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COMMENTARY

Cyclic Nucleotide Phosphodiesterase (PDE) Inhibitors and Immunomodulation

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ABSTRACT. Intracellular levels of cyclic nucleotide second messengers are regulated predominantly by the complex superfamily of cyclic nucleotide phosphodiesterase (PDE) enzymes. Recent advances in our understanding of the molecular pharmacology of these enzymes has led to their identification as biologic regulators of certain disease states and the development of isozyme-selective inhibitors as potential therapeutic agents. A large body of *in vitro* and preclinical data suggests the therapeutic utility of PDE4 inhibitors as potent anti-inflammatory agents. Early clinical trials with selective PDE inhibitors substantiate this approach while highlighting pharmacodynamic and toxicologic pitfalls inherent to the inhibition of specific PDE isozymes. This commentary will review our current understanding of PDE inhibitors as immunomodulatory agents. BIOCHEM PHARMACOL **57**;9:965–973, 1999. © 1999 Elsevier Science Inc.

KEY WORDS. cyclic AMP; cyclic nucleotide phosphodiesterase; immunosuppression; inflammation

The medicinal and social use of cyclic nucleotide PDE† inhibitors dates back many centuries. The most common of these agents, caffeine, was first widely advocated in the medical literature by Henry Hyde Salter in his monograph of 1860 entitled "On asthma: Its pathology and treatment." In that work, Salter described asthma as "a morbid proclivity of the musculonervous system of (the) bronchial tubes to be thrown into a state of activity; the stimulus may be either immediate or remotely applied, but in either case would not normally be attended by any such result." Salter suggested "two breakfast cups of strong coffee . . . given on an empty stomach" as a remedy that "in many cases is more efficacious than any other." While deceptively simple, this dose, timing, and route of administration optimize absorption, achieve high peak serum levels, specifically target the exaggerated diurnal variations in pulmonary function characteristic of asthma, and may account for why two-thirds of Salter's patients benefited from the intake of coffee [1].

Our mechanistic understanding of PDE inhibitors began with a series of publications in the late 1950s by Sutherland and Rall. These investigators described the properties of a cyclic adenine ribonucleotide later called cAMP, reporting an increased formation of cAMP by substances such as epinephrine and glucagon, as well as inhibition of the enzymes hydrolyzing cAMP by sodium fluoride and caffeine [2, 3]. As the involvement of cyclic nucleotide second messengers in cell signaling and homeostasis became established in the 1960s, regulation of this pathway by PDE inhibitors became an area of considerable interest. However, it was not until the early 1970s, in a series of reports by Lichtenstein and colleagues, that the immunomodulatory properties of cAMP and the anti-inflammatory potential of PDE inhibitors were first demonstrated clearly [4–6]. Coincident with the reports by Lichtenstein and colleagues was the discovery that PDE activity from tissue homogenates could be fractionated, that these fractions contained pharmacokinetically distinct PDEs, and that the relative amounts of these PDE activities varied among different tissues [7–9]. These findings indicated the presence of a complex family of PDE enzymes, and suggested the potential utility of selectivity in the design of PDE inhibitors.

This commentary will describe the molecular biology and pharmacology of the superfamily of cyclic nucleotide PDEs and review the *in vitro* and *in vivo* evidence for immunomodulatory activity associated with inhibition of specific PDE isozymes.

CLASSIFICATION AND STRUCTURAL BIOLOGY

The current classification scheme for cyclic nucleotide phosphodiesterases is shown in Table 1. This classification encompasses seven distinct families, differentiated on the basis of substrate specificity and sensitivity to endogenous and exogenous regulators [10, 11]. Many of the families include more than one gene product, often located on different chromosomes [12, 13]. Among specific gene products, multiple splice variants often exist [14, 15]. Thus, specific human PDE isoforms number well over 30. A few of

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† *Abbreviations*: PDE, phosphodiesterase; cAK, cAMP-dependent protein kinase; cAMP, adenosine 3′, 5′-cyclic monophosphate; cGK, cGMP-dependent protein kinase; cGMP, guanosine 3′, 5′-cyclic monophosphate; Ig, immunoglobulin; IL, interleukin; LT, leukotriene; LPS, lipopolysaccharide; and PAF, platelet-activating factor.

