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COMMENTARY

COULD ISOENZYME-SELECTIVE PHOSPHODIESTERASE INHIBITORS RENDER BRONCHODILATOR THERAPY REDUNDANT IN THE TREATMENT OF BRONCHIAL ASTHMA?

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Bronchial asthma is predominantly a disease of industrialized countries. Recently compiled statistics indicate that greater than 5% of the population suffers from asthma and that, unlike other common treatable conditions [1], the prevalence and severity of asthma are increasing [2] as is the number of reported cases of fatal asthma [3, 4]. These are particularly alarming statistics given the marked increase in the prescribing of various anti-asthma therapies [5, 6]. At least two explanations can be advanced to explain why currently available drugs fail to control asthma adequately: (1) they may be inactive or provide an inappropriate therapy, or (2) while efficacious, they are not being used optimally. It is the view of the author that in many cases the drugs prescribed while providing symptomatic relief are essentially inactive at controlling the progression of the disease. This may be due to the more recent realization that bronchial asthma is a chronic inflammatory disorder [7, 8] rather than an intrinsic or acquired abnormality of airways smooth muscle function. Indeed, widespread submucosal inflammation, characterized by the infiltration of pro-inflammatory cells in particular eosinophils and *T*-lymphocytes together with epithelial sloughing, has been reported not only in individuals who have died from asthma [9] but in patients with only mild symptoms of the disease [10, 11]. Furthermore, the chronicity of the inflammation in asthma may lead eventually to a state of irreversible airways obstruction brought about by structural changes in the lung notably subepithelial fibrosis and airways smooth muscle hypertrophy. Since drugs such as β-adrenoceptor agonists exert probably only an acute anti-inflammatory influence *in vivo*, it is not surprising that their use is limited to treating only the symptoms of the disease not the disease process itself. As a consequence, chronic use of such drugs could potentially exacerbate asthma and even increase mortality [12–14].

It has been advocated [7] that, in view of the now recognized inflammatory basis of bronchial asthma, inhaled steroids should be considered the first line of therapy. While these drugs do indeed suppress inflammation effectively, their non-specific nature, even when administered by inhalation, may give rise eventually to undesirable side-effects including bronchial tumors, epithelial atrophy and candidiasis which obviously preclude their routine use [15]. It is only too clear, therefore, that current therapies for the treatment of asthma are not entirely satisfactory. New drugs with greater selectivity and fewer side-effects are required. It is the purpose of this commentary to provide evidence that selective cyclic nucleotide phosphodiesterase (PDE†) inhibitors provide perhaps the most promising new approach for the *prophylaxis* of bronchial asthma by acting principally on those cell types responsible for promoting airways inflammation. Implicit in this hypothesis is that the suppression of the inflammatory response should, in theory, significantly reduce (or even eliminate) the need for bronchodilator therapy since the disease process itself is being treated.

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† Abbreviations: PDE, phosphodiesterase; cyclic AMP, adenosine 3′,5′-cyclic monophosphate; cyclic GMP, guanosine 3′,5′-cyclic monophosphate; LTB_4, leukotriene B_4; PAF, platelet-activating factor; H_2O_2, hydrogen peroxide; O_2^-, superoxide anion; fMLP, *N*-formyl-methionyl-leucyl-phenylalanine; MBP, major basic protein; ECP, eosinophil cationic protein; EDN, eosinophil-derived neurotoxin; MHC, major histocompatibility complex; IL, interleukin; LPR, late phase reaction; BAL, broncho-alveolar lavage; PGE_2, prostaglandin E_2; TNFα, tumour necrosis factor-α; GMCSF, granulocyte macrophage colony-stimulating factor; ICAM, intercellular adhesion molecule; ELAM, endothelial leukocyte adhesion molecule; VCAM, vascular cell adhesion molecule; IBMX, 3-isobutyl-1-methylxanthine; and OZ, opsonized zymosan.

WHAT IS INFLAMMATION?

The classical definition of inflammation is the body's reaction to an injurious insult. In the context of bronchial asthma, airways inflammation is considered to be precipitated by irritants (e.g. trimetallic anhydride) which damage directly various elements of the airways or, more usually, by allergen which, in sensitized individuals, activates pro-inflammatory cells normally resident in the lung (e.g. macrophages, mast cells) which express the low-affinity receptor ($Fc_\varepsilon RII$/CD23) for IgE. This leads to the release of various mediators, proteolytic enzymes and reactive oxygen radicals which are toxic to airways cells culminating in an *acute* inflammatory response.

Characteristically, the initial insult is followed by vasodilatation at the level of the tracheobronchial arterioles and a consequent increase in blood flow. This is evoked by various *acute* pro-inflammatory mediators including histamine, 5-hydroxytryptamine (5-HT), tachykinins and prostaglandins. There is an increase in capillary and microvenular pressure arising from the arteriolar vasodilatation together with the liberation of other acute pro-inflammatory mediators exemplified by platelet-activating factor (PAF) and the peptidoleukotrienes which contract directly post-capillary microvenular endothelial cells. Together, these effects increase microvenular permeability and permit the loss of plasma proteins from the vascular compartment. The increase in the osmotic pressure following the loss of solute from the circulation leads also to marked fluid exudation and ultimately to oedema. In addition, there is an associated increase in blood viscosity which may be exacerbated by erythrocyte rouleaux formation together with adherence and margination of leukocytes and platelets to the post-capillary microvenular endothelium. Finally, the marginated leukocytes, particularly eosinophils, *T*-lymphocytes, macrophages and neutrophils, leave the microvenules by diapedesis and are attracted into the lung by chemotactic factors released by cells at the site of damage.

If the initiating stimulus is eliminated or is controlled adquately, a process of repair ensues where damaged tissues are returned to their normal organization and function. Failure to remove or control the initiating stimulus, however, leads to a continuing or *chronic* state of inflammation which can be self-perpetuating if the damaged tissue generates auto-antibodies or a cell-mediated immune response. Chronic airways inflammation in asthma is, in addition, associated with the release of a plethora of so-called *chronic* inflammatory mediators typically the cytokines which can prime cells to mediators that are ordinarily inert or poorly active. Thus, it is now recognized that in addition to chronic inflammatory disorders such as silicosis and tuberculosis the pathological changes seen in bronchial asthma are the result of a specialized chronic inflammatory response exhibiting specific well-defined characteristics (see below) which, if untreated, may give rise eventually to a state of *irreversible* airways obstruction.

PHOSPHODIESTERASE MULTIPLICITY

Cyclic nucleotide PDEs (EC 3.1.4.17) are a family of enzymes which hydrolyse the 3′-ribose phosphate bond of the naturally occurring second messenger nucleotide 3′,5′-cyclic monophosphates to form the biologically inert 5′-nucleoside monophosphates. With respect to cyclic AMP and cyclic GMP, five isoenzyme classes have been defined biochemically according to a number of criteria including substrate specificity, inhibitor sensitivity, allosteric modulation and Ca^{2+}- and calmodulin-dependence (Table 1 and see Refs. 16–18 for further details). More recently, primary protein and cDNA sequencing techniques have corroborated this biochemical PDE classification with the identification of at least five gene families that code for cyclic nucleotide PDEs. It is noteworthy, that each of these gene families appears to contain two or more sub-families, many of which can give rise to multiple mRNAs through alternate splicing. With the advent of molecular biology, evidence is now available to suggest the presence of 20–30 different mammalian PDE isoenzymes. For more details on the biochemistry, pharmacology and physical characteristics of PDE isoenzymes the reader is directed towards recent reviews by Beavo [16], Beavo and Reifsnyder [17] and Nicholson *et al.* [18].

