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REVIEW ARTICLE

Cyclic Nucleotide Phosphodiesterases: Properties, Activators, Inhibitors, Structure–Activity Relationships, and Possible Role in Drug Development

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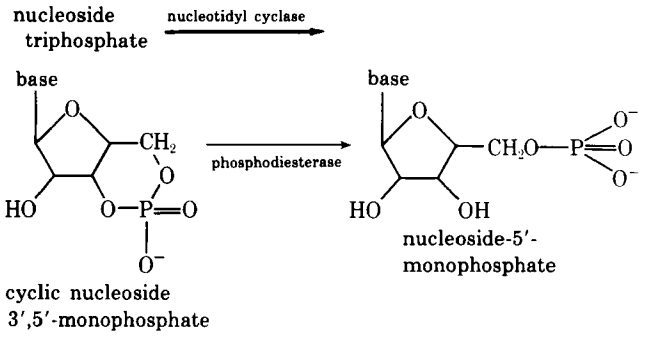
Keyphrases □ Phosphodiesterases, cyclic nucleotide—properties, activators, inhibitors, structure–activity relationships, possible role in drug development, review □ Cyclic nucleotide phosphodiesterases—properties, activators, inhibitors, structure–activity relationships, possible role in drug development, review □ Structure–activity relationships—cyclic nucleotide phosphodiesterases, review □ Nucleotide phosphodiesterases, cyclic—review of properties, possible role in drug development

CONTENTS

The central importance of cyclic nucleotide phosphodiesterases in drug development stems from the significant role that they must play in the control of intracellular cyclic nucleotide levels coupled with their apparent sensitivity to a large variety of chemical compounds (1, 2). Although two review articles on phosphodiesterases appeared recently (3, 4), aspects of these enzymes directly related to drug development have largely been ignored.

The purposes of this review are to cover these aspects in greater detail and to evaluate critically most major trends in this rapidly expanding area. However, due to the immense explosion in cyclic nucleotide research, an arbitrary selection of published reports had to be made. Many additional references may be found in the previous review articles (3, 4).

Phosphodiesterase catalyzes the apparently nonreversible (5) hydrolysis of the 3′-bond in the cyclic nu-



Scheme I

cleotides to produce their noncyclic 5′-monophosphate derivatives (Scheme I). Phosphodiesterase-catalyzed hydrolysis is the major physiological pathway for the termination of the intracellular effects of the cyclic nucleotides; there is not much support for other pathways for the degradation of cyclic AMP (6, 7).

Abnormal levels or forms of phosphodiesterase are associated with, and appear to contribute to, a number of disease states. Furthermore, various hormones may act by modulating phosphodiesterase activity in their target tissues. The enzyme also appears to be directly involved in the regulation of the chemotactic response of cellular slime molds (8–15) and may be implicated in receptors for taste (16–18), dopamine (19), and light (20) as well.

In a number of situations, the intracellular levels of the cyclic nucleotides and their related effects appear to be correlated closely with the magnitude of phosphodiesterase activity. The enzyme also translates into cellular events the actions of many drugs. It appears that phosphodiesterase represents more than just an off-switch for the cyclic nucleotide system and that it shares with the cyclases, the enzymes catalyzing the formation of the cyclic nucleotides, the important function of the delicate control of the intracellular cyclic nucleotide levels and consequently their myriad of important effects (21–23).

Although the cyclases, at least theoretically, present a suitable and possibly superior locus for the modulation of intracellular cyclic nucleotide levels, they presently occupy a position secondary to phosphodiesterases in efforts to develop new drugs that act *via* the cyclic nucleotide system. This stems primarily from the strict structural requirements for compounds that can directly affect cyclase activities. This may be due, at least in part, to the highly discriminative nature of these enzymes, since they appear to be primarily designed to distinguish only a limited number of the natural information-transferring molecules (hormones), depending upon the tissue in question. Only close structural relatives of those ligands can directly influence cyclase activity, thus severely limiting the range of chemical classes that can be developed for the purpose.

This limitation, however, may not include the compounds that can specifically block the stimulatory effects of the natural hormones on the cyclase enzymes. Here, a slightly wider selection of chemical entities may allow for the development of still better and possibly more selective blockers of hormonal effects on the cyclase enzymes (24). Conversely, phosphodiesterases, owing to their apparent sensitivity to a wider selection of chemical structures, present a more fertile field for development of new drugs with wider ranges of activity and selectivity. Since the cyclic nucleotides appear to be involved in nearly all facets of cellular activity in both healthy and disease states, there is no limit to the range of pharmacological effects that phosphodiesterase modifiers may produce, hence the central importance of phosphodiesterase for modern drug development.

This review is divided into two main sections. The first section describes the general aspects of the phosphodiesterase enzymes. The second section deals primarily with the activators and inhibitors of the enzyme and structure–activity relationships of the latter.

GENERAL CONSIDERATIONS

Phosphodiesterases occur widely in biological systems. They are present in nearly all mammalian tissues (3, 4, 25, 26), with the possible exceptions of red blood cells (27) and isolated rat adrenal cells (28). These two cellular systems provide excellent models for studying cyclic nucleotide synthesis uncomplicated by the interference of phosphodiesterases. These enzymes have been found also in bacteria (29–32), yeast (33), insects (34–37), higher plants (38–42), and several marine organisms (43, 44). In general, they are found wherever the cyclic nucleotides exist. Phosphodiesterase activity is detected early in embryonic development (20, 45–47), increases up to maturity, seems to decrease with age (48–50), and varies in activity with the cell cycle (51).

The central nervous system (CNS) seems to contain the highest levels of phosphodiesterase activity (27, 35, 52–55), a good portion of which appears to be particulate and may be associated with nerve endings, postsynaptic membranes (56–58), and synaptosomes (59–61). The enzyme from almost all other tissues is usually soluble, although there are definite indications that it may be at least partly particulate in intact cells and is solubilized during the isolation procedures. The particulate nature of phosphodiesterase and its possible plasma membrane localization are of the utmost importance to the design of drugs that can modulate the activity of the enzyme. The localization of the enzyme on the outside of cellular membranes is an obligatory assumption to provide necessary support for the effects on the enzyme activity in intact cells of some large peptide derivatives that cannot cross cellular membranes, *e.g.*, insulin–cepharose complexes that appear to act extracellularly (62, 63).

The general and kinetic properties of phosphodiesterases were recently reviewed (3, 4). Most studies in this area were carried out on the soluble enzymes, with few investigations of the particulate phosphodiesterases. Whether the soluble phosphodiesterases are identical structurally and/or functionally to the

particulate forms is not known. Thus, it may prove dangerous to extrapolate data obtained with the soluble enzymes to the particulate forms. This point cannot be overstressed, since the particulate forms may represent the more important *in vivo* forms of the enzyme and appear to respond differently to drugs.

Soluble phosphodiesterases are remarkably stable enzymes in the crude form, with an approximate molecular weight in the range of about 100,000–500,000 (3, 39, 64–66). They require a divalent cation for activity, preferably Mg^{+2}, and have a pH optimum between 7.5 and 8.5 (44, 67–71). Some unusual metal-ion requirements were reported for specific rat liver (72) and *Escherichia coli* enzymes (29). Some plant enzymes appear to have widely different properties and varying sensitivities to pH, ions, and inhibitors (30, 42, 73, 74). Plant phosphodiesterases also appear to exhibit unusual sensitivity to phosphate (73, 75) and can preferentially hydrolyze the 5′-bond of cyclic AMP to produce the 3′-monophosphate nucleoside, in obvious contrast to animal phosphodiesterases (42, 74, 76–78).

With mild techniques of isolation, phosphodiesterases seem to be associated with the cyclase complexes, appearing usually in the same fraction as the cyclases (56, 79–81). The association between phosphodiesterases and nucleotidyl cyclases can still be detected in partially purified preparations of the latter (82). It is possible that at least one phosphodiesterase form may represent a part of the cyclase complex. Phosphodiesterase contamination often presents a difficulty in cyclase assays, necessitating the addition of large quantities of theophylline to inhibit phosphodiesterase activity.

Kinetically, the enzyme presents a complex picture even when present in a reasonable state of purity. This observation led to the suggestion that the enzyme exhibits negative (79, 83) or even positive (84, 85) cooperative behavior. Such a conclusion, however, cannot be convincingly reached before greater purification of the enzyme is achieved.

Preparation, Purification, and Assay—The methods used for the preparation, purification, and assay of phosphodiesterase were recently reviewed (86, 87). These methods generally follow the leading efforts from the laboratory of Earl Sutherland, where the first methods to prepare, partially purify, and assay the enzyme were developed (52). The method selected for the assay of phosphodiesterase should be sensitive enough to measure the activity of the important, low K_m forms of the enzyme. Since the enzyme appears to exist in at least two forms, varying widely in K_m values, the substrate levels used in the assay do largely determine which form of the enzyme is being assayed.

There are several good methods for the assay of phosphodiesterase activity (88–91) (for review, see 3, 4, 86, 87, and 92). Although the colorimetric and titrimetric methods are simpler and more convenient to use than other methods, their low sensitivity makes them unsuitable for the assay of the high affinity, low K_m forms of the enzyme.

The most widely used methods at present are based on the use of labeled cyclic nucleotides as substrates. The noncyclic nucleotides formed by the action of the enzyme are converted into nucleosides by the addition of a nucleotidase (crude snake venom). These nucleosides are then separated and counted as a measure of phosphodiesterase activity (59, 93, 94). These methods are precise and sensitive enough to allow measurement of the enzyme activity at very low substrate concentrations similar to those encountered *in vivo*. They suffer, however, from the disadvantage of high blank values. Chromatography on more selective ion-exchange resins produces better separation of the labeled nucleosides, improving their recovery and allowing increased sensitivity and lower blank values (95, 96).

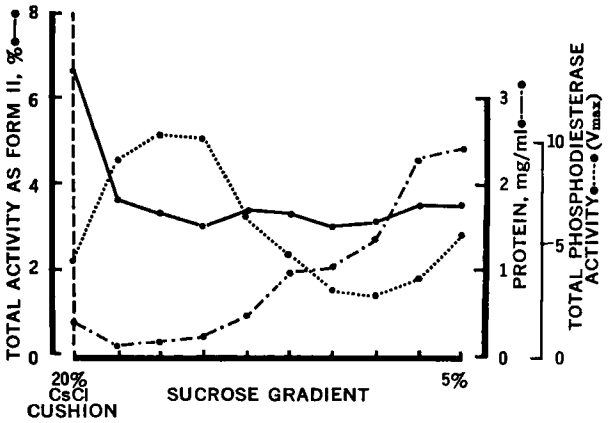


Figure 1—*Sucrose gradient fractionation of platelet whole homogenate. The fractions were collected by puncturing the bottom of the tube and were dialyzed against 0.05* M *potassium phosphate buffer, pH 7.5, and analyzed for protein and for phosphodiesterase activity. Phosphodiesterase assays were carried out at 15 substrate concentrations covering the range from* 3×10^{-8} *to* 8×10^{-3} M *to allow for the calculation of the relative proportion of activity attributable to either form of the enzyme. The percentage of the total activity present as Form II was calculated after the method of Cleland (138); (v) is expressed in μmoles hydrolyzed/0.5 ml/10 min at 30°.*

Histological assays have also been described for the study of phosphodiesterase (97), as have methods for the localization of the enzyme in tissues (98, 99).

Different Forms of Phosphodiesterase—Multiple forms of phosphodiesterase have been shown to exist under a wide variety of conditions and from a large number of sources (47, 55, 100–111). These forms appear to differ mainly in their substrate affinities (65, 92, 102, 112–119), heat and cation sensitivities (14, 101, 120–124), substrate specificities (59, 101, 125, 126), chromatographic and electrophoretic mobilities (102, 109), subcellular localization (20, 21, 59, 127), and, possibly, function (20, 128). The presence of multiple forms of the enzyme may explain its frequently anomalous kinetic behavior. It also allows for speculation as to the mode and manner in which these forms may be involved in the moment-to-moment control of phosphodiesterase activity and, consequently, the moment-to-moment control of the basal cyclic nucleotide levels.

The separation of the different forms of the enzyme has been accomplished by various methods (66, 102, 106, 129, 130). Sucrose gradient fractionation ap-

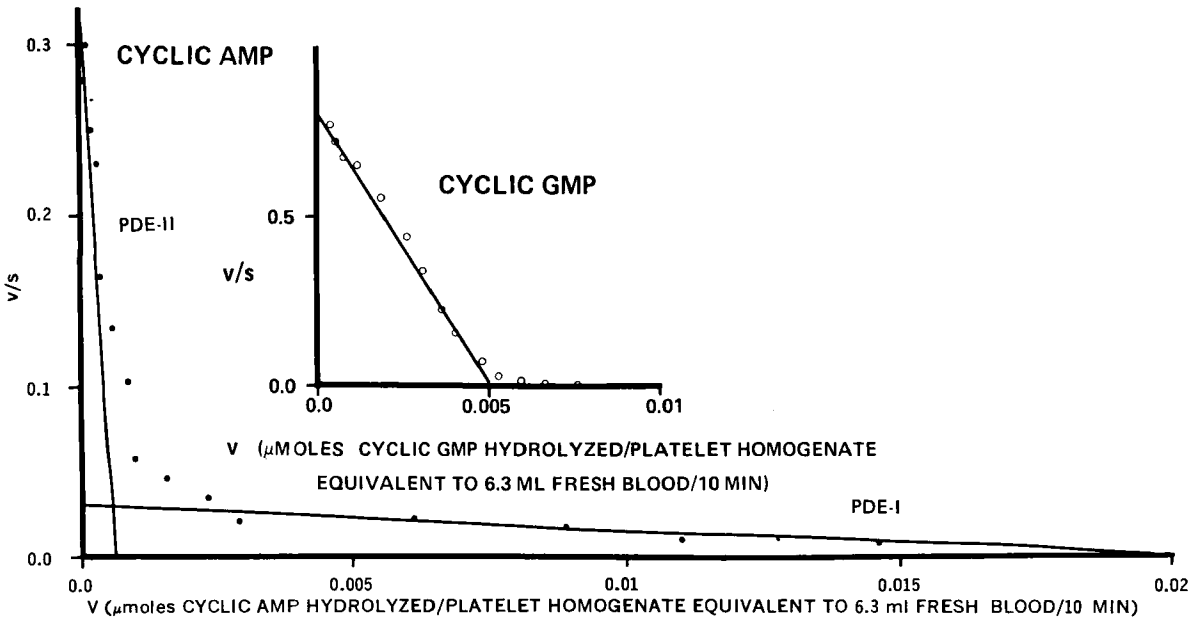


Figure 2—*Kinetics of hydrolysis of cyclic AMP and GMP by platelet whole homogenate. Enzyme assays were carried out on the peak tubes obtained by chromatography (DEAE) representing activities of Forms I and II. v = initial velocity (μmoles hydrolyzed/2 mg protein/min). s = substrate concentration (millimoles).*

pears to represent the simplest, most gentle, and convenient method for the crude separation of the different forms (79, 115). An example of the use of this method on platelet homogenates is shown in Fig. 1. Other methods may produce slightly "cleaner" fractions but generally do not improve the resolution significantly (115, 129).

Different Forms in Same Cell Line—Most investigators in this area agree that two main forms of the enzyme appear to exist in any homologous tissue preparation, multiple forms possibly being an artifact of the heterogeneity of the tissues used (119, 131). A case in point is the work of Uzunov and Weiss (106, 132) who found at least six forms of the enzyme in rat brain. However, when homologous preparations of rat astrocytoma cells were examined, only two forms were found (133).

The two forms differ in a number of aspects but primarily in their affinity for the substrates. Since there are two naturally occurring substrates for these enzymes, cyclic adenosine-3′,5′-monophosphate (cyclic AMP) and cyclic guanosine-3′,5′-monophosphate (cyclic GMP), two forms of the enzyme may exist for each substrate. Some tissues, however, appear to contain one form of the enzyme with a definite substrate specificity (134–137). For example, human blood platelets and rat brain seem to contain only one cyclic GMP phosphodiesterase (88, 115) (Fig. 2).

The resolution of the two forms is best achieved by kinetic analysis (81, 111, 129) at a wide range of substrate concentrations, particularly if coupled by physical separation. Table I summarizes the kinetic parameters of the enzyme forms present in a number of tissues obtained by kinetic analysis combined with chromatography[1] (129). The methods used are quite simple and can easily detect the presence of the two forms of the enzyme.

The presence of multiple forms of the enzymes stimulated speculation as to which, if either, of the two forms is more important in the control of the intracellular cyclic nucleotide levels. Since the intracellular levels of the cyclic nucleotides are in the micromolar range or lower (21, 139), the low K_m, high affinity phosphodiesterase (Form II) is favored for this role. In most tissues, Form II has a K_m value close to 10^{-6} M (4, 88), at least two orders of magnitude lower than that of the high K_m, low affinity Form I (3).

Only recently has the importance of Form II in the control of intracellular cyclic nucleotide levels in intact cells become more apparent (140). In tissues from a variety of hypertensive rats, the intracellular levels of cyclic nucleotides are inversely correlated with the Form II activity present (141–143). No such correlation existed with Form I activity, which was also measured in these same studies. The role, if any, played by Form I is open to question; studies utilizing high substrate concentrations that would preferably measure Form I activity are of questionable significance. At the present time, studies utilizing low substrate concentrations, in the micromolar range, that would preferentially reflect Form II activity appear to be more valuable because they may reflect the activity of the enzyme most probably involved in the *in vivo* control of intracellular cyclic nucleotide levels.

The significance of the presence of two main enzymes with two widely varying affinities for the hydrolysis of either cyclic nucleotide is not entirely clear. There are indications that the two forms are interconvertible. This makes it possible to speculate that the two dimorphs of phosphodiesterase could provide adequate means of controlling phosphodies-

[1] DEAE.