TABLE 1. Human cyclic nucleotide PDE isoxymes

Family (No. of genes)	Characteristics	K_m (μM) (cAMP; cGMP)	Primary tissue distribution	Examples of inhibitors
PDE1 (3)	Ca^{2+}/calmodulin-stimulated	1-30; 3	Heart, brain, lung, smooth muscle	KS-505a Vinpocetine
PDE2 (1)	cGMP-stimulated	50; 50	Adrenal gland, heart, lung, liver, platelets	EHNA (MEP-1)
PDE3 (2)	cGMP-inhibited cAMP>>cGMP specific	0.2; 0.3	Heart, lungs, liver, platelets, adipose tissue, immunocytes	Cilostazol Enoxamone Milrinone Siguazodan
PDE4 (4)	cAMP-specific cGMP-insensitive	4; >3000	Sertoli cells, kidney, brain, liver, lung, immunocytes	CDP840 Rolipram SB 207499 Tibenelast
PDE5 (1)	cGMP-specific	150; 1	Lung, platelets	Dipyridamole MY-5445 Sildenafil Zaprinast
PDE6 (4)	cGMP-specific	2000; 60	Photoreceptors	Dipyridamole Zaprinast
PDE7 (1)	cAMP-specific High affinity	0.2; >1000	Skeletal muscle, heart, kidney, brain, pancreas, T lymphocytes	None available

the more commonly used selective inhibitors of each of the families are listed in Table 1; non-selective inhibitors include theophylline, pentoxifylline, and 3-isobutyl-1-methylxanthine (IBMX) [16].

All PDE isoforms contain three functional domains: a regulatory N-terminus, a central catalytic domain, and a regulatory C-terminus. While the catalytic domains are highly homologous across families (>50% sequence identity at the amino acid level), the N-terminal and C-terminal domains are heterologous; these domains impart the characteristics of each PDE family to the particular isoform [16, 17]. While the N-terminus is involved in allosteric regulation (calmodulin binding in PDE1; cGMP binding in PDE2; phosphorylation sites in PDE1, PDE3, PDE4, and PDE5) and membrane targeting, recent data suggest that the C-terminus may be involved in dimerization [18–25].

PHARMACOLOGY

Figure 1 depicts the general schema for the formation and degradation of cyclic nucleotides. cAMP and cGMP are formed from their respective triphosphates by the catalytic activity of adenylyl or guanylyl cyclase, respectively. These enzymes are activated in response to either transmembrane receptor ligation (such as G protein coupled receptors) or direct engagement (such as forskolin). All PDEs inactivate their cyclic nucleotide targets by hydrolytic cleavage of the 3′-phosphodiester bond, resulting in the formation of the corresponding, inactive 5′-monophosphate. PDE inhibitors block this activity, resulting in the "passive" accumulation of specific cyclic nucleotides. While activation of adenylyl or guanylyl cyclase would be expected to induce "active" generation of cyclic nucleotides, steady-state cyclic nucleotide concentrations are only transiently increased due to compensatory increases in PDE activity [26–28]. Concomitant cyclase activation and PDE inhibition produces synergistic effects on cyclic nucleotide steady-state levels [26, 29]. Cyclic nucleotides exert their effects on cellular processes through the activity of cyclic nucleotide-dependent protein kinases (cAK and cGK), families of serine-threonine kinases with multiple cellular targets including transcription regulators, ion channels, and signaling proteins [30, 31].

