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Isoenzyme selective phosphodiesterase inhibitors: potential clinical uses

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Introduction

Naturally occurring xanthine derivatives have been used in the treatment of asthma and as stimulants of the central nervous system for at least 2 millennia. The major advance in the understanding of their mechanism of action was made in 1962 when Butcher and Sutherland demonstrated that the methyl xanthines were able to inhibit the hydrolysis of cyclic AMP (cAMP) by tissue phosphodiesterases (Butcher & Sutherland, 1962). The realisation that alteration of tissue cyclic nucleotide levels produced profound physiological effects on cell function led to a search for other agents capable of inhibiting phosphodiesterase activity. Although some drugs of therapeutic value were found such as theophylline and papaverine–which proved to have inhibitory effects upon phosphodiesterase activity, many of the xanthine derivatives identified at that time were relatively ineffective and had poor therapeutic windows. Recent advances in the field of cyclic nucleotide research have, however, rekindled interest in agents acting as phosphodiesterase inhibitors.

It has become clear that the hydrolysis of cAMP and cyclic GMP (cGMP) by phosphodiesterases is not dependent on a single enzyme but on the activities of a range of structurally related isoenzymes. Molecular biological and pharmacological characterisation of these isoenzymes have revealed marked differences in their distribution, both at the tissue and at the intracellular levels. This has led to a search for selective inhibitors of individual phosphodiesterase isoenzymes with the aim of producing a new group of therapeutic agents which have more potent effects upon specific tissues than the non-selective phosphodiesterase inhibitors such as theophylline. A large group of isoenzyme selective phosphodiesterase inhibitors have now been identified and this review describes the mechanisms through which these agents are believed to have their effect at the cellular level and indicates potential clinical uses of these drugs.

Cyclic nucleotides and cell responses

cAMP and cGMP are the two intracellular second messengers formed following stimulation of adenylyl cyclase and guanylyl cyclase respectively. The effects of a wide range of regulatory hormones are mediated through specific receptors coupled to these two enzymes. The classical model describing coupling of the receptor to adenylyl cyclase is that of the β-adrenoceptor, where stimulation of the receptor results in dissociation of the coupled G (for GTP binding) protein (G_s). G_s exists as a heterotrimer of alpha, beta and gamma subunits: following stimulation of the beta receptor the subunits dissociate, and the free alpha subunits so formed are responsible for stimulating adenylyl cyclase (see Figure 1, also see Gilman, 1987). The breakdown of cAMP and cGMP to 5′AMP and 5′GMP respectively is regulated by the activity of phosphodiesterases.

The effects of elevation of cyclic nucleotide levels varies from tissue to tissue and the relative importance of these two intracellular second messengers in the control of cell responses is also dependent upon the cell type under consideration. An illustration of this difference can be seen in nonskeletal muscle types. Whereas in airway smooth muscle elevation of cAMP produces relaxation, in vascular smooth muscle elevation of cGMP levels is important in the relaxant response induced by many agents. In contrast elevation of tissue cAMP content in cardiac muscle does not relax the tissue but instead produces a positive inotropic effect. Because the phosphodiesterase isoenzyme(s) physiologically important in controlling tissue cyclic nucleotide content vary in different tissues, it follows that selective inhibitors of the isoenzymes should produce at least partially tissue specific effects.

In some tissues cAMP and cGMP co-regulate physiological responses. Although, as mentioned above, elevation of cAMP content in airway smooth muscle is the major mechanism through which relaxation is

