4D are not completely shared by other PDE4 isoenzymes,[168] raising the real possibility that the targeting of a specific PDE4 gene family is a viable concept for drug development.

Table II. Phosphodiesterase (PDE)4 subtypes identified in human airways smooth muscle and pro-inflammatory cells by reverse transcription-polymerase chain reaction (RT-PCR) [Data compiled from Engels et al.,[162] Gantner et al.,[163,164] Verghese et al., [165] Seybold et al.,[50] Giembycz et al.,[56] Fuhrmann et al.,[166] and Wright et al.[167]]

Cell/tissue	PDE isogene expression			
	HSPDE4A	HSPDE4B	HSPDE4C	HSPDE4D
T lymphocyte	+	+	–	+
B lymphocyte	+	+	–	+
Eosinophil	+	+	–	+
Neutrophil	+	+	–	+
Monocyte	+	+	–	+
Epithelial cell	+	–	+	+
Trachea	+	+	+	+

+ indicates mRNA present; – indicates mRNA absent.

Intriguingly, the magnitude of allergen-induced pulmonary leukocyte infiltration and cell composition is identical in sensitised PDE4D -/- and wild type mice[169] questioning the role PDE4D in this response. However, the ability of muscarinic agonists to promote bronchoconstriction in knockout animals *in vivo* and to inhibit adenylyl cyclase activity in lung is abolished. This effect is not due to down-regulation of receptor number implying that PDE4D controls muscarinic M_2 and M_3 receptor signalling in the lung. With respect to emesis, perhaps the best PDE4 gene family to avoid is PDE4C.[43] Representatives of this isoenzyme family generally are not present in pro-inflammatory cells (table II), but are abundantly expressed in the CNS[170] where PDE4 inhibitors are believed to promote many of their adverse effects. However, whether this is through inhibition of PDE4C is unknown.[161]

6.2 Exploitation of Conformational States of PDE4

An alternative approach (patented by SmithKline-Beecham) is based on the ability of certain PDE4 isoforms to adopt at least 2 non-interconvertible or slowly interconvertible conformations, $PDE4_H$ and $PDE4_L$, for which rolipram has high and low affinity, respectively.[171-173] Significantly, the rank order of potency of a variety of compounds to inhibit $PDE4_H$ and $PDE4_L$ is distinct, thus enabling a specific conformational state of PDE4 to be selectively targeted. The additional finding that the relative amounts of each conformer vary considerably between cells and tissues, and that inhibition of $PDE4_L$ and $PDE4_H$ are associated with a number of anti-inflammatory and adverse responses, respectively, has provided a rational basis for designing new compounds with a high $PDE4_H/PDE4_L$ ratio.[171-173] *In vitro* and *in vivo* studies have established that inhibition of $PDE4_L$ is linked to the suppression of the NADPH oxidase in eosinophils,[174] IL-2 release from splenocytes,[175] and TNF-α generation from monocytes.[91,176] Conversely, emesis (perhaps the major dose-limiting adverse effect of these drugs)[153] and gastric acid secretion[177] are believed to result exclusively from inhibition of $PDE4_H$. It is worth noting that certain functional responses, which might be considered desirable are evoked following inhibition of $PDE4_H$ such as bronchodilatation[178] and degranulation of human neutrophils.[91] In addition, other effects that are not apparently related to inhibition of either $PDE4_H$ and $PDE4_L$ have been described suggesting that additional conformations of PDE4 might exist.

These findings notwithstanding, compounds have been synthesised that have a considerably increased $PDE4_H/PDE4_L$ ratio compared with rolipram (H/L = 0.01 to 0.001) such as CDP-840 (H/L = 0.27),[173] piclamilast (H/L = 3)[74,171] and SB-207499 (H/L = 1.1)[92,179] with the hope of retaining anti-inflammatory activity while reducing adverse effects. Indeed, SB-207499 was selected for clinical development based on a markedly improved $PDE4_H/PDE4_L$ ratio and its negative charge at physiological pH, which should reduce penetration across the blood-brain barrier and, thus lower the potential for adverse effects. However, Phase IIb clinical trials have established that, although apparently free of cardiovascular effects, SB-207499 (15mg orally) is emetic. Interestingly, this adverse reaction was produced with only the first and second doses suggesting that the mechanisms governing emesis desensitise rapidly.[180] Based upon these clinical data it would appear that $PDE4_H/PDE4_L$ ratios considerably greater than 1 may be necessary to provide an acceptable therapeutic index. Ironically, it has seemingly been difficult to synthesise compounds with this property, although Pfizer[181,182] and the then Rhône-Poulenc Rorer[183] have reported some success with novel series of oxindoles, catechol benzimidazoles and quaternary substituted γ-lactams. For example, CP-146523 inhibits PDE4 with an IC_{50} of approximately 400 nmol/L but is relatively weak at displacing [^{3}H]rolipram from rat brain cortex, a tissue rich in $PDE4_H$. Similarly, CP-293121 has reduced emetic potential due to its high $PDE4_H/PDE4_L$ ratio.[144,151]

6.3 Pharmacokinetic Strategies and Alternative Routes of Administration

A primary objective of the pharmaceutical industry is to synthesise orally active PDE4 inhibitors that display *in vivo* efficacy in humans with an acceptable therapeutic ratio. Current experience with PDE4 inhibitors suggests that this goal can prove difficult to achieve when adverse effects are simply an extension of the pharmacology of these compounds. However, adverse effects may be limited by identifying methods of delivery that improve the pharmacokinetic behaviour of existing PDE4 inhibitors. One possibility is to administer the drug of choice as a slow-release formulation such that the peak concentration achieved in the plasma is lowered relative to overall systemic exposure.[184] This approach has been successfully adopted for pentoxifylline, a non-selective PDE inhibitor, which allows for the administration of higher doses before adverse effects become manifest.[185] Alternatively, direct application of PDE4 inhibitors to the airways as an inhaled formulation might be the preferred route of administration. Indeed, preliminary data suggests that this approach should retain the desired therapeutic activity while minimising adverse effects.[186-188]

6.4 Development of Hybrid Inhibitors

In many immune and pro-inflammatory cells such as T lymphocytes and macrophages, which are believed to drive eosinophilic inflammation, PDE3 is widely expressed (table I). However, inhibitors of this isoenzyme family generally are inactive or poorly active *in vitro* and in *in vivo* models of allergic inflammation, but reproducibly potentiate the effect of inhibitors of PDE4.[16,17] This has been demonstrated in several cells types including human T lymphocytes, basophils and lung microvascular endothelial cells.[95,109,189]

Based on these findings it has been proposed that compounds that inhibit both PDE3 and PDE4 should be less likely to produce adverse effects than selective PDE4 inhibitors since activity would be expected at lower doses.[190] While this approach certainly appears attractive from a superficial perspective, it is not without potential problems when the clinical pharmacology of PDE3 inhibitors is considered. Indeed, these drugs originally were developed for the therapy of congestive heart failure and, therefore, certain predications can be made regarding their adverse effect profile. Of particular concern is their potential arrhythmogenic and vasodilator activities together with their ability to produce positive inotropism and chronotropism in the heart (see section 4).[191,192] Although this approach has not been rigorously tested in human volunteers, many researchers are of the opinion that the cardiovascular complications of PDE3 inhibitors preclude the development of hybrid inhibitors for asthma. Furthermore, logic dictates that if inhibitors of PDE3 and PDE4 act synergistically in the resolution of inflammation, they could also synergise in the production of adverse effects. Nevertheless, the knowledge that the PDE3 in cardiac muscle (PDE3A) is different from the isoform (PDE3B) expressed by pro-inflammatory cells such as T lymphocytes,[50] provides an opportunity to engineer molecules with reduced activity against PDE3A. Although PDE3B-selective compounds have not yet been described, the PDE3 inhibitor vesnarinone is 10-fold more potent against PDE3A than PDE3B,[193] indicating that PDE3 isogene inhibitors theoretically can be synthesised.