Specific PDE isoforms have highly selective cellular and subcellular localizations [32, 33]. Moreover, gene products within a single PDE family may demonstrate differential cellular and subcellular localization [18, 34, 35]. Thus, compartmentalization of PDE activity may play an important role in the local regulation of intracellular cyclic

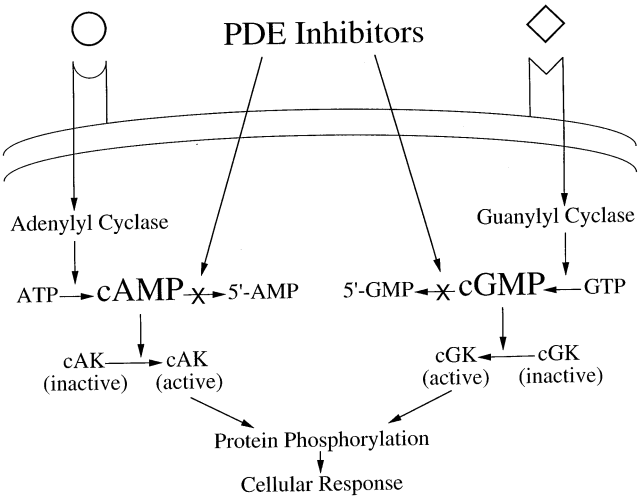

FIG. 1. Cyclic nucleotide homeostasis. Abbreviations: cAK, cAMP-dependent protein kinase; and cGK, cGMP-dependent protein kinase.

TABLE 2. cAMP-PDE expression and significance in human inflammatory cells

Cell	cAMP-PDEs expressed	Effects of inhibition
Basophil	PDE3, PDE4	Decreased histamine release Decreased leukotriene (LTC_4) generation
B lymphocyte	PDE4, (PDE3)	Decreased IgE synthesis
Eosinophil	PDE4, (PDE3)	Decreased CD11b expression Decreased chemotaxis Decreased degranulation Decreased superoxide generation Decreased leukotriene (LTC_4) generation
Mast cell	PDE3, PDE4	Decreased histamine release
Monocyte/Macrophage	PDE4, (PDE3)	Decreased TNFα ± IL-1β (not IL-6 or IL-8) generation Decreased arachidonic acid release Decreased leukotriene (LTB_4 and LTC_4) generation Decreased phagocytosis
Neutrophil	PDE4	Decreased CD11b/CD18 expression Decreased degranulation Decreased superoxide generation Decreased leukotriene generation
T lymphocyte	PDE3, PDE4, PDE7	Decreased blastogenesis Decreased proliferative response Decreased cytokine (IL-2, IL-4, IL-5, IL-13, IFN-γ, GM-CSF) generation

nucleotide content. Moreover, a variety of mechanisms allow rapid, short-term regulation of the activity of PDE isoforms. First, a local shift in cyclic nucleotide content through the activity of one PDE family may affect the activity of other PDE families through allosteric regulation or active site competition [36, 37]. Second, during cellular signaling, individual splice variants are differentially expressed; since these isoforms may be confined to specific compartments, local cyclic nucleotide homeostasis may be affected [38–41]. Finally, the activity of specific PDE isoforms may be regulated by cAK-, cGK-, and protein tyrosine kinase-mediated protein phosphorylation [21, 22, 42–44]. This mechanism is of particular interest due to the potential for creating feedforward and feedback loops that modulate intracellular signal transduction. Thus, a complex network of homeostatic controls has evolved for the rapid and selective regulation of intracellular cyclic nucleotides.

IN VITRO EFFECTS

The inflammatory response is exquisitely sensitive to modulations in steady-state levels of cyclic nucleotides. Target cells for these effects include both immune cells and accessory cells, such as airway smooth muscle, epithelial cells, endothelial cells, and nerves. In immune cells, the primary regulatory cyclic nucleotide is cAMP. Elevation of intracellular cAMP, mediated predominantly through inhibition of PDE4, results in a wide range of anti-inflammatory effects on all cell types, as summarized in Table 2.

Basophils and Mast Cells

While human basophils contain PDE3, PDE4, and PDE5, only PDE4 inhibitors have shown efficacy as single agent immunomodulators. Inhibition of PDE4 has been shown to down-regulate both anti-IgE-induced histamine release and LTC_4 generation, as well as PAF-induced histamine release from human basophils [45–48]. While PDE3 and PDE5 inhibitors show no independent efficacy in regulating these functions, PDE3 inhibitors potentiate the activity of PDE4 inhibitors on both modulation of intracellular cAMP concentrations and inhibition of mediator release [47, 48]. To date, there are no studies delineating the effects of selective PDE inhibitors on cytokine generation from human basophils.