Given the apparent multiplicity of PDE isoenzymes, it is perhaps not surprising that many cell types express more than a single PDE and that the distribution of these isoenzymes between different cells varies markedly. Thus, although the precise significance of this PDE isoenzyme multiplicity is poorly understood, it provides a unique therapeutic opportunity for the development of highly isoenzyme family-selective (second generation), and, theoretically, sub-family-selective (third generation) PDE inhibitors which may permit the discrete and selective manipulation of various pathophysiological processes including bronchial asthma.

Until relatively recently, research into the potential utility of PDE inhibitors in asthma was confined almost entirely to airways smooth muscle. The hypothesis posed in this article, however, is that to effect bronchodilatation may not remain a general therapeutic goal for asthma therapy if the inflammatory response is effectively suppressed. In this respect, therefore, the most salient question, addressed below, is: are PDE inhibitors anti-inflammatory and, if so, are they active in asthma. Readers interested in the airways smooth muscle actions of PDE inhibitors should consult recent critiques on this subject [19, 20].

ANTI-INFLAMMATORY ACTIONS OF PHOSPHODIESTERASE INHIBITORS IN ISOLATED CELLS

Mast cells

Although mast cells were originally thought to participate only in the immediate asthmatic response (predominantly bronchoconstriction), there is now evidence that these cells are involved also in the late phase reaction (LPR) [see Ref. 21]. Indeed, mast cell degranulation is accompanied by a respiratory burst evolving highly reactive toxic oxygen species which are believed to promote epithelial shedding, goblet cell hyperplasia and consequent mucous hyper-secretion, factors that undoubtedly contribute to the LPR. Moreover, at the First Congress of the European Respiratory Society in 1991, evidence was provided that mast cells produce the cytokine IL-4, an effective stimulant of IgE production. This observation has important implications for the LPR which is clearly IgE-dependent. Taken together, therefore, mast cells should not be ignored when considering new drugs for the treatment of asthma.

Phosphodiesterase isoenzyme profile. No reports on the PDE isoenzyme profile of *human* mast cells have been published but some information is available on mast cells obtained from the thoracic cavity and peritoneum of the rat [22], and from

Table 1. Cyclic nucleotide phosphodiesterase multiplicity: Isoenzymes, substrate specificity and selective inhibitors

PDE family	Isoenzyme	Preferred substrate	Selective inhibitor(s)
Iα*	Ca^{3+}/Calmodulin-stimulated	Cyclic AMP	Vinpocetine
II	Cyclic GMP-stimulated	No preference	No inhibitors
III†	Cyclic GMP-inhibited	Cyclic AMP	SKF 94120 Siguazodan Motapizone Imazodan Org 9935 Anegrelide CI-930 OPC 3689 Amrinone Cilostamide
IV†	Cyclic AMP-specific	Cyclic AMP	Rolipram Ro 20-1724 Tibenelast Debufylline
V	Cyclic GMP-specific	Cyclic GMP	Zaprinast SKF 96231

* Evidence for a Ca^{2+}/calmodulin-stimulated isoenzyme family (PDE Iβ) with kinetic characteristics distinct from PDE Iα is available in human airways smooth muscle.

† A number of PDE inhibitors are available which do not readily discriminate between the PDE III and PDE IV isoenzymes. These so-called hybrid inhibitors include benzafentrine, zardaverine and Org 30029.

murine bone marrow [see Ref. 19]. Bergstrand and colleagues [22] reported that the PDE activity from an enriched population (>90%) of rat mast cells is predominantly (~80%) soluble in origin. Gel filtration of a high speed supernatant fraction over DEAE-Sephadex resolved three peaks of PDE activity which, based upon limited kinetic data and sensitivity to zaprinast, may tentatively be designated PDE V, PDE II and PDE III (given in order of elution) [22]. There may be some species difference in PDE profile, however, since the predominant cyclic AMP PDE in murine mast cells has the characteristics of a PDE IV [see Ref. 19]. The nature and identity of the particulate PDE(s) in rat mast cells are unknown.

Functional effects. Although cyclic AMP elevating drugs seem to inhibit various indices of mast cell activation [see, for example, Ref. 23], few studies have examined the functional effect of PDE inhibitors in these cells. The apparent lack of a PDE IV in rat mast cells implied by the data of Bergstrand *et al.*, [22] is supported by the finding that rolipram does not inhibit IgE-induced histamine release from these cells [24]. This contrasts with the inhibitory effect of zaprinast and suggests that cyclic GMP-elevating agents may exert anti-inflammatory activity in the mast cell [24]. Unfortunately, inhibitors of PDE III were not examined in this study so a modulatory role of cyclic AMP in this cell response cannot be excluded.

Eosinophils

Recent evidence suggests that activated eosinophils play an important role in the pathogenesis of bronchial asthma through their ability to promote localized inflammatory lesions within the bronchial mucosa which may contribute ultimately in the development of non-specific airways hyper-responsiveness. Indeed, activated eosinophils generate highly reactive oxygen radicals and elaborate a wide range of potentially cytotoxic mediators (e.g. lipoxygenase and cyclooxygenase products, cytokines, PAF) and basic granule constituents [major basic protein (MBP), eosinophil cationic protein (ECP), eosinophil-derived neurotoxin (EDN)] which are toxic to both airways nerves and epithelial cells. Research conducted over the last decade has led to the view that the inflammation of the bronchial mucosa seen in asthma is eosinophil-based orchestrated in part by CD4+ *T*-lymphocytes (see below). To reduce or inhibit the pro-inflammatory activity of eosinophils, therefore, appears a particularly important site for asthma therapy. It is not surprising, in this respect, that there has been much research lately into the basic biochemistry and pharmacology of eosinophils.

Phosphodiesterase isoenzyme profile. Guinea pig peritoneal eosinophils [25, 26] and eosinophils purified from human venous blood [our unpublished observations] express predominantly, if not exclusively, a PDE IV which is tightly membrane bound [25, 26]. There is no enzymological evidence for either soluble or particulate PDEs I, II, III or V [25, 26]. It is noteworthy, however, that guinea pig eosinophils do synthesize cyclic GMP by a guanylyl cyclase that is apparently insensitive to sodium nitroprusside [our unpublished observations]. This suggests, therefore, that the cyclic GMP level in these cells is regulated by (a) PDE IV, (b) another PDE isoenzyme the activity of which was not readily detected, or (c) a pump which transports cyclic GMP out of the cell.

Curiously, the kinetics of cyclic AMP hydrolysis catalysed by the PDE IV in both guinea pig [25, 26] and human eosinophil membranes [our unpublished observations] are non-linear and separable into high and low affinity components. This may indicate that these cells contain more than one PDE IV or a single PDE isoform which can undergo reversible covalent modification (e.g. phosphorylation) such that its catalytic activity is altered (see section on *T*-lymphocytes and neutrophils).