6.5 Development of Inhibitors of Other Isoenzyme Families

An additional concern about the development of selective PDE4 inhibitors is the report that rats given rolipram repeatedly for 2 weeks displayed a profile of adverse effects similar to the toxicology of PDE3 inhibitors.[194] In particular, rolipram produced cardiac fibrosis, degeneration and epicarditis a similar histopathology seen with milrinone and ICI-153110.[195,196] Another effect produced by rolipram normally associated with the administration of PDE3 inhibitors was arteritis of the abdominal vasculature.[194] Although these lesions are believed to occur only in rodents, the recent discovery of new PDE families that are expressed

in cells and tissues relevant to the pathogenesis of asthma provide alternative targets for increasing cAMP with potential therapeutic opportunity. In addition to PDE3 and PDE4, 3 other isoenzyme families have been discovered that regulate the cAMP content. In 1993, a gene isolated from a human glioblastoma cDNA library was expressed in a cAMP PDE-deficient strain of the yeast, *Saccharomyces cerevisiae*.[7] This gene, originally named *HCP-1* (High affinity, Cyclic AMP-specific Phosphodiesterase 1) encodes a cAMP-specific PDE which is insensitive to cGMP and inhibitors of the PDE3 and PDE4 isoenzyme families, and does not hydrolyse cGMP. Furthermore, *HCP-1* does not share extensive homology to the *Drosophila* dunce cAMP PDE (i.e. PDE4) and, therefore, represents a member of a novel PDE family that has been designated PDE7.[7] Human PDE7 is currently believed to be encoded by a single gene that is localised to the q13 region of chromosome 8,[46,197] from which at least 2 splice variants (PDE7A1, PDE7A2) can be derived.[8,9]

Northern blot analyses have identified an abundance of PDE7 mRNA in human skeletal muscle. In addition, transcripts of identical size are present in human heart and kidney.[7] In the context of allergic diseases, Bloom and Beavo[198] identified high levels of PDE7 mRNA in the human T lymphocyte line, HUT 78 and, more recently, evidence has emerged that PDE7 mRNA and protein are ubiquitously expressed throughout mammalian tissues including human peripheral blood $CD4^+$ and $CD8^+$ T lymphocytes,[9,95] epithelial cells,[166,167] human monocytes, neutrophils, eosinophils, and airways smooth muscle (unpublished observations). Selective inhibitors of PDE7 have not yet been described and so the functional role of these enzymes is undefined. However, recent antisense studies indicate that PDE7 is involved in the regulation of T lymphocyte proliferation in response to ligation of CD3/CD28.[199] Inevitably, the discovery of compounds that selectively inhibit PDE7 will provoke a considerable research effort to determine whether PDE7 represents a viable therapeutic target.

Another cAMP PDE family was discovered in 1998 by expressed sequence tag data base searching and was denoted PDE8 to distinguish it from PDE3, 4 and 7.[11,13] Two genes have so far been identified,[11,13,200] PDE8A and PDE8B, that have a discrete tissue distribution. At the mRNA level, PDE8A is abundantly expressed in the human testis, ileum, colon and ovary with lower levels in the heart, brain, kidney and pancreas.[13] In contrast, PDE8B mRNA is expressed in the thyroid gland.[200] Although pro-inflammatory and immune cells have not been systematically screened, PDE8 mRNA is present in human monocytes, the epithelial cell line A549, and airways smooth muscle (unpublished observations). Understanding the functions that these novel PDE isoenzymes subserve has to await the discovery of selective inhibitors, but the possibility that these proteins could be exploited therapeutically is one that, almost certainly, will be examined.

The latest additions to the PDE supergene family, for which information is available, are PDE9A[10,14] and PDE10A,[12,15] which were also discovered by database searching for expressed sequence tags. The former family exclusively hydrolyse cGMP[10,14] and is not discussed here. In contrast, PDE10A is a dual specificity enzyme that degrades cAMP and cGMP.[12,15] Northern and dot blot analyses have established that human PDE10A mRNA transcripts are abundantly expressed in the brain, in particular the putamen and caudate nucleus, but are absent in the lung, trachea and peripheral blood leucocytes,[15] suggesting that the enzyme represents an unlikely target for novel anti-inflammatory pharmaceuticals. The human PDE10A gene, HSPDE10A1, has been mapped to chromosome 6q26,[15] which features a locus for juvenile Parkinson's diseases in 6q25.2-q27. Given that PDE10A is enriched in the putamen and caudate nucleus, where dopamine receptors are expressed, a possible genetic linkage between PDE10A and juvenile Parkinson's diseases has been suggested.[15]

6.6 Theophylline Revisited

Until relatively recently, the therapeutic efficacy of theophylline in asthma was attributed to its weak bronchodilator activity resulting from the inhibition of cyclic nucleotide PDEs in airways smooth muscle cells. However, there is now increasing evidence that theophylline exerts an immunomodulatory action at plasma concentrations that do not effect airways smooth muscle tone.[201,202] Several lines of investigation have lead to this conclusion. In essentially all studies that have been conducted, theophylline protects against the late asthmatic response following allergen provocation implying that the emigration of pro-inflammatory and immunocompetent cells from the circulation into the lung and/or their subsequent activation is suppressed. In a study by Ward et al.,[63] theophylline at a mean plasma concentration of 7.8 mg/L, inhibited the late phase reaction in patients with asthma in response to allergen and the typical increase in CD4+ and CD8+ T lymphocytes. Similarly, it has been reported that the number of CD8+ T lymphocytes in the peripheral blood of children with asthma is suppressed compared with normal individuals and that the degree to which this occurs correlates with the severity of the disease.[203,204] Significantly, treatment of those children for 1 month with theophylline restored the T lymphocyte count to the level found in the control group. Further support for an immunomodulatory effect of theophylline has been derived from studies examining the clinical effects of controlled withdrawal in patients on high dose inhaled steroids.[205,206] Such intervention is associated with a deterioration in symptoms and lung function, a reduction in activated CD4+ and CD8+ T lymphocytes in the peripheral blood and a commensurate increase in the number of these cells in the lung.[205,206] Recently, it was reported that in patients with moderate asthma and persistent symptoms, theophylline, at a dose below the recommended therapeutic range, in combination with low-dose budesonide, produced clinical benefits equivalent to high-dose budesonide given as a monotherapy.[207] Thus, in addition to the economic implications of reducing steroid usage, these data would suggest that theophylline is steroid sparing.[201]

In addition to T lymphocytes, theophylline also modulates other pro-inflammatory and immune cells. In children with asthma treated with theophylline for 10 days, both neutrophil and macrophage activity (chemotaxis, superoxide anion generation, bacterial killing) assessed *ex vivo* is suppressed, and the degree of this suppression correlates positively with the concentration of theophylline measured in the BAL fluid.[208,209] Similar experiments have demonstrated that the number of EG2+ (activated) eosinophils and CD4+ T lymphocytes are reduced in allergic subjects given low dose theophylline (mean plasma concentration 6.6 mg/L) for 6 weeks,[64,210] and that this might relate to the ability of theophylline to promote eosinophil apoptosis.[211] At the mediator level, oral administration of theophylline (mean level 10.9 mg/L) to atopic patients with moderately severe asthma has been shown to reduce the number of cells (mostly mast cells) staining for IL-4 and IL-5, thereby implying that theophylline may repress transcription of the IL-4 and IL-5 genes.[65,212] Moreover, Mascali and colleagues[213] reported an increase in the elaboration of the anti-inflammatory cytokine IL-10 from peripheral blood mononuclear cells harvested from 24 patients with asthma.

The molecular mechanism(s) underlying the immunomodulatory actions of theophylline is far from clear, but several activities have been considered that could act in concert. The most attractive of these is through the inhibition of cAMP PDEs, which provides a logical rationale for the further development of second and third generation 'theophyllines' (see section 2). However, the concentration of theophylline in the blood necessary to produce anti-inflammatory effects generally is less than 10 mg/L, which has a negligible effect on cAMP hydrolysis and, accordingly, has resurrected the proposal of a cAMP-independent mechanism of action. Although several possibilities have been advanced including adenosine receptor antagonism, the inhibition of Ca^{2+} influx into target cells and the elaboration of catecholamines, none satisfactory

account for the results described above. It is of considerable interest that theophylline was recently shown to inhibit the activation of the transcription factor, nuclear factor κB (NFκB), in human mast cells at therapeutic concentrations (6 to 18 mg/L or 30 to 100 μmol/L) that are below those required to inhibit cAMP hydrolysis.[214] Potentially, this is a significant finding as many pro-inflammatory genes relevant to asthma pathogenesis are believed to be regulated by NFκB including TNFα, IL-1β, GM-CSF and the growth factor RANTES (Regulated on Activation, Normal T cell Expressed and Secreted).[215]

If theophylline does, indeed, owe its therapeutic activity to a mechanism other that PDE inhibition, then a dedicated chemistry effort around the alkylxanthine structure could result in compounds with enhanced therapeutic activity and reduced adverse effects that, paradoxically, might be achieved by reducing the ability of such compounds to inhibit PDE. In this respect, the xanthine derivative arofylline (LAS-31025) has now entered Phase III clinical trials for the treatment of asthma based on encouraging Phase II studies in which a dose of 20mg significantly improved FEV_1 after oral administration.[216,217] Arofylline is a relatively weak inhibitor of PDE4 but, nevertheless, displays an anti-inflammatory profile in animal models of asthma similar to rolipram. It has been reported that arofylline is free of cardiovascular and CNS adverse effects in animals and is considerably less emetic in dogs than rolipram with a 10-times greater therapeutic index (see Norman[144]). Results from further clinical trials are eagerly awaited.