Human mast cells contain both PDE3 and PDE4; unlike basophils, inhibitors of either isozyme possess independent efficacy in down-regulating IgE-mediated histamine release from both connective tissue- and mucosal-type mast cells [46, 49, 50]. The effects of PDE inhibitors on mast cell-derived cytokine generation have not been reported.

Eosinophils

While human eosinophils contain both PDE3 and PDE4, the majority of cAMP hydrolysis may be attributed to the activity of a combination of soluble and particulate fraction PDE4 isoforms [51]. PDE4 inhibitors block a variety of eosinophil functions, including opsonized zymosan-stimulated superoxide generation, PAF- and C5a-induced LTC_4 production, PAF- and eotaxin-induced CD11b/CD18 up-regulation, and PAF-, C5a-, and eotaxin-induced chemotaxis [52–56]. Inhibitors of PDE3 and PDE1 show no independent efficacy in down-regulating eosinophil functions. While the evidence for synergy between adenylyl cyclase activators and PDE4 inhibitors in down-regulating various eosinophil functions is conflicting, at least one

group of investigators has shown that inhibitors of cAK are able to reverse the effects of PDE4 inhibitors on freshly isolated human eosinophils [52, 54, 56]. The effects of PDE4 inhibitors on eosinophil-derived cytokine generation have not been reported.

Lymphocytes

While B lymphocytes contain both PDE3 and PDE4, the preponderance of cAMP hydrolysis can be attributed to PDE4 activity in the soluble fraction of these cells [51, 57]. The relationship between steady-state cyclic nucleotide levels and IgE synthesis is complex. While elevations of intracellular cAMP appear to augment IgE synthesis in the presence of suboptimal levels of IL-4, inhibition of IgE synthesis is seen in the presence of optimal concentrations of IL-4 [58]. Moreover, low concentrations of non-selective PDE inhibitors appear to enhance IgE synthesis, while higher concentrations inhibit IgE synthesis; this effect may be due to preferential inhibition of cGMP-PDE and the IgE enhancing effects of intracellular cGMP [58, 59]. Interestingly, while PDE4 inhibitors have been shown to reduce spontaneous IgE release from mononuclear cells isolated from allergic subjects, these effects have not been observed with purified B cells [60, 61]. The effects of PDE inhibition on the synthesis of other immunoglobulin classes and B cell activation/differentiation have not been reported.

In addition to PDE3 and PDE4, T lymphocytes possess PDE1, PDE5, and PDE7; functional studies with PDE7 have not been reported due to a lack of specific inhibitors. The distribution of PDE3 and PDE4 activity is nearly identical in $CD4^+$ and $CD8^+$ lymphocytes, with the majority of PDE4 activity localized to the soluble fraction and the majority of PDE3 activity localized to the particulate fraction [51, 62, 63]. Interestingly, elevated levels of PDE4 mRNA have been reported in the $CD4^+$ cells from atopic versus nonatopic subjects, and differential expression of PDE4 isoforms has been reported in Th1 versus Th2 clones [27, 51]. In general, PDE4 inhibitors down-regulate mitogen-, antigen-, and allogeneic HLA class II-induced blastogenesis and T cell proliferation [62, 64–66]. While PDE3 inhibitors show little or no independent efficacy in these models, they do augment the efficacy of PDE4 inhibitors. Finally, while PDE4 inhibitors down-regulate the mitogen- or antigen-induced production of a variety of proinflammatory cytokines including IL-2, IL-4, IL-5, IL-13, interferon-γ (IFNγ), and granulocyte-macrophage colony-stimulating factor (GM-CSF), up-regulation of IL-10, an anti-inflammatory cytokine, has been reported [62, 65, 67]. Once again, while PDE3 inhibitors show no independent efficacy in these models, they do augment the efficacy of PDE4 inhibitors. The effect of PDE inhibitors on T lymphocyte adhesion molecule expression has not been reported.