Functional effects. Drugs which have been categorized previously as PDE IV (rolipram and denbufylline) or PDE III/IV inhibitors (zardaverine, Org 30029) reduce markedly H_2O_2 generation (index of respiratory burst activity) and thromboxane biosynthesis induced by both soluble [leukotriene B_4 (LTB_4), PAF] and particulate [opsonized zymosan (OZ)] stimuli ([25] and our unpublished observations). Consistent with these data is the observation that over the same range at which functional responses are inhibited, rolipram potently abrogates PAF-induced Ca^{2+} mobilization in these cells and increased the cyclic AMP content and activates cyclic AMP-dependent protein kinase [26]. Selective inhibitors of PDE V (e.g. zaprinast) and PDE III (e.g. SKF 94120, Org 9935, imazodan), in contrast, are inactive in these functional and biochemical tests at concentrations at which they retain isoenzyme selectivity [25, 26]. Although eosinophils produce cyclic GMP, the functional effect(s) of increasing the cell content of this cyclic nucleotide is unknown. The observation that neither zaprinast nor dibutyryl-cyclic GMP inhibit OZ-induced H_2O_2 generation [25, 26] may indicate that cyclic GMP does not modulate respiratory burst activity in eosinophils.

Although β-adrenoceptor agonists, chloera toxin and hydrophobic cyclic AMP analogues suppress human eosinophil activation [27], only two studies have examined the potential inhibitory activity of PDE inhibitors in these cells [27, 28]. In one of these Kita *et al.* [27] reported that IgG- and secretory IgA-induced EDN release was inhibited by 3-isobutyl-1-methylxanthine (IBMX). That this was mediated via a cyclic AMP-dependent mechanism is suggested by the observation that IBMX also potentiated the inhibitory action of isoprenaline and salbutamol [27]. The PDE isoenzyme inhibited by IBMX in human eosinophils is probably a PDE IV given the recent observation of Dent *et al.* [28] that rolipram and zardaverine but not SKF 94120 effectively antagonize OZ-induced H_2O_2 generation.

T-*Lymphocytes*

Convincing evidence is now available for the involvement of activated *T*-lymphocytes in allergic asthma in particular a population which are CD4+ [i.e. express receptors for the class II major histocompatibility complex (MHC)]. Indeed, a close association between CD4+ *T*-lymphocytes and eosinophilia has been demonstrated and it is now believed that the airways eosinophilia seen in asthma is orchestrated, in part, by *T*-lymphocytes. More recently a functional subset of CD4+ cells, *Th2*, has been identified which secretes a number of cytokines (IL-4, IL-5) that may give rise to eosinophil-rich bronchial mucosal inflammation which characterizes clinical asthma. It would seem from this evidence, therefore, that to dampen down the activity of CD4+ *T*-lymphocytes could be particularly desirable for the treatment of asthma.

Phosphodiesterase isoenzyme profile. In 1976 Thompson and his colleagues [29] reported the presence of a single PDE activity in a mixed population (i.e. *T*- and *B*-cells) of human lymphocytes that had kinetic characteristics consistent with, what is now called, a PDE III isoenzyme. This classification was also suggested by the work of Takemoto *et al.* [30] who provided evidence that >80% of the soluble cyclic AMP PDE activity in an enriched population of *T*-lymphocytes was inhibited by micromolar concentrations of cyclic GMP. More recently, Robicsek *et al.* [31] reported on the PDE isoenzyme(s) present in the soluble fraction of an enriched (>88%) population of *platelet-free T*-lymphocytes resolved by HPLC anion-exchange chromatography. One peak of cyclic GMP hydrolytic activity eluting at low ion strength, and presumably representing PDE I and/or PDE V, and three peaks of cyclic AMP hydrolytic activity were resolved by the column. Of the cyclic AMP hydrolysing activities two were inhibited by Ro 20-1724 but curiously none of them was affected by CI-930 (PDE III-selective inhibitor) [31]. Multiple PDE IVs may, therefore, be present in this cell type. The nature and identity of the CI-930-resistant soluble activity are unknown. It is not clear why the results of Robicsek *et al.* [31] conflict with those published by both Thompson *et al.* [29] and Takemoto *et al.* [30] other than to postulate platelet (rich source of PDE III) contamination in these earlier studies.

Subsequent experiments by Robicsek and his colleagues [32] with Ro 20-1724 and CI-930 suggest that *T*-lymphocytes do, in fact, express a PDE III that is located predominantly in the particulate fraction of cell homogenates. This contrasts with the subcellular distribution of PDE IV which is enriched in the cytosolic fraction [32]. The functional significance of this subcellular distribution is not clear at the present time.

To date, all PDE studies in *T*-lymphocytes have been performed on a mixed population of CD4+ and CD8+ (i.e. cells which express receptors for class I MHC) cells. Given the apparent significance of CD4+ *T*-lymphocytes in bronchial asthma, an intriguing question to be addressed is whether the PDE isoenzyme profile differs between *T*-lymphocyte subsets and, indeed, between the *Th1* and *Th2* CD4+ *T*-lymphocyte variants.

Functional effects. To date the effects of cyclic nucleotide PDE inhibitors on various functional indices of *T*-lymphocyte activation have not been examined in detail. There is evidence, however, that agonist-induced increases in cyclic AMP correlate with inhibition of *T*-lymphocyte activation and proliferation [33]. In keeping with this, Ro 20-1724 suppresses *T*-lymphocyte cytotoxicity [34] and produces some inhibition of phytohaemagglutinin-induced blastogenesis [32]. Evidence that both soluble PDE IV *and* particulate PDE III act in concert to control *T*-lymphocyte activity is provided from the observation that Ro 20-1724 and CI-930

act synergistically in the inhibition of blastogenesis [32].

Macrophages

A consistent finding in patients with allergic asthma is that the number of CD23+ alveolar macrophages is increased approximately 3-fold compared to non-asthmatic individuals. This is consistent with the finding that up-regulation of the $Fc_{\varepsilon}RII$ receptor is associated with all pathologies and experimental situations where IgE levels are elevated. An increase in $Fc_{\varepsilon}RII$ receptor expression on alveolar macrophages, together with the finding that IgE activation of these cells results in the release of a plethora of potentially pro-inflammatory mediators and cytotoxic species, implicates this cell type in allergy and asthma and, so, provides a site at which drugs may act to effectively suppress IgE-dependent activation.

Phosphodiesterase isoenzyme profile. There are no data, to the author's knowledge, on the PDEs present in *alveolar* macrophages obtained either from humans or from laboratory animals. It is known, however, that murine *peritoneal* macrophages contain both soluble and particulate cyclic AMP hydrolytic activity; cyclic GMP is also readily hydrolysed by these cells [35]. Partial purification of the soluble PDEs in murine macrophages by DEAE-cellulose anion-exchange chromatography suggests the presence of five PDE isoenzymes (one from each of the gene families—see Introduction and Table 1) [17]. In contrast to many other cells, however, the PDE elution profile is seemingly atypical. In particular, an activity having the characteristics of a PDE IV is eluted at the same ionic strength as PDE I and PDE V [35].