7. Where Do We Go From Here?

In the preceding sections, evidence is provided that second generation PDE4 inhibitors, exemplified by SB-207499, may improve, albeit to a limited extent, lung function in patients with asthma. However, despite an extensive research effort, 'proof of concept' studies in human volunteers designed to assess if PDE4 inhibitors exert an *anti-inflammatory* influence in clinical asthma still are not available. Given the number of clinical trials that have been conducted, one can conclude that at the maximum tolerated doses no evidence for an anti-inflammatory effect has been found. How then can the anti-inflammatory potential of PDE4 inhibitors be evaluated in humans if acute adverse effects (e.g. nausea, vomiting) preclude the administration of higher, potentially therapeutically-active, doses? One possibility is through the use of antiemetic drugs such as prochlorperazine or ondansetron, which could allow inhibitors to be given to volunteers with asthma in doses sufficient to inhibit PDE4 activity *in vivo*.

In the absence of a 'proof of concept', what are the ways forward? Assuming that the overall idea is correct, then the identification of PDE4 inhibitors with markedly improved therapeutic indices is clearly desirable if these drugs are to be used as a monotherapy in asthma. However, in light of the success in combining a β_2-adrenoceptor agonist (salmeterol) with a steroid (fluticasone) as a single formulation (Seretide™), it is tempting to ask if similar benefit would be derived in combining low dose PDE4 inhibitors with existing therapies. Indeed, synergy might be predicted at the level of cAMP accumulation with a combination of a β_2-adrenoceptor agonist and a PDE4 inhibitor, and, so theoretically, this combination could be more efficacious than either drug alone. The idea of combining a PDE4 inhibitor with a steroid is also attractive since it could be steroid-sparing.

If the inflammation that underlies asthma is ultimately shown not to respond adequately to PDE4 inhibitors and the modest improvement in lung function observed in clinical trials merely reflects an action on airways smooth muscle, then it is important to appreciate that other diseases such as rheumatoid arthritis,[184] atopic dermatitis[218] and COPD,[151,219] which have a different inflammatory basis, may be sensitive to intervention with these drugs. In this respect, a recent trial of SB-207499 in patients with COPD provides optimism that PDE4 inhibitors do, indeed, have potential in the treatment of certain respiratory disorders. At a conference held in London in September 1998, it was reported that in a group of patients with moderate

COPD (mean FEV_1 = 47% of predicted; mean smoking history 39.7 pack years), SB–207499 15mg twice daily improved FEV_1 by 160ml (11% of the initial FEV_1) over a 6-week treatment period compared with placebo (see Rogers and Giembycz[144]). Similar improvements, relative to placebo, were observed in forced vital capacity and peak expiratory flow rate after 6 weeks treatment. These results have been published in abstract form.[220] However, it is not known if SB–207499 is acting as an anti-inflammatory agent or a smooth muscle relaxant. If SB–207499 does impact upon the neutrophilic inflammation that characterises COPD, then a critical question is why this drug is relatively inactive at the same doses in clinical trials for asthma. One explanation for this apparent paradox is that inflammatory processes in COPD are more sensitive to SB–207499. If this is true, then a prediction is that PDE4 inhibitors in general should provide clinical benefit in this disease. Alternatively, the selectivity of SB–207499 for the PDE4D isoenzyme might be a critical determinant of efficacy (but see section 6.1) necessitating the development of third generation inhibitors to optimise isoenzyme selectivity. Given these possibilities, a quantitative comparison of the role of PDE4 inhibitors, and of the functional significance of PDE4D and the other gene families, in regulating pro-inflammatory responses in cells central to the pathology of COPD (e.g. neutrophils, alveolar macrophages) and asthma (e.g. CD4+ T lymphocytes, eosinophils) could be instructive.

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References

1. Flemming DM, Crombie DL. Prevalence of asthma and hay fever in England and Wales. BMJ 1987; 294: 279-83
2. Sly RM. Increases in death from asthma. Ann Allergy 1984; 53: 2-25
3. Barnes PJ. Asthma deaths: a continuing problem. In: Sheppard M, editor. Advanced medicine. London: Bailliere Tindall, 1988: 53-61
4. Keating G, Mitchell EA, Jackson R, et al. Trends in the sales of drugs for asthma in New Zealand, Australia and the United Kingdom. BMJ 1983; 289: 348-51
5. Hay IFC, Higgenbotham TW. Has the management of asthma improved? Lancet 1987; II: 609-11
6. Beavo JA, Conti M, Heaslip RJ. Multiple cyclic nucleotide phosphodiesterases. Mol Pharmacol 1994; 46: 399-405
7. Michaeli T, Bloom TJ, Martins T, et al. Isolation and characterization of a previously undetected human cAMP phosphodiesterase by complementation of cAMP phosphodiesterase-deficient *Saccharomyces cerevisiae*. J Biol Chem 1993; 268: 12925-32
8. Han P, Zhu X, Michaeli T. Alternative splicing of the high affinity cAMP-specific phosphodiesterase (PDE7A) mRNA in human skeletal muscle and heart. J Biol Chem 1997; 272: 16152-7
9. Bloom TJ, Beavo JA. Identification and tissue-specific expression of PDE7 phosphodiesterase splice variants. Proc Natl Acad Sci U S A 1996; 93: 14188-92
10. Soderling SH, Bayuga SJ, Beavo JA. Identification and characterization of a novel family of cyclic nucleotide phosphodiesterases. J Biol Chem 1998; 273: 15553-8
11. Soderling SH, Bayuga SJ, Beavo JA. Cloning and characterization of a cAMP-specific cyclic nucleotide phosphodiesterase. Proc Natl Acad Sci U S A 1998; 95: 8991-6
12. Soderling TR, Bayuga SJ, Beavo JA. Isolation and characterization of a dual substrate phosphodiesterase gene family: PDE10A. Proc Natl Acad Sci U S A 1999; 96: 7071-6
13. Fisher DA, Smith JF, Pillar JS, et al. Isolation and characterization of PDE8A, a novel human cAMP-specific phosphodiesterase. Biochem Biophys Res Commun 1998; 246: 570-7
14. Fisher DA, Smith JF, Pillar JS, et al. Isolation and characterization of PDE9A, a novel human cGMP-specific phosphodiesterase. J Biol Chem 1998; 273: 15559-64
15. Fujishige K, Kotera J, Michibata H, et al. Cloning and characterization of a novel human phosphodiesterase that hydrolyses both cAMP and cGMP (PDE10A). J Biol Chem 1999; 274: 18438-45
16. Giembycz MA, Dent G, Souness JE. Theophylline and isoenzyme-selective phosphodiesterase inhibitors. In: Kay AB, editor. Allergy and allergic diseases. Oxford: Blackwell Scientific, 1997: 531-67
17. Torphy TJ. Phosphodiesterase isozymes: molecular targets for novel antiasthma agents. Am J Respir Crit Care Med 1998; 157: 351-70
18. Torphy TJ, Undem BJ. Phosphodiesterase inhibitors: new opportunities for the treatment of asthma. Thorax 1991; 46: 512-23
19. Torphy TJ, Murray KJ, Arch JRS. Selective phosphodiesterase isoenzyme inhibitors. In: Page CP, Metzger WJ, editors. Drugs and the lung. New York: Raven Press, 1994: 397-477
20. Giembycz MA. Could isoenzyme-selective phosphodiesterase inhibitors render bronchodilator therapy redundant in the treatment of bronchial asthma? Biochem Pharmacol 1992; 43: 2041-51
21. Giembycz MA, Dent G. Prospects for selective cyclic nucleotide phosphodiesterase inhibitors in the treatment of bronchial asthma. Clin Exp Allergy 1992; 22: 337-44
22. Raeburn D, Souness JE, Tomkinson A, et al. Isoenzyme-selective cyclic nucleotide phosphodiesterase inhibitors: biochemistry, pharmacology and therapeutic potential in asthma. Prog Drug Res 1993; 40: 9-31
23. Dent G, Giembycz MA. Phosphodiesterase inhibitors: Lily the Pink's medicinal compound for asthma? Thorax 1996; 51: 647-9