Table I—Cyclic AMP and Cyclic GMP Phosphodiesterases from a Number of Tissues[a]

Tissue	Cyclic AMP (cAMP)				Cyclic GMP (cGMP)				Total V_m cAMP / Total V_m cGMP
	K_m[b] Values, M		V_m[c]	% V_mII[d]	K_m[b] Values, M		V_m[c]	% V_mII[d]	
	I	II			I	II			
Human blood platelets[e]	6.1×10^{-4}	3.2×10^{-6}	0.025	4.2	1.6×10^{-5}		0.009	—	2.84
Monkey brain (sediment)	1.7×10^{-2}	1.7×10^{-5}	0.081	30.3	1.4×10^{-4}		0.019	—	4.20
Monkey brain (supernate)	3.0×10^{-4}		0.282	—	3.2×10^{-4}		0.478	—	0.59
Rabbit brain (sediment)	6.6×10^{-3}	5.6×10^{-5}	0.130	21.1	1.7×10^{-4}	3.7×10^{-6}	0.073	19.4	1.78
Rabbit brain (supernate)	1.9×10^{-3}	9.8×10^{-5}	0.390	8.7	6.6×10^{-5}	2.9×10^{-6}	0.122	7.8	3.20
Rabbit fundic mucosa	4.7×10^{-4}	1.2×10^{-6}	0.139	14.3	2.7×10^{-4}	5.4×10^{-7}	0.185	13.7	0.75
Rabbit gallbladder	4.0×10^{-3}	6.0×10^{-6}	0.059	14.6	4.0×10^{-3}	3.0×10^{-7}	0.028	12.6	2.10
Rabbit heart	1.7×10^{-2}	4.1×10^{-6}	0.104	13.0	2.0×10^{-4}	3.3×10^{-6}	0.023	21.4	4.47
Rabbit intestines	5.9×10^{-5}	1.0×10^{-6}	0.048	26.6	4.6×10^{-4}	3.4×10^{-6}	0.137	4.5	0.35
Rabbit kidneys	5.1×10^{-4}	5.5×10^{-6}	0.456	5.7	2.2×10^{-3}	2.0×10^{-5}	0.328	5.9	1.39
Rabbit liver	6.9×10^{-5}	4.0×10^{-7}	0.358	3.1	7.2×10^{-5}	4.0×10^{-6}	0.416	19.5	0.86
Rabbit pyloric mucosa	2.7×10^{-3}	8.7×10^{-6}	0.117	24.3	1.1×10^{-5}	1.2×10^{-6}	0.041	17.2	2.85

[a] Cyclic AMP and cyclic GMP phosphodiesterase activities were determined (102) in 4000 ×*g* supernates at 15 substrate concentrations (129). The incubation mixture contained ^{3}H-cyclic AMP or ^{3}H-cyclic GMP (2×10^{-11}–2×10^{-6} mole), 0.9 μmole $MgSO_4$, and 25 μmoles tromethamine buffer at pH 7.5 in a total volume of 0.5 ml. [b] Michaelis–Menten constant. [c] Enzyme maximal velocity = μmoles hydrolyzed/30 min at 30°/20 mg wet tissue. [d] Percent of total V_m as Form II, calculated as described by Cleland (138). [e] 10,000 ×*g* supernate of platelet homogenates was used. Platelets from 6.3 ml of blood were used per assay.

terase activity *in vivo*. Form I may represent a large store of the enzyme which is quickly convertible to the higher affinity Form II when the need arises. It may also serve the function of handling sudden surges of cyclic nucleotide concentrations (114). Form II would be the form normally concerned with the control of the *in vivo* cyclic nucleotide levels.

Most of the reported phosphodiesterase activity in different tissues was determined at relatively high substrate concentrations and would, under most experimental conditions, represent the form that is probably less important *in vivo* (Form I). This led to the somewhat misleading conclusion that phosphodiesterase activity may be 100 times the adenylyl cyclase activity in some tissues (52, 139, 144–147).

In a number of studies, the particulate nature of Form II was clearly stressed (12, 32, 79–81, 85, 98, 115, 148–156). Its presence on the external surface of cells has been well documented (156–158). This, in combination with its apparent calcium and hormonal sensitivity (140, 156, 159–161), supports the possible role of Form II as a hormonal receptor. The effects of calcium ions on phosphodiesterase activity from rabbit liver are shown in Fig. 3. As can be seen, no effect of calcium ions on Form I activity is observed in contrast to the large stimulatory effect on Form II activity. There are some reports, however, of an inhibitory effect of calcium ions on phosphodiesterase activity in certain tissues (119, 162).

The presence of soluble and particulate forms of phosphodiesterase should lead to a reconsideration of the role of the enzyme in the control of cyclic nucleotide levels. The occurrence of soluble and particulate forms of the same enzyme differing in kinetic parameters is not unknown. For example, malate dehydrogenase occurs in both soluble and particulate forms in *Neurospora* (163). It could be hypothesized that the active form of phosphodiesterase is membrane bound and in equilibrium with a soluble, low affinity form. Association or dissociation from the membranes would represent activation or inactivation of the enzymes, respectively. The membranous site for the attachment of the enzyme could then represent a receptor site for the action of ligands affecting intracellular cyclic nucleotide levels by activating or inactivating phosphodiesterase. More gentle methods for the preparation and assay of phosphodiesterase should be used to avoid the transfer of activity to the soluble fraction and the consequent obliteration of induced effects. Some recent studies with insulin support this mechanism (156, 164, 165).

The structural relationships between the two main forms of the enzyme are far from clear. Studies on the molecular structure of the two forms must await further purification of the different phosphodiesterases. Furthermore, it is not completely settled whether cyclic AMP hydrolysis is carried out by the same set of enzymes that also catalyzes the hydrolysis of other cyclic nucleotides. There are some indications in certain tissues that one of the two forms of the enzyme may be an artifact of the isolation procedures employed (126, 149, 166).

The importance of the two forms of the enzyme is underlined by the apparent absence of one form in human glial cell tumor (167) and in transformed cells (168).

From the point of view of drug development, the sensitivities of the two forms of phosphodiesterases to activators or inhibitors appear to be different (120, 135, 169–173). This is a very important point, since it is doubtful that agents acting on only the high K_m forms of the enzymes would be useful. It appears that screening compounds for possible activity against phosphodiesterases should stress the importance of the low K_m forms, since these are likely to be the more critical. An impressive example of drug selectiv-

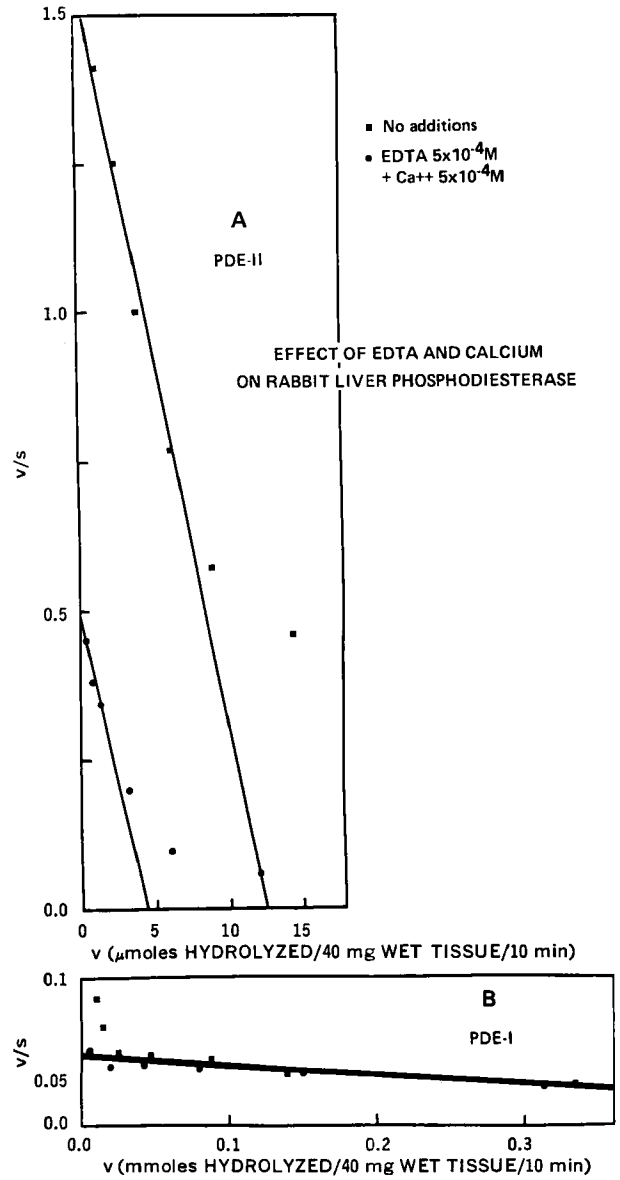


Figure 3—*Eadie plots for the effects of Ca^{+2} on the activity of cyclic AMP phosphodiesterase in rabbit liver. A represents the results obtained at low substrate concentrations (Form II) and B represents the results obtained at high substrate levels (Form I).*

ity for the low K_m phosphodiesterase is that of adenosine on the enzymes from human blood platelets. As can be seen in Fig. 4, adenosine inhibited the Form II activity with no effect on the Form I activity present in the platelet homogenates (174).

Different Forms in Different Cell Lines—One prime requirement in drug development is the tissue specificity of the drug in question. The differences between the phosphodiesterases from different tissues appear to be large enough to support the use of this enzyme as a target for the development of new drugs (55, 122, 175–180). There appear to be both structural and kinetic differences between the phosphodiesterases isolated from different tissues (121). These differences are reflected in the differential sensitivities to drugs of the enzymes from different sources (122, 157, 172, 174, 175, 177, 178, 181–193)

Table II—Inhibitor Constants (K_i) of Theophylline on Form II Activity from Guinea Pig Lung and Stomach Using Cyclic AMP or Cyclic GMP as Substrates[a]

Substrate	K_i[b]	
	Lung	Stomach
Cyclic AMP	4×10^{-4}	4×10^{-4}
Cyclic GMP	2×10^{-3}	1.3×10^{-4}

[a] Assayed according to Thompson and Appleman (102) at substrate concentrations of 1 and 2 μM and six drug concentrations. [b] Molar, determined as described in Ref. 211.

and even from neighboring areas of the brain (194). Therefore, testing the responses of phosphodiesterases from different tissues may provide preliminary clues to the possible tissue selectivity of the drug in question.

Cyclic GMP versus *Cyclic AMP Phosphodiesterases*—Although several pyrimidine and purine cyclic nucleotides are hydrolyzed by different tissue preparations (3, 55, 72, 117, 125, 195, 196), the significance of this observation is unclear since only two cyclic nucleotides appear to occur in nature, cyclic AMP and cyclic GMP, both of which are purines. The hydrolysis of the two naturally occurring cyclic nucleotides was thought at one time to be catalyzed by the same set of enzymes (197). Present evidence, however, points to the possible existence of two different sets of enzymes with widely different affinities for the two naturally occurring cyclic nucleotides (85, 126, 130, 160, 198, 199).

Recent studies seem to emphasize the differences between cyclic AMP and cyclic GMP phosphodiesterases in subcellular localization, cation requirements (166), and response to drugs (177, 181, 200–202). Since the two naturally occurring cyclic nucleotides appear to mediate different, if not antagonistic, events in many systems (203–210), it is of paramount importance to recognize the functionally different natures of cyclic AMP and cyclic GMP phosphodiesterases. The selective effects of drugs on these enzymes will greatly determine their biological effects on the tissues examined. This is exemplified by the effects of theophylline on phosphodiesterase preparations from the guinea pig stomach and lung, using each cyclic nucleodtide as a substrate (Table II). Theophylline shows greater selectivity for cyclic AMP phosphodiesterase than for cyclic GMP phosphodiesterase in the lung than in the stomach. This may at least partially explain the effects of theophylline in the two tissues.

Not only do the selective effects of a drug on cyclic AMP and cyclic GMP phosphodiesterases vary from tissue to tissue but also the effects in the same tissue vary with different drugs (177, 181, 212–215). The effects of the three phosphodiesterase inhibitors, theophylline, papaverine, and glycyrrhetinic acid, on the enzymes from the fundic mucosa of three species (Table III) serve to illustrate the latter point. Greater selectivity for cyclic AMP phosphodiesterase is correlated with greater inhibitory effect on gastric acid secretion. The three compounds have profoundly different selectivities for the specific phosphodiesteras-

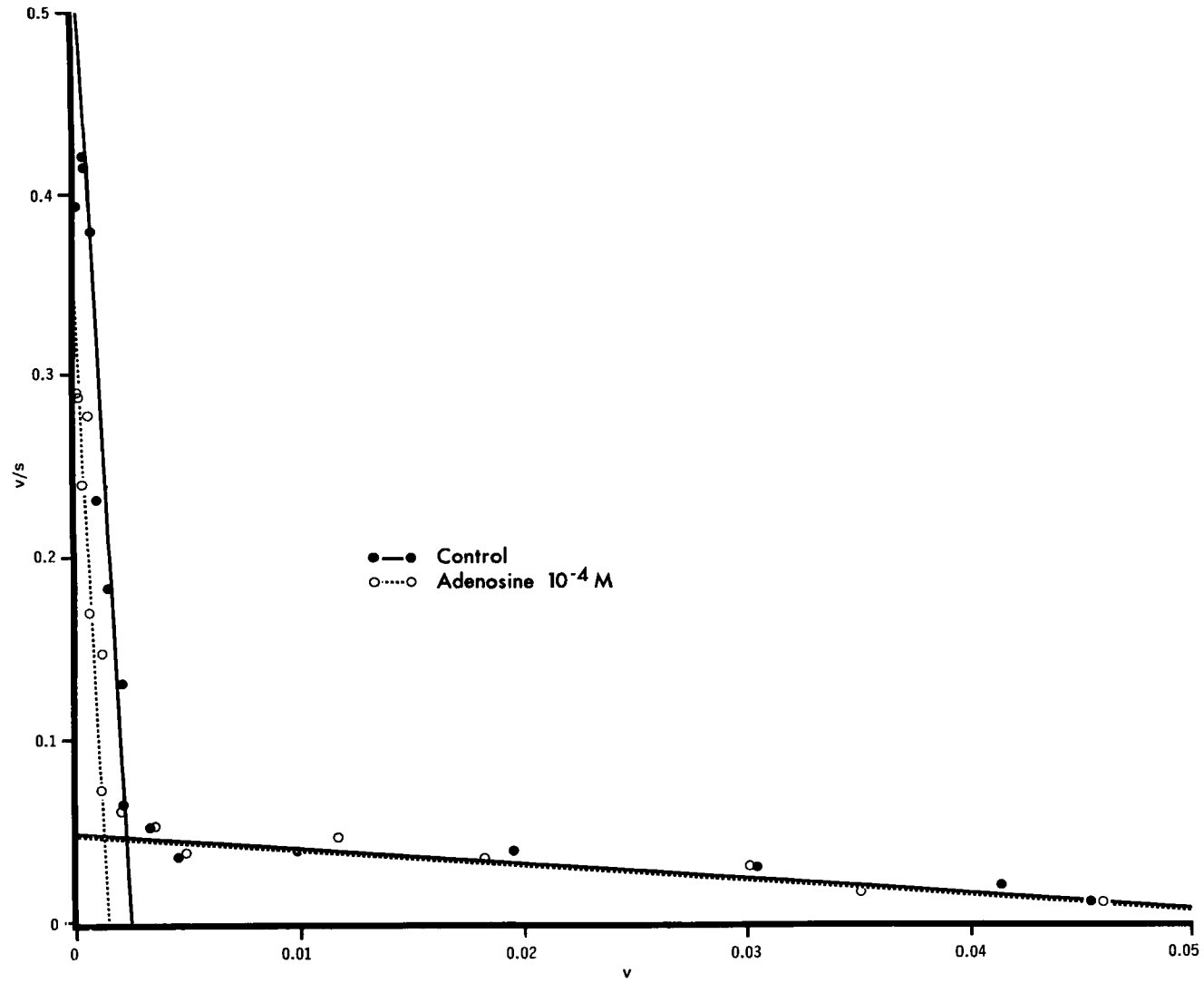


Figure 4—*Eadie plots for the effects of adenosine (10^{-4} M) on phosphodiesterase activity from human blood platelet membrane-supplemented whole homogenates. Note the selective inhibition by adenosine on Form II while no effect is evident on Form I (174).*

es and produce different effects on the output of the system (204, 212).

The realization of the possible importance of substrate selectivity in the use of phosphodiesterases as targets for the development of new drugs is quite recent. Little screening work has been reported where different substrates for the enzymes were used. This matter will probably attract more and more attention.

One important point to stress in this respect is the ability of each naturally occurring cyclic nucleotide to influence the rate of hydrolysis of the other (112, 130, 140, 167, 186, 188, 214–218). Thus, compounds selectively affecting the specific phosphodiesterase for either nucleotide will have indirect effects on the activity of the other. This is complicated by the ability of either cyclic nucleotide to both stimulate and inhibit the rate of hydrolysis of the other, depending on its concentration. This may provide an explanation for the nonconforming effects of exogenously applied cyclic nucleotides in some systems, as in the stimulatory effects of cyclic AMP on the contraction of some smooth muscles (219). It also may explain the antagonistic effects of some drugs on the levels of the two cyclic nucleotides (218).

A model enzyme system composed of specific subunits with affinity for cyclic AMP and others for cyclic GMP has been proposed (4, 215). It is quite apparent that cyclic GMP may be involved as an *in vivo* regulator of phosphodiesterase activity (130, 216). The greater sensitivity of the cyclic GMP phosphodiesterase to calcium ions (101) strongly emphasizes this possibly important role.

In general, there is greater affinity of phosphodiesterases for cyclic GMP (136, 141, 214, 215) than for cyclic AMP. This may be related to the generally lower *in vivo* concentrations of cyclic GMP.

The previously unsuspected selectivity of phosphodiesterase inhibitors for either cyclic AMP or cyclic GMP enzymes may provide an explanation for the anomalous behavior of some of these compounds *in vivo*. For example, papaverine, although far more potent than theophylline as an inhibitor of cyclic AMP phosphodiesterase, is less potent than expected in elevating plasma free fatty acids and blood glucose (220).