Functional effects. In intact murine macrophages, Ro 20-1724 and rolipram potentiate prostaglandin E_2 (PGE_2)-stimulated cyclic AMP accumulation [35]. Similar data are obtained with anagrelide and OPC 3689 (selective PDE III inhibitors) [35]. Both PDE isoenzymes, therefore, can regulate the cyclic AMP content in these cells. Selective PDE inhibitors are also active functionally although the data vary markedly between species: in *guinea pig* peritoneal macrophages, for example, superoxide generation under basal conditions is inhibited by rolipram and Ro 20-1724 but not by SKF 94120 [36], indicating either the absence of PDE III in these cells or compartmentalization of PDE III action. PDE IV may, therefore, be the sole isoform responsible for regulating macrophage respiratory burst in this species. This may not necessarily be true for human alveolar macrophages, however, given the recent report that Ro 20-1724 has no effect on human alveolar macrophage activation whereas forskolin, in contrast, does produce some suppression [37].

Cyclic GMP may also regulate Ca^{2+}-dependent functional responses in macrophages since cyclic GMP activates the Ca^{2+}-transport ATPase that straddles the endoplasmic reticulum so accelerating the rate at which agonist-induced Ca^{2+} transients decay [38]. Inhibitors of PDE I or PDE V may, therefore, be expected to act similarly.

Neutrophils

In some animal models of asthma, the development of airways hyper-reactivity is, in part, neutrophil dependent. However, despite reports documenting increases in the percentage of neutrophils in bronchoalveolar lavage (BAL) fluid taken from asthmatic individuals following antigen challenge and the suggestion that some forms of occupational asthma seem to have a neutrophil-dependent component, the role of the neutrophil in asthma pathogenesis is still uncertain. Nevertheless, the potential inhibitory activity of selective PDE inhibitors at the level of this cell type has been evaluated.

Phosphodiesterase isoenzyme profile. Recently, the PDE isoenzymes in the human neutrophil were partially characterized [39–41]. Like that found in the eosinophil, the predominant species in this cell is a PDE IV [39–41], although it is unclear at present whether it is predominantly a soluble [see Ref. 41] or membrane-associated [see Ref. 39] enzyme. Anion-exchange chromatography of human neutrophil cytosol [41] and Triton X-100-treated intact cells [40] indicates also a PDE V. Analysis of cyclic AMP hydrolysis by PDE IV in human neutrophils has yielded inconsistent results where both simple [40] and complex [39] kinetics have been reported, the latter being similar to those described for eosinophil PDE IV [25, 26]. This may be due to the presence of multiple PDE IVs in these cells, a postulate strengthened by the observation that two peaks of rolipram-sensitive PDE activity were resolved from these cells by anion-exchange chromatography [41].

Functional effects. The suggestion that PDE IV may be important functionally in human neutrophils is supported by the observation that *N*-formyl-methionyl-leucyl-phenylalanine (fMLP)-evoked respiratory burst is inhibited by Ro 20-1724, rolipram and tibenelast [39, 40, 42] but not by cilostamide, amrinone (PDE III-selective) or zaprinast [39, 40]. Curiously, and in complete contrast to that found in guinea pig and human eosinophils, OZ-induced superoxide generation has been reported to be completely resistant to PDE IV inhibitors [39, 40]. The reason for this apparent stimulus-dependent effect is not clear, but it may be due to functional antagonism as described recently for this effect of OZ in human granulocytes [43] given the evidence provided by Schudt and co-workers that rolipram and Ro 20-1724 are effective inhibitors of OZ-induced $\cdot O_2^-$ generation [44].

In human neutrophils, PDE inhibitors attenuate also fMLP- and calcimycin-induced biosynthesis of both PAF and LTB_4 [45] and chemotaxis induced by butanol-extracted *Escherichia coli* [46], fMLP and LTB_4 [47]. Lysozyme release evoked from cytochalasin B-treated cells is also inhibited by agents which increase the intracellular cyclic AMP content [48].

The role of PDE V inhibitors in modulating neutrophil function is not well documented. Of the studies reported, zaprinast does not inhibit calcimycin-induced respiratory burst activity [40]. However, a role for cyclic GMP in regulating

neutrophil activity is suggested by the finding that dibutyryl cyclic GMP inhibits PAF and fMLP-induced O_2^- generation (although inexplicably potentiates the same response evoked by the complement fragment C5a) [49]. Furthermore, molsidomine, a nitrovasodilator believed to act through soluble guanylyl cyclase, and its metabolites, 3-morpholinosydnonimine (SIN-1) and *N*-nitroso-*N*-morpholinoaminoacetonitrile (SIN-1A), are reported to attenuate fMLP-stimulated β-glucuronidase release [50], indicating that the exocytotic response can be modulated by a cyclic GMP-dependent mechanism.

Endothelial cells

Perhaps the most important site of action for anti-inflammatory drugs is at those vascular sites where pro-inflammatory cells and plasma proteins leave the circulation. Current evidence now favours, the post-capillary microvenules of the tracheobronchial circulation as the principal locus where this occurs. At least two anti-inflammatory actions of PDE inhibitors at this level could be desirable: first to avert the adhesion of pro-inflammatory cells to the venular endothelium and second to prevent these cells, together with plasma proteins and hence water, from leaving the circulation.

In human cultured endothelial cells, it has been reported that many inflammatory mediators including PAF and the peptidoleukotrienes promote endothelial cell contraction with the resultant formation of physical pores between adjacent cells through which pro-inflammatory cells and plasma proteins can pass. Since *in vitro* pharmacological studies have shown β-adrenoceptor agonists to inhibit microvenular permeability when administered by inhalation, selective PDE inhibitors may similarly share this potentially beneficial property.

Phosphodiesterase isoenzyme profile. The PDE isoforms present in *microvenular* endothelial cells are unknown. However, recent biochemical studies have established that porcine and bovine *aortic* endothelial cells contain predominantly two soluble cyclic nucleotide PDEs namely a PDE II and a PDE IV which, in the intact cell, regulate the cyclic GMP and cyclic AMP content, respectively [51, 52]. A PDE III activity in the particulate fraction of porcine pulmonary endothelial cells has also been identified.*

Functional effects. Only one study to the author's knowledge has examined the effect of selective PDE inhibitors on endothelial cell permeability *in vitro* [53]. Consistent with the PDE isoenzyme profiles in endothelial cells described above, PDE III (motapizone), PDE IV (rolipram) and PDE III/IV (zardaverine) inhibitors at isoenzyme-selective concentrations abolish H_2O_2-induced enhanced permeability of endothelial cells derived from porcine pulmonary artery. Moreover, zardaverine and PGE_1 at concentrations which are not by themselves inhibitory act synergistically when used in combination at attenuating endothelial cell permeability [53]. Microvascular leakage and in theory oedema may, therefore, be suppressed by inhibition of either PDE III or PDE IV (see below). It is not known if PDE V inhibitors and cyclic GMP-elevating agents are active in this test system although data recently reported by Raeburn and Karlsson [54] suggest that this might be so (see section on "*Microvascular leakage*" below).