Monocytes/Macrophages

Human monocytes possess both PDE3 and PDE4 activity; PDE3 is confined to the particulate fraction, and PDE4 is confined to the soluble fraction [51]. While PDE4 predominates by a factor of 5 in monocytes, PDE3 activity in macrophages increases to nearly the level of PDE4; PDE1 and PDE5 activities become evident as well [51, 68]. Interestingly, both increases in steady-state cAMP levels and activation by LPS have been shown to increase PDE4 activity, with different kinetics of transcriptional activation observed with each of the PDE4 isoforms [26, 39, 69]. Functionally, PDE4 inhibitors down-regulate LPS-induced tumor necrosis factor-α (TNFα) production, formylmethionyl-leucyl-phenylalanine (fMLP)-induced arachidonic acid release, and calcium ionophore-induced LT generation [45, 70–73]. Interestingly, PDE4 inhibitors do not modulate superoxide, nitric oxide, IL-6, or IL-8 production from human monocytes [74–77]. While the evidence for down-regulation of IL-1β production is conflicting, a number of investigators have reported up-regulation of IL-10 by PDE4 inhibitors [77–80]. PDE3 inhibitors are less efficacious in down-regulating TNF production and demonstrate variable efficacy in modulating other monocyte/macrophage functions [70, 75, 78]. This variability may be attributable, in part, to variations in experimental design resulting in different levels of cellular activation/differentiation. The effects of PDE inhibitors on adhesion molecule expression and antigen presentation have not been reported, although pretreatment of monocytes with PDE4 inhibitors has been shown to down-regulate antigen-driven IL-2 production from autologous T lymphocytes [81].

Neutrophils

The primary cAMP hydrolyzing activity in neutrophils is attributable to PDE4. PDE4 inhibitors down-regulate superoxide production in response to noncognate stimuli; interestingly, cognate stimuli are resistant to the effects of PDE4 inhibitors [82–85]. Moreover, PDE inhibitors decrease neutrophil degranulation, LT production, and adhesion to endothelial cells through down-regulation of CD11b [45, 86–88]. While PDE3 inhibitors show little or no efficacy in modulating these cellular functions, adenylyl cyclase inducers act in an additive or synergistic fashion with PDE4 inhibitors in down-regulating most neutrophil cellular functions [82, 86, 88].

IN VIVO EFFECTS

The *in vitro* profiles of non-selective and selective PDE inhibitors in immune and non-immune cells suggest potential therapeutic utility as anti-depressants, anti-proliferative/immunomodulatory agents, tocolytics, cardiac inotropes/chronotropes, and cytoprotective agents [89–93]. The safety and efficacy of isozyme-selective PDE inhibitors as immunomodulators have been modelled preclinically in

both rodents and non-human primates. While PDE1, PDE3, and PDE5 inhibitors show little or no independent efficacy for this indication, PDE4 inhibitors have demonstrated activity in inhibiting antigen-induced bronchoconstriction and airway hyper-responsiveness, antigen-induced eosinophil infiltration, and local cytokine generation, using a variety of models for pulmonary allergic inflammation [94–103]. While the clinical development of PDE3 inhibitors for congestive heart failure was abandoned due to a concomitant increase in the incidence of cardiac arrhythmias, PDE4 and a combination of PDE3/4 inhibitors show promise as anti-inflammatory agents in the treatment of allergic diseases [16, 104, 105]. Recently, a PDE5 inhibitor was licensed for the treatment of male sexual dysfunction [106].