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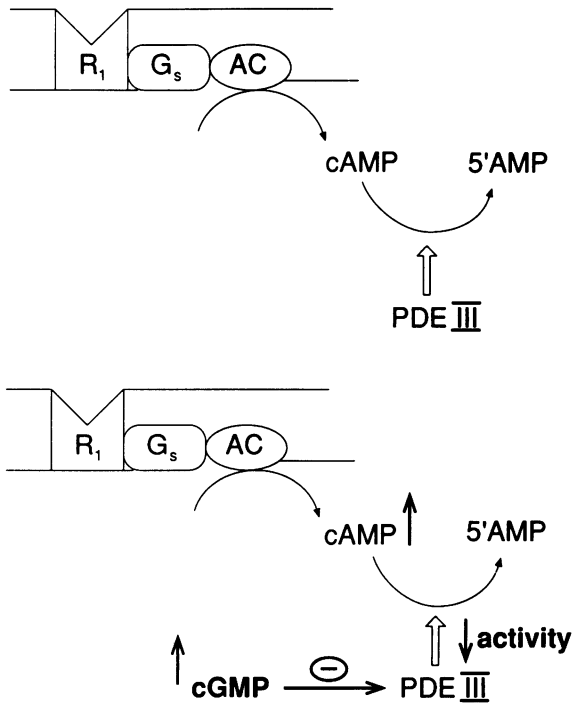

Figure 1 Site of action of phosphodiesterase (here the type III isoenzyme) in hydrolysis of cyclic AMP to 5'AMP. Agonist stimulation of a receptor (R_1) positively coupled via a G protein (G_s) to adenylyl cyclase (AC) results in formation of cyclic AMP (cAMP). Phosphodiesterases (PDE) are responsible for the breakdown of cAMP (upper panel). In a tissue where the type III isoenzyme is important in control of the rate of hydrolysis of cAMP, inhibition of the activity of the type III isoenzyme by elevation in tissue cyclic GMP (cGMP) content can potentially increase cAMP levels (lower panel).

believed to occur, elevation of cGMP content produces an additional relaxant response which is additive (but not synergistic) to the response to cAMP (Heaslip *et al.*, 1987). An added complication *in vivo* is that changes in tissue cGMP content can modulate the activity of one family of cyclic AMP hydrolysing phosphodiesterase isoenzymes (the type III family, q.v.) with the consequence that physiological alteration of cell responses mediated through elevation of cAMP levels can be potentially regulated by alteration in tissue cGMP content (see Figure 1b).

The true situation *in vivo* is likely to be even more complex in view of the increasing recognition that regulatory interactions ('cross talk') occurs between the different intracellular signal transduction mechanisms (Hill & Kendall, 1989). For example, because the activity of some of the type I phosphodiesterase isoenzymes can be modulated by changes in intracellular calcium content, agents stimulating phosphoinositidase C linked receptors could potentially alter cyclic nucleotide responses by increasing intracellular calcium content via the formation of inositol 1, 4, 5 trisphosphate. A detailed account of the regulation of the individual isoenzymes can be found elsewhere (Thompson, 1991). However, despite these complicated intracellular homeostatic mechanisms, alteration of tissue cyclic nucleotide levels and cell responses can be observed following inhibition of individual phosphodiesterase isoenzymes in a given tissue providing the potential for the development of novel therapeutic agents.

Molecular biology of phosphodiesterase isoenzymes

Characterisation of mammallian phosphodiesterase activities using primary protein and cDNA sequence data has revealed the presence of at least five distinct gene families coding for cyclic nucleotide phosphodiesterase isoenzymes (for review see Beavo & Reifsnyder, 1990). Each of these families contains a minimum of two sub families. In addition many of the genes can potentially give rise to two or more alternately spliced mRNAs further increasing the potential diversity of these families of isoenzymes. At present there are at least 25 tentatively identified phosphodiesterase isoenzymes but this number is likely to increase. Members of each family share approximately 25% sequence identity with members of other families, with the majority of identity occurring in the catalytic domain. Sequences of members of individual sub families are highly homologous, showing greater than 70% identity. These families are summarised in Table 1.

One problem that readers of the phosphodiesterase literature must be aware of is the lack of a consistent nomenclature, particularly in the early literature. In many of the earlier papers investigators defined phosphodiesterase activities on the basis of column elution profiles which resulted in much confusion. This problem has been resolved by the adoption of a classification based upon the molecular biological characterisation of individual phosphodiesterase isoenzyme as summarised in Table 1, and it is this nomenclature which is used throughout this review.