A number of inducible adhesion molecules such ELAM-1, ICAM-1 and VCAM-1 are expressed on the luminal surface of vascular endothelial cells (and indeed on epithelial cells) during inflammation which act to enhance leukocyte margination. Since adherence appears to be an obligatory step in the migration of leukocytes from the microvenules to the airways, preventing the expression of these molecules may provide an effective way of reducing pulmonary leukocyte accumulation. To date, however, no *in vitro* evidence is available on the effect of selective PDE inhibitors or other cyclic AMP-elevating drugs on the expression of these endothelial cell adhesion molecules. Similarly, it is currently unknown if selective PDE inhibitors can prevent pro-inflammatory cell adhesion to vascular endothelial cells.

ANTI-INFLAMMATORY ACTIONS OF PHOSPHODIESTERASE INHIBITORS *IN VIVO*

Microvascular leakage

Recently, the effect of a range of isoenzyme selective PDE inhibitors was examined on microvascular leakage (using fluorescene isothiocyanate-labelled dextran as a plasma protein marker) induced by intratracheal administration of PAF in normal guinea pigs and in response to allergen in sensitized animals [54–56]. Inhibitors of PDE IV (rolipram) but *not* PDE I (vinpocetine) or PDE III (siguazodan) were found to be effective. This latter observation is curious in view of the *in vitro* data of Schudt and his colleagues [53] who identified a PDE III in endothelial cells which when inhibited by motapizone attenuated H_2O_2-induced enhanced permeability. The reason for this discrepancy is unclear but may be due to a difference in species, vessel type, nature of the leak-evoking stimulus, or site of PDE action.

The effect of cyclic GMP-elevating drugs on the integrity of the microvenular endothelium has received little attention. It might be anticipated, however, that drugs which increase vascular smooth muscle cyclic GMP content such as zaprinast could, by virtue of their marked vasodilator action, exacerbate rather than alleviate microvenular permeability by increasing tracheobronchial blood flow. This hypothesis, however, may be incorrect given the preliminary report of Raeburn and Karlsson [54] who documented that zaprinast attenuates markedly PAF-induced leakage in the guinea-pig under conditions where arteriolar blood flow was presumably increased.

Pro-inflammatory cell infiltration

A number of studies have evaluated the effect of PDE III/IV inhibitors on infiltration of pro-inflammatory cells into the airways lumen of guinea pigs in response to PAF and allergen [57–59]. In a study by Schudt *et al.* [57] pretreatment of sensitized animals with zardaverine significantly attenuated

* Schudt C, personal communication, cited with permission.

allergen-induced infiltration of pro-inflammatory cells into the BAL fluid. Differential cell counts revealed that allergen increased, above control, the number of macrophages, eosinophils and neutrophils and that the recovery from BAL of all three cell types was suppressed by zardaverine to a level comparable to that produced by dexamethasone. Similar inhibitory data have been reported for the PDE III/IV inhibitor benzafentrine on allergen- [59] and PAF-induced [58] pulmonary eosinophil accumulation in guinea pigs.

Although PAF does, indeed, elicit a selective pulmonary eosinophilia, Morley and his colleagues [60] have pointed out that the eosinophil count in the airways lumen is considerably less than that seen following antigen provocation. This observation together with the fact that PAF antagonists do not abrogate the eosinophilia due to allergen implicates mediators other than PAF in pulmonary eosinophil recruitment. It is now known that a number of the so-called *chronic* inflammatory mediators in particular IL-3, IL-5, granulocyte macrophage colony-stimulating factor (GMCSF) and tumour necrosis factor-α (TNFα) are involved in the pulmonary accumulation and activation of eosinophils. Given the putative role of these cytokines in initiating and perpetuating the inflammatory response in chronic asthma it is important to know if their actions can be suppressed by selective PDE inhibitors. Recently, Kings *et al.* [61] reported that subcutaneous and intraperitoneal injection of human recombinant GMCSF, IL-3 and mouse TNFα elicited a selective accumulation of eosinophils into the pulmonary airways of guinea pigs and that this effect was effectively prevented by prior administration of benzafentrine to the animals. This is a particularly important observation since it implies that selective PDE inhibitors are active at blocking the deleterious actions of both acute (e.g. PAF, LTB_4) *and* chronic (e.g. cytokines) inflammatory mediators.

It is important to include a caveat in this section since although PDE inhibitors can apparently modulate endothelial cell function [53], it is difficult to identify whether inhibition of leakage or pulmonary leukocyte accumulation observed *in vivo* is due to an action of PDE inhibitors at this or another site. This is especially true when these effects are elicited in sensitized animals or subjects by allergen.

OTHER POTENTIAL CELLULAR SITES OF ACTION

In addition to the cell types discussed above, PDE inhibitors suppress also the activity of monocytes (e.g. Refs. 62 and 63], basophils [e.g. Refs. 64 and 65] and platelets [e.g. Refs. 66 and 67] which may be involved to some extent in asthma pathogenesis. An action of PDE inhibitors on fibroblasts, airways nerves and, in particular, on secretory and epithelial cells is also likely.

VERDICT

The available data suggest a potential therapeutic role for selective PDE inhibitors in the prophylaxis of bronchial asthma. This is indicated by the finding that these drugs not only elicit bronchodilatation [see Refs. 20 and 21] *but* evoke widespread inhibition of essentially all cells that may participate in the inflammatory response seen in asthma. Furthermore, current *in vivo* data show such drugs to be as effective as steroids at suppressing pro-inflammatory cell infiltration into the airways of laboratory animals. Given the recent report that cyclic AMP-elevating drugs suppress mitogenesis in airways smooth muscle [68], it is the author's contention that highly selective PDE inhibitors have the required pharmacological profile to provide a new approach for the treatment of not only mild (essentially asymptomatic) asthma but also moderate and severe forms of the disease where structural changes to the airways are occurring.

WHICH ISOENZYME INHIBITOR?

A number of factors must be considered in the selection of an isoenzyme-selective PDE inhibitor with anti-inflammatory activity relevant to asthma therapy. Whilst the PDE isoform(s) must obviously be present in the target cell(s), it needs to be established if isoenzyme inhibition leads to the desired functional response in *intact* cells and, if so, whether it is active *in vivo*. In addition, the preferred route of administration, bioavailability/pharmacokinetics, duration of action and potential side-effects need to be evaluated.

The data listed in Table 2 show that with the possible exception of the platelet, a high-affinity cyclic AMP PDE IV is present in all cell types that have been implicated in asthma pathogenesis. Moreover, functional studies *in vitro* have shown inhibitors of PDE IV to suppress endothelial cell permeability and the activity of essentially all pro-inflammatory cells (Table 2), whilst *in vivo* stimulus-evoked microvascular leak and pulmonary eosinophilia are ameliorated by PDE IV inhibitors. The observation that drugs such as rolipram and Ro 20-1724 are, in addition, bronchodilators in laboratory animals [see Refs. 19 and 20] suggests that, if active in humans, PDE IV inhibition would be predicted to exert not only a "*blanket*" anti-inflammatory influence but to act also directly on aiways smooth muscle to improve lung function. These data are complemented by the finding that PDE IV-selective drugs do not elicit the marked cardiovascular side-effects noted with PDE III-selective inhibitors [see Ref. 20].