24. Nicholson CD, Shahid M. Inhibitors of cyclic nucleotide phosphodiesterase isoenzymes: their potential utility in the therapy of asthma. Pulmon Pharmacol 1994; 7: 1-17
25. Torphy TJ, Barnette MS, Hay DW, et al. Phosphodiesterase IV inhibitors as therapy for eosinophil-induced lung injury in asthma. Environ Health Perspect 1994; 102 Suppl. 10: 79-84
26. Dent G, Giembycz MA. Interaction of PDE4 inhibitors with enzymes and cell functions. In: Schudt C, Dent G, Rabe K, editors. Handbook of immunopharmacology: phosphodiesterase inhibitors. London: Academic Press, 1996: 111-26
27. Giembycz MA, Souness JE. Phosphodiesterase IV inhibitors as potential therapeutic agents in allergic disease. In: Townley RG, Agarwal DK, editors. Immunopharmacology of allergic disease. New York: Marcel-Dekker, 1996: 523-59
28. Skoyles JR, Sherry KM. Pharmacology, mechanisms of action and uses of selective phosphodiesterase inhibitors. Br J Anaesth 1992; 68: 293-302
29. Leeman M, Lejeune P, Melot C, et al. Reduction in pulmonary hypertension and in airway resistance by enoximone (MDL 17,043) in decompensated COPD. Chest 1987; 91: 662-6
30. Fujimura M, Kamio Y, Saito M, et al. Bronchodilator and bronchoprotective effects of cilostazol in humans *in vivo*. Am J Respir Crit Care Med 1995; 151: 222-5
31. Bardin PG, Dorward MA, Lampe FC, et al. Effect of selective phosphodiesterase 3 inhibition on the early and late asthmatic responses to inhaled allergen. Br J Clin Pharmacol 1998; 45: 387-91
32. Conti M, Swinnen JV. Structure and function of the rolipram-sensitive, low Km cyclic AMP phosphodiesterase: a family of highly related proteins. In: Houslay MD, Beavo J, editors. Molecular pharmacology of cell regulation: cyclic nucleotide phosphodiesterase structure and drug action. New York: Wiley, 1990: 243-66
33. Bolger G, Michaeli T, Martins T, et al. A family of human phosphodiesterases homologous to the *dunce* learning and memory gene product of *Drosophila melanogaster* are potential targets for antidepressant drugs. Mol Cell Biol 1993; 13: 6558-71
34. Colicelli J, Birchmeier C, Michaeli T, et al. Isolation and characterization of a mammalian gene encoding a high-affinity cAMP phosphodiesterase. Proc Natl Acad Sci U S A 1989; 86: 3599-603
35. Davis RL, Takayasu H, Eberwine M, et al. Cloning and characterization of mammalian homologs of the Drosophila dunce$^+$ gene. Proc Natl Acad Sci U S A 1989; 86: 3604-8
36. Swinnen JV, Joseph DR, Conti M. The mRNA encoding a high-affinity cAMP phosphodiesterase is regulated by hormones and cAMP. Proc Natl Acad Sci U S A 1989; 86: 8197-201
37. Swinnen JV, Joseph DR, Conti M. Molecular cloning of rat homologues of the *Drosophila melanogaster* dunce cAMP phosphodiesterase: evidence for a family of genes. Proc Natl Acad Sci U S A 1989; 86: 5325-9
38. Chen CN, Denome S, Davis RL. Molecular analysis of cDNA clones and the corresponding genomic coding sequences of the Drosophila dunce$^+$ gene, the structural gene for cAMP phosphodiesterase. Proc Natl Acad Sci U S A 1986; 83: 9313-7
39. Livi GP, Kmetz P, McHale MM, et al. Cloning and expression of cDNA for a human low-Km, rolipram-sensitive cyclic AMP phosphodiesterase. Mol Cell Biol 1990; 10: 2678-86
40. McLaughlin MM, Cieslinski LB, Burman M, et al. A low-Km, rolipram-sensitive, cAMP-specific phosphodiesterase from human brain. Cloning and expression of cDNA, biochemical characterization of recombinant protein, and tissue distribution of mRNA. J Biol Chem 1993; 268: 6470-6
41. Obernolte R, Bhakta S, Alvarez R, et al. The cDNA of a human lymphocyte cyclic-AMP phosphodiesterase (PDE IV) reveals a multigene family. Gene 1993; 129: 239-47
42. Sullivan M, Egerton M, Shakur Y, et al. Molecular cloning and expression, in both COS-1 cells and *S. cerevisiae*, of a human cytosolic type-IVA, cyclic AMP specific phosphodiesterase (hPDE-IVA-h6.1). Cell Signal 1994; 6: 793-812
43. Obernolte R, Ratzliff J, Baecker PA, et al. Multiple splice variants of phosphodiesterase PDE4C cloned from human lung and testis. Biochim Biophys Acta 1997; 1353: 287-97
44. Baecker PA, Obernolte R, Bach C, et al. Isolation of a cDNA encoding a human rolipram-sensitive cyclic AMP phosphodiesterase (PDE IVD). Gene 1994; 138: 253-6
45. Engels P, Sullivan M, Muller T, et al. Molecular cloning and functional expression in yeast of a human cAMP-specific phosphodiesterase subtype (PDE IV-C). FEBS Lett 1995; 358: 305-10
46. Milatovich A, Bolger G, Michaeli T, et al. Chromosome localizations of genes for five cAMP-specific phosphodiesterases in man and mouse. Somat Cell Mol Genet 1994; 20: 75-86
47. Szpirer C, Szpirer J, Riviere M, et al. Chromosomal localization of the human and rat genes (PDE4D and PDE4B) encoding the cAMP-specific phosphodiesterases 3 and 4. Cytogenet Cell Genet 1995; 69: 11-4
48. Conti M, Nemoz G, Sette C, et al. Recent progress in understanding the hormonal regulation of phosphodiesterases. Endocr Rev 1995; 16: 370-89
49. Houslay MD, Sullivan M, Bolger GB. The multienzyme PDE4 cyclic adenosine monophosphate-specific phosphodiesterase family: intracellular targeting, regulation, and selective inhibition by compounds exerting anti-inflammatory and antidepressant actions. Adv Pharmacol 1998; 44: 225-342
50. Seybold J, Newton R, Wright L, et al. Induction of phosphodiesterases 3B, 4A4, 4D1, 4D2, and 4D3 in Jurkat T- cells and in human peripheral blood T-lymphocytes by 8-bromo-cAMP and Gs-coupled receptor agonists: potential role in β_2-adrenoreceptor desensitization. J Biol Chem 1998; 273: 20575-88
51. Monaco L, Vicini E, Conti M. Structure of two rat genes coding for closely related rolipram-sensitive cAMP phosphodiesterases: multiple mRNA variants originate from alternative splicing and multiple start sites. J Biol Chem 1994; 269: 347-57
52. Houslay MD, Milligan G. Tailoring cAMP-signalling responses through isoform multiplicity. Trends Biochem Sci 1997; 22: 217-24
53. Shakur Y, Pryde JG, Houslay MD. Engineered deletion of the unique N-terminal domain of the cyclic AMP-specific phosphodiesterase RD1 prevents plasma membrane association and the attainment of enhanced thermostability without altering its sensitivity to inhibition by rolipram. Biochem J 1993; 292: 677-86
54. Smith KJ, Scotland G, Beattie J, et al. Determination of the structure of the N-terminal splice region of the cyclic AMP-specific phosphodiesterase RD1 (RNPDE4A1) by ^{1}H NMR and identification of the membrane association domain using chimeric constructs. J Biol Chem 1996; 271: 16703-11
55. Scotland G, Houslay MD. Chimeric constructs show that the unique N-terminal domain of the cyclic AMP phosphodiesterase RD1 (RNPDE4A1A; rPDE-IVA1) can confer membrane association upon the normally cytosolic protein chloramphenicol acetyltransferase. Biochem J 1995; 308: 673-81
56. McPhee I, Pooley L, Lobban M, et al. Identification, characterization and regional distribution in brain of RPDE-6 (RNPDE4A5), a novel splice variant of the PDE4A cyclic