Table III—Selectivity of Theophylline, Papaverine Hydrochloride, and Glycyrrhetinic Acid for Low K_m Cyclic AMP and Cyclic GMP Phosphodiesterase from the Fundic Mucosa of Three Species (210) and Their Effects on Acid Secretion[a]

Substrate	Dog			Rat			Rabbit			Effect on Gastric Acid Secretion
	Substrate			Substrate			Substrate			
	Cyclic AMP	Cyclic GMP	G/A[b]	Cyclic AMP	Cyclic GMP	G/A[b]	Cyclic AMP	Cyclic GMP	G/A[b]	
	Inhibitor Constants (K_i), Molar[c]									
Theophylline	50	26	0.5	38	17	0.5	44	17	0.4	Potentiates
Papaverine	2	2.6	1.3	2.5	2.8	1.1	2.5	3	1.2	No effect
Glycyrrhetinic acid	7	13	1.9	4.0	10	2.5	7.5	15	2.0	Inhibits

[a] Enzyme activity was determined by a radioactive assay technique (102). [b] G/A = K_i(cyclic GMP as substrate)M/K_i(cyclic AMP as substrate)M; higher numbers indicate greater selectivity for cyclic AMP phosphodiesterase. [c] $\times 10^{-5}$. Determined by the method in Ref. 211 at two substrate (0.8 and 1×10^{-6} M cyclic AMP and 1 and 2×10^{-6} M cyclic GMP) and six drug concentrations bracketing the K_i value.

Interconvertibility of Phosphodiesterases—As mentioned earlier, there is some support for the possible interconvertibility of the two main forms of phosphodiesterase (79, 129, 198, 221). A measure of the change in the proportion of either form of the enzyme present in a particular experimental situation can provide an estimate of the effects of different agents on the interconvertibility of the two forms. The results with some prostaglandins, epinephrine, and aspirin are shown in Table IV. Although the effects are small, they point to a possible mechanism for the control of phosphodiesterase activity. Final confirmation of this mechanism must await further purification of the enzymes.

Two apparently interconvertible forms of the enzyme were isolated from cellular slime molds (157). The importance of sulfhydryl groups for the interconversion is apparent in this system, as evidenced by the effects of dithiothreitol. However, the latter compound has little effect in mammalian systems (222).

Natural Intracellular Activators and Inhibitors—Protein activators for phosphodiesterase were isolated from a number of tissues (84, 107, 159, 161, 222–229). These activators appear to mediate the stimulatory effects of calcium ions on the enzyme activity from the brain and may prove quite important as natural regulators of the enzyme activity in that tissue. Natural protein inhibitors for phosphodiesterase have also been described (228, 230, 231) and in some systems may have other functions (232).

A heat-stable, nondialyzable phosphodiesterase inhibitor was also isolated from soybeans (73) and slime mold (233), and an activator was isolated from the latter (100). The possible physiological role, if any, of these natural activators or inhibitors in the control of the enzyme activity has not been established. Whether interference with the effects of these activators or inhibitors can present a mechanism for possible drug effects on phosphodiesterase remains to be seen. Also it is not clear whether these activators or inhibitors are collectively similar or different (234).

ATP, pyrophosphate (235), adenosine (71, 174), citrate, isocitrate, pyruvate, oxalate, malate, and tartrate (67) were reported to inhibit the enzyme activity. The possible role of any or all of these intermediates in the control of phosphodiesterase activity *in vivo* remains to be determined.

Cyclic nucleotides appear to activate (236) and to control the rate of synthesis of phosphodiesterase (168, 237–239). Evidence primarily from tissue cultures and bacteria strongly support a role for cyclic AMP in the control of phosphodiesterase synthesis, probably *via* cyclic nucleotide-dependent protein kinase (240).

Effects of Hormones on Phosphodiesterase—Several hormones, including insulin (79, 151, 154, 156, 164, 165, 241, 242) (for review, see 3 and 4), ACTH (243), growth hormone (79), cholecystokinin and gastrin (129, 244–246), aldosterone (96), histamine (32, 247–249), epinephrine (133, 250–253), prostaglandins (110), corticoids (117, 154, 254–256), thyroxine (117, 254, 257), and plant cytokinesins (258) have been reported to affect phosphodiesterase activities in their target tissues. In most of these situations, the forms of the enzyme affected consistently appear to be the low K_m, high affinity forms. These effects tend to bolster the possible hormonal receptor function of phosphodiesterase, particularly its low K_m forms.

In none of these situations, however, has the importance of phosphodiesterase effects been unequivocally established in the mechanisms of action of these hormones. It is not clear whether the effects observed on phosphodiesterase are primary or secondary effects of the hormones studied, since similar effects are produced by cyclic GMP (112, 259) or its hydrolysis product (222), high salt solutions (260), and, possibly, lipids (261). It is not clear whether these hor-

Table IV—Effects of Compounds on the Percent Form II Activity in Membrane-Supplemented Whole Homogenate of Human Blood Platelets[a]

Drug	Drug Concentration	Form II, %
Control	—	7.1
Epinephrine	10^{-5} M	14.3
Aspirin	10^{-5} M	3.8
PGE_1	1.4×10^{-6} M	2.0
$PGF_{2\alpha}$	2×10^{-4} M	5.5
PGE_2	2×10^{-4} M	7.0

[a] Phosphodiesterase activity was determined as described for Table I. The percent Form II was calculated *via* the use of a computer program developed after the treatments of Cleland (138). The assays were done as described for Table I.

monal effects could lend themselves to modulation *via* the use of drugs. However, these effects remain potentially important.

Diseases Associated with Altered Phosphodiesterase Levels—In some situations, as in hypertension (141–143), hepatomas (262), diabetes (263), and obesity (263), increased activity of the low K_m, high affinity phosphodiesterase may be an important part of the etiology of the disease state, although this may not always be the case (20, 264). In these conditions, the use of appropriate phosphodiesterase inhibitors could alleviate the deficiency in intracellular cyclic AMP by the most desired mechanism for any drug, namely, by correcting the basic biological defect. On the other hand, there is also evidence for decreased phosphodiesterase activity in diabetes (137, 165, 265, 266) and hepatomas (267).

In some diseases, the lowered cyclic AMP levels are associated with decreased sensitivity of adenylyl cyclase to stimulation. The most feasible approach to correct the cyclic AMP deficiency under these circumstances is the inhibition of phosphodiesterase. Examples of these conditions are hypertension (141–143, 268), asthma (269), psoriasis (270), and, possibly, cancer (271, 272).

Phosphodiesterase Inhibition, Cyclic Nucleotide Accumulation, and Pharmacological Effects—Many compounds thought to act *via* phosphodiesterase inhibition and the consequent cyclic nucleotide accumulation are "uncomfortably" weak inhibitors of the enzyme, with K_i values often in the millimolar range. In most of these situations, it is doubtful that the compound could attain the high concentrations needed *in vivo*. Often, insignificant or only marginal *in vivo* phosphodiesterase inhibition can be demonstrated; yet some accumulation of the cyclic nucleotides may occur (93, 273, 274). The latter may become evident only if accompanied with cyclase stimulation.

A popular example of this is theophylline, a weak phosphodiesterase inhibitor with a K_i value of about 1 mmole or greater in most systems. It is doubtful that it reaches this high concentration *in vivo* when administered in the usual doses. No *in vivo* phosphodiesterase inhibition by theophylline can be demonstrated in most systems, yet theophylline by itself sometimes produces small elevations of cyclic nucleotide tissue levels when administered *in vivo*. It is widely believed, however, that phosphodiesterase inhibition and the consequent cyclic nucleotide elevations represent the main mechanism for its effects. The potentiation of hormonal effects by theophylline is one main criterion required to determine the possible mediator role of cyclic nucleotides in the mechanism of action of the hormones (275, 276).

Most phosphodiesterase inhibitors, including the more active ones, cause only small accumulations of cyclic nucleotides when compared with the amounts generally produced *via* adenylyl or guanylyl cyclase stimulation. In many systems, a small cyclic nucleotide elevation is all that is needed for maximal stimulation of the physiological response (274). It is not clear what role, if any, the extremely high cyclic nucleotide levels, which are sometimes produced *via* cyclase stimulation, play in the physiological effect, since, in most instances, the effect is usually essentially complete at much lower intracellular levels of the cyclic nucleotides (277).

It should always be remembered that phosphodiesterase inhibition may represent only one of several effects that phosphodiesterase inhibitors can produce. These other effects may be totally independent of the cyclic nucleotide system and may, in certain instances, be responsible for the major portion of the activity of these agents. The other effects of the methylxanthines are clearly summarized (213, 274, 278) and could serve as an example of other responses that can be produced by phosphodiesterase inhibitors, apparently unrelated to their effects on cyclic nucleotide metabolism.

Other properties of drugs may play a role in determining their effects on cellular biochemical events, *e.g.*, the ability of the drug to reach the enzyme. Membrane permeability may have been important in the case of a xanthine derivative, where striking differences between its *ex vivo* and *in vitro* potency on phosphodiesterase were demonstrated (191).

ACTIVATORS AND INHIBITORS: STRUCTURE–ACTIVITY RELATIONSHIPS

In this section, the structure–activity relationships of compounds that have been reported to affect phosphodiesterase activity will be discussed. Since most of the reported studies utilized cyclic AMP as the substrate, the activities measured were those of cyclic AMP phosphodiesterases. Not every reported compound is listed, but most entities that fall into common structural (preferably) or utility classes are included. Several miscellaneous structures may be found in the references cited. The two main objectives were: *(a)* to determine the structural features or groups that are common among compounds showing activity on phosphodiesterases, and *(b)* to determine the extent to which variation of structural moieties has allowed improved potency and/or selectivity.

Discussion of this section is complicated by the heterogeneity of the enzyme preparations used, the wide variations in the assay conditions, the different levels of substrate employed, making it difficult to determine what form of the enzyme was assayed, and the different methods used in determining the potency of the agents employed.

To present a uniform discussion of the results, it was essential to unify the method of presentation. Those studies employing a substrate concentration of 10^{-5} M or less were assumed to measure Form II (low K_m) activity, while those at higher substrate levels were assumed to estimate Form I (high K_m) activity. The enzyme source was mentioned whenever practical.

For quantification of the results, five terms were employed in the following order of preference whenever possible:

a. Inhibitor constant (K_i), in micromoles per liter.

b. Inhibitor concentration$_{50}$ (I_{50}), in micromoles per liter.

c. Percent inhibition (100 minus percent of control).

d. Potency relative to theophylline (based on K_i or I_{50} values) if the latter was employed as a standard in the same system.

e. Potency relative to papaverine (based on K_i or I_{50} values) if the latter was employed as a standard in the same system.

In the absence of either K_i or I_{50} values, no accurate relative potency could be determined. Therefore, the following four additional terms were used to give a rough estimate of potency whenever possible:

f. The compound produced greater percent inhibition than theophylline in the same system.

g. The compound produced less percent inhibition than theophylline in the same system.

h. The compound produced greater percent inhibition than papaverine in the same system.

i. The compound produced less percent inhibition than papaverine in the same system.

These letters were used as superscripts throughout the tables to qualify the potency values reported. In some instances, more than one superscript was used to provide more adequate information on the activity of the compound in question. Although I_{50} and K_i values are often similar, they are not necessarily identical, particularly with competitive inhibitors (279, 280), and were listed separately.

Activators—Compounds reported to stimulate phosphodiesterase are not as numerous as those that appeared to inhibit it. Since the most common abnormalities in cyclic nucleotide metabolism usually result in lower levels of these important mediators, inhibition of their degradation is the more desirable therapeutic target. Nevertheless, activators are of interest in diseases associated with elevated cyclic nucleotide levels, *e.g.*, cyclic AMP in diabetes (263, 281, 282), cholera (283), alcoholism (284), and mania (285) and cyclic GMP in psoriasis (286) and hypertension (287).

Several compounds of widely different structures were reported to activate phosphodiesterase from a variety of tissues (Table V). These activators include nitroglycerin and papaverine, which also inhibit the insoluble enzyme in rabbit colon homogenate, and hydralazine, which inhibits the enzyme activity in bovine mesenteric artery (289). However, the effects of these compounds on phosphodiesterase could be indirect. In most systems, crude preparations of the enzyme were used and effects on cyclic GMP synthesis and/or metabolism cannot be ruled out. Cyclic GMP has repeatedly been shown to have significant effects on cyclic AMP phosphodiesterase activity (66, 85, 111, 112, 188, 214, 222, 259).

Imidazole (70, 111, 129, 230, 288, 290, 291) is most frequently quoted as a stimulator of phosphodiesterase, although high concentrations are generally needed. It has been shown to have no effect on some phosphodiesterase systems (74, 215), to have negligible effects on rat brain phosphodiesterase (288), and possibly to even inhibit at concentrations of 10–50 m*M* (215). Its stimulating effects appear to depend on the presence of Mn^{+2} (70).

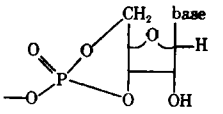
Table V—Activators of Phosphodiesterase

Name	Enzyme Source	Reference
Acetamide	*D. discoideum*	10
L-Arginine	Mouse brain	186
L-Aspartic acid	Mouse brain	186
Catecholamines	Rat adrenal	251
Cyclic GMP	Rat thymic lymphocytes	112
	Rat liver	85
Cysteine	Alaska pea seedlings	74
Dithiothreitol	Alaska pea seedlings	74
Gastrin and related peptides	Rabbit tissues (misc.)	129
Glutathione	*D. discoideum*	12
Glycine	Mouse brain	186
5′-GMP	Beef heart	222
Histamine analogs and metabolites	Rat brain	288
	Bullfrog gastric mucosa	217
Hydralazine	Bovine artery	289
Imidazole	Mouse pancreatic islet	111
	Guinea pig heart	290
	Alaska pea seedlings	74
	Rat brain	288
	Bovine brain	70
	Rat heart	291
Insulin	Rat liver	151
Lipids	Rat brain	261
Nicotine	Bovine tongue epithelium	17
Nicotinic acid	Adipose tissue	292
Nitroglycerin	Rabbit colon cytoplasm	289
Papaverine	Rabbit colon cytoplasm	289
	Cat cerebral cortex	191
Phenylalanine	Bovine tongue epithelium	17
Propylthiouracil	*D. discoideum*	10
Protein activators	Rat brain	227
	Bovine brain	234
	Bovine heart	84
	Porcine brain	84
	Human brain	84
	Several rat tissues	234
	Bovine brain cerebra	223
	Rat cerebellum	106
	Hamster islet cell tumor	107
Quinine	Bovine tongue epithelium	17
Strychnine	Bovine tongue epithelium	17
Thioacetamide	*D. discoideum*	10
Thiourea	*D. discoideum*	10
Urea	Bovine tongue epithelium	17
Others	Adipose tissue	292
	Rat mast cells	293

A number of sulfhydryl compounds significantly stimulate phosphodiesterase from a variety of sources. This fact led to the speculation that free sulfhydryl groups are essential for phosphodiesterase activity (3, 189).

In view of the possible interconvertibility of the two forms of phosphodiesterase, as discussed previously, the substrate concentrations used could significantly influence the effect produced by the compound studied. A compound that stimulates the hydrolysis of low concentrations of cyclic AMP could act *via* a shift of activity from Form I to Form II. Decreased phosphodiesterase activity at high substrate concentrations would be expected; thus, the same compound could be described as both a stimulant and an inhibitor, depending on the substrate concentration used. Examples of this duality include cholecystokinin, epinephrine, and cyclic GMP on cyclic AMP hydrolysis. This shift may underlie the obvious controversy concerning the effects of bitter taste

Table VI—Cyclic Nucleotides of Natural Bases



Name	Base	Potency*	Enzyme Source	Inhibitor Concentration, moles/liter	Phosphodiesterase Form	Reference
Cyclic GMP	Guanine	47[c]	Mouse pancreatic islets	7.9×10^{-6}	II	111
		22[c]	Pea seedlings	1×10^{-4}	II	74
		100[a]	Rat liver	—	II	85
		18[a]	Frog erythrocytes	—	I	214
		40[a]	Rat thymic lymphocytes	1×10^{-4}	II	112
		1.0[b]	Cat heart	—	II	188
		700[b]	Rat brain	—	II	188
		120[a]	Beef heart	1×10^{-3}	I	222
Cyclic IMP	Hypoxanthine	1.8[a], 3.8[b], 34.2[d]	Beef heart	—	II	185
		24[b], 9.6[d]	Beef brain	—	II	185
		60[a], 80[b], 3.1[d]	Rabbit lung	—	II	185
		22[b], 7.3[d]	Rabbit kidney	—	II	185
		24[a]	Frog erythrocytes	5×10^{-5}	I	66
		2.0[b]	Cat heart	—	II	188
		940[b]	Rat brain	—	II	188
		210[a]	Beef heart	1×10^{-3}	I	222
Cyclic CMP	Cytosine	19[c]	Pea seedlings	1×10^{-4}	II	74
		Inactive	Cat heart	—	II	188
		Inactive	Rat brain	—	II	188
Cyclic UMP	Uracil	Inactive	Rat brain	—	II	188
		1600[b]	Cat heart	—	II	188
Cyclic TMP	Thymine	2000[b]	Cat heart	—	II	188
		Inactive	Rat brain	—	II	188

* Superscript italic letters refer to the terms discussed under *Activators and Inhibitors: Structure–Activity Relationships.*

principles on phosphodiesterase activity in the tongue epithelium (17, 18).

Inhibitors—*Cyclic Nucleotide Derivatives*—Although only two cyclic nucleotides are known to exist in nature, their universal and important effects have stimulated the synthesis of a large variety of cyclic phosphate derivatives of both natural and synthetic bases. Two basic biological targets are easily discernible behind this tremendous synthetic effort: *(a)* to produce compounds that could mimic the effects of the naturally occurring messengers on their target enzymes (cyclic nucleotide-dependent protein kinases) while resisting phosphodiesterase hydrolysis, and *(b)* to inhibit phosphodiesterase activity and thus raise the intracellular concentrations of the two naturally occurring cyclic nucleotides. Although other biological targets should also be considered (1), only these two have been consistently pursued.

Due to the extremely large number of compounds that have been studied for their effects on phosphodiesterase, only selected examples of inhibitor action will be presented here. Detailed lists of these compounds and their activities can be found elsewhere (177, 183, 294–299).