Table 1 Characteristics of the major phosphodiesterase isoenzyme families

Family	*Affinity* *cAMP*	*cGMP*	*Features*	*Distribution*
I	low	high	Ca^{2+}/calmodulin dependent	CNS, liver, adipocytes
	high	high	as above	heart, kidney
II	high	high	cGMP stimulated	CNS, adrenal cortex, airway smooth muscle, heart, kidney
III	low	low	cGMP inhibited	heart, platelets, smooth muscle, adipocytes, CNS, kidney, liver
IV	low	—	no known regulator	smooth muscle, inflammatory cells, brain, liver heart, kidney
V	—	high/low		retina, smooth muscle, platelets

Tissue distribution of phosphodiesterase isoenzymes

Studies of the tissue distribution of individual isoenzymes have revealed that there are marked regional differences in the distribution of phosphodiesterase isoenzymes, with some isoenzymes being widespread but others having a limited distribution. For example, the type V_{B1} isoenzyme is only present in high concentration in the outer segments of retinal rod cells where it is involved in photoreceptor responses (Hurwitz *et al.*, 1985). Other isoenzymes are much more widely distributed: for example the type III isoenzyme is found as a physiologically important component of tissue phosphodiesterase activity in heart, platelets, some smooth muscle types, adipocytes and liver (Harris *et al.*, 1989; Silver *et al.*, 1988; Simpson *et al.*, 1988).

The analysis of tissue homogenates has revealed that many cell types contain several different phosphodiesterase activities. Canine airway smooth muscle, for example, contains at least four phosphodiesterase isoenzymes from families I, III, IV and V (Torphy, 1988). However, it is important to realise that the demonstration of the presence of a tissue phosphodiesterase isoenzyme *in vitro* using antisera or by examining cyclic nucleotide hydrolytic activity of tissue homogenates does not necessarily imply the presence of a physiologically important enzyme activity in whole cells. In airway smooth muscle for example, although 85% of cAMP hydrolytic activity is accounted for by the type 1c isoenzyme in tissue homogenates, with type III and IV isoenzymes accounting for 10% and 5% respectively, it is the type IV isoenzyme which appears to be most important for the control of cAMP levels and muscle tone in intact tissue (Hall *et al.*, 1989, 1990b; Harris *et al.*, 1989; Torphy & Undem, 1991). It seems highly likely that compartmentalisation of the different isoenzymes within the cell accounts for at least some of these apparent discrepancies.

Selective inhibitors of phosphodiesterase isoenzymes

The recognition of the existence of multiple phosphodiesterase isoenzymes has led to the development of a large number of inhibitors which demonstrate selectivity for individual isoenzymes, with obvious therapeutic potential for selective effects on individual tissues (Nicholson *et al.*, 1991). The initial characterisation of these agents depended on examining their effects on whole cell tissue cyclic nucleotide levels and their ability to inhibit cyclic nucleotide hydrolysis in homogenates. The development of new compounds will be eased by the availability of cDNA clones for many of the isoenzymes which can be expressed in systems with low constitutive background phosphodiesterase activity. Selective inhibitors are available for all of the families so far identified apart from the type II cGMP stimulated family, although by far the largest group of inhibitors are those of the type III family reflecting the interest of the pharmaceutical industry in identifying novel positive inotropic agents. Examples of some of the better characterised inhibitors available are shown in Table 2, although many other agents exist, some with selective actions on a single isoenzyme, and some with actions on more than one isoenzyme family.