AMP phosphodiesterase family. Biochem J 1995; 310: 965-74
57. Houslay MD, Scotland G, Pooley L, et al. Alternative splicing of the type-IVA cyclic AMP phosphodiesterase gene provides isoform variants with distinct N-terminal domains fused to a common, soluble catalytic unit: 'designer' changes in Vmax, stability and membrane association. Biochem Soc Trans 1995; 23: 393-8
58. Houslay MD. The N-terminally alternately spliced regions of PDE4A cAMP-specific phosphodiesterases determine intracellular targeting and regulation of catalytic activity. Biochem Soc Trans 1996; 24: 980-6
59. Houslay MD, Scotland G, Erdogan S, et al. Intracellular targeting, interaction with Src homology 3 (SH3) domains and rolipram-detected conformational switches in cAMP-specific PDE4A phosphodiesterase. Biochem Soc Trans 1997; 25: 374-81
60. Yarwood SJ, Steele MR, Scotland G, et al. The RACK1 signalling scaffold protein selectively interacts with the cAMP-specific phosphodiesterase PDE4D5 isoform. EMBO J 1999; 274: 14909-17
61. Conti M. Subcellular localization of PDE4 variants: interactions with scaffold/adapter proteins [abstract]. Gordon Research Conference on Cyclic Nucleotide Phosphodiesterases, 1999
62. Bolger GB. Molecular biology of the cyclic AMP-specific cyclic nucleotide phosphodiesterases: a diverse family of regulatory enzymes. Cell Signal 1994; 6: 851-9
63. Ward AJM, McKenniff M, Evans JM, et al. Theophylline: an immunomodulatory role in asthma. Am Rev Respir Dis 1993; 147: 518-23
64. Sullivan PJ, Bekir S, Jaffar Z, et al. The effect of low dose theophylline on the bronchial wall infiltrate after antigen challenge. Lancet 1994; 343: 1006-8
65. Djukanovic R, Finnerty JP, Lee C, et al. The effect of theophylline on mucosal inflammation in asthmatic airways: biopsy results. Eur Resp J 1995; 8: 831-3
66. Louis R, Bury T, Corhay JL, et al. LY186655, a phosphodiesterase inhibitor, inhibits histamine release from human basophils, lung and skin fragments. Int J Immunopharmacol 1992; 14: 191-4
67. Peachell PT, Undem BJ, Schleimer RP, et al. Preliminary identification and role of phosphodiesterase isozymes in human basophils. J Immunol 1992; 148: 2503-10
68. Weston MC, Anderson N, Peachell PT. Effects of phosphodiesterase inhibitors on human lung mast cell and basophil function. Br J Pharmacol 1997; 121: 287-95
69. Cooper KD, Kang K, Chan SC. Phosphodiesterase inhibition by Ro 20-1724 reduces hyper-IgE synthesis by atopic dermatitis *in vitro*. J Invest Dermatol 1985; 84: 477-82
70. Dent G, Giembycz MA, Rabe KF, et al. Inhibition of eosinophil cyclic nucleotide PDE activity and opsonised zymosan-stimulated respiratory burst by 'type IV'-selective PDE inhibitors. Br J Pharmacol 1991; 103: 1339-46
71. Dent G, Giembycz MA, Evans PM, et al. Suppression of human eosinophil respiratory burst and cyclic AMP hydrolysis by inhibitors of type IV phosphodiesterase: interaction with the beta adrenoceptor agonist albuterol. J Pharmacol Exp Ther 1994; 271: 1167-74
72. Souness JE, Carter CM, Diocee BK, et al. Characterization of guinea-pig eosinophil phosphodiesterase activity. Assessment of its involvement in regulating superoxide generation. Biochem Pharmacol 1991; 42: 937-45
73. Souness JE, Villamil ME, Scott LC, et al. Possible role of cyclic AMP phosphodiesterases in the actions of ibudilast on eosinophil thromboxane generation and airways smooth muscle tone. Br J Pharmacol 1994; 111: 1081-8
74. Souness JE, Maslen C, Webber S, et al. Suppression of eosinophil function by RP 73401, a potent and selective inhibitor of cyclic AMP-specific phosphodiesterase: comparison with rolipram. Br J Pharmacol 1995; 115: 39-46
75. Hatzelmann A, Tenor H, Schudt C. Differential effects of non-selective and selective phosphodiesterase inhibitors on human eosinophil functions. Br J Pharmacol 1995; 114: 821-31
76. Berends C, Dijkhuizen B, Demonchy JGR, et al. Inhibition of PAF-induced expression of CD11b and shedding of L-selectin on human neutrophils and eosinophils by the type IV selective PDE inhibitor, rolipram. Eur Respir J 1997; 10: 1000-7
77. Kaneko T, Alvarez R, Ueki IF, et al. Elevated intracellular cyclic AMP inhibits chemotaxis in human eosinophils. Cell Signal 1995; 7: 527-34
78. Tenor H, Hatzelmann A, Church MK, et al. Effects of theophylline and rolipram on leukotriene C_4 (LTC_4) synthesis and chemotaxis of human eosinophils from normal and atopic subjects. Br J Pharmacol 1996; 118: 1727-35
79. Schudt C, Tenor H, Hatzelmann A. PDE isoenzymes as targets for anti-asthma drugs. Eur Respir J 1995; 8: 1179-83
80. Seldon PM, Barnes PJ, Meja K, et al. Suppression of lipopolysaccharide-induced tumor necrosis factor-α generation from human peripheral blood monocytes by inhibitors of phosphodiesterase 4: interaction with stimulants of adenylyl cyclase. Mol Pharmacol 1995; 48: 747-57
81. Semmler J, Wachtel H, Endres S. The specific type IV phosphodiesterase inhibitor rolipram suppresses tumor necrosis factor-α production by human mononuclear cells. Int J Immunopharmacol 1993; 15: 409-13
82. Molnar Kimber K, Yonno L, Heaslip R, et al. Modulation of TNFα and IL-1β from endotoxin-stimulated monocytes by selective PDE isozyme inhibitors. Agents Actions 1993; 39: C77-9
83. Prabhakar U, Lipshutz D, Bartus JO, et al. Characterization of cAMP-dependent inhibition of LPS-induced TNFα production by rolipram, a specific phosphodiesterase IV (PDE IV) inhibitor. Int J Immunopharmacol 1994; 16: 805-16
84. Griswold DE, Webb EF, Breton J, et al. Effect of selective phosphodiesterase type IV inhibitor, rolipram, on fluid and cellular phases of inflammatory responses. Inflammation 1993; 17: 333-44
85. Derian CK, Santulli RJ, Rao PE, et al. Inhibition of chemotactic peptide-induced neutrophil adhesion to vascular endothelium by cAMP modulators. J Immunol 1995; 154: 308-17
86. Wright CD, Kuipers PJ, Lobylarz-Singer D, et al. Differential inhibition of human neutrophil functions: role of cyclic AMP-specific and cyclic GMP-insensitive phosphodiesterase. Biochem Pharmacol 1990; 40: 699-707
87. Schudt C, Winder S, Forderkunz S, et al. Influence of selective phosphodiesterase inhibitors on human neutrophil functions and levels of cAMP and Ca_i. Naunyn Schmiedebergs Arch Pharmacol 1991; 344: 682-90
88. Ottonello L, Morone MP, Dapino P, et al. Cyclic AMP-elevating agents down-regulate the oxidative burst induced by granulocyte/macrophage colony-stimulating factor (GM-CSF) in adherent neutrophils. Clin Exp Immunol 1995; 101: 502-6
89. Ottonello L, Marone G, Dapino G, et al. Tumour necrosis factor α-induced oxidative burst in neutrophils adherent to fibronectin: effects of cyclic AMP-elevating agents. Br J Haematol 1995; 91: 566-70