In contrast to the apparent ubiquity of PDE IV in pro-inflammatory cells, the distribution of PDE I, PDE II, PDE III and PDE V varies from cell type to cell type (Table 2). Inhibitors of these isoforms, therefore, would be expected to be more discrete in their actions. It seems unlikely that drugs which selectively inhibit PDE III will provide a rational approach for the treatment of sub-mucosal inflammation in asthma since although a PDE III has been identified in the platelet, mast cell, *T*-lymphocyte, basophil and endothelial cell, and that PDE III-selective inhibitors are functionally active, their cardiovascular side-effects (e.g. tachycardia, hypotension) will almost certainly preclude their routine use. However, the utility of hybrid drugs which do not discriminate between PDE III and PDE IV (e.g.

Table 2. Summary of phosphodiesterase isoenzymes in pro-inflammatory cells implicated in asthma pathogenesis and the effects of selective inhibitors

Cell type	Phosphodiesterase isoenzyme profile	Functional effect of selective inhibitors*	Reference(s)
Mast cell (rat)†	II, III, V	↓ Histamine release (V)	[22, 24]
Mast cell (murine)	IV	Not known	[see 19]
Eosinophil (guinea pig)‡	IV	↓ Respiratory burst (IV)	[25, 26]
Eosinophil (human)‡	IV	↓ Respiratory burst (IV)	[28]
		↓ EDN release (NS)	[27]
T-Lymphocyte (human)‡	I(?),§ III, IV, V(?)	↓ Blastogenesis (III, IV)	[32]
		↓ Cytotoxicity (IV)	[34]
Macrophage (murine)	I, II, III, IV, V	Not known	
Macrophage (guinea pig)	Not investigated	↓ Respiratory burst (IV)	[36]
Macrophage (human)	Not investigated	↔Thromboxane release (IV)	[37]
Neutrophil (human)‡	IV, V	↓ Respiratory burst (IV)	[39–42, 44]
		↓ Lipid biosynthesis (IV)	[45]
		↓ Chemotaxis (NS)	[46, 47]
Endothelial cell (bovine/porcine aortic)	II, IV	Not known	[51, 52]
Endothelial cell (porcine pulmonary)	II, III, IV	↓ Permeability (III, IV)	[53]
Platelet (human)	III, V	↓ Aggregation (III)	[66]
		↓ Secretion (III)	[67]
Basophil (human)	III, IV	↓ Histamine release (IV)	[64, 65]
		↓ Leukotriene C_4 release (IV)	
Monocyte (human)	IV	↓ Arachidonic acid metabolism (NS)	[62, 63]
Airways epithelial cell	Not investigated	Not known	
Airways secretory cell	Not investigated	Not known	
Airways nerves	Not investigated	Not known	
Fibroblasts	Not investigated	Not known	

*Roman letter in parentheses indicates isoenzyme selectivity of PDE inhibitor used in functional studies. NS = non-selective PDE inhibitor used. Key: (↓) Inhibition; and (↔) No effect.

† Isoenzyme profile based upon limited kinetic data and sensitivity to zaprinast.

‡ Multiple PDE IV isoenzymes may be present.

§ Isoenzyme not identified with certainty.

benzafentrine, zardaverine and Org 30029) is currently under clinical evaluation. The rationale for adopting a mixed inhibitor approach is to provide optimal anti-inflammatory (PDE IV-mediated) and bronchodilator (PDE III-mediated) activity in the same molecule. Practically, however, such drugs would have to be administered by inhalation to reduce the systemic consequences of PDE III inhibition.

At the present time the functional effects of inhibitors of PDE I, PDE II and PDE V in cells which express these isoforms are unclear. This is due almost entirely to the limited availability of selective drugs. Nevertheless, cyclic GMP-dependent mechanisms do seem to inhibit functional responses in certain leukocytes (Table 2) and possess also some spasmolytic activity [see Refs. 19 and 20]. PDE V, in this respect, may provide an appropriate target for drug action.

On balance the currently available data suggest that drugs which can selectively inhibit PDE IV or both PDE III and PDE IV (or even PDE IV and PDE V, if they can be synthesized)—so-called hybrid inhibitors—exhibit the most promising pharmacology with respect to addressing the inflammatory basis of bronchial asthma. The role of the other isoenzymes, in particular PDE II, in modulating cell function will be made clear only when selective inhibitors become available.

CONCLUSION

The answer to the question posed in this article, *Could isoenzyme-selective phosphodiesterase inhibitors render bronchodilator therapy redundant in the treatment of bronchial asthma*?, is, in theory, yes. Logic dictates that to suppress effectively the inflammation seen in asthma will lessen the need for bronchodilator therapy, and PDE IV, PDE III/IV and possibly PDE V-selective drugs do exhibit the required pharmacology to be efficacious in this respect. Thus, if PDE inhibitors are ultimately shown to be active in humans, they may represent a kind of *deus ex machina* in the treatment of bronchial asthma combining tissue selectively with reduced side-effects.