From a theoretical standpoint, the ability of cyclic nucleotide derivatives to act as substrates for phosphodiesterase should reflect their ability to act as inhibitors of the enzyme, since they will compete for the same site on the enzyme (60). However, this relationship does not seem to hold true in a number of situations. On the other hand, structural requirements for good phosphodiesterase inhibition in cyclic nucleotide derivatives appear to be generally similar to those needed to produce good activation of the cyclic nucleotide protein kinases (183). The latter enzymes are generally believed to translate changes in cyclic nucleotide levels into biochemical events by phosphorylating key enzymes and thus activating or inhibiting them (300).

1. Cyclic nucleotides of natural bases: Perhaps the simplest group of cyclic AMP analogs includes those compounds in which the base moiety is not adenine but another biologically occurring purine or pyrimidine derivative (Table VI). The less familiar cyclic nucleotides and their respective bases are the following: cyclic GMP, guanine; cyclic inosine monophosphate (cyclic IMP), hypoxanthine; cyclic cytidine monophosphate (cyclic CMP), cytosine; cyclic uridine monophosphate (cyclic UMP), uracil; and cyclic thymidine monophosphate (cyclic TMP), thymine. The last two are often inactive or only weakly active as phosphodiesterase inhibitors compared to their biocongeners (66, 188). The potency of cyclic IMP is often found to be of the same magnitude as that of cyclic GMP (66, 188, 222); cyclic CMP is sometimes ineffective as an inhibitor (66, 188) but is nearly equal in potency to cyclic GMP as an inhibitor of Form I from pea seedlings (74).

2. Cyclic nucleotides of synthetic bases: Several types of cyclic AMP analogs have been studied on phosphodiesterases from a variety of tissues (Table VII). Compounds of two series (Groups A and B) were generally more potent than theophylline on rabbit lung and bovine heart Form II (184). Three other groups, including imidazole nucleotides (Group I), N^1,N^6-ethenonucleotides (Group H), and substituted inosine nucleotides (Group E), were more variable in their inhibitory potency (184). Data from a similar study (302) indicated relatively little variation in I_{50} values within a series of 8-substituted analogs of cy-

Table VII—Cyclic Nucleotides of Synthetic Bases

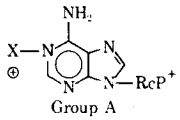
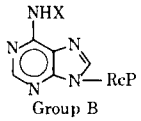
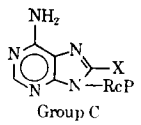
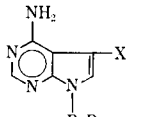
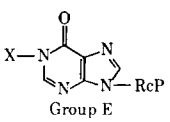
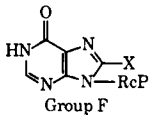
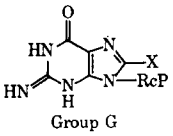
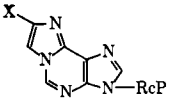
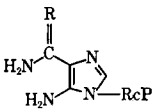
Substitution	Base	Potency*	Enzyme Source	Inhibitor Concentration, moles/liter	Phospho-diesterase Form	Reference
X = O	 Group A	35[b], 7.1[d]	Rabbit lung	—	II	184
		50[b], 2.6[d]	Bovine heart	—	II	184
X = OCH_3		150[b], 1.7[d]	Rabbit lung	—	II	184
		90[b], 1.4[d]	Bovine heart	—	II	184
X = OC_2H_5		45[b], 5.5[d]	Rabbit lung	—	II	184
		120[b], 1.1[d]	Bovine heart	—	II	184
X = $OCH_2C_6H_5$		35[b], 7.1[d]	Rabbit lung	—	II	184
		80[b], 1.6[d]	Bovine heart	—	II	184
X = CH_3		65[b], 3.8[d]	Rabbit lung	—	II	184
		80[b], 1.6[d]	Bovine heart	—	II	184
X = $CH_2C_6H_5$	 Group B	67[c]	*D. discoideum*	2×10^{-4}	I	10
X = COC_6H_5		72[c]	*D. discoideum*	2×10^{-4}	I	10
X = COC_3H_7-*n*		46[b], 5.4[d]	Rabbit lung	—	II	172
		110[a]	Rabbit lung	—	II	301
X = OH		13[b], 19.2[d]	Rabbit lung	—	II	184
		150[b], 0.87[d]	Bovine heart	—	II	184
X = OCH_3		100[b], 2.5[d]	Rabbit lung	—	II	184
		80[b], 1.6[d]	Bovine heart	—	II	184
X = OC_2H_5		42[b], 6.0[d]	Rabbit lung	—	II	184
		68[b], 1.9[d]	Bovine heart	—	II	184
X = $OCH_2C_6H_5$		190[b], 1.3[d]	Rabbit lung	—	II	184
		240[b], 0.54[d]	Bovine heart	—	II	184
X = $CONHCH_3$		260[b], 0.9[d]	Rabbit lung	—	II	183
X = $CONHC_3H_7$-*n*		330[b], 0.7[d]	Rabbit lung	—	II	183
X = $CONHC_6H_5$		160[b], 1.4[d]	Rabbit lung	—	II	183
X = $COOC_2H_5$		210[b], 1.1[d]	Rabbit lung	—	II	183
X = SH	 Group C	540[b]	Rat brain	—	II	188
		300[b]	Cat heart	—	II	188
		20[b]	Rabbit lung	—	I	302
X = SCH_3		125[b]	Rat brain	—	II	188
		39[b]	Cat heart	—	II	188
		24[b]	Beef heart	—	I	302
X = SC_2H_5		52[b]	Rat brain	—	II	188
		41[b]	Cat heart	—	II	188
X = SCH_2CH_2OH		200[b]	Rat brain	—	II	188
		44[b]	Cat heart	—	II	188
X = $SCH_2C_6H_5$		40[b]	Rat brain	—	II	188
		24[b]	Cat heart	—	II	188
		22[b], 5.9[d]	Beef heart	—	I	183
		60[b], 3.8[d]	Rabbit lung	—	I	183
X = NH_2		400[b]	Rat brain	—	II	188
		23[b]	Cat heart	—	II	188
X = N_3		150[b]	Rat brain	—	II	188
		90[b]	Cat heart	—	II	188
X = $NHCH_3$		700[b]	Rat brain	—	II	188
		160[b]	Cat heart	—	II	188
X = $N(CH_3)_2$		2300[b]	Rat brain	—	II	188
		3300[b]	Cat heart	—	II	188
X = $NHCH_2CH_2OH$		4400[b]	Rat brain	—	II	188
		270[b]	Cat heart	—	II	188
X = OH		Inactive	Rat brain	—	II	188
		Inactive	Cat heart	—	II	188
X = OCH_3		600[b]	Rat brain	—	II	188
		130[b]	Cat heart	—	II	188
X = Br		67[b]	Rat brain	—	II	188
		16[b]	Cat heart	—	II	188
X = Br		52[c]	*D. discoideum*	2×10^{-4}	I	10
X =		20[c]	*D. discoideum*	2×10^{-4}	I	10
X = S—C_6H_4—*p*-Cl		200[b]	Rabbit lung	—	I	302
		22[b]	Beef heart	—	I	302
X = H	 Group D	20[b]	Beef heart	—	I	303
		12[b]	Rabbit lung	—	I	303
X = CN		2.0[b]	Beef heart	—	I	303
		2.4[b]	Rabbit lung	—	I	303
X = $CONH_2$		5.0[b]	Beef heart	—	I	303
		6.7[b]	Rabbit lung	—	I	303
X = H	 Group E	80[b], 3.1[d]	Rabbit lung	—	II	184
		3.8[b], 34.2[d]	Bovine heart	—	II	184
X = OH		330[b], 0.76[d]	Rabbit lung	—	II	184
		830[b], 0.16[d]	Bovine heart	—	II	184

Table VII—(*Continued*)

Substitution	Base	Potency*	Enzyme Source	Inhibitor Concentration, moles/liter	Phospho-diesterase Form	Reference
X = $NHCH_2C_6H_5$	 Group F	11[c]	*D. discoideum*	2×10^{-4}	I	10
X = Br		28[c]	*D. discoideum*	2×10^{-4}	I	10
X = $SCH_2C_6H_5$		67[b]	Rabbit lung	—	I	302
		13[b]	Beef heart	—	I	302
X = $NHCH_2C_6H_5$	 Group G	19[c]	*D. discoideum*	2×10^{-4}	I	10
X = Br		32[c]	*D. discoideum*	2×10^{-4}	I	10
		150[b]	Rabbit lung	—	I	302
		48[b]	Beef heart	—	I	302
X = H	 Group H	280[b], 0.9[d]	Rabbit lung	—	II	184
		590[b], 0.22[d]	Bovine heart	—	II	184
X = C_6H_5		160[b], 1.6[d]	Rabbit lung	—	II	184
		73[b], 0.18[d]	Bovine heart	—	II	184
R = O	 Group I	100[b], 2.5[d]	Rabbit lung	—	II	184
		170[b], 0.76[d]	Bovine heart	—	II	184
R = S		80[b], 3.1[d]	Rabbit lung	—	II	184
		60[b], 2.2[d]	Bovine heart	—	II	184

* See footnote to Table VI. † RcP = β-D-ribofuranosyl cyclic 3,5-phosphate.

clic AMP (Group C), with the possible exception of 8-*p*-chlorophenylthio cyclic AMP which showed considerable selectivity for beef heart over rabbit lung enzyme.

Other workers (188), examining a series of cyclic AMP derivatives with substituents in the 8-position (Group C), showed that 8-bromo and 8-benzylthio cyclic AMP were the most potent inhibitors of the series on cat heart and rat brain Form II, respectively. The former enzyme appeared more sensitive to inhibition.

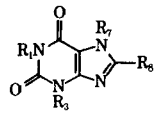
In a study on *Dictyostelium discoideum* Form I (10) (Groups B and C), the 8-substituted cyclic AMP derivatives were somewhat less potent inhibitors than the 6-substituted amino congeners. This effect was attributed to a greater steric hindrance for binding of the 8-substituted compounds, although neither N^6- nor 8-substitution profoundly affected the rate

Table VIII—Variations in the Sugar-Phosphate Moiety of Cyclic Nucleotides

Name	Potency*	Enzyme Source	Inhibitor Concentration, moles/liter	Phospho-diesterase Form	Reference
2′,3′-Cyclic AMP	1700[b]	Rat brain	—	II	188
	600[b]	Cat heart	—	II	188
	5[c]	Pea seedlings	1×10^{-4}	II	74
2′,3′-Cyclic GMP	210[b]	Cat heart	—	II	188
	Inactive	Rat brain	—	II	188
	15[c]	Rat thymic erythrocyte	1×10^{-4}	II	112
2′,3′-Cyclic IMP	2000[b]	Cat heart	—	II	188
2′,3′-Cyclic UMP	Inactive	Cat heart	—	II	188
	Inactive	Rat brain	—	II	188
	Inactive	Pea seedlings	1×10^{-4}	II	74
2′,3′-Cyclic CMP	Inactive	Rat brain	—	II	188
2′-*O*-Acetyl cyclic AMP	5[b], 5.0[d]	Rabbit lung	—	II	172
2′-*O*-Methyl cyclic AMP	4.8[b], 52.1[d]	Rabbit lung	—	II	172
2′-*O*-(2,4-DNP) cyclic AMP	25[b], 10.0[d]	Rabbit lung	—	II	172
2′-*O*-Butyryl cyclic AMP	5.6[b], 44.6[d]	Rabbit lung	—	II	172
	26[a]	Bovine thyroid	—	II	301
2′-Deoxy cyclic AMP	11[b], 22.7[d]	Rabbit lung	—	II	172
N^6,2′-*O*-Dibutyryl cyclic AMP	230[b], 1.1[d]	Rabbit lung	—	II	172
	160[a]	Bovine thyroid	—	II	301
	650[b]	Rat brain	—	II	188
	100[b]	Cat heart	—	II	188
5′-Methylene-5′-deoxy cyclic AMP	Inactive	Rabbit brain	1×10^{-4}	II	305
5′-Thio-5′-deoxy cyclic AMP	15[a], 12[b], 10.8[d]	Beef heart	—	II	185
	3.5[a], 2.4[b], 104[d]	Rabbit lung	—	II	185
5′-*N*-*n*-Octylamino-5′-deoxy cyclic AMP	200[a]	Beef heart	—	I	306
$S^{4'}$-Cyclic AMP	56[c]	Bovine heart	1×10^{-3}	II	307

* See footnote to Table VI.

Table IX—Xanthine Derivatives



Name	R_1	R_3	R_7	R_8	Potency*	Enzyme Source	Inhibitor Concentration, moles/liter	Phosphodiesterase Form	Reference
Caffeine	CH_3	CH_3	CH_3	H	27[c,f]	Cat cerebral cortex	2.5×10^{-3}	I	191
					38[c,g]	Rabbit skeletal muscle	1×10^{-3}	II	199
					3000[a], 0.6[d]	Rat adrenals	—	I	312
					31[c,g]	Beef brain	1.2×10^{-2}	I	313
					150[b], 0.8[d]	Rat brain	—	II	297
					<1.0[d]	Rat fat cell	5×10^{-4}	II	93
					48[c,g]	Bovine taste papillae	3×10^{-3}	I	18
					1000[a]	Mouse pancreatic islet	5.3×10^{-3}	II	111
					18[c,g]	Cockroach brain	1×10^{-4}	II	35
					2400[a]	Beef heart	—	II	213
					Inactive	Pea seedlings	1×10^{-4}	II	74
					930[b], 0.62[d]	Rat cerebral cortex	—	II	19
					69[c,g]	Mouse brain	1×10^{-2}	I	186
Theobromine	H	CH_3	CH_3	H	28[c]	Rabbit skeletal muscle	1×10^{-3}	II	199
					4500[a], 0.4[d]	Rat adrenals	—	I	312
					41[c,g]	Bovine taste papillae	3×10^{-3}	I	18
					150[b], 0.8[d]	Rat brain	—	II	297
					<1.0[d]	Rat fat cell	5×10^{-4}	II	93
					38[c,g]	Mouse brain	1×10^{-2}	I	186
	C_2H_5	C_2H_5	H	H	200[b], 0.13[e]	Hamster islet cell tumor	—	II	107
					>1.0[d]	Rat fat cell	4×10^{-4}	II	93
SC 2964	CH_3	C_4H_9-*i*	H	H	~15.0[d]	Rat fat cell	5×10^{-5}	II	93
					89[c]	Mouse pancreatic islet	8×10^{-4}	II	111
	C_5H_{11}-*n*	C_2H_5	H	H	~1.0[d]	Rat fat cell	4×10^{-4}	II	93
	CH_3	CH_3	H	SH	<1.0[d]	Rat fat cell	5×10^{-4}	II	93
	CH_3	CH_3	H	CH_3	160[a], 2.6[d]	Beef heart	5×10^{-4}	I	314
	CH_3	CH_3	H	C_2H_5	100[a], 4.2[d]	Beef heart	5×10^{-4}	I	314
	CH_3	CH_3	H	C_3H_7-*n*	210[a], 2.0[d]	Beef heart	5×10^{-4}	I	314
	CH_3	CH_3	H	C_4H_9-*n*	70[a], 6.0[d]	Beef heart	5×10^{-4}	I	314
	CH_3	CH_3	H	C_5H_{11}-*n*	40[a], 10.5[d]	Beef heart	5×10^{-4}	I	314
	CH_3	CH_3	H	C_6H_{13}-*n*	20[a], 21.0[d]	Beef heart	5×10^{-4}	I	314
	CH_3	CH_3	H	C_7H_{15}-*n*	Inactive	Beef heart	5×10^{-4}	I	314
	CH_3	CH_3	H	$CH_2CH_2CH_2CH_2OH$	Inactive	Beef heart	5×10^{-4}	I	314
	CH_3	CH_3	H	C_3H_7-*i*	50[a], 8.4[d]	Beef heart	5×10^{-4}	I	314
	CH_3	CH_3	H	C_4H_9-*i*	150[a], 2.8[d]	Beef heart	5×10^{-4}	I	314
	CH_3	CH_3	H	C_3H_5-*c*	60[a], 7.0[d]	Beef heart	5×10^{-4}	I	314
	CH_3	CH_3	H	C_4H_7-*c*	90[a], 4.7[d]	Beef heart	5×10^{-4}	I	314
	CH_3	CH_3	H	C_5H_9-*c*	210[a], 2.0[d]	Beef heart	5×10^{-4}	I	314
Etamiphylline	CH_3	CH_3	$CH_2CH_2N(C_2H_5)_2$	H	10.5[c,g]	Beef brain	6.25×10^{-3}	I	200
Oxyphylline	CH_3	CH_3	CH_2CH_2OH	H	22.5[c,g]	Beef brain	6.25×10^{-3}	I	200
Diprophylline	CH_3	CH_3	$CH_2CHOHCH_2OH$	H	22.8[c,g]	Beef brain	6.25×10^{-3}	I	200
Etamiphylline iodomethylate	CH_3	CH_3	$CH_2CH_2\overset{+}{N}CH_3(C_2H_5)_2I^-$	H	Inactive	Beef brain	6.25×10^{-3}	I	200
	CH_3	CH_3	$CH_2CH_2NH_2$	H	12.1[c,g]	Beef brain	6.25×10^{-3}	I	200
	CH_3	CH_3	$CH_2CH_2NHCH_2C_5H_5$	H	30.8[c,g]	Beef brain	6.25×10^{-3}	I	200
	CH_3	CH_3	$CH_2CH_2\overset{+}{N}(CH_3)_2CH_2C_6H_5I^-$	H	Inactive	Beef brain	6.25×10^{-3}	I	200

	CH_3	CH_3	CH_2CH_2OH		53[c,g]	Beef brain	1.2×10^{-2}	I	313
	CH_3	CH_3	$CH_2CHOHCH_2OH$		50[c,g]	Beef brain	1.2×10^{-2}	I	313
	CH_3	CH_3	CH_2COOH		Inactive	Beef brain	1.2×10^{-2}	I	313
	CH_3	CH_3	$CH_2CH_2N(CH_3)_2$		45[c,g]	Beef brain	1.2×10^{-2}	I	313
	CH_3	CH_3	$CH_2CH_2CH_2NH_2$		48[c,g]	Beef brain	1.2×10^{-2}	I	313
				H	25[c]	Human umbilical artery	1.8×10^{-4}	I	192
Pentoxifylline	$CH_3CO(CH_2)_4$	CH_3	CH_3		50[c]	Human umbilical vein	1.8×10^{-4}	I	192
				H	36[c]	Human umbilical artery	1.8×10^{-4}	I	192
SK 7	$CH_3(CH_2)_5$	CH_3	CH_3		56[c]	Human umbilical vein	1.8×10^{-4}	I	192

* See footnote to Table VI.

of hydrolysis of these derivatives by phosphodiesterase. 8-Bromo and 8-benzylamino derivatives of cyclic GMP and cyclic IMP (Groups G and F, respectively) were also examined on *D. discoideum* phosphodiesterase and found to be generally less effective than the cyclic AMP derivatives, although only the 8-bromo substituent was common to all three types of cyclic nucleotides.