90. Nourshargh S, Hoult JRS. Inhibition of human neutrophil degranulation by forskolin in the presence of phosphodiesterase inhibitors. Eur J Pharmacol 1986; 122: 205-12
91. Barnette MS, Bartus JO, Burman M, et al. Association of the anti-inflammatory activity of phosphodiesterase 4 (PDE4) inhibitors with either inhibition of PDE4 catalytic activity or competition for [^{3}H]rolipram binding. Biochem Pharmacol 1996; 51: 949-56
92. Barnette MS, Christensen SB, Essayan DM, et al. SB 207499 (Ariflo), a potent and selective second-generation phosphodiesterase 4 inhibitor: *in vitro* anti-inflammatory actions. J Pharmacol Exp Ther 1998; 284: 420-6
93. Nielson CP, Vestal RE, Sturm RJ, et al. Effects of selective phosphodiesterase inhibitors on the polymorphonuclear leukocyte respiratory burst. J Allergy Clin Immunol 1990; 86: 801-8
94. Fonteh AN, Winkler JD, Torphy TJ, et al. Influence of isoproterenol and phosphodiesterase inhibitors on platelet-activating factor biosynthesis in the human neutrophil. J Immunol 1993; 151: 339-50
95. Giembycz MA, Corrigan CJ, Seybold J, et al. Identification of cyclic AMP phosphodiesterases 3, 4 and 7 in human $CD4^+$ and $CD8^+$ T-lymphocytes: role in regulating proliferation and the biosynthesis of interleukin-2. Br J Pharmacol 1996; 118: 1945-58
96. Essayan DM, Huang S, Undem BJ, et al. Modulation of antigen- and mitogen-induced proliferative responses of peripheral blood mononuclear cells by non-selective and isozyme-selective cyclic nucleotide phosphodiesterase inhibitors. J Immunol 1994; 153: 3408-13
97. Essayan DM, Huang S, Kagey Sobotka A, et al. Effects of non-selective and isozyme selective cyclic nucleotide phosphodiesterase inhibitors on antigen-induced cytokine gene expression in peripheral blood mononuclear cells. Am J Respir Cell Mol Biol 1995; 13: 692-702
98. Banner KH, Roberts NM, Page CP. Differential effect of phosphodiesterase 4 inhibitors on the proliferation of human peripheral blood mononuclear cells from normals and subjects with atopic dermatitis. Br J Pharmacol 1995; 116: 3169-74
99. Van Wauwe J, Aerts F, Walter H, et al. Cytokine production by phytohemagglutinin-stimulated human blood cells: effect of corticosteroids, T-cell immunosuppressants and phosphodiesterase IV inhibitors. Inflamm Res 1995; 44: 400-5
100. Anastassiou ED, Paliogianni F, Balow JP, et al. Prostaglandin E_2 and other cyclic AMP-elevating agents modulate IL-2 and IL-2α gene expression at multiple levels. J Immunol 1992; 148: 2845-52
101. Chan SC, Li SH, Hanifin JM. Increased interleukin-4 production by atopic mononuclear leukocytes correlates with increased cyclic adenosine monophosphate-phosphodiesterase activity and is reversible by phosphodiesterase inhibition. J Invest Dermatol 1993; 100: 681-4
102. Crocker IC, Townley RG, Khan MM. Phosphodiesterase inhibitors suppress proliferation of peripheral blood mononuclear cells and interleukin-4 and -5 secretion by human T-helper type 2 cells. Immunopharmacol 1996; 31: 223-35
103. Crocker IC, Ohia SE, Church MK, et al. Phosphodiesterase type 4 inhibitors, but not glucocorticoids, are more potent in suppression of cytokine secretion by mononuclear cells from atopic than nonatopic donors. J Allergy Clin Immunol 1998; 102: 797-804
104. Essayan DM, Kagey Sobotka A, Lichtenstein LM, et al. Regulation of interleukin-13 by type 4 cyclic nucleotide phosphodiesterase (PDE) inhibitors in allergen-specific human T-lymphocyte clones. Biochem Pharmacol 1997; 53: 1055-60
105. Kaminuma O, Mori A, Wada K, et al. A selective type 4 phosphodiesterase inhibitor, T-440, modulates intracellular cyclic AMP level and interleukin-2 production of Jurkat cells. Immunopharmacol 1998; 38: 247-52
106. Kaminuma O, Mori A, Suko M, et al. Interleukin-5 production by peripheral blood mononuclear cells of asthmatic patients is suppressed by T-440: relation to phosphodiesterase inhibition. J Pharmacol Exp Ther 1996; 279: 240-6
107. Seldon PM, Barnes PJ, Giembycz MA. Interleukin-10 does not mediate the inhibitory effect of PDE4 inhibitors and other cAMP-elevating drugs on lipopolysaccharide-induced tumor necrosis factor-α generation from human peripheral blood monocytes. Cell Biochem Biophys 1998; 29: 179-201
108. Blease K, Burke-Gaffney A, Hellewell PG. Modulation of cell adhesion molecule expression and function on human lung microvascular endothelial cells by inhibitors of phosphodiesterases 3 and 4. Br J Pharmacol 1998; 124: 229-37
109. Kuehl FA, Zanetti ME, Soderman DD, et al. Cyclic AMP-dependent regulation of lipid mediators in white cells: a unifying concept for explaining the efficacy of theophylline in asthma. Am Rev Respir Dis 1987; 136: 210-3
110. Howell RE, Sickles BD, Woeppel SL. Pulmonary anti-allergic and bronchodilator effects of isozyme-selective phosphodiesterase inhibitors in guinea-pigs. J Pharmacol Exp Ther 1993; 264: 609-15
111. Underwood DC, Osborn RR, Novak LB, et al. Inhibition of antigen-induced bronchoconstriction and eosinophil infiltration in the guinea pig by the cyclic AMP-specific phosphodiesterase inhibitor, rolipram. J Pharmacol Exp Ther 1993; 266: 306-13
112. Raeburn D, Underwood SL, Lewis SA, et al. Anti-inflammatory and bronchodilator properties of RP 73401, a novel and selective phosphodiesterase type IV inhibitor. Br J Pharmacol 1994; 113: 1423-31
113. Hughes B, Howat D, Lisle H, et al. The inhibition of antigen-induced eosinophilia and bronchoconstriction by CDP 840, a novel stereo-selective inhibitor of phosphodiesterase type 4. Br J Pharmacol 1996; 118: 1183-91
114. Gozzard N, Herd CM, Blake AM, et al. Effect of theophylline and rolipram on antigen-induced airway responses in neonatally immunised rabbits. Br J Pharmacol 1996; 117: 1405-12
115. Turner CR, Andreson CJ, Smith WB, et al. Effects of rolipram on responses to acute and chronic antigen exposure in monkeys. Am J Respir Crit Care Med 1994; 149: 1153-9
116. Turner CR, Cohan VL, Cheng JB, et al. The *in vivo* pharmacology of CP-80,633, a selective inhibitor of phosphodiesterase 4. J Pharmacol Exp Ther 1996; 278: 1349-55
117. Nagai H, Takeda H, Iwama T, et al. Studies on anti-allergic activity of AH-21-132, a novel isozyme-selective phosphodiesterase inhibitor in airways. Jap J Pharmacol 1995; 67: 149-56
118. Danahay H, Broadley KJ. Effects of inhibitors of phosphodiesterase, on antigen-induced bronchial hyperreactivity in conscious sensitized guinea-pigs and airway leukocyte infiltration. Br J Pharmacol 1997; 120: 289-97
119. Danahay H, Broadley KJ. PDE4 inhibition and a corticosteroid in chronically antigen exposed conscious guinea-pigs. Clin Exp Allergy 1998; 28: 513-22
120. Elwood W, Sun J, Barnes PJ, et al. Inhibition of allergen-induced lung eosinophilia by type IV and combined type III- and IV-selective phosphodiesterase inhibitors in Brown Norway rats. Inflammation Res 1995; 44: 83-6