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REFERENCES

1. Charlton JR and Valez R, Some international comparisons of mortality amenable to medical intervention. *Br Med J* **292**: 295–301, 1986.
2. Fleming DM and Crombie DL, Prevalence of asthma and hay fever in England and Wales. *Br Med J* **294**: 279–283, 1987.
3. Sly RM, Increases in death from asthma. *Ann Allergy* **53**: 2–25, 1984.
4. Barnes PJ, Asthma deaths: A continuing problem. In: *Advanced Medicine* (Ed. Sheppard M), Vol. 24, pp. 53–61. Bailliere Tindall, London, 1988.
5. Keating G, Mitchell EA, Jackson R, Beaglehole R and Rea H, Trends in sales of drugs for asthma in New Zealand, Australia and the United Kingdom. *Br Med J* **289**: 348–351, 1984.
6. Hay IFC and Higenbottam TW, Has the management of asthma improved? *Lancet* **2**: 609–611, 1987.
7. Barnes PJ, New concepts in the pathogenesis of bronchial hyper-responsiveness and asthma. *J Allergy Clin Immunol* **83**: 1013–1026, 1989.
8. Morley J and Smith D, Lung inflammation, its significance for asthma therapy. *Agents Actions* **26**: 31–39, 1989.
9. Dunhill MS, The pathology of asthma with special reference to changes in the bronchial mucosa. *J Clin Pathol* **13**: 27–33, 1960.
10. Laitinen LA, Heino M, Laitinen A, Kava T and Haahtela T, Damage of the airway epithelium and bronchial reactivity in patients with asthma. *Am Rev Respir Dis* **131**: 599–606, 1985.
11. Beasley R, Roche WR, Roberts JA and Holgate ST, Cellular events in the bronchi in mild asthma and after bronchial provocation. *Am Rev Respir Dis* **139**: 806–817, 1989.
12. Sears MR, Taylor DR, Print CG, Lake DC, Li Q, Flannery EM, Yates DM, Lucas MK and Herbison GP, Regular inhaled beta-agonist treatment in bronchial asthma. *Lancet* **336**: 1391–1396, 1990.
13. Crane J, Pearce N, Flatt A, Burgess C, Jackson R, Kwong T, Ball M and Beasley R, Prescribed fenoterol and death from asthma in New Zealand, 1981–83. Case-control study. *Lancet* **i**: 917–922, 1989.
14. Pearce N, Grainger J, Atkinson M, Crane J, Burgess C, Culling C, Windom B and Beasley R, Case-controlled study of prescribed fenoterol and death from asthma in New Zealand, 1977–1981. *Thorax* **45**: 170–175, 1990.
15. Seal JP and Compton MR, Side-effects of corticosteroid agents. *Med J Aust* **144**: 139–142, 1986.
16. Beavo JA, Multiple isozymes of cyclic nucleotide phosphodiesterase. *Adv Second Messenger Phosphoprotein Res* **22**: 1–38, 1988.
17. Beavo JA and Reifsnyder DH, Primary sequence of cyclic nucleotide phosphodiesterase isozymes and the design of selective inhibitors. *Trends Pharmacol Sci* **11**: 150–155, 1990.
18. Nicholson CD, Challiss RAJ and Shahid M, Differential modulation of tissue function and therapeutic potential of selective inhibitors of cyclic nucleotide phosphodiesterase isoenzymes. *Trends Pharmacol Sci* **12**: 19–27, 1991.
19. Torphy TJ and Undem BJ, Phosphodiesterase inhibitors: New opportunities for the treatment of asthma. *Thorax* **46**: 512–523, 1991.
20. Giembycz MA and Dent G, Prospects for selective cyclic nucleotide phosphodiesterase inhibitors in the treatment of bronchial asthma. *Clin Exp Allergy* **22**: 337–344, 1992.
21. White MV and Kaliner MA, Mast cells and asthma. In: *Lung Biology in Health and Disease* (Eds. Kaliner MA, Barnes PJ and Persson CGA), Vol. 49, pp. 409–440. Marcel Dekker, New York, 1991.
22. Bergstrand H, Lundqvist B and Schurmann A, Rat mast cell high affinity cyclic nucleotide phosphodiesterases: Separation and inhibitory effects of two anti-allergic agents. *Mol Pharmacol* **14**: 848–855, 1978.
23. Marone G, Columbo M, Triggiani M, Cirillo R, Genovese A and Formisano S, Inhibition of IgE-mediated release of histamine and peptide leukotriene from human basophils and mast cells. *Biochem Pharmacol* **36**: 13–20, 1987.
24. Frossard N, Laudry Y, Pauli G and Ruckstuhl M, Effects of cyclic AMP- and cyclic GMP-phosphodiesterase inhibitors on immunological release

of histamine and on lung contraction. *Br J Pharmacol* **73**: 933–938, 1981.
25. Dent G, Giembycz MA, Rabe KF and Barnes PJ, Inhibition of eosinophil cyclic nucleotide PDE activity and opsonised zymosan-stimulated respiratory burst by 'type IV'-selective PDE inhibitors. *Br J Pharmacol* **103**: 1339–1346, 1991.
26. Souness JE, Carter CM, Diocee BK, Hassall GA, Wood LJ and Turner NC, Characterization of guinea-pig eosinophil phosphodiesterase activity. Assessment of its involvement in regulating superoxide generation. *Biochem Pharmacol* **42**: 937–945, 1991.
27. Kita H, Abu-Ghazaleh RI, Gleich GJ and Abraham RT, Regulation of Ig-induced eosinophil degranulation by adenosine 3′,5′-cyclic monophosphate. *J Immunol* **146**: 2712–2718, 1991.
28. Dent G, Evans PM, Chung KF and Barnes PJ, Zardaverine inhibits respiratory burst activity in human eosinophils. *Am Rev Respir Dis* **141**: A878, 1989.
29. Thompson WJ, Ross CP, Pledger WJ, Strada SJ, Banner RL and Hersh EM, Cyclic adenosine 3′:5′-monophosphate phosphodiesterase. Distinct forms in human lymphocytes and monocytes. *J Biol Chem* **251**: 4922–4929, 1976.
30. Takemoto DJ, Lee W-NP, Kaplan SA and Appleman MM, Cyclic AMP phosphodiesterase in human lymphocytes and lymphoblasts. *J Cyclic Nucleotide Res* **4**: 123–132, 1978.
31. Robicsek SA, Krzanowski JJ, Szentivanyi A and Polson JD, High pressure liquid chromatography of cyclic AMP phosphodiesterase from purified human T-lymphocytes. *Biochem Biophys Res Commun* **163**: 554–560, 1989.
32. Robicsek S, Blanchard DK, Djeu J, Krzanowski JJ, Szentivanyi A and Polson JB, Multiple high-affinity cAMP phosphodiesterases in human T-lymphocytes. *Biochem Pharmacol* **42**: 869–877.
33. Mary D, Aussel C, Ferrua B and Fehlmann M, Regulation of interleukin 2 synthesis by cAMP in human T cells. *J Immunol* **139**: 1179–1184, 1987.
34. Plaut M, Marone G and Gillespie E, The role of cyclic AMP in modulating cytotoxic T lymphocytes. II. Sequential changes during culture in responsiveness of cytotoxic lymphocytes to cyclic AMP-active agents. *J Immunol* **131**: 2945–2952, 1983.
35. Okonogi K, Gettys TW, Uhing RJ, Tarry WC, Adams DO and Prpic V, Inhibition of prostaglandin E_2-stimulated cAMP accumulation by lipopolysaccharide in murine peritoneal macrophages. *J Biol Chem* **266**: 10305–10312, 1991.
36. Turner NC and Guermy T, The effect of phosphodiesterase inhibitors on macrophage superoxide generation. *Thorax* **46**: P107, 1991.
37. Fuller RW, O'Malley G, Baker AJ and MacDermot J, Human alveolar macrophage activation: Inhibition by forskolin but not β-adrenoceptor stimulation or phosphodiesterase inhibition. *Pulmonary Pharmacol* **1**: 101–106, 1989.
38. Randriamampita C, Ciapa B and Trautman A, Cyclic GMP-dependent refilling of calcium stores in macrophages. *Pflügers Arch* **417**: 633–637, 1991
39. Wright CD, Kuipers PJ, Kobylarz-Singer D, Devall LJ, Klinkefus BA and Weishaar RE, Differential inhibition of human neutrophil functions: Role of cyclic AMP-specific, cyclic GMP-insensitive phosphodiesterase. *Biochem Pharmacol* **40**: 699–707, 1990.
40. Nielson CP, Vestal RE, Sturm RJ and Heaslip R, Effect of selective phosphodiesterase inhibitors on the polymorphonuclear leukocyte respiratory burst. *J Allergy Clin Immunol* **86**: 801–808, 1990.
41. Schudt C, Winder S, Forderkunz S, Hatzelmann A and Ullrich V, Influence of selective phosphodiesterase inhibitors on human neutrophil functions and levels of cAMP and Ca_i. *Naunyn Schmiedebergs Arch Pharmacol* **344**: 682–690, 1991.
42. Ho PPK, Wang LY, Towner RD, Hayes SJ, Pollock D, Bowling N, Wyss V and Panetta JA, Cardiovascular effect and stimulus-dependent inhibition of superoxide generation from human neutrophils by tibenelast, 5,6-diethoxybenzo(*b*)thiophene-2-carboxylic acid, sodium salt (LY 186655). *Biochem Pharmacol* **40**: 2085–2092, 1990.
43. Turner NC, Souness JE, Wood LJ, Diocee BK, Hassal GA and Scott L, Effect of phosphodiesterase inhibitors on granylocyte superoxide generation. *Br J Pharmacol* **102**: 39P, 1991.
44. Schudt C, Winder S, Müller B and Ukena D, Zardaverine as a selective inhibitor of phosphodiesterase isozymes. *Biochem Pharmacol* **42**: 153–162, 1991.
45. Chilton FH, Schmidt D, Torphy TJ, Goldman D and Undem B, cAMP inhibits platelet activating factor (PAF) biosynthesis in the human neutrophil. *FASEB J* **3**: A308, 1989.
46. Rivkin I and Neutze JA, Influence of cyclic nucleotides and a phosphodiesterase inhibitor on *in vitro* human blood neutrophil chemotaxis. *Arch Int Pharmacodyn Ther* **228**: 196–204, 1977.
47. Harvath L, Robbins JD, Russell AA and Seamon KB, cAMP and human neutrophil chemotaxis. Elevation of cAMP differentially affects chemotactic responsiveness. *J Immunol* **146**: 224–232, 1991.
48. Zurier RB, Weishmann G, Hoffstein S, Kemmerman S and Tai HH, Mechanisms of lysosomal enzyme release from human leukocytes. II. Effects of cAMP and cGMP, autonomic agonists, and agents which affect microtubule function. *J Clin Invest* **53**: 297–309, 1974.
49. Ervens J, Schultz G and Seifert R, Differential inhibition and potentiation of chemoattractant-induced superoxide formation in human neutrophils by cell-permeant analogue of cyclic GMP, N^2,2′-*O*-dibutyryl guanosine 3′:5′-cyclic monophosphate. *Naunyn Schmiedebergs Arch Pharmacol* **343**: 370–376, 1991.
50. Schröder H, Ney P, Woditsch I and Schrör K, Cyclic GMP mediates SIN-1-induced inhibition of human polymorphonuclear leukocytes. *Eur J Pharmacol* **182**: 211–218, 1990.
51. Souness JE, Diocee BK, Martin W and Moodie SA, Pig aortic endothelial cell cyclic nucleotide phosphodiesterase. Use of phosphodiesterase inhibitors to evaluate their roles in regulating cyclic nucleotide levels in intact cells. *Biochem J* **266**: 127–132, 1990.
52. Lugnier C and Schini VB, Characterization of cyclic nucleotide phosphodiesterases from cultured bovine aortic endothelial cells. *Biochem Pharmacol* **39**: 75–84, 1990.
53. Suttorp N, Welsch T, Weber U, Richter U and Schudt C, Activation of cAMP-dependent protein kinase blocks hydrogen peroxide-induced enhanced endothelial permeability *in vitro*. *Am Rev Respir Dis* **143**: A572, 1991.
54. Raeburn D and Karlsson J-A, Comparison of the effects of isoenzyme-selective phosphodiesterase inhibitors and theophylline on PAF-induced plasma leak in the guinea-pig airways *in vivo*. *Am Rev Respir Dis*, in press.
55. Raeburn D, Woodman V, Buckley G and Karlsson J-A, Inhibition of PAF-induced microvascular leakage in the guinea-pig lung *in vivo*: The effect of rolipram and theophylline. *Eur Respir J* **4**(Suppl 14): 590s, 1991.
56. Raeburn D, Woodman VR, Buckley GP and Karlsson J-A, Effect of phosphodiesterase inhibitors and salbutamol on ovalbumin-induced plasma extravasation in the anaesthetized, sensitized guinea-pig. *Respir Med* **86**: 85–86, 1992.