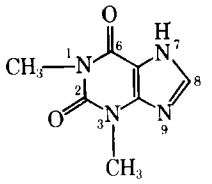
A series of cyclic AMP derivatives examined on beef heart and rabbit lung phosphodiesterase included an N^6-ethoxycarbonyl and several N^6-carbamoyl derivatives (Group B). The N^6-(N-methylcarbamoyl) and the N^6-ethoxycarbonyl derivatives were similar to theophylline in potency and selectivity, while the propylcarbamoyl and phenylcarbamoyl analogs selectively inhibited the rabbit lung and beef heart enzymes, respectively (183).

Tubercidin cyclic phosphate (Group D, X = H) is a 7-position carbon isostere of cyclic AMP; it and two closely related compounds, toyocomycin (X = CN) and sangivomycin (X = $CONH_2$), were all very good inhibitors of beef heart and rabbit lung Form I. The corresponding heterocyclic bases were also good inhibitors, but the analogous nucleosides and nucleoside 5′-phosphates were generally much weaker (303).

As might be anticipated, the most potent cyclic nucleotide inhibitors of phosphodiesterase generally have intact purine rings which resemble adenine, guanine, or hypoxanthine rather closely. Analogs of cyclic AMP having substituents in the 1- or 8-position or attached to the 6-amino group may be quite potent, depending on the nature of the substituent. Compounds with substituents bonded to the 8-position through a nitrogen or oxygen atom are often less potent than those having an 8-sulfur attachment. Isosteric substitutions (304) in the heterocyclic skeletons of cyclic nucleotides also seem to offer considerable promise for the development of potent phosphodiesterase inhibitors.

3. Variation of sugar phosphate moiety of cyclic nucleotides: Many cyclic nucleotides have been prepared that incorporate variations of the normal β-D-ribofuranosyl cyclic 3′,5′-phosphate moiety; representative examples are included in Table VIII. One such class of compounds is the 2′,3′-cyclic nucleoside phosphates, some of which were 0.001–0.005 as potent as the corresponding 3′,5′-congeners on Form II from cat heart and rat brain (188). In other systems, they were either weak or noninhibitors (66, 74).

An examination of several 2′-*O*-substituted cyclic AMP derivatives on Form II led to the conclusion that the beef heart and rabbit lung enzymes were more sensitive than similar beef brain and rabbit kidney systems to inhibition by compounds having relatively small groups in the 2′-*O*-position. Beef brain enzyme was found to be more sensitive to inhibitors bearing the bulkier 2′-*O*-(2,4-dinitrophenyl) and 2′-*O*-butyryl groups (172). The 2′-*O*-butyryl derivative is generally more potent than the N^6-butyryl (see also Table VII, Group B), which is more potent than the familiar N^6,2′-*O*-dibutyryl derivative (172, 301). The usefulness, however, of dibutyryl cyclic AMP as a tool for studying biochemical mechanisms far sur-



III

passes its utility as an inhibitor of phosphodiesterase. Its better cell penetration and slower metabolism compared to the parent cyclic AMP make it an excellent example of drug latentiation (308); nevertheless, its effects on intact biological systems must be viewed with caution (309, 310).

A number of excellent reports dealing with sugar phosphate variations in cyclic nucleotides are not discussed here (10, 183, 185, 311).

Xanthine Derivatives—Theophylline (III) is the most common standard reference agent used in studies of phosphodiesterase inhibitors. In a variety of guinea pig and rat tissues, it shows a moderate amount of tissue selectivity which appears to be species dependent (175, 176).

Caffeine is not consistently more or less active than theophylline, but commonly varies from 0.5 to 2 times as potent (Table IX). Theobromine, however, is less potent than theophylline in a variety of tissues.

Numerous xanthine derivatives have been synthesized, many of which are substituted at the 8-position as well as on the three commonly substituted secondary nitrogens. Several theophylline derivatives with substituted ethyl groups in the 7-position were less inhibitory than the parent structure on beef brain Form I, although etamiphylline is described as a better respiratory stimulant than theophylline (200). Etamiphylline iodomethylate, which has no inhibitory properties on phosphodiesterase, also has interesting *in vivo* properties. Highest potency was observed with an *N*-benzylaminoethyl function in the 7-position, while a quaternary nitrogen on the 7-substituent was detrimental (200). Another group of 7-substituted xanthines having a 3-pyridylmethyl function in the 8-position was also less potent than theophylline on beef brain Form I (313).

Two vasoactive substances are 1-substituted theobromine derivatives. SK 7 was more potent than pentoxifylline[2] as an inhibitor of umbilical phosphodiesterases, but their potencies relative to theophylline were not reported (192).

A series of 8-alkyl theophyllines showed interesting properties on beef heart Form I. 8-*n*-Hexyltheophylline was the most potent member, being 21 times as active as theophylline. Surprisingly, the 8-*n*-heptyl homolog was not effective. Equally unexpected was the fact that the 8-ω-hydroxybutyl derivative was ineffective, although the 8-butyl congener was 6 times as potent as theophylline. Compounds having four- and five-carbon branched and cycloalkyl groups in the 8-position were less effective than their straight chain counterparts; however, the C_3 groupings of isopropyl and cyclopropyl yielded analogs that were several times more potent than the *n*-propyl analog (314).

[2] BL 191.

Table X—Inhibitor Constants of Papaverine on Form II Activity from Selected Guinea Pig and Rat Tissue (175)

Tissue		K_i Value, μM[a]
Guinea Pig:	Brain	100
	Heart	180
	Kidneys	150
	Liver	30
	Lungs	50
Rat:	Brain	50
	Heart	40
	Kidneys	50
	Liver	100
	Lungs	25

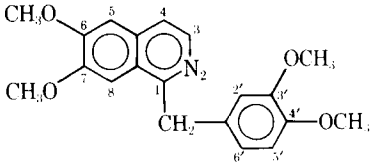
[a] Determined as described for Table II.

The 3-isobutyl analog of theophylline, SC 2964, was 15 times as potent as theophylline on rat fat cell Form II, a system in which substantial increases in lipolysis were associated with only partial phosphodiesterase inhibition. Close agreement was obtained between potencies as lipolytic agents and phosphodiesterase inhibitors. Substitution of a sulfhydryl group in the 8-position of theophylline did not improve its potency (93).

Replacing the 3-methyl of theophylline with more bulky, lipophilic groups yields compounds with improved potency, while increasing alkyl chain length up to hexyl at the 8-position is quite advantageous. Compounds with substituted alkyl substituents in the 7-position offer no improvement over theophylline, although an additional 8-substituent may be helpful.

Papaverine and Related Isoquinolines—The utility of the opium alkaloid papaverine as a standard reference for phosphodiesterase inhibition is well established. It effectively inhibits both Form I (18, 312, 315–321) and Form II (19, 107, 121, 171, 175, 181, 273, 322) from a variety of tissues. However, papaverine appears to activate phosphodiesterase from the cytoplasmic fraction of rabbit colon (289) and the cat cerebral cortex (191). While commonly exhibiting a potency from 10 to 1000 times that of theophylline, papaverine rarely shows appreciable tissue selectivity in its actions (Table X).

Replacing the 7-methoxy of papaverine with a hydroxy reduced its potency substantially on beef heart phosphodiesterase (Table XI). A similar replacement in the 3′-position (Ro 20-0518) did not affect its potency significantly; but at the 4′-position, the introduction of a hydroxy group resulted in a compound 2–3 times as potent as papaverine (315). When all four methoxys of papaverine were replaced with ethoxys, potency was not appreciably altered (317, 318,



papaverine

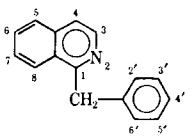
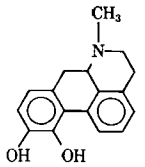
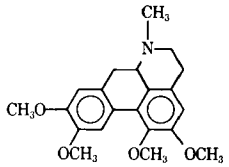
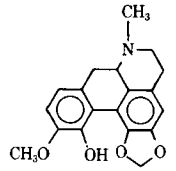


Table XI—Benzylisoquinoline Derivatives

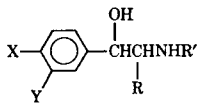
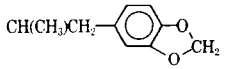
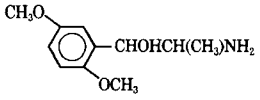
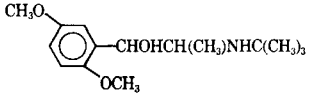
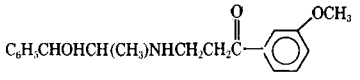
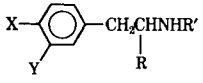
Name	Substituents at Position 3	6	7	3′	4′	6′	Potency*	Enzyme Source	Inhibitor Concentration, moles/liter	Phosphodiesterase Form	Reference
Ro 20-0518	H	CH_3O	HO	CH_3O	CH_3O	H	9[c,i]	Beef heart	1×10^{-5}	I	315
	H	CH_3O	CH_3O	HO	CH_3O	H	32[c,h]	Beef heart	1×10^{-5}	I	315
	H	CH_3O	CH_3O	CH_3O	HO	H	66[c,h]	Beef heart	1×10^{-5}	I	315
Ethaverine	H	C_2H_5O	C_2H_5O	C_2H_5O	C_2H_5O	H	5.6[a], 0.7[e]	Beef heart	—	?	323
							40[c]	Human platelets	5×10^{-5}	I	317
							15[a], 0.3[e]	Human platelets	—	I	318
Papaveroline	H	HO	HO	HO	HO	H	22[a], 0.2[e]	Beef heart	—	?	323
							Negligible	Guinea pig brain	1×10^{-4}	I	319
	C_2H_5	CH_3O	CH_3O	CH_3O	CH_3O	H	0.8[a], 2.5[e]	Rabbit ileum	—	II	324
6-Bromopapaverine	H	CH_3O	CH_3O	CH_3O	CH_3O	Br	61[c,i]	Guinea pig brain	2×10^{-4}	I	319
PV_2	H	CH_3O	CH_3O	H	Cl	H	12[a], 2.8[e]	Beef heart	—	I	320
Dioxyline	CH_3	CH_3O	CH_3O	CH_3O	C_2H_5O	H	59[a], 0.08[e]	Human platelets	—	I	318
	H	CH_3O	CH_3O	H	H	H	6.5[a], 0.6[e]	Beef heart	—	?	323
	H	H	H	CH_3O	CH_3O	H	22[a], 0.2[e]	Beef heart	—	?	323
	C_2H_5	CH_3O	CH_3O	H	H	H	48[e,f,h]	Human platelets	5×10^{-5}	I	317
Eupaverine	CH_3	OCH_2O		OCH_2O		H	25[a], 0.9[e]	Beef heart	—	I	316
							34[a], 1.0[e]	Beef heart	—	I	320
							0.6[a], 3.3[e]	Rabbit ileum	—	II	324
							7[a], 0.8[e]	Beef coronary artery	—	I	325, 94
							30[a], 1.3[e]	Rat adrenal	—	I	312
	H	H	H	H	H	H	Negligible	Beef heart	1×10^{-5}	?	323
Related Alkaloids											
Apomorphine							15[b], 0.8[e]	Dog cerebral cortex	—	II	121, 122
							15[b], 0.67[e]	Rat cerebral cortex	—	II	19
							220[b], 0.1[e]	Dog red blood cell	—	II	122
							170[b], 0.07[e]	Rat red blood cell	—	II	121, 122
(−)-Glaucine							36[a], 0.1[e]	Beef heart	—	?	323
(+)-Glaucine							39[a], 0.1[e]	Beef heart	—	?	323
Bulbocapnine							46[b], 0.22[e]	Rat cerebral cortex	—	II	19

* See footnote to Table VI.

323) but four hydroxys were clearly detrimental (319, 323). Replacement of both the 3′- and 4′-methoxys with hydrogen retained activity but potency decreased when the 6- and 7-methoxys were removed. Deletion of the entire 1-veratryl group of papaverine affords 6,7-dimethoxyisoquinoline, which retained only about 3% of the potency of papaverine on beef heart phosphodiesterase (323). Removal of the 3′- and 4′-methoxy groups and addition of a 3-ethyl function did not change the inhibitory potency of papaverine on human platelet Form I (317). Addition of a bromine atom at the 6′-position of either papaverine or papaverinol resulted in a more potent inhibitor of both the soluble and particulate forms of guinea pig brain Form I (319).

Replacement of both pairs of methoxys with methylenedioxy functions and addition of a 3-methyl group produces a compound (eupaverine) that was

Table XII—Adrenergic Amines and Related Compounds

Name	X	Y	R	R′	Potency*	Enzyme Source	Inhibitor Concentration, moles/liter	Phosphodiesterase Form	Reference
A. Phenethanolamines 									
l-Norepinephrine	OH	OH	H	H	48[c]	Beef heart	5×10^{-5}	I	252
					~17[c]	Guinea pig lung	1×10^{-4}	I	250
					~5[c]	Guinea pig lung	1×10^{-4}	II	250
					>2000[b]	Rat cerebral cortex	—	II	19
l-Epinephrine	OH	OH	H	CH_3	7[b], 42[c,f]	Beef heart	5×10^{-5}	I	252
					~30[c]	Guinea pig lung	1×10^{-4}	I	250
					Inactive	Guinea pig lung	1×10^{-4}	II	250
					12[c,g,i]	Beef heart	5×10^{-5}	II	252
					>2000[b]	Rat cerebral cortex	—	II	19
l-Isoproterenol	OH	OH	H	$CH(CH_3)_2$	46[c]	Beef heart	5×10^{-5}	I	252
					~37[c]	Guinea pig lung	1×10^{-4}	I	250
					Inactive	Guinea pig lung	1×10^{-4}	II	250
d,l-Isoproterenol	OH	OH	H	$CH(CH_3)_2$	45[c]	Beef heart	5×10^{-5}	II	252
Albuterol	OH	CH_2OH	H	$C(CH_3)_3$	7[c]	Beef heart	5×10^{-5}	I	252
Protokylol	OH	OH	H		47[c]	Beef heart	5×10^{-5}	I	252
Nylidrin	OH	H	CH_3	$CH(CH_3)C_2H_4C_6H_5$	10,000[a], 0.08[d]	Human platelets	—	I	318
Isoetharine	OH	OH	C_2H_5	$CH(CH_3)_2$	10,000[a], 0.08[d]	Human platelets	—	I	318
l-Phenylephrine	H	OH	H	CH_3	Inactive	Beef heart	5×10^{-5}	I	252
l-Dichloroisoproterenol	Cl	Cl	H	$CH(CH_3)_2$	Inactive	Beef heart	5×10^{-5}	I	252
Methoxamine					Inactive	Guinea pig lung	1×10^{-4}	II	250
					~16[c]	Guinea pig lung	1×10^{-4}	I	250
Butoxamine					Inactive	Guinea pig lung	1×10^{-4}	II	250
					Inactive	Guinea pig lung	1×10^{-4}	I	250
Oxyfedrine					1800[a], 0.07[d]	Beef coronary artery	—	I	94
B. Phenethylamines 									
Dopamine	OH	OH	H	H	46[c]	Beef heart	5×10^{-5}	I	252
					750[b], 0.01[e]	Rat cerebral cortex	—	II	19
Tyramine	OH	H	H	H	Inactive	Beef heart	5×10^{-5}	I	252
3-Methoxytyramine	OH	OCH_3	H	H	Inactive	Beef heart	5×10^{-5}	I	252
					>2000[b]	Rat cerebral cortex	—	II	19
l-Dopa	OH	OH	COOH	H	53[c]	Beef heart	5×10^{-5}	I	252

l-Tyrosine	OH	H	COOH	H	Inactive	Beef heart	5×10^{-5}	I	252
Amphetamine	H	H	CH_3	H	>1000[b]	Rat brain	—	II	287
					32[c]	Mouse brain	1×10^{-2}	I	186
p-Chlorophenylalanine	Cl	H	COOH	H	>1000[b]	Rat brain	—	II	287
Prenylamine	H	H	CH_3	$CH_2CH_2CH(C_6H_5)_2$	330[e], 0.7[d]	Beef heart	—	I	326
					80[a], 0.6[d]	Beef coronary artery	—	I	94
Verapamil (iproveratril)					1000–3000[b]	Rat brain, heart; rabbit heart, artery	—	?	327
					3300[a], 0.24[d]	Human platelets	—	I	318
					530[a], 0.43[d]	Beef heart	—	I	94
3,4-Dimethoxyphenethylamine	OCH_3	OCH_3	H	H	>2000[b]	Rat cerebral cortex	—	II	19

C. Miscellaneous Catechol Derivatives

R				
3,4-Dihydroxy-				
—H	Inactive	Beef heart	5×10^{-5}	I	252
—CHO	5[c]	Beef heart	5×10^{-5}	I	252
$—CH_2COOH$	28[c]	Beef heart	5×10^{-5}	I	252
	>10,000[b], <0.82[d]	Rat erythrocyte	—	II	121
	630[b], 0.95[d]	Canine cerebral cortex	—	II	121
—CHOHCOOH	34[c]	Beef heart	5×10^{-5}	I	252
$—CH_2CH_2COOH$	28[c]	Beef heart	5×10^{-5}	I	252

* See footnote to Table VI.

equipotent to papaverine on purified Form I from beef heart (316, 320). Replacing the 1-veratryl group of papaverine with a *p*-chlorobenzyl function increased the inhibitory effects on Form I of beef heart (320). The 3-methyl-4′-ethoxy analog of papaverine (dioxyline) was considerably less potent than papaverine on human platelet Form I (318).