121. Howell RE, Jenkins LP, Fielding LE, et al. Inhibition of antigen-induced pulmonary eosinophilia and neutrophilia by selective inhibitors of phosphodiesterases types 3 or 4 in Brown Norway rats. Pulmon Pharmacol 1995; 8: 83-9
122. Sturm RJ, Osborne MC, Heaslip RJ. The effect of phosphodiesterase inhibitors on pulmonary inflammatory cell influx in ovalbumin-sensitized guinea-pigs. J Cell Biochem 1990; 14: 337
123. Underwood DC, Matthews JK, Osborn RR, et al. The influence of endogenous catecholamines on the inhibitory effects of rolipram against early- and late-phase response to antigen in the guinea pig. J Pharmacol Exp Ther 1997; 280: 210-9
124. Underwood DC, Bochnowicz S, Osborn RR, et al. Antiasthmatic activity of the second-generation phosphodiesterase 4 (PDE4) inhibitor SB 207499 (Ariflo) in the guinea pig. J Pharmacol Exp Ther 1998; 287: 988-95
125. Lagente V, Moodley I, Perrin S, et al. Effects of isozyme-selective phosphodiesterase inhibitors on eosinophil infiltration in the guinea-pig lung. Eur J Pharmacol 1994; 255: 253-6
126. Lagente V, Pruniaux MP, Junien JL, et al. Modulation of cytokine-induced eosinophil infiltration by phosphodiesterase inhibitors. Am J Respir Crit Care Med 1995; 151: 1720-4
127. Santing RE, Olymulder CG, Van der Molen K, et al. Phosphodiesterase inhibitors reduce bronchial hyperreactivity and airway inflammation in unrestrained guinea pigs. Eur J Pharmacol 1995; 275: 75-82
128. Howell RE, Woeppel SL, Howell DE, et al. Pulmonary antiallergic and antiinflammatory effects of a novel, orally-active phosphodiesterase IV inhibitor (WAY-127093B) in guinea pigs and rats. Inflamm Res 1995; 44 Suppl. 2: S172-3
129. Holbrook M, Gozzard N, James T, et al. Inhibition of bronchospasm and ozone-induced airway hyperresponsiveness in the guinea-pig by CDP840, a novel phosphodiesterase type 4 inhibitor. Br J Pharmacol 1996; 118: 1192-200
130. Raeburn D, Karlsson J-A. Effect of isoenzyme-selective inhibitors of cyclic nucleotide phosphodiesterase on microvascular leak in guinea-pig airways *in vivo*. J Pharmacol Exp Ther 1993; 267: 1147-52
131. Ortiz J, Cortijo J, Valles JM, et al. Rolipram inhibits airway microvascular leakage induced by platelet-activating factor, histamine and bradykinin in guinea-pig. J Pharmacol 1993; 45: 1090-2
132. Ortiz JL, Valles JM, Marticabrera M, et al. Effects of selective phosphodiesterase inhibitors on platelet-activating factor- and antigen-induced airway hyperreactivity, eosinophil accumulation, and microvascular leakage in guinea pigs. Naunyn-Schmiedeberg's Arch Pharmacol 1996; 353: 200-6
133. Souness JE, Giembycz MA. Cyclic nucleotide phosphodiesterases in airways smooth muscle. In: Raeburn D, Giembycz MA, editors. Airways smooth muscle: biochemical control of contraction and relaxation. Basel: Birkhauser Verlag AG, 1994: 271-308
134. Myou S, Fujimura M, Kamio Y, et al. Bronchodilator effect of inhaled olprinone, a phosphodiesterase 3 inhibitor, in asthmatic patients. Am J Resir Crit Care Med 1999; 160: 817-20
135. Foster RW, Rakshi K, Carpenter JR, et al. Trials of the bronchodilator activity of the isoenzyme-selective phosphodiesterase inhibitor, AH 21-132 in healthy volunteers during methacholine challenge test. Br J Clin Pharmacol 1992; 34: 527-34
136. Brunnee T, Engelstatter R, Steinijans VW, et al. Bronchodilatory effect of inhaled zardaverine, a phosphodiesterase III and IV inhibitor, in patients with asthma. Eur Respir J 1992; 5: 982-5
137. Ukena D, Rentz K, Reiber C, et al. Effects of the mixed phosphodiesterase III/IV inhibitor, zardaverine, on airway function in patients with chronic airflow obstruction. Respir Med 1995; 89: 441-4
138. Evans DJ, Aikman SL, Kharitanov SA, et al. Inhaled tolafentrine, a PDE III/IV inhibitor: acute effect on histamine- and AMP-induced bronchoconstriction and exhaled NO in mild asthma [Abstract]. Am J Respir Crit Care Med 1996; 153: A347
139. Israel EP, Mathur PN, Tashkin D, et al. LY 186655 prevents bronchospasm in asthma of moderate severity. Chest 1988; 91: 715-8
140. Jonker GJ, Tijhuis GJ, De Monchy JGR. RP 73401 (a phosphodiesterase IV inhibitor) single dose does not prevent allergen-induced bronchoconstriction during the early phase reaction in asthma [abstract]. Eur Respir J 1996; 9: 82S
141. McGrath JL, Aikman SL, Cook RM, et al. Six weeks treatment with inhaled RP 73401, a PDE IV inhibitor: effect on airway hyperresponsiveness and exhaled nitric oxide in mild to moderate asthma [abstract]. Am J Respir Crit Care Med 1997; 155: A660
142. Perry MJ, O'Connell J, Walker C, et al. CDP840: a novel inhibitor of PDE-4. Cell Biochem Biophys 1998; 29: 113-32
143. Harbinson PL, MacLeod D, Hawksworth R, et al. The effect of a novel orally active selective PDE4 isoenzyme inhibitor (CDP840) on allergen-induced responses in asthmatic subjects. Eur Respir J 1997; 10: 1008-14
144. Norman P. PDE4 Inhibitors 1998. Exp Opin Ther Pat 1998; 8: 771-84
145. Nieman RB, Fisher BD, Amit O, et al. SB 207499 (Ariflo™), a second generation, selective oral phosphodiesterase type 4 (PDE4) inhibitor, attenuates exercise-induced bronchoconstriction in patients with asthma [abstract]. Am J Respir Crit Care Med 1998; 157: A413
146. Compton CH, Cedar E, Nieman RB, et al. Ariflo™ improves pulmonary function in patients with asthma: results of a study in patients taking inhaled corticosteroids [abstract]. Am J Respir Crit Care Med 1999; 159: A522
147. Krause W, Kuhne G. Pharmacokinetics of rolipram in the rhesus and cynomolgous monkeys, the rat and the rabbit: studies on species differences. Xenobiotica 1988; 18: 561-71
148. Data on file (WO9723460). Celltech Therapeutics Ltd, 1997
149. Data on file (WO9723461). Celltech Therapeutics Ltd, 1997
150. Rogers DF, Giembycz MA. Asthma therapy for the 21st century. Trends Pharmacol Sci 1998; 19: 160-4
151. Society for Medicines Research. Trends in Medicinal Chemistry Meeting Report. London: Society for Medicines Research, December 1996
152. Escott KJ, Birrell M, Webber SE, et al. Efficacy versus toxicity of PDE4 inhibitors [abstract]. Am J Respir Crit Care Med 1998; 157: A413
153. Duplantier AJ, Biggers MS, Chambers RJ, et al. Biarylcarboxylic acids and -amides: inhibition of phosphodiesterase type IV versus [^{3}H]rolipram binding activity and their relationship to emetic behavior in the ferret. J Med Chem 1996; 39: 120-5
154. Gale DD, Landells LJ, Spina D, et al. Pharmacodynamic-pharmacokinetic (PD/PK) profile of the phosphodiesterase (PDE) 4 inhibitor, V11294A, in human volunteers [Abstract]. Am J Respir Crit Care Med 1999; 159: A108
155. Muller T, Engels P, Fozard JR. Subtypes of the type 4 cAMP phosphodiesterases: structure, regulation and selective inhibition. Trends Pharmacol Sci 1996; 17: 294-8
156. Bushnik T, Conti M. Role of multiple cAMP-specific phosphodiesterase variants. Biochem Soc Trans 1996; 24: 1014-9
157. Manning CD, Burman M, Christensen SB, et al. Suppression of human inflammatory cell function by subtype-selective PDE4 inhibitors correlates with inhibition of PDE4A and PDE4B. Br J Pharmacol 1999; 128: 1393-8

158. Torphy TJ, Christensen SB, Barnette MS, et al. Molecular basis for an improved therapeutic index of SB 207499, a second generation phosphodiesterase 4 inhibitor [abstract]. Eur Respir J 1997; 10: S313
159. Cavalla D, Gale DD, Spina D, et al. Activity of V11294A, a novel phosphodiesterase 4 (PDE4) inhibitor, in cellular and animal models of asthma [abstract]. Am Rev Respir Crit Care Med 1997; 155: A660
160. Cavalla D, Gale D. A case history in successful virtual research. Drugs News Perspect 1997; 10: 470-6
161. Robichaud A, Tattersall FD, Choudhury I, et al. Emesis induced by inhibitors of type IV cyclic nucleotide phosphodiesterase (PDE IV) in the ferret. Neuropharmacol 1999; 38: 289-97
162. Engels P, Fichtel K, Lubbert H. Expression and regulation of human and rat phosphodiesterase type IV isogenes. FEBS Lett 1994; 350: 291-5
163. Gantner F, Tenor H, Gekeler V, et al. Phosphodiesterase profiles of highly purified human peripheral blood leukocyte populations from normal and atopic individuals: a comparative study. J Allergy Clin Immunol 1997; 100: 527-35
164. Gantner F, Gotz C, Gekeler V, et al. Phosphodiesterase profile of human B lymphocytes from normal and atopic donors and the effects of PDE inhibition on B cell proliferation. Br J Pharmacol 1998; 123: 1031-8
165. Verghese MW, McConnell RT, Lenhard JM, et al. Regulation of distinct cyclic AMP-specific phosphodiesterase (phosphodiesterase type 4) isozymes in human monocytic cells. Mol Pharmacol 1995; 47: 1164-71
166. Fuhrmann M, Jahn HU, Seybold J, et al. Identification and function of cyclic nucleotide phosphodiesterase isoenzymes in airway epithelial cells. Am J Respir Cell Mol Biol 1999; 20: 292-302
167. Wright LC, Seybold J, Robichaud A, et al. Phosphodiesterase expression in human epithelial cells. Am J Physiol 1998; 275: L694-700
168. Jin S-LC, Richard FJ, Kuo W-P, et al. Impaired growth and fertility of cyclic AMP-specific phosphodiesterase PDE4D-deficient mice. Proc Natl Acad Sci U S A 1999; 96: 11998-12003
169. Conti M, Jin C, Hansen G. Role of PDE4 in cell signalling: new insights from PDE4 Knockout mice. William Harvey Research Conferences - PDE inhibitors: drugs with an expanding range of therapeutic uses. Nice, 1999
170. Engels P, Abdel'Al S, Hulley P, et al. Brain distribution of four rat homologues of the Drosophila dunce cAMP phosphodiesterase. J Neurosci Res 1995; 41: 169-78
171. Souness JE, Rao S. Proposal for pharmacologically distinct conformers of PDE4 cyclic AMP phosphodiesterases. Cell Signal 1997; 9: 227-36
172. Barnette MS, Christensen SB, Underwood DC, et al. Phosphodiesterase 4: biological underpinnings of the design of improved inhibitors. Pharmacol Rev Commun 1997; 8: 65-73
173. Hughes B, Owens R, Perry M, et al. PDE4 inhibitors: the use of molecular cloning in the design and development of novel drugs. Drug Disc Today 1997; 2: 89-101
174. Barnette MS, Manning CD, Cieslinski LB, et al. The ability of phosphodiesterase IV inhibitors to suppress superoxide production in guinea pig eosinophils is correlated with inhibition of phosphodiesterase IV catalytic activity. J Pharmacol Exp Ther 1995; 273: 674-9
175. Souness JE, Houghton C, Sardar N, et al. Evidence that cyclic AMP phosphodiesterase inhibitors suppress interleukin-2 release from murine splenocytes by interacting with a "low affinity" phosphodiesterase 4 conformer. Br J Pharmacol 1997; 121: 743-50
176. Souness JE, Griffin M, Maslen C, et al. Evidence that cyclic AMP phosphodiesterase inhibitors suppress TNFα generation from human monocytes by interacting with a 'low-affinity' phosphodiesterase 4 conformer. Br J Pharmacol 1996; 118: 649-58
177. Barnette MS, Grous M, Cieslinski LB, et al. Inhibitors of phosphodiesterase IV (PDE IV) increase acid secretion in rabbit isolated gastric glands: correlation between function and interaction with a high-affinity rolipram binding site. J Pharmacol Exp Ther 1995; 273: 1396-402
178. Harris AL, Connell MJ, Ferguson EW, et al. Role of low Km cyclic AMP phosphodiesterase inhibition in tracheal relaxation and bronchodilation in the guinea pig. J Pharmacol Exp Ther 1989; 251: 199-206
179. Christensen SB, Guider A, Forster CJ, et al. 1,4-Cyclohexanecarboxylates: potent and selective inhibitors of phosphodiesterase 4 for the treatment of asthma. J Med Chem 1998; 41: 821-35
180. Murdoch RD, Cowley H, Upward J, et al. The safety and tolerability of Ariflo™ (SB 207499), a novel and selective phosphodiesterase 4 inhibitor, in healthy male volunteers [abstract]. Am J Respir Crit Care Med 1998; 157: A409
181. Masamune H, Cheng JB, Cooper K, et al. Discovery of micromolar PDE IV inhibitors that exhibit much reduced affinity for the [^{3}H] rolipram binding site; 3-Norboryl-4-methoxyphenylmethylene oxindoles. Bioorganic Med Chem Letts 1995; 5: 1965-8
182. Cheng JB, Cooper K, Duplantier AJ, et al. Synthesis and *in vitro* profile of a novel series of catechol benzimidazoles: the discovery of potent, selective phosphodiesterase type IV inhibitors with greatly attenuated affinity for the [^{3}H] rolipram binding site. Bioorganic Med Chem Letts 1995; 5: 1969-72
183. Hulme C, Moriarty K, Huang FC, et al. Quaternary substituted PDE IV inhibitors II: the synthesis and *in vitro* evaluation of a novel series of y-lactams. Bioorg Med Chem Lett 1998; 8: 399-404
184. Souness JE, Foster M. Potential of phosphodiesterase type 4 inhibitors in the treatment of rheumatoid arthritis. Curr Res Rheum Arthr 1998; 2: 255-68
185. Ward A, Clissold SP. Pentoxifylline: a review of its pharmacokinetic properties, and its therapeutic efficacy. Drugs 1987; 34: 50-97
186. Data on file (Eur.Pat. 3200032). Kamijo S, Imai J, 1989
187. Data on file (Eur.Pat. 319902). Kamijo S, Imai J, Kodaira H, 1989
188. Data on file (Eur.Pat. 350913). Masakatsu K, Ohashi M, 1990
189. Robicsek SA, Blanchard DK, Djeu JY, et al. Multiple high affinity cyclic AMP phosphodiesterases in human T-lymphocytes. Biochem Pharmacol 1991; 42: 869-77
190. Tenor H, Schudt C. Analysis of PDE isoenzyme profiles in cells and tissues by pharmacological methods. In: Schudt C, Dent G, Rabe K, editors. Handbook of immunopharmacology: phosphodiesterase inhibitors. London: Academic Press, 1996: 21-40
191. Wood MA, Hess ML. Long term therapy of congestive heart failure with phosphodiesterase inhibitors. J Am Med Sci 1989; 297: 105-13
192. Naccarelli GV, Goldstein RA. Electrophysiology of phosphodiesterase inhibitors. Am J Cardiol 1989; 63: 35A-40A
193. Masuoka H, Ito M, Sugioka M, et al. Two isoforms of cGMP-inhibited cyclic nucleotide phosphodiesterases in human tissues distinguished by their responses to vesnarinone, a new cardiotonic agent. Biochem Biophys Res Commun 1993; 190: 412-7