Although the majority of the effects of these phosphodiesterase isoenzyme inhibitors at the tissue level are believed to be due to alteration in tissue cyclic nucleotide content, it should be remembered that some agents may have additional sites of action which need to be considered when trying to predict the physiological effects of a given drug. For example, the xanthine derivatives theophylline and 3-iso-butyl-1-methylxanthine (IBMX) which are extensively used *in vitro* as nonselective phosphodiesterase inhibitors are also potent antagonists at adenosine receptors (Green & Stanberry, 1977; Schwabe & Trost, 1980). Another agent used clinically is dipyridamole which as well as inhibiting the type V phosphodiesterase isoenzyme is a potent inhibitor of adenosine uptake (Marangos *et al.*, 1985). Xanthine derivatives may also exert effects by inhibiting Gi, the G protein involved in coupling of receptors which exert inhibitory effects upon adenylyl cyclase (Ramkumar & Stiles, 1988). Finally, phosphodiesterase inhibitors may modulate other cell responses mediated through phosphodiesterase like enzymes. For example, there is some evidence from airway smooth muscle studies that IBMX and the type IV isoenzyme inhibitor rolipram can directly inhibit phosphoinositidase C, the enzyme responsible for the production of the intracellular second messengers inositiol 1,4,5 trisphosphate and diacylglycerol in response to agonist stimulation (Hall *et al.*, 1990a).

Table 2 Isoenzyme selective phosphodiesterase inhibitors

Family	*Inhibitors*	*Potential uses*
I	Vinpocetine[1]	vascular smooth muscle relaxant
II	None known	
III	SK&F 94120, SK&F 94836, cilostamide milrinone, enoximone, ICI 118233[2–8]	bronchodilator, antithrombotic inotropic vasodilator
IV	rolipram, RO 20–1724[5,9,10]	bronchodilators, anti-inflammatories, antidepressants,
V	dipyridamole, zaprinast[11]	antithrombotic

References: 1 Ahn *et al.* (1989); 2 Elks & Mangianello (1984); 3 Kauffman *et al.* (1986, 1987); 4 Pyne *et al.* (1987); 5 Reeves *et al.* (1987); 6 Silver *et al.* (1987, 1988); 7 Torphy *et al.* (1988); 8 Gristwood *et al.* (1986); 9 Harris *et al.* (1989); 10 Torphy (1989); 11 Lugnier *et al.* (1986).

Biological effects of isoenzyme selective phosphodiesterase inhibitors

The effect of a given isoenzyme inhibitor in a specific tissue depends on two factors. The first is the direct effect of the inhibitor on a given isoenzyme to produce elevation of the relevant cyclic nucleotide level. The second factor is the feedback effect upon phosphodiesterase activity that may occur due to the effect of the change in cyclic nucleotide levels upon the activity of tissue phosphodiesterases. For example, an inhibitor of the cGMP specific isoenzyme would be expected to elevate cGMP levels in a tissue expressing an enzyme of the type V family. If that tissue also expresses significant activity of a type III cGMP inhibited isoenzyme, the elevation in cGMP content would be expected to indirectly elevate cAMP levels due to the inhibitory effect of increased cGMP levels on the activity of isoenzymes of the type III family (see Figure 1b).

The major potential clinical uses which have been proposed for isoenzyme selective phosphodiesterase inhibitors are as positive inotropic agents for use in heart failure and cardiac surgery, vasodilators, inhibitors of platelet aggregation, antidepressants, anti-inflammatories and bronchodilators (Nicholson *et al.*, 1991). At present the majority of information concerning the use of these agents is in animal models. However, a number of agents are currently undergoing evaluation for use in humans and the amount of published data will increase rapidly over the next few years.