3,4-Dihydropapaverine had negligible activity on both the soluble and particulate forms of guinea pig brain Form I, while 3,4-dihydroethaverine retained some of the activity of papaverine in both platelet (317) and purified beef heart Form I (316). Tetrahydropapaveroline was not effective on guinea pig brain Form I (319) or beef heart Form I (325) and was a weak inhibitor on rabbit ileum Form II (although it retained good spasmolytic action in the last system) (324).

A variety of isoquinolines with groups other than benzyl (or substituted benzyl) in the 1-position was ineffective on guinea pig brain Form I, as were those bearing representative pyridylmethyl functions in the same position (319); nevertheless, quinoparine, the desmethylene analog of papaverine, was more potent than papaverine on beef heart Form I (320).

The quaternary salt, papaverine methiodide, was not effective on beef heart phosphodiesterase (323).

These data support the conclusion (323) that the 6,7-dimethoxy substituents contribute more to the effectiveness of papaverine as a phosphodiesterase inhibitor than do the methoxy functions in the benzyl group. Furthermore, alkyl substitution in the 3-position of the heterocyclic ring retains activity, as does hydroxylation of the methylene group, but substitution of hydroxy for methoxy groups on the isoquinoline rings is generally undesirable. Partial or complete saturation of the *N*-containing ring of papaverine analogs does not provide a clear trend in phosphodiesterase inhibition, but quaternization of the nitrogen is clearly detrimental.

Although a direct comparison is lacking, morphine and codeine appear much less effective than apomorphine as inhibitors of Form II (122, 297). The apomorphine congeners, glaucine (323) and bulbocapnine (19), are effective inhibitors but both are less potent than papaverine.

Adrenergic Amines and Related Compounds—The catechol system is important to phosphodiesterase inhibition in both phenethylamines and phenethanolamines. Although catechol, resorcinol, and hydroquinone (50 μM) do not themselves inhibit phosphodiesterase activity (252), significant potency in phenethylamines favors the 3,4-dihydroxy configuration (Table XII). Replacement of the 3-hydroxy group on *l*-dopa with hydrogen (*l*-tyrosine) destroyed all activity; but if the carboxy function was changed to hydrogen (dopamine), little change was observed. Methylation of the 3-hydroxy group of dopamine destroyed activity (252).

Although they are not as potent as their amine-containing congeners, several nitrogen-free catechol derivatives also showed considerable activity on beef heart Form I (252). Those derivatives showing activi-

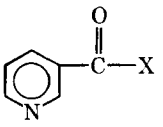
ty contained either an aldehyde or a carboxylic acid function, although benzaldehyde was remarkably weak.

The presence of the β-OH group is clearly not essential for phosphodiesterase inhibition in catecholamines (*e.g.*, dopamine *versus* norepinephrine); furthermore, the optical configuration about the β-carbon of isoproterenol is not critical, nor is the size of the amine substituent (252). As in the case of phenethylamines, anything other than 3,4-dihydroxy substitution on phenethanolamines resulted in drastically reduced potency in the system studied. Slight activity in the case of albuterol[3] may be attributed to the "homo-catechol" system (252).

Inhibition of phosphodiesterase by the adrenergic hormones occurs preferentially at high substrate concentrations. Two studies referred to in Table XII as Form II studies (250, 252) used a substrate concentration of 10^{-5} *M*, which is marginal for testing Form II activity. When phosphodiesterase studies using lower concentrations of cyclic AMP were carried out (253), a stimulant effect of these hormones on phosphodiesterase activity from rat liver was observed. This phosphodiesterase stimulant action was suggested as the intracellular mechanism for the α-adrenergic effects of these catechols, which were repeatedly shown to be associated with decreased intracellular levels of cyclic AMP (139, 276, 328, 329). This strongly reemphasizes the importance of the selection of the substrate concentration used for the type of effects observed.

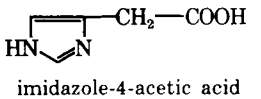
Pyridine and Quinoline Derivatives—Of several pyridine dicarboxylic acids tested on Form I from *Brevibacterium liquefaciens*, dipicolinic acid was clearly superior at very high inhibitor concentrations (71) (Table XIII). Nicotinic acid is included here as an inhibitor, although it also stimulated phosphodiesterase from adipose tissue (292). As an inhibitor of rat liver Form I (330), nicotinic acid was considerably inferior in potency to nicotinamide, *N,N*-diethylnicotinamide, and 3-acetylpyridine. Analogs of nicotinic acid having longer side chains were weak or inactive on rat fat cell Form II (331).

Other nicotinamide analogs (IV), retaining the 3-pyridylcarbonyl moiety and varying the other residue (X), were described as potent inhibitors of the rat liver soluble enzyme. Ethyl nicotinate (X = OC_2H_5), the strongest inhibitor among the agents investigated, was also able to increase hepatic cyclic AMP levels following intraperitoneal injection (332).

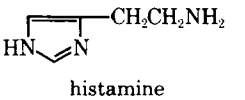


IV: X = CH_3, OCH_3, OC_2H_5, NH_2, NHC_2H_5, and $N(C_2H_5)_2$

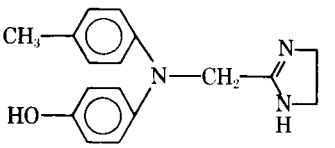
Although only a few quinoline derivatives have been investigated, their potencies as inhibitors of phosphodiesterase appear to be minimal, at least on



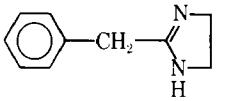
imidazole-4-acetic acid
V



histamine
VI



phentolamine
VII



tolazoline
VIII

Form I. 8-Hydroxyquinoline, as well as dipicolinic acid, probably does not actually inhibit phosphodiesterase but rather inactivates it by metal-ion chelation (71). In fact, a variety of metal-chelating carboxylic acids has been reported to inhibit phosphodiesterase activity from a number of sources (3, 67, 296).

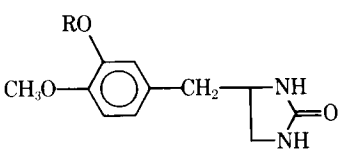
Indole Derivatives—Serotonin weakly inhibited phosphodiesterase activity in beef heart (252) (Table XIV) while reserpine more substantially inhibited the enzyme from the brains of trout (44), rat (297), and cockroach (35). A synthetic derivative of reserpine, 1-diethylaminoethylreserpine, was 200 times more potent than theophylline as an inhibitor of rabbit brain cortex Form I (333).

Dihydroergotamine was about twice as potent as theophylline on cat cerebral cortex Form I (191) but showed a uniform, pronounced inhibition of phosphodiesterase in other organs only at high, unphysiological concentrations (10^{-3} *M*) (190). Only in cat brain phosphodiesterase did both dihydroergotamine and an hydrogenated ergot alkaloid mixture[4] induce an accumulation of cyclic AMP at physiological dose levels (190). It has been reported that dihydroergotamine inhibits both adenyl cyclase and phosphodiesterase; thus it antagonizes catecholamine-stimulated lipolysis by adenyl cyclase inhibition and potentiates the effects of other lipolytic agents *via* phosphodiesterase inhibition (336).

Nine dihydroergotamine derivatives were approximately equal in potency to caffeine and theophylline on cat brain gray matter Form I (334). Brucine and strychnine were weak inhibitors (18) of Form I while the latter was also reported as an activator (17) of the enzyme.

2-Bromolysergic acid diethylamide was 5 times as potent as theophylline *in vitro* (290) and 15–40 times as potent as theophylline on guinea pig heart phosphodiesterase *ex vivo* (335).

Imidazole Derivatives—Imidazole itself is most often reported as an activator of both Forms I and II

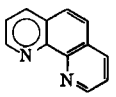


IX: R = CH_3, Ro 7-2956
X: R = n-C_4H_9, Ro 20-1724

[3] AH 3365.

[4] Hydergine.

Table XIII—Pyridine and Quinoline Derivatives

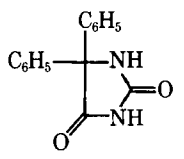
Name	Substituent(s)	Potency*	Enzyme Source	Inhibitor Concentration, moles/liter	Phosphodiesterase Form	Reference
		Pyridines 				
	3-Acetyl	~70[c]	Rat liver	?	I	330
	3-*N*,*N*-Diethylcarboxamido	~70[c]	Rat liver	?	I	330
	3—CH=CH—COOH	Weak	Rat fat cells	1×10^{-4}	II	331
	3-$(CH_2)_n$COOH (n = 0,1,2,3)	Inactive	Rat fat cells	1×10^{-4}	II	331
	3-$(CH_2)_4$COOH	Weak	Rat fat cells	1×10^{-4}	II	331
	2,3-Dicarboxyl	16.0[c]	*B. liquefaciens*	1×10^{-2}	I	71
	2,4-Dicarboxyl	38.5[c]	*B. liquefaciens*	1×10^{-2}	I	71
	2,5-Dicarboxyl	21.5[c]	*B. liquefaciens*	1×10^{-2}	I	71
Dipicolinic acid	2,6-Dicarboxyl	94.8[c]	*B. liquefaciens*	1×10^{-2}	I	71
α,α'-Dipyridyl	2-(2-Pyridyl)	8.6[c]	*B. liquefaciens*	1×10^{-2}	I	71
Nicotinic acid	3-Carboxyl	~20[c]	Rat liver	4×10^{-2}	I	330
Nicotinamide	3-Carboxamido	~70[c]	Rat liver	4×10^{-2}	I	330
NADP	—	Weak	Rat liver	2×10^{-2}	I	330
		Quinolines				
8-Hydroxyquinoline		60[c]	*B. liquefaciens*	1×10^{-2}	I	71
ortho-Phenanthroline		25[c]	Bovine brain	1×10^{-4}	I	70
Quinoline		17[c]	Bovine brain	1×10^{-4}	I	70

* See footnote to Table VI.

but occasionally does not significantly affect the enzyme (74, 215). Imidazole-4-acetic acid (V) (217) and histamine (VI) (10^{-2} *M*) (248, 281, 337) also showed stimulant effects on the enzyme (see *Activators*), although the latter did not affect the activity from beef heart (252), bullfrog gastric mucosa (217), or cyclic GMP phosphodiesterase from rat brain (53).

The α-blocking agent phentolamine (VII) effectively inhibited Form I from mouse brain (186) and rat brain (288) at high concentrations, although the structurally related α-blocker, tolazoline (VIII), was considerably weaker in one system (288) and possibly stimulatory in the other. Low concentrations of phentolamine did not inhibit beef heart Form I (252). While evidence does not support the conclusion that α-adrenergic blocking agents as a class inhibit phosphodiesterase, it has been suggested (288) that phosphodiesterase inhibition as well as α-adrenergic blockade may be involved in the actions of phentolamine.

Several members (IX and X) of a class of 4-(3,4-dialkoxybenzyl)-2-imidazolidinones were reported to be surprisingly potent inhibitors of phosphodiesterase. The prototype of the series, Ro 7-2956, was 42 times as potent as theophylline in this rat red blood cell system. Within this family of compounds, activity was greatly dependent upon the nature and position of the aromatic ring substituents. Inhibition increased with the length of the *meta*-alkoxy group and decreased with the length of the *para*-alkoxy function (338).



XI: diphenylhydantoin

Addition of a carboxy group to the 4-position of the heterocyclic ring in Ro 7-2956 decreased activity markedly, as did changing the aromatic group to naphthyl. Opening the heterocyclic ring to form a diamine reduced activity to nearly that of theophylline. Resolution of Ro 7-2956 showed that the *l*-enantiomer had more than 3 times the potency of the *d*-form and nearly twice that of the racemic compound. Both theophylline and Ro 20-1724 inhibited rat erythrocyte phosphodiesterase in a mixed manner, with both competitive and noncompetitive aspects (177). Subsequent work (121, 122) pointed out pronounced differences in the sensitivity of phosphodiesterases from a variety of tissues to Ro 20-1724 when compared with papaverine and theophylline. Ro 20-1724 also selectively inhibited hydrolysis of cyclic AMP compared to cyclic GMP (177).

The imidazolidinedione diphenylhydantoin (XI) was reported to be one-fourth as potent as the theophylline on rat brain Form II (297) and less than 2% as active as papaverine on the enzyme from the hamster pancreatic islet cell tumor (107).

Table XIV—Indole Derivatives

Name	Substituent	Potency*	Enzyme Source	Inhibitor Concentration, moles/liter	Phosphodiesterase Form	Reference
Serotonin		Weak	Beef heart	5×10^{-5}	I	252
Reserpine		170[b], 0.7[d]	Rat brain	—	II	297
		39[c,f]	Cockroach brain	1×10^{-4}	II	35
		23[c]	Trout brain	5×10^{-4}	I	44
	1-Diethylaminoethyl	0.55[a], 200[d]	Rabbit brain cortex	—	I	333
Dihydroergotamine		29[c,f]	Cat cerebral cortex	2.5×10^{-5}	I	191
Dihydroergotamine analogs (a total of nine)		~1.0[d]	Cat brain gray matter	2.5×10^{-5}	I	334
Lysergide (lysergic acid diethylamide)	2-Bromo	100[a], 5.0[d]	Guinea pig heart	—	?	290
		15–40[d]	Guinea pig heart (*ex vivo*)	3×10^{-4}	?	335
		68[a], 2.6[d]	Beef heart	—	I	326
Strychnine nitrate		40[c,g]	Bovine taste papillae	6×10^{-4}	I	18
Brucine		39.2[c,g]	Bovine taste papillae	6×10^{-4}	I	18

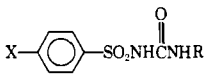
* See footnote to Table VI.

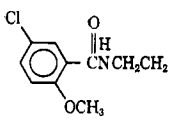
Sulfonylureas—When comparisons are made to caffeine or theophylline, the sulfonylureas are invariably less potent, even in tissues other than pancreas. Chlorpropamide and tolbutamide are most often reported to inhibit phosphodiesterase but neither is consistently the more potent. It would appear from the data in Table XV that the nature of the alkyl and ring substituents is not very important in structure–activity relationships, but an insufficient number of derivatives has been reported for any clear relationships to be outlined.

One attempt was made to show that the phosphodiesterase inhibitory action of the sulfonylureas depends on the intact structure since a mixture of toluenesulfonic acid and butylurea had very little inhibitory activity (341). This should not imply that dividing the molecule at some other point (*e.g.*, *p*-toluenesulfonamide and *N*-butylformamide) would necessarily produce the same result.

Benzothiadiazines and Related Compounds—All benzothiadiazine derivatives cited (Table XVI) as phosphodiesterase inhibitors are of the 1,1-dioxide group and only diazoxide is not of the diuretic class, which almost by definition bears a sulfamoyl group in the 7-position. Diazoxide and six thiazide diuretics were compared with caffeine and theophylline on beef heart Form I, and all compounds tested inhibited the enzyme but were less potent than caffeine and less than one-half as potent as theophylline (342). Most of the potency differences between the ben-

Table XV—Sulfonylureas



Name	X	R	Potency*	Enzyme Source	Inhibitor Concentration, moles/liter	Phosphodiesterase Form	Reference
Tolbutamide	CH_3	C_4H_9-*n*	23.0[c]	Rat kidney	5×10^{-3}	II	339
			59[c]	Rat adrenals	1×10^{-2}	?	340
			28[c,g], 3000[a]	Bovine thyroid	5×10^{-3}	I	301
			53[c]	Hamster islet cell tumor	1×10^{-2}	II	341
			3000[b]	Hamster islet cell tumor	—	II	107
Toluenesulfonic acid + butylurea			15[c,g]	Hamster islet cell tumor	1×10^{-2}	II	341
Chlorpropamide	Cl	C_3H_7-*n*	25.7[c]	Rat kidney	5×10^{-3}	II	339
			47[c]	Rat adrenals	1×10^{-2}	?	340
			27[c,g]	Bovine thyroid	5×10^{-3}	I	301
Acetohexamide	CH_3CO		46[c]	Rat adrenals	1×10^{-2}	?	340
Tolazamide	CH_3		57[c]	Rat adrenals	1×10^{-2}	?	340
Glyburide (glibenclamide)		H	20[c]	Mouse pancreatic islet	—†	II	111

* See footnote to Table VI. † Inhibitor concentration, 8.7 μg/ml.

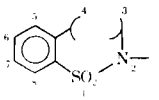
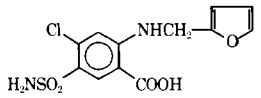
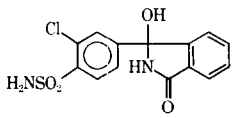


Table XVI—Benzothiadiazine Derivatives and Related Compounds

(4-3) Moiety	Substituent at Position 2	6	7	Name	Potency*	Enzyme Source	Inhibitor Concentration, moles/liter	Phosphodiesterase Form	Reference
—N=C(CH$_3$)—	H	H	Cl	Diazoxide	0.28[d]	Beef heart	$\sim10^{-3}$	I	342
					11[c]	Bovine artery	10^{-3}	I	289
					450[a]	Beef heart	—	I	343
					1200[b]	Hamster islet cell tumor	—	II	107
—N=CH—	H	Cl	SO_2NH_2	Chlorothiazide	0.31[d]	Beef heart	$\sim10^{-3}$	I	342
					5200[a], 0.54[d]	Guinea pig kidney	—	II	175
					2900[a], 0.45[d]	Rat kidney	—	II	175
—NHCH$_2$—	H	Cl	SO_2NH_2	Hydrochlorothiazide	0.02[d]	Beef heart	$\sim10^{-3}$	I	342
					1500[a]	Beef heart	—	I	343
—NH—CH(CHCl$_2$)—	H	Cl	SO_2NH_2	Trichlormethiazide	0.11[d]	Beef heart	$\sim10^{-3}$	I	342
—NH—CH$_2$—	H	CF_3	SO_2NH_2	Hydroflumethiazide	0.08[d]	Beef heart	$\sim10^{-3}$	I	342
—NH—CH(CH$_2$C$_6$H$_5$)—	H	CF_3	SO_2NH_2	Bendroflumethiazide	0.05[d]	Beef heart	$\sim10^{-3}$	I	342
—NH—CH(CH$_2$SCH$_2$CF$_3$)—	CH_3	F	SO_2NH_2	Polythiazide	0.41[d]	Beef heart	$\sim10^{-3}$	I	342
				Furosemide	500[a]	Beef heart	—	I	343
				Chlorthalidone	2500[a], 0.06[d]	Beef heart	—	I	344

* See footnote to Table VI.

zothiadiazine derivatives are not great, making it hazardous to extrapolate structure–activity relationships in too much detail; however, a few points seem worth mentioning.