57. Schudt C, Winder S, Lutze M, Kilian U and Beume R, Zardaverine: A cyclic AMP specific PDE III/IV inhibitor. *Agents Actions* **34**: 161–177, 1991.
58. Sanjar S, Aoki S, Boubekeur K, Chapman ID, Smith D, Kings MA and Morley J, Eosinophil accumulation in pulmonary airways of guinea-pigs induced by exposure to an aerosol of platelet-activating factor: Effect of anti-asthma drugs. *Br J Pharmacol* **99**: 267–272, 1990.
59. Sanjar S, Aoki S, Kristersson A, Smith D and Morley J, Antigen challenge induces pulmonary airway eosinophil accumulation and airway hyperreactivity in sensitized guinea-pigs: The effect of anti-asthma drugs. *Br J Pharmacol* **99**: 679–686, 1990.
60. Aoki S, Boubekeur K, Kristersson A, Morley J and Sanjar S, Is allergic airway hyperreactivity of the guinea-pig dependent on eosinophil accumulation in the lung? *Br J Pharmacol* **94**: 365P, 1988.
61. Kings MA, Chapman I, Kristersson A, Sanjar S and Morley J, Human recombinant lymphokines and cytokines induce pulmonary eosinophilia in the guinea-pig which is inhibited by ketotifen and AH 21-132. *Int Arch Allergy Appl Immunol* **91**: 354–361, 1990.
62. Godfrey RW, Manzi RM, Genarro DE and Hoftstein ST, Phospholipid and arachidonic acid metabolism in zymosan-stimulated human monocytes: Modulation by cAMP. *J Cell Physiol* **131**: 384–392, 1987.
63. Whyte JR, Torphy TJ, Christensen SB, Lee JA and Mong S, Partial purification of the rolipram-sensitive phosphodiesterase from human monocytes. *FASEB J* **4**: A1987, 1990.
64. Klein-Tebbe J, Wicht L, Friese A, Schunack W, Schudt C and Kunkel G, Inhibition of IgE-mediated histamine release from basophils by selective phosphodiesterase inhibitors. *Allergology* **12**(Suppl): P1 01.20, 1989.
65. Peachell PT, Undem BJ, Schleimer RP, Lichtenstein LM and Torphy TJ, Action of isozyme-selective phosphodiesterase (PDE) inhibitors on human basophils. *FASEB J* **4**: A639, 1990.
66. Murray KJ, England PJ, Hallam TJ, Maguire J, Moores K, Reeves ML, Simpson AWM and Rink TJ, The effect of siguazodan, a selective phosphodiesterase inhibitor, on human platlet function. *Br J Pharmacol* **99**: 612–616, 1990.
67. Muggli R, Tschopp TB, Mittelholzer E and Baumgartner HR, 7-Bromo-1,5-dihydro-3,6-dimethylimidazo-[2,1-*b*]quinazolin-2(3H)-one (Ro 15-2041), a potent antithrombotic agent that selectively inhibits platelet cyclic AMP-phosphodiesterase. *J Pharmacol Exp Ther* **235**: 212–219, 1985.
68. Panettieri RA, Rubinstein NA, Feuerstein B and Kotlikoff MI, Beta-adrenergic inhibition of airway smooth muscle cell proliferation. *Am Rev Respir Dis* **143**: A608, 1991.