Phosphodiesterase isoenzyme inhibitors as inotropic vasodilators

By far the largest literature on the potential clinical value of phosphodiesterase isoenzyme inhibitors concerns inhibitors of the type III isoenzyme family as positively inotropic vasodilators. Selective inhibitors of the type III family would be expected to relax vascular smooth muscle, inhibit platelet aggregation and have a positive inotropic action, all due to the increases in cAMP levels in the relevant tissues. The positive inotropic action of cAMP is believed to be mediated through an increase in intracellular calcium levels due to phosphorylation of a cardiac muscle calcium channel. Two phosphodiesterase type III isoenzyme inhibitors in current use are enoximone and milrinone. Short term administration of these agents to patients with congestive cardiac failure produces an increase in cardiac index and reduction in ventricular filling pressures, both of about 50% (Jaski *et al.*, 1985; Kereiakes *et al.*, 1984). Because of their action as smooth muscle relaxants both cause a fall in blood pressure and are relatively contraindicated in hypotensive patients. These agents play a potential role as short term intravenous therapies for normotensive patients with severe cardiac failure refractory to other treatment, and are also extensively used for inotropic support in patients undergoing cardiac surgery or heart-lung transplantation (Curfman, 1991). The relative contribution to their clinical action of their positive inotropic effects and their vasodilator effects remains unclear.

Longer term studies have been disappointing and recently a 2 year study of oral milrinone in the treatment of heart failure was prematurely terminated due to an excess mortality of 27% in the treatment group after an average follow up of 8 months (Packer *et al.*, 1991). The explanation for this is uncertain although it may be related to pro-arrhythmic effects secondary to increased cardiac automaticity due to elevated cAMP content in cardiac tissue. Other than arrhythmias adverse effects of these agents are relatively rare but include thrombocytopenia with both drugs and impaired liver function tests with enoxomine (Anderson *et al.*, 1987; Anon, 1991; Kereiakes *et al.*, 1984).

One possible therapeutic approach which has not been extensively studied is the combined effect of a type III phosphodiesterase isoenzyme inhibitor with an agent such as dobutamine which would stimulate the adenylyl cyclase activity through a cell surface receptor. The potential benefit of this approach would be that the equivalent effects on cAMP levels in the targeted cell type would be achieved with lower concentrations of each of the two drugs when used in combination. This would potentially reduce the incidence of side effects of these agents which are unrelated to their effect upon cyclic nucleotide levels, but would obviously not be expected to alter effects related directly to changes in tissue cyclic nucleotide content.

Antithrombotic agents

The main family of phosphodiesterase isoenzymes and in platelets is the type III cGMP inhibited form (Simpson *et al.*, 1988). In animal studies inhibitors of this isoenzyme family elevate cyclic AMP levels and inhibit platelet aggregation and similar effects may be observed in human isolated platelets (Murray *et al.*, 1990). At present, however, there are no data on the use of selective inhibitors of the type III family in preventing thrombotic events in humans.

One agent which has been studied in humans as a potential antithrombotic agent is dipyridamole which is a reasonably potent inhibitor of isoenzymes of the cGMP specific type V isoenzyme family. By elevating cGMP levels in platelets dipyridamole might also be expected to elevate platelet cAMP indirectly due to the inhibitory effect of intrinsic cGMP levels on this isoenzyme (see Figure 1b). Certainly, elevation of rabbit platelet cyclic AMP levels can be demonstrated by agents which regulate platelet cyclic GMP content (Maurice & Haslam, 1990). However, there is no convincing evidence that this effect of dipyridamole occurs *in vivo* or is of clinical value.

Antidepressant agents

One of the earliest observed effects of xanthines in humans was that of mild central nervous system (CNS) stimulation. Histochemical studies have revealed abundant amounts of the type II and type IV isoenzyme families in the CNS and in addition high concentrations of the 61kDa calcium calmodulin dependent isoenzyme (1_{A2}) in the dendritic fields of Purkinje and pyramidal

cells of cerebral cortex (Hansen & Beavo, 1982; Kincaid *et al.*, 1986–7, 1987). In animal models rolipram, an inhibitor of the type IV isoenzyme present in brain, produces characteristic behavioural alterations (Schneider *et al.*, 1986; Watchel, 1983; Watchel *et al.*, 1980). Human studies have shown antidepressant properties for rolipram in double-blind studies, with efficacy being similar to conventional tricyclic agents (Bennie *et al.*, 1988; Bobon *et al.*, 1988). However, whether the antidepressant effect of rolipram is related to its effect upon tissue cyclic nucleotide content, or to its ability to enhance noradrenaline availability by stimulating tyrosine hydroxylase activity and noradrenaline release (Kehr *et al.*, 1985) remains unclear.