Saturation of the 3,4-double bond of chlorothiazide decreased potency by a factor of 15 on beef heart Form I; other 3,4-saturated derivatives, except polythiazide, were less potent than chlorothiazide. The effects of alkyl or substituted alkyl groups in the 3-position appear to be minimal. Obviously, the 7-sulfamoyl function with the accompanying diuretic activity is not essential for phosphodiesterase inhibition, as shown by the case of diazoxide. Substitution in the 6-position does not appear to be of special significance where the substituent is H, Cl, or CF_3. The influence on phosphodiesterase activity of the 6-fluoro and 2-alkyl substituents of polythiazide, the most potent member of the series on beef heart Form I, remains to be elucidated.

Table XVII—Inhibitor Constants of Chlorothiazide on Phosphodiesterase Activity from Selected Guinea Pig and Rat Tissues (175)

Tissue	K_i Form II, mM[a]
Guinea Pig:	
Kidneys	5.2
Lungs	13.0
Brain, heart, liver	>85.0
Rat:	
Kidneys	2.9
Liver	85.0
Brain, heart, lungs	>85.0

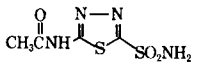
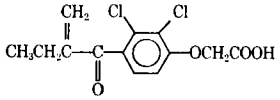
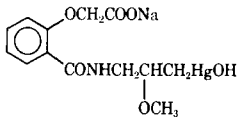
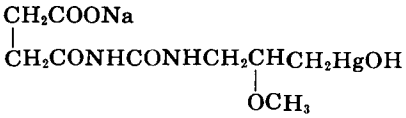
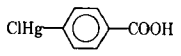
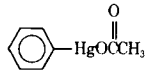
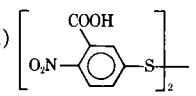
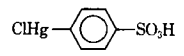
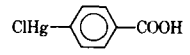
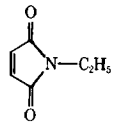
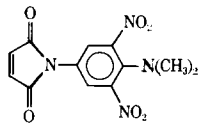
[a] Determined as described for Table II.

Although the thiazide diuretics are not potent phosphodiesterase inhibitors, the inhibitor constants (K_i) for one derivative, chlorothiazide, are lower for Form II from kidney tissue (guinea pig and rat) than for any of the other tissues studied (Table XVII), indicating marked selectivity for the kidney enzyme.

Two other diuretics, structurally related to the benzothiadiazines, were also reported to inhibit phosphodiesterase. Chlorthalidone (344) had about one-fifteenth the potency of theophylline, while furosemide (343) was three times as potent as hydrochlorothiazide on beef heart Form I.

Miscellaneous Diuretics and Sulfhydryl-Binding Agents—The importance of sulfhydryl groups for phosphodiesterase activity, as discussed earlier, is

Table XVIII—Miscellaneous Diuretics and Sulfhydryl-Binding Agents

Name	Structure	Potency*	Enzyme Source	Inhibitor Concentration, moles/liter	Phosphodiesterase Form	Reference
Acetazolamide		6000[a], 0.027[d]	Beef heart	—	I	343, 344
Ethacrynic acid		400[a], 0.4[d] 35[c]	Beef heart Guinea pig heart	— 2×10^{-3}	I ?	343, 344 189
Mersalyl		72[c]	Guinea pig heart	2×10^{-3}	?	189
Meralluride		67[c]	Guinea pig heart	2×10^{-3}	?	189
p-Chloromercuribenzoic acid		65[c]	Guinea pig heart	2×10^{-3}	?	189
Phenylmercuric acetate		96[c]	Guinea pig heart	2×10^{-3}	?	189
Methylmercuric chloride	CH_3HgCl	76[c]	Guinea pig heart	2×10^{-3}	?	189
Mercuric chloride	$HgCl_2$	92[c]	Guinea pig heart	2×10^{-3}	?	189
Iodoacetic acid	ICH_2COOH	12.4[c]	Bullfrog gastric mucosa	1×10^{-3}	I	217
		5[c]	Guinea pig heart	2×10^{-3}	?	189
5,5′-Dithiobis(2-nitrobenzoic acid)		27.0[c]	Bullfrog gastric mucosa	1×10^{-3}	I	217
p-Chloromercuribenzenesulfonic acid		58.0[c]	Bullfrog gastric mucosa	1×10^{-3}	I	217
p-Chloromercuribenzoic acid		61.0[c]	Bullfrog gastric mucosa	1×10^{-3}	I	217
N-Ethylmaleimide		5[c]	Guinea pig heart	2×10^{-3}	?	189
N-(4-Dimethylamino-3,5-dinitrophenyl)-maleimide		100[c]	Bullfrog gastric mucosa	1×10^{-3}	I	217

* See footnote to Table VI.

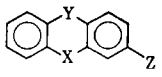
reemphasized by the results shown in Table XVIII. Several mercurial diuretics and other sulfhydryl-binding agents appear to inhibit strongly the enzyme from a number of sources (189). However, the incomplete reversal of inhibition by dithiothreitol throws some doubt on sulfhydryl binding as the only mechanism by which these compounds inhibit phosphodiesterase (189). Furthermore, acetazolamide and ethacrynic acid, with no apparent sulfhydryl-binding ability, still inhibit the enzyme (343, 344).

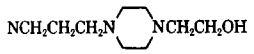
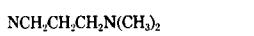
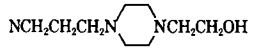
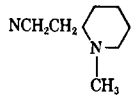
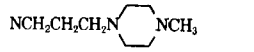
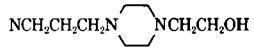
Psychotropic Agents—The reported ability of the vast majority of psychotropic agents to inhibit phosphodiesterase from a variety of tissues must be tempered by some additional observations:

1. Phenothiazine derivatives have also been reported to inhibit adenyl cyclase in brain (345) as well as other tissues (346).

2. Three tricyclic antidepressants, imipramine, desipramine, and amitriptyline, have been found to be potent stimulators of cyclic AMP formation in

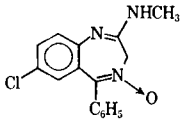
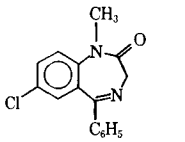
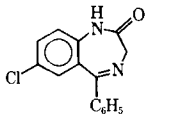
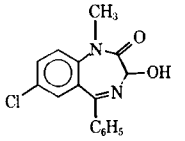
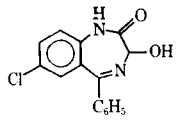
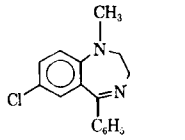
Table XIX—Psychotropic Agents: Tricyclic Derivatives



Name	X	Y	Z	Potency*	Enzyme Source	Inhibitor Concentration, moles/liter	Phosphodiesterase Form	Reference
Chlorpromazine	$NCH_2CH_2CH_2N(CH_3)_2$	S	Cl	170[b], 0.7[d]	Rat brain	—	II	297
				14[c]	Beef heart	5×10^{-5}	I	333
				50[c]	Rabbit brain	5×10^{-5}	I	333
				85[c]	Ox heart	1×10^{-3}	I	357
				80[a]	Rat brain	—	II	358, 359
				69[c]	Mouse brain	1×10^{-3}	I	186
Promazine	$NCH_2CH_2CH_2N(CH_3)_2$	S	H	2[c]	Beef heart	5×10^{-5}	I	333
				24[c]	Rabbit brain	5×10^{-5}	I	333
Fluphenazine		S	CF_3	48[b], 2.5[d]	Rat brain	—	II	297
				18[c]	Beef heart	5×10^{-5}	I	333
				64[c]	Rabbit brain	5×10^{-5}	I	333
Trifluprom azine		S	CF_3	300[b], 0.4[d]	Rat brain	—	II	297
				130[a]	Rat brain	—	II	358, 359
Perphenazine		S	Cl	14[c]	Beef heart	5×10^{-5}	I	333
				60[c]	Rabbit brain	5×10^{-5}	I	333
Thioridazine		S	SCH_3	180[b]	Rat brain	—	II	297
Prochlorperazine		S	Cl	14[c]	Beef heart	5×10^{-5}	I	333
				64[c]	Rabbit brain	5×10^{-5}	I	333
Chlorprothixene	$C{=}CHCH_2CH_2N(CH_3)_2$	S	Cl	170[a]	Rat brain	—	II	358, 359
Imipramine	$NCH_2CH_2CH_2N(CH_3)_2$	CH_2CH_2	H	222[a], 0.4[d]	Rat adipose	—	II	171
				765[a], 1.0[d]	Rat adipose	—	I	171
				430[d]	Rat brain	—	II	358, 359
				40[c]	Ox heart	1×10^{-3}	I	357
				700[b], 0.2[d]	Rat brain	—	II	297
				90[c,f]	Human brain	1×10^{-3}	I	178
				58[c]	Human platelets	1×10^{-3}	I	178
				36[c]	Mouse brain	5×10^{-3}	I	186
Desipramine	$NCH_2CH_2CH_2NHCH_3$	CH_2CH_2	H	45[c]	Ox heart	1×10^{-3}	I	357
				480[a]	Rat brain	—	II	358, 359
				36[c]	Mouse brain	5×10^{-3}	I	186
Trimepramine	$NCH_2CH(CH_3)CH_2N(CH_3)_2$	CH_2CH_2	H	55[c]	Ox heart	1×10^{-3}	I	357
Nortriptyline	$C{=}CHCH_2CH_2NHCH_3$	CH_2CH_2	H	70[c]	Ox heart	1×10^{-3}	I	357
				200[b], 0.6[d]	Rat brain	—	II	297
				86[c,f]	Human brain	1×10^{-3}	I	178
				34[c,g]	Human platelets	1×10^{-3}	I	178
				66[c]	Mouse brain	1×10^{-3}	I	186
Amitriptyline	$C{=}CHCH_2CH_2N(CH_3)_2$	CH_2CH_2	H	70[c]	Ox heart	1×10^{-3}	I	357
				460[b], 0.3[d]	Rat brain	—	II	297
				450[a]	Rat brain	—	II	358, 359
Opipramol		CH=CH	H	86[c,f]	Human brain	1×10^{-6}	I	178
				25[c,g]	Human platelets	1×10^{-6}	I	178
				80[c]	Ox heart	1×10^{-3}	I	357
Protriptyline	$CHCH_2CH_2CH_2NHCH_3$	CH=CH	H	65[c]	Ox heart	1×10^{-3}	I	357
Doxepin	$C{=}CHCH_2CH_2N(CH_3)_2$	O—CH_2	H	>1000[b]	Rat brain	—	II	297

* See footnote to Table VI.

Table XX—Psychotropic Agents: Benzodiazepine Derivatives (181)

Name	Structure	Potency*	Enzyme Source	Inhibitor Concentration, moles/liter	Phosphodiesterase Form
Chlordiazepoxide		29[c] 15[c] 18[c]	Cat temporal cortex Cat hippocampus Cat cerebellum	5×10^{-5} 5×10^{-5} 5×10^{-5}	II II II
Diazepam		55[c] 44[c] 42[c]	Cat temporal cortex Cat hippocampus Cat cerebellum	5×10^{-5} 5×10^{-5} 5×10^{-5}	II II II
N-Demethyldiazepam		34[c] 22[c] 15[c]	Cat temporal cortex Cat hippocampus Cat cerebellum	5×10^{-5} 5×10^{-5} 5×10^{-5}	II II II
N-Methyloxazepam		33[c] 19[c] 27[c]	Cat temporal cortex Cat hippocampus Cat cerebellum	5×10^{-5} 5×10^{-5} 5×10^{-5}	II II II
Oxazepam		15[c] 11[c] 11[c]	Cat temporal cortex Cat hippocampus Cat cerebellum	5×10^{-5} 5×10^{-5} 5×10^{-5}	II II II
Medazepam		18[b], 11.1[d] 25[b], 32[d] 15[b], 67[d]	Cat cortex Rat brain Neuroblastoma	— — —	II II II

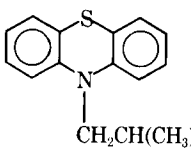
* See footnote to Table VI.

guinea pig cerebral slices (347) at doses where their phosphodiesterase inhibition is marginal; chlorpromazine and perphenazine had no significant effect on cyclic AMP levels. Theophylline at 10^{-3} *M* inhibited the effect of imipramine by >90% on cerebral slices (347). A similar situation was observed with adenosine (348–354). This antagonism may involve a competition between theophylline on the one hand and antidepressants or adenosine on the other for the receptor sites on phosphodiesterase, allowing the intracellular levels of cyclic AMP to rise through other mechanisms. Other workers reported that of nortriptyline, anitriptyline, desipramine, and imipramine, only the last had significant activity on ox heart Form II and it was less potent than caffeine (355). It is also possible that these compounds are more active on the activated enzyme, *i.e.*, in presence of the protein activator shown to be present in the brain and discussed previously.

3. Regarding a causal relationship between phosphodiesterase inhibition and the action of tricyclic antidepressants, it has been cautioned that, while levels of cyclic AMP in brain tissue may be of importance in regulating mental states, inhibition of brain phosphodiesterase by a drug does not necessarily confer antidepressant activity. A case in point is that of promethazine (XII), which is as potent an inhibitor of rat brain phosphodiesterase as certain tricyclic antidepressants but has no antidepressant effect *in vivo* (356).

A point-by-point interpretation of structure *versus* activity for the psychotropic agents would probably be fruitless as well as confusing. However, nearly all tricyclic psychotropic agents (Table XIX) have shown respectable potency as inhibitors of phosphodiesterase in most systems, depending on the concentration used.

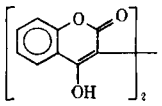
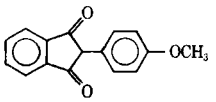
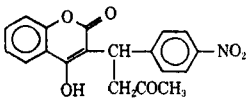
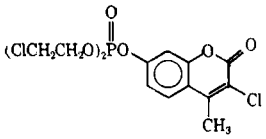
The minor tranquilizers of the chlordiazepoxide type (Table XX) have not been studied extensively as phosphodiesterase inhibitors but show considerable inhibition at low concentrations on the enzymes from the cat CNS (181). Haloperidol, a substituted butyrophenone, has invariably shown little potency on phosphodiesterases (297, 333, 358, 359).



$CH_2CH(CH_3)N(CH_3)_2$

XII: promethazine

Table XXI—Anticoagulants (363)

Name	Structure	Potency*	Enzyme Source	Phosphodiesterase Form
Dicumarol		35[b]	Human platelets	II
Anisindione		240[b]	Human platelets	II
Acenocoumarol		270[b]	Human platelets	II
Haloxan		10[b]	Human platelets	II

* See footnote to Table VI.

The relationship of cyclic AMP levels to brain function has been extensively reviewed (54, 177, 360–362).

Anticoagulants—Anticoagulants of the type shown in Table XXI may inhibit phosphodiesterase and raise platelet cyclic AMP levels (363); this has been shown to mediate platelet-aggregation inhibition (318, 364). Thus, these compounds may inhibit the formation of fibrin clots by their effects on prothrombin metabolism, and they may inhibit platelet aggregation by virtue of their phosphodiesterase inhibitory action.

Miscellaneous Heterocyclic Compounds of Synthetic Origin—A number of heterocyclic compounds with important pharmacological profiles have been found to be strong inhibitors of phosphodiesterase (Table XXII). Dipyridamole, a derivative of pyrimido[5,4-*d*]pyrimidine which was introduced several years ago as a coronary vasodilator, is a potent inhibitor of platelet aggregation and a very effective phosphodiesterase inhibitor. Nevertheless, it is generally less potent than papaverine (94, 181, 316, 318, 325). Although it showed considerable selectivity for human platelet over human brain phosphodiesterase (178), dipyridamole was less potent than its congener RA 233 on the human platelet enzyme (365). RA 233, which lacks one of the two equivalent piperidino moieties found in dipyridamole, was particularly effective against the human platelet enzyme (322, 365).

A thieno[3,2-*d*]pyrimidine derivative[5] was equipotent to dipyridamole on human platelet Form I but was less potent than the dipyridamole relative, RA 233. There was no correlation between the action of any one of these three pyrimidine derivatives and the inhibitory effect on ADP-induced platelet aggregation (365).

[b] VK 744.

SQ 20,009, a substituted pyrazolo[3,4-*b*]pyridine, is a potent inhibitor of rat brain Form II (213). Being considerably more potent on brain phosphodiesterase than on the heart, lipocyte, or adrenal enzymes (213), it is perhaps not surprising that it possesses marked antianxiety activity (297).

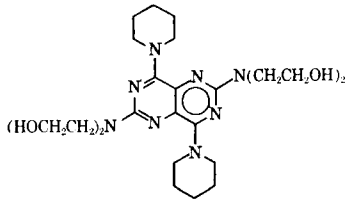
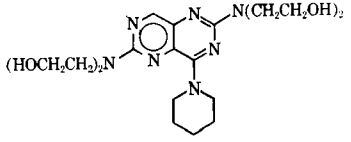
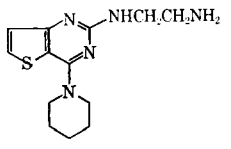
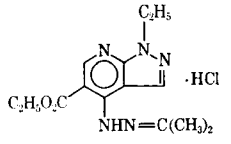
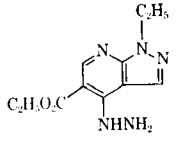
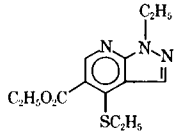
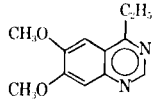
Two close relatives of SQ 20,009 also possess the pyrazolopyridine skeleton. SK 20,006, which lacks only the 4-isopropylidine moiety, was less potent than SQ 20,009 on rat brain and rat adrenal phosphodiesterase but more potent on the rat lipocyte enzyme (213). The 4-ethylthio analog, SQ 65,442, was more potent than theophylline on cockroach brain Form II (35).