Non-selective Inhibitors

Despite a recent decline in popularity, theophylline (1,3-dimethylxanthine) remains the most commonly prescribed PDE inhibitor in the world and a central member in the therapeutic armamentarium for asthma and chronic obstructive pulmonary disease [107]. While theophylline functions primarily as a non-selective PDE inhibitor, it is also a non-selective adenosine receptor antagonist, a potentiator of catecholamine release from the adrenal medulla, and a modulator of transmembrane calcium ion fluxes [108]. Clinically, theophylline acts as a bronchodilator (predominantly through inhibition of PDE3 and PDE4), an anti-inflammatory agent (predominantly through inhibition of PDE4), an enhancer of diaphragmatic contractility (presumably through inhibition of PDE3), and a stimulator of mucociliary clearance; notwithstanding earlier data to the contrary, studies have demonstrated that theophylline does not increase the hypercapnic or hypoxic ventilatory response [109–113]. Theophylline-mediated potentiation of catecholamine secretion may augment adenylyl cyclase activity, steady-state cAMP levels, and PDE3/4-mediated bronchodilatation and anti-inflammatory efficacy. Antagonism of adenosine receptors by theophylline contributes to many of the renal, cardiac, gastrointestinal, and central nervous system adverse events; moreover, inhibition of PDE3 directly contributes to both gastrointestinal and cardiac adverse events [108]. Thus, the narrow therapeutic window seen with theophylline administration is a direct result of these opposing pharmacodynamic effects.

Selective Inhibitors

Despite considerable *in vitro* and preclinical data, published results of clinical trials with selective PDE inhibitors remain quite limited. While enoxamone, a PDE3 inhibitor, induced moderate bronchodilatation and improvement in pulmonary vascular hemodynamics in a study of 19 subjects with chronic obstructive pulmonary disease, significant adverse events (primarily cardiac) occurred in nearly half of the study subjects [114]. Cilostazol, a newer PDE3 inhibitor, has demonstrated bronchodilatory and bronchoprotective efficacy in adult subjects with a lower incidence of adverse events [115, 116]. While zaprinast, a PDE5 inhibitor, blunted the exercise-induced fall in FEV_1 in a cohort of adult exercise-induced asthmatics, this effect was not seen in a cohort of pediatric subjects, and histamine-induced bronchoconstriction remained unchanged in both cohorts [117, 118].

The interpretation of clinical studies with PDE3/4 and pure PDE4 inhibitors is somewhat more complicated. For example, studies with combined PDE3/4 inhibitors such as benzafentrine and zardaverine have focused primarily on bronchodilatation as a clinical endpoint. The results of these studies have ranged from negative to mild bronchodilatory efficacy roughly equivalent to that of a β2 agonist; anti-inflammatory endpoints have not been evaluated in these studies, and significant adverse events characteristic of PDE3 inhibitors have limited the utility of these agents [119, 120]. Tibenelast, a weak PDE4 inhibitor, was only minimally effective in improving pulmonary function in a cohort of 40 asthmatic subjects treated in a double-blind, placebo-controlled crossover study; however, the adverse event profile in these subjects showed little evidence of systemic PDE4 inhibition, and the dosing regimen and duration of the study precluded assessment of anti-inflammatory contributions to clinical efficacy [121]. However, when used topically on symmetrically involved disease sites over a 28-day period in a double-blind and paired comparison, CP80,633, a PDE4 inhibitor, induced clinical improvement in 20 of 20 subjects with atopic dermatitis [122].

Recently, advances in our understanding of the molecular pharmacology of PDE4 isozymes have led to the development of conformationally selective PDE4 inhibitors that preserve the anti-inflammatory efficacy of these agents while reducing the incidence of emesis (the characteristic, dose-limiting toxicity of PDE4 inhibitors) [123–125]. A number of such "second generation" PDE4 inhibitors are currently in clinical trials. Preliminary results from one double-blind, placebo-controlled trial of one of these agents, CDP840, administered orally for more than a week, demonstrated significant ablation of the asthmatic late phase response following allergen challenge without significantly altering the immediate response to histamine challenge, suggesting an anti-inflammatory mechanism of action; of note, dose-limiting toxicity was not seen in this study [126].

CONCLUSIONS

Advances in our understanding of the molecular pharmacology of cyclic nucleotide PDE isozymes have led to the development of first and second generation selective inhibitors for many of the PDE families. These inhibitors are being evaluated as therapeutics for a variety of clinical indications. A substantial body of *in vitro* and preclinical data supports the development of PDE4 inhibitors as potent anti-inflammatory agents whose clinical potential may soon be realized.

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