Bronchodilators

The non-selective phosphodiesterase inhibitor theophylline has been extensively used in clinical practice as a bronchodilator but is not very potent and has a poor therapeutic index, its use often being limited by gastrointestinal or CNS side effects. Inhibitors of the type IV and to a lesser extent type III isoenzyme families have been demonstrated to have relaxant properties when tested on airway smooth muscle preparations from a range of animal species (Hall *et al.*, 1990b; Nicholson *et al.*, 1990; see also Torphy & Undem, 1991 for a comprehensive review; Torphy *et al.*, 1988). In anaesthetized guinea pigs both rolipram and SK&F 94836 (inhibitors of the type IV and type III isoenzyme families respectively) are able to inhibit bronchoconstriction induced by a range of agonists and are considerably more potent than theophylline (Harris *et al.*, 1989; Torphy *et al.*, 1988). There are less data on the use of selective phosphodiesterase inhibitors in normal human subjects or patients with asthma. The effects of short term administration of the type III inhibitor enoximone have been examined in patients with chronic airflow obstruction, with a small fall in pulmonary resistance and a concomitant rise in dynamic lung compliance being noted (Leeman *et al.*, 1987). One study with the mixed type III and type IV inhibitor AH 21–132 demonstrated a bronchodilator response in normal subjects (Forster & Rakshi, 1990). Further studies are clearly required to examine the potential role of such agents in the treatment of asthma. Inhibitors of the type IV isoenzyme are of particular interest as these agents also have potential anti-inflammatory properties which might be expected to be of value in the treatment of asthma (Giembycz, 1992) (see also below).

Phosphodiesterase inhibitors as potential anti-inflammatory agents

Elevation of tissue cAMP content inhibits activation of a wide range of inflammatory cells including mast cells, basophils, neutrophils, macrophages and probably eosinophils (Dent *et al.*, 1990; Nielson *et al.*, 1990; Plant *et al.*, 1983; Wright *et al.*, 1990). *In vitro* theophylline derivatives can be demonstrated to inhibit a range of inflammatory cells responses such as mast cell degranulation, although whether these effects occur *in vivo* remains to be established. The isoenzyme(s) important for control of tissue cyclic nucleotide levels in inflammatory cells *in vivo* are now being established, with the type IV isoenzyme appearing to play a major role in many cell types (Dent *et al.*, 1990; Rubicsek *et al.*, 1989). Preliminary studies in animals suggest that isoenzyme selective phosphodiesterase inhibitors may be potentially useful for inhibiting inflammatory responses.

Summary and future directions

The recognition of the existence of the large number of phosphodiesterase isoenzymes and their differential tissue localisation provides an opportunity to develop novel agents designed at manipulating physiological responses dependent on changes in cyclic nucleotide levels in a tissue specific manner. Such agents are likely to have important clinical roles as positive inotropic drugs, bronchodilators, antidepressants and anti-inflammatory drugs. Future research will be directed to develop more selective inhibitors of the individual isoenzymes, and to define in greater detail the tissue distribution of each family of enzymes. In addition the effects of combined therapy using an isoenzyme selective phosphodiesterase inhibitor in conjunction with an agonist designed to simulate adenylyl or guanylyl cyclase via a receptor dependent mechanism in a given tissue will provide the opportunity for developing an even greater degree of tissue selectivity. The availability of cDNA clones encoding for many of the known isoenzymes will provide additional information on tissue distribution and the expression of such clones in cell lines with low background phosphodiesterase activity will aid screening of novel compounds. The use of isoenzyme selective phosphodiesterase inhibitors will further our knowledge of the mechanisms underlying regulation of phosphodiesterase activity in different cell types, and may be of value in defining the pathophysiological abnormalities important in the pathogenesis of diseases in which abnormal cell signalling plays a role.

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