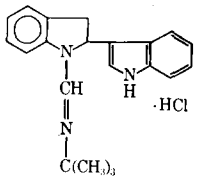
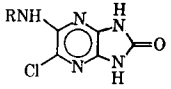
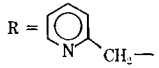
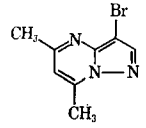
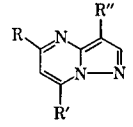
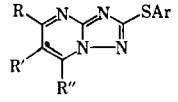
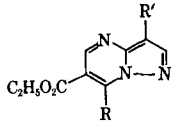
The bronchodilator–vasodilator compound known generically as quazodine is an effective inhibitor of phosphodiesterase from a number of tissues. It has a pharmacological profile similar to that of theophylline and was approximately twice as potent as the latter on phosphodiesterase from bovine thyroid (301) and a variety of rabbit tissues (176); it was more than 6 times as potent as theophylline on the enzyme from bovine brain (176) and 16 times more potent on human platelet Form I (318).

The interesting diuretic properties of MJ 8592-1, an indolylindoline (376), prompted a study of its activity on phosphodiesterase. Although it was not especially potent on phosphodiesterase from a variety of guinea pig and rat tissues, it preferentially inhibited the kidney enzyme in both species. This selective inhibition of phosphodiesterase was proposed as a mechanism for the diuretic effects of MJ 8592-1 (175).

CEIP, CDIP, and a pyridylmethyl analog are imidazo[4,5-*b*]pyrazine derivatives which were studied as hypotensives and inhibitors of phosphodiesterase (366). CEIP, which also has bronchodilator, cardiac stimulant, and peripheral vasodilator properties, is

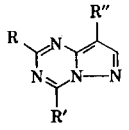
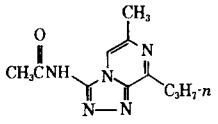
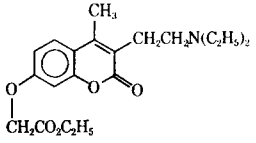
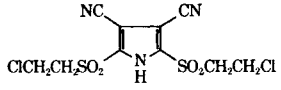
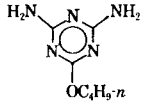
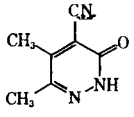
Table XXII—Miscellaneous Heterocyclic Compounds of Synthetic Origin

Name	Structure	Potency	Enzyme Source	Inhibitor Concentration, moles/liter	Phosphodiesterase Form	Reference
Dipyridamole		22[c,g]	Human brain	1×10^{-5}	I	178
		85[c,f]	Human platelets	1×10^{-5}	I	178
		>1.0[d]	Ox heart, rabbit brain, human platelets	—	II	322
		45[c]	Human platelets	1.2×10^{-4}	I	365
		62[a], 0.4[e]	Beef heart	—	I	316
		17[a], 0.3[e]	Human platelets	—	I	318
		15[b], 0.8[e]	Cat cortex	—	II	181
		12[b], 0.3[e]	Rat brain	—	II	181
		1.5[b], 3.7[e]	Neuroblastoma cell	—	II	181
		16[a], 0.35[e]	Beef heart	—	I	94, 325
RA 233		>1.0[d]	Ox heart, rabbit brain, human platelets	—	II	322
		55[c]	Human platelets	1.2×10^{-4}	I	365
VK 744		45[c]	Human platelets	1.2×10^{-4}	I	365
SQ 20,009		2[a]	Rat brain	—	II	213
		2[b]	Rat brain	—	II	297
		4.8[b]	Rabbit brain	—	II	213
		20[b]	Rat adrenal	—	II	213
		21[b]	Rat lipocytes	—	II	213
		64[a]	Cat heart	—	II	213
SQ 20,006		~10[b]	Rat lipocytes	—	II	213
		100[b], 0.1[e]	Cat cortex	—	II	181
		30[b], 0.2[e]	Neuroblastoma cell	—	II	181
		53[b], 0.07[e]	Rat brain	—	II	181
		50[b]	Cat heart	—	II	213
SQ 65,442		80[c,f]	Cockroach brain	1×10^{-4}	II	35
Quazodine		361[a], 2.5[d]	Rabbit lung	—	I	176
		1331[a], 1.7[d]	Rabbit heart	—	I	176
		181[a], 1.8[d]	Rabbit liver	—	I	176
		1540[a], 1.2[d]	Rabbit spleen	—	I	176
		800[a], 1.9[d]	Rabbit kidney	—	I	176
		1290[a], 1.7[d]	Rabbit pyloric mucosa	—	I	176
		773[a], 1.3[d]	Rabbit fundic mucosa	—	I	176

		116[a], 6.5[d]	Bovine brain	—	I	176
		59[c,f]	Bovine thyroid	1×10^{-4}	II	301
		50[a], 0.1[e]	Human platelets	—	I	318
MJ 8592-1		2700[a], 0.5[d]	Guinea pig brain	—	II	175
		Inactive	Guinea pig heart	—	II	175
		2000[a], 1.4[d]	Guinea pig kidney	—	II	175
		Inactive	Guinea pig liver	—	II	175
		11,000[a], 0.9[d]	Guinea pig lung	—	II	175
		60,000[a], 0.03[d]	Rat brain	—	II	175
		Inactive	Rat heart	—	II	175
		3400[a], 0.38[d]	Rat kidney	—	II	175
		10,000[a], 0.1[d]	Rat liver	—	II	175
		Inactive	Rat lung	—	II	175
						
CEIP	R = C_2H_5—	70[c,f]	Bovine heart	1×10^{-3}	I	366
		60[c,f]	Bovine heart	1×10^{-3}	I	182
		52[c,f]	Rabbit uterus	1×10^{-3}	I	182
		62[c,f]	Bovine pancreas	1×10^{-3}	I	182
		52[c,f]	Rat lung	1×10^{-3}	I	182
		54[c,f]	Rat heart	1×10^{-3}	I	182
CDIP	R = $(CH_3)_2NCH_2CH_2$	19[c,g]	Bovine heart	1×10^{-3}	I	366
		7[c,g]	Rabbit uterus	1×10^{-3}	I	182
		Inactive	Rat lung	1×10^{-3}	I	182
		8[c,g]	Rat heart	1×10^{-3}	I	182
		28[c,f]	Bovine heart	1×10^{-3}	I	366
ICN 3009		123[b]	Rabbit kidney	—	II	367
		140[b]	Rabbit lung	—	II	367
		60[b]	Bovine brain	—	II	367
		210[b]	Beef heart	—	II	367
3,5,7-Trisubstituted pyrazolo[1,5-*a*]-pyrimidines		≫1.0[d]	Rabbit lung, beef heart	—	II	368
2-Aralkylthio-*s*-triazolo-[1,5-*a*]pyrimidines		>1.0[d]	Rabbit lung, rabbit kidney	—	II	369
6-Carbethoxy-3,7-di-substituted pyrazolo-[1,5-*a*]pyrimidines		≫1.0[d]	Rabbit lung, rabbit kidney	—	II	370

(*continued*)

Table XXII—(*Continued*)

Name	Structure	Potency	Enzyme Source	Inhibitor Concentration, moles/liter	Phospho-diesterase Form	Reference
3,5,7-Trisubstituted pyrazolo[1,5-*a*]1,3,5-triazines		≫1.0[d]	Rabbit lung	—	II	371
ICI 58,301		62[a], 4.2[d]	Bovine heart	—	I	372, 373
Chromonar (Carbocromene)		71[c,f] 1400[a], 0.004[e]	Rat heart Beef coronary artery	1×10^{-3} —	I I	291, 324 94, 325
A		75[c,f]	Rat adipose tissue	1×10^{-3}	I	375
B		24[c,g]	Rat adipose tissue	5×10^{-3}	I	375
C		21[c,g]	Rat adipose tissue	5×10^{-3}	I	375

* See footnote to Table VI.

Table XXIII—Miscellaneous Purine and Pyrimidine Bases, Nucleosides, and Nucleotides

Name	Potency*	Enzyme Source	Inhibitor Concentration, moles/liter	Phosphodiesterase Form	Reference
		Bases and Derivatives			
Purine	12[c]	Toad bladder epithelium	5×10^{-3}	I	379
Adenine	200[b]	Rat skeletal muscle	—	I	380
	44[c,g]	Rat adipose tissue	1×10^{-2}	I	381
	~10[c,g]	Beef heart	1×10^{-3}	I	222
	35[c]	Toad bladder epithelium	2.25×10^{-3}	I	379
	112[a]	Rat skeletal muscle	—	I	380
	29[c]	Isolated rat fat cell	1×10^{-3}	II	383
	21[c,g]	Rabbit skeletal muscle	1×10^{-3}	II	199
6-Hydroxypurine	14[c,g]	Rabbit skeletal muscle	1×10^{-3}	II	199
6-Dimethylaminopurine	49[c,g]	Rabbit skeletal muscle	1×10^{-3}	II	199
	~800[a]	Rat diaphragm	—	I	382
Puromycin	800[a]	Rat diaphragm	—	I	382
Mercaptopurine	76[c,f]	Human platelets	1×10^{-3}	I	178
	76[c,f]	Human brain	1×10^{-3}	I	178
Guanine	10[c]	Beef heart	1×10^{-3}	I	222
Hypoxanthine	210[b]	Rat skeletal muscle	—	I	380
Xanthine	142[b]	Rat skeletal muscle	—	I	380
		Nucleosides			
Purine riboside	Inactive	Rat skeletal muscle	—	I	380
Adenosine	30[c]	Rat fat cells	1×10^{-3}	II	383
	52[c]	Toad bladder epithelium	5×10^{-3}	I	379
	46[c,g]	Rat adipose tissue	1×10^{-2}	I	381
	10[c]	Beef heart	1×10^{-3}	I	222
	200[b]	Rat skeletal muscle	—	I	380
	25[c,g]	Rabbit skeletal muscle	1×10^{-3}	II	199
	Inactive	Pea seedlings	1×10^{-4}	II	74
	19[c]	Mouse brain	1×10^{-3}	I	185
	21[c,h]	Rat fat cells	7×10^{-5}	II	384
Deoxyadenosine	55[c]	Rat fat cells	1×10^{-3}	II	383
	59[c,f]	Rat adipose tissue	1×10^{-2}	I	381
	24[c]	Mouse brain	1×10^{-3}	I	186
	31[c,h]	Rat fat cells	7×10^{-5}	II	384
2-Fluoroadenosine	26[c,h]	Rat fat cells	7×10^{-5}	II	384
Guanosine	24[c]	Rat fat cells	5×10^{-4}	II	383
	33[c,g]	Rat adipose tissue	2×10^{-3}	I	381
	10[c]	Beef heart	1×10^{-3}	I	222
	43[c]	Mouse brain	1×10^{-3}	I	186
Desoxyguanosine	73[c]	Mouse brain	1×10^{-3}	I	186
Inosine	342[b]	Rat skeletal muscle	—	I	380
	23[c,g]	Rat adipose tissue	2×10^{-3}	I	381
	10[c]	Toad bladder epithelium	5×10^{-3}	I	379
	24[c]	Mouse brain	1×10^{-3}	I	185
Xanthosine	162[b]	Rat skeletal muscle	—	I	380
	14[c,g]	Rat adipose tissue	2×10^{-3}	I	381
Thymidine	22[c,g]	Rat adipose tissue	1×10^{-2}	I	381
Uridine	15[c,g]	Rat adipose tissue	1×10^{-2}	I	381
Cytidine	8[c,g]	Rat adipose tissue	1×10^{-2}	I	381
		Nucleotides			
5′-GMP	Activates	Beef heart	1×10^{-3}	I	222
5′-AMP	Inactive	Beef heart	1×10^{-3}	I	222
5′-IMP	Inactive	Beef heart	1×10^{-3}	I	222
ATP	18[c]	Toad bladder epithelium	5×10^{-3}	I	379
ADP	18[c]	Toad bladder epithelium	5×10^{-3}	I	379

* See footnote to Table VI.

reportedly 2–5 times more potent than theophylline as an inhibitor of phosphodiesterase from several sources; *ex vivo* it produced up to a 10-fold increase in the cyclic AMP content of rat heart. Analogs of CEIP with more basic substituents were generally less potent (182). Although CDIP is less active than theophylline as a phosphodiesterase inhibitor *in vitro*, its *in vivo* vasodilator and bronchodilator activities are of interest (182).

Several structurally related series of fused-ring heterocycles were recently reported as inhibitors of phosphodiesterase (368–371, 377). A number of 3,5,7-trisubstituted pyrazolo[1,5-*a*]pyrimidines were considerably more potent than theophylline as inhibitors of Form II from rabbit lung and beef heart (368). One member of this series, ICN 3009, was reported to have interesting cardiotropic properties (267). Members of a series of 6-carbethoxy-3,7-disubstituted pyrazolo[1,5-*a*]pyrimidines were also considerably more potent than theophylline on Form II from rabbit lung and rabbit kidney (370).

Many members of a series of 2-aralkylthio-*s*-triazolo[1,5-*a*]pyrimidines showed significant activity on Form II of rabbit lung and kidney (369). Various 3,5,7-trisubstituted pyrazolo[1,5-*a*]-1,3,5-triazines were found to be 50–150 times more active than theophylline on Form II from rabbit lung (371); some exhibited a specificity toward lung enzyme and several

were more potent as inhibitors of cyclic AMP phosphodiesterase than of cyclic GMP phosphodiesterase (377).

ICI 58,301 is a *sym*-triazolo[4,3-*a*]pyrazine derivative and a potent inhibitor of bovine heart Form I *in vitro* (372, 373); preliminary *ex vivo* experiments with orally dosed guinea pigs indicated greater selectivity for the lung than for the heart enzyme. The effects of ICI 58,301 in reducing the degree of bronchospasm in guinea pigs could be attributed to increased levels of cyclic AMP due to phosphodiesterase inhibition (373).

Chromonar, a coronary vasodilator, is a coumarin derivative with inhibitory properties on Form I from rat heart (291, 374) and beef coronary artery (94, 325). A metabolite[6] of chromonar is only weakly active on the rat heart enzyme (291, 374).

Three lipolytic agents of widely differing structure (Compounds A, B, and C, Table XXII) have shown activity on Form I from rat adipose tissue (375).

Two benzo[*b*]thiophene derivatives, 5-chloro-3-(2-dimethylaminoethyl)benzo[*b*]thiophene hydrochloride and *N*-(4-bromobenzo[*b*]thien-2-yl)guanidinium *p*-toluenesulfonate (not tabulated), are representatives of a number of benzo[*b*]thiophene derivatives with secondary or tertiary amine side chains which reportedly show marked noncompetitive inhibition of ox heart Form I (378).

Miscellaneous Purine and Pyrimidine Bases, Nucleosides, and Nucleotides—Table XXIII includes a number of miscellaneous nucleoside derivatives that are frequently present or added to incubation mixtures where phosphodiesterase activity is measured. Their effects on phosphodiesterase assays are often disregarded, in spite of their sometimes strong inhibitory actions on the enzyme.

CONCLUSIONS

A brief exposure to the structure–activity relationships of phosphodiesterase inhibitors might tempt one to conclude that all useful drugs inhibit phosphodiesterases. While this is certainly not the case (296–298), there appears to be a correlation, if not a causal relationship, between the *in vitro* phosphodiesterase activities of a great many drugs and their *in vivo* effects. It is obvious, after scanning the tabulated material, that no single structural skeleton or moiety is sufficient to confer the action of phosphodiesterase inhibition on a substance, Rather, it is clear that compounds of widely differing structures may show phosphodiesterase inhibition. It is, however, also readily apparent that certain structural moieties, particularly amidine (N—C=N), guanidine [N—C(=N)—N], amidrazone (N—N—C=N), and 3,4-disubstituted phenethylamine, which appear so frequently in useful drug entities, are also common to a great many inhibitors of phosphodiesterases.

This preponderant phosphodiesterase inhibition among various drug classes is perhaps not unexpected since cyclic nucleotides appear to be involved in the control of nearly all facets of cellular activity and metabolism in both healthy and disease states. The cyclic nucleotides, therefore, understandably present a worthy target for the action of drugs. The association between drugs and cyclic nucleotides is strong and vital to the successful mission of both in maintaining health.

[6] Intensainsäure.

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RESEARCH ARTICLES

Decomposition of *p*-Aminosalicylic Acid in the Solid State

J. T. CARSTENSEN [x] and PAKDEE POTHISIRI

Abstract □ Aminosalicylic acid was used as a test substance to show that a solid-state decomposition governed by nucleation kinetics can give rise to first-order kinetics. The effect of sorbed moisture at a low degree of coverage was tested. Sorbed moisture theories proposed previously apply when it is realized that the rate constants in the presence of the sorbed layer may differ from the values of these parameters under anhydrous conditions.

Keyphrases □ Decomposition—aminosalicylic acid in solid state giving rise to first-order kinetics, sorbed moisture theories discussed □ Aminosalicylic acid—decomposition in the solid state, first-order decomposition patterns □ Stability—decomposition of aminosalicylic acid in solid state, sorbed moisture theories discussed, first-order decomposition patterns □ Moisture—decomposition of aminosalicylic acid in solid state

The problems of stability of solid dosage forms have been discussed, although not with great frequency, in the pharmaceutical literature. Reference is made in this report to two basic articles (1, 2). For more complete references, the reader is referred to reviews on the subject (3, 4).

The problem of how to extrapolate pharmaceutical stability data is of importance in excess determination and in label statements on expiration dating; to this end, it is necessary to know prior to extrapolation [or to determine from the data (5)] if the decomposition is zero or first order.

The stability of the drug without excipients and the stability of the drug in the presence of moisture (6) (but no other excipients) throw some light on the theoretical order of reaction and the actual mode of decomposition. In the absence of moisture, topochemical- and nucleation-governed reactions occur (3, 7, 8); of these, *some* topochemical patterns may be approximated by first-order decay (3, 9). In the absence of moisture, the more common decomposition