194. Larson JL, Pino MV, Geiger LE, et al. The toxicity of repeated exposures to rolipram, a type IV phosphodiesterase inhibitor, in rats. Pharmacol Toxicol 1996; 78: 44-9
195. Alousi A, Fabian RJ, Baker JF, et al. Milrinone. In: Scriabine A, editor. New drugs annual: cardiovascular drugs. New York: Raven Press, 1985: 245-83
196. Westwood FR, Iswaran TJ, Greaves P. Pathologic changes in blood vessels following administration of an inotropic vasodilator (ICI 153,110) to the rat. Fund Appl Toxicol 1990; 14: 797-809
197. Han P, Fletcher CF, Copeland NG, et al. Assignment of the mouse PDE7A gene to the proximal region of chromosome 3 and of the human PDE7A gene to chromosome 8q13. Genomics 1998; 48: 275-6
198. Bloom TJ, Beavo JA. Identification of PDE VII in HUT78 T-lymphocyte cells [abstract]. FASEB J 1994; 8: A372
199. Li L, Yee C, Beavo JA. CD3- and CD28-dependent induction of PDE7 required for T cell activation. Science 1999; 283: 848-51
200. Hayashi M, Matsushima K, Ohashi H, et al. Molecular cloning and characterization of human PDE8B, a novel thyroid-specific isozyme of 3′,5′-cyclic nucleotide phosphodiesterase. Biochem Biophys Res Commun 1998; 250: 751-6
201. Markham A, Faulds D. Theophylline: a review of its potential steroid sparing effects in asthma. Drugs 1998; 56: 1081-91
202. Chung KF. Theophylline in chronic asthma: evidence for disease-modifying properties. Clin Exp Allergy 1996; 26 Suppl. 2: 22-7
203. LaHat N, Nir E, Horenstein L, et al. Effect of theophylline on the proportion and function of T-suppressor cells in asthmatic children. Allergy 1985; 40: 453-7
204. Shohat B, Volovitz B, Varsano I. Induction of suppressor T-cells in asthmatic children by theophylline treatment. Clin Allergy 1983; 13: 487-93
205. Brenner M, Berkowitz R, Marshall N, et al. Need for theophylline in severe steroid-requiring asthmatics. Clin Allergy 1988; 18: 143-50
206. Kidney JC, Dominguez M, Taylar P, et al. Immunomodulation by theophylline: demonstration by withdrawal of therapy. Am J Respir Crit Care Med 1995; 151: 1907-14
207. Evans DJ, Taylor DA, Zetterstrom O, et al. A comparison of low-dose inhaled budesonide plus theophylline and high-dose inhaled budesonide for moderate asthma. N Engl J Med 1997; 337: 1412-8
208. O'Neill SJ, Sitar DS, Klass DJ, et al. The pulmonary disposition of theophylline and its influence on human macrophage bactericidal function. Am Rev Respir Dis 1986; 134: 1225-8
209. Condino-Neto A, Vilela MM, Cambiucci EC, et al. Theophylline therapy inhibits neutrophil and mononuclear cell chemotaxis from chronic asthmatic children. Br J Clin Pharmacol 1991; 32: 557-61
210. Jaffar Z, Sullivan P, Page C, et al. Low-dose theophylline modulates T-lymphocyte activation in allergen-challenged asthmatics. Eur Respir J 1996; 9: 456-62
211. Ohta K, Sawamoto S, Nakajima M, et al. The prolonged survival of human eosinophils with interleukin-5 and its inhibition by theophylline via apoptosis. Clin Exp Allergy 1996; 26: 10-5
212. Finnerty JP, Lee C, Wilson S, et al. Effects of theophylline on inflammatory cells and cytokines in asthmatic subjects: a placebo-controlled parallel group study. Eur Respir J 1996; 9: 1672-7
213. Mascali JJ, Cvietusa P, Negri J, et al. Anti-inflammatory effects of theophylline: modulation of cytokine production. Ann Allergy Asthma Immunol 1996; 77: 34-8
214. Coward WR, Sagara H, Church MK. Asthma, adenosine, mast cells and theophylline. Clin Exp Allergy 1998; 28 Suppl. 3: 42-6
215. Blackwell TS, Christman JW. The role of nuclear factor-κB in cytokine gene regulation. Am J Respir Cell Mol Biol 1997; 17: 3-9
216. LAS 31025. Clin Trials Monitor 1997; 69: 25-6
217. Ferrer P, Dihn-Xuan T, Chanal I, et al. Bronchodilator activity of LAS 31025, a new selective phosphodiesterase inhibitor [abstract]. Am J Respir Crit Care Med 1997; 155: A660
218. Hanifin JM, Chan SC, Cheng JB, et al. Type 4 phosphodiesterase inhibitors have clinical and *in vitro* anti-inflammatory effects in atopic dermatitis. J Invest Dermatol 1996; 107: 51-6
219. Barnes PJ. Chronic obstructive pulmonary disease: new opportunities for drug development. Trends Pharmacol Sci 1998; 19: 415-23
220. Compton CH, Gubb J, Cedar E, et al. The efficacy of Ariflo™ (SB 207499), a second generation, oral PDE4 inhibitor, in patients with COPD [abstract]. Am J Respir Crit Care Med 1999; 159: A806

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