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Perspective

A New Generation of Phosphodiesterase Inhibitors: Multiple Molecular Forms of Phosphodiesterase and the Potential for Drug Selectivity

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In 1960, Sutherland first described the role of cyclic AMP as a "second messenger", mediating the response of cells to a variety of hormones and neurotransmitters.[1] Sutherland and his contemporaries subsequently demonstrated the importance of cyclic AMP in the regulation of a variety of metabolic processes, including cardiac and smooth muscle contractility, glycogenolysis, platelet aggregation, secretion, and lipolysis.[2-9] The reader desiring additional information on the involvement of cyclic AMP with these processes is directed to any of several excellent reviews.[10-13] The physiological importance of cyclic GMP, on the other hand, remains largely a mystery, and early suggestions that cyclic GMP acts in opposition to cyclic AMP (the "Yin-and-Yang" hypothesis[14]) have in many cases been discounted.[15-17]

The importance of Sutherland's observation was quickly recognized by many medicinal chemists and pharmacologists who subsequently synthesized and evaluated a variety of cyclic AMP analogues, β-receptor stimulants, and inhibitors of phosphodiesterase, the enzyme that is responsible for cyclic AMP degradation within the cell, in an attempt to discover agents that would mimic the effects of cyclic AMP.[18-24] In most instances these agents have been of more theoretical than practical value, since they typically reproduced the desirable as well as the undesirable effects of cyclic AMP, and, in the case of the phosphodiesterase inhibitors, many of the agents that have been synthesized have lacked potency as well as specificity. Thus, although over two decades have passed since Sutherland's reports were first published, there are at the present time only a limited number of useful therapeutic agents available that are obvious extensions of the original principle.

Within the last few years several discoveries have prompted a renewal of interest in the area of modulators of cyclic nucleotide metabolism as therapeutic agents. This resurgence has been stimulated in large part by the work of Thompson, Hidaka, Wells, and others, who demonstrated that rather than existing as a single intracellular enzyme, there are actually several distinct molecular forms of phosphodiesterase present within the cell, which vary according to substrate specificity (cyclic AMP vs. cyclic GMP), intracellular location (soluble vs. membrane bound), and response to calmodulin.[25-31] With several

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(1) Sutherland, E. W.; Rall, T. W. *Pharmacol. Rev.* **1960**, *12*, 265.
(2) Rodbell, M. *J. Biol. Chem.* **1964**, *239*, 375.
(3) Levine, R. A. *Gastroenterology* **1970**, *59*, 280.
(4) Sulzman, E. W.; Levine, L. *J. Clin. Invest.* **1971**, *90*, 131.
(5) Montague, W.; Howell, S. L. *Biochem J.* **1972**, *129*, 551.
(6) Pöch, G.; Kukovetz, W. R. *Adv. Cyclic Nucleotide Res.* **1972**, *1*, 195.
(7) Sobel, B. E.; Mayer, S. E. *Circ. Res.* **1973**, *32*, 407.
(8) Phillis, J. W. *Can. J. Neurol. Sci.* **1977**, *4*, 153.
(9) Butcher, R. W.; Sneyd, J. G. T.; Park, C. R.; Sutherland, E. W. *J. Biol. Chem.* **1966**, *241*, 1651.
(10) Fain, J. N. In "Cyclic Nucleotides: Mechanisms of Action"; Cramer, H., Schultz, J., Eds.; Wiley: London, 1977.
(11) Rasmussen, H.; Goodman, D. B. P. *Physiol. Rev.* **1977**, *57*, 421.
(12) Tsien, R. W. *Adv. Cyclic Nucleotide Res.* **1977**, *8*, 363.
(13) Adelstein, R. S.; Hathaway, D. R. *Am. J. Cardiol.* **1979**, *44*, 783.
(14) Goldberg, N. D.; Haddox, M. K.; Nicol, S. E.; Glass, D. B.; Sanford, C. H.; Kuehl, F. A.; Estensen, R. *Adv. Cyclic Nucleotide Res.* **1975**, *5*, 307.
(15) Murad, F. *Adv. Cyclic Nucleotide Res.* **1979**, *11*, 175.
(16) Diamond, J.; Teneick, R. E.; Trapani, A. J. *Biochem. Biophys. Res. Commun.* **1977**, *79*, 912.
(17) Keely, S. L.; Lincoln, T. M.; Corbin, J. D. *Am. J. Physiol.* **1978**, *234*, 432.
(18) Weinryb, I.; Chasin, M.; Free, C. A.; Harris, D. N.; Goldberg, H.; Michel, I. M.; Pail, V. S.; Phillips, M.; Samaniego, S.; Hess, S. M. *J. Pharmacol. Sci.* **1972**, *61*, 1556.
(19) Simon, L. N.; Shuman, D. A.; Robins, R. K. *Adv. Cyclic Nucleotide Res.* **1973**, *3*, 225.
(20) Meyer, R. B.; Miller, J. B. *Life Sci.* **1974**, *14*, 1019.
(21) Lippman, W. *Experimentia* **1974**, *30*, 237.
(22) Hoefle, M. L.; Hastings, S. G.; Meyer, R. F.; Corey, R. M.; Holmes, A.; Stratton, C. D. *J. Med. Chem.* **1975**, *18*, 148.
(23) Evans, D. B.; Parham, C. S.; Schenck, M. T.; Laffan, R. J. *J. Cyclic Nucleotide Res.* **1976**, *2*, 307.
(24) Berndt, S. F.; Schulz, H.-U.; Stock, K. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1976**, *294*, 271.
(25) Thompson, W. J.; Appleman, M. M. *Biochemistry* **1971**, *10*, 311.
(26) Beavo, J. A.; Hardman, J. G.; Sutherland, E. W. *J. Biol. Chem.* **1971**, *246*, 3841.
(27) Wells, J. N.; Baird, C. E.; Wu, Y. J.; Hardman, J. G. *Biochim. Biophys. Acta* **1975**, *384*, 430.

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Table I. Kinetic Characterization of Phosphodiesterases from Guinea Pig Left Ventricular Muscle[a]

PDE type	substrate	K_m, μM		V_{max}, pmol min^{-1} μg^{-1}		effect of calmodulin
		high affinity	low affinity	high affinity	low affinity	
I	cyclic AMP	0.8	63.8	5.2	30.6	calmodulin stimulates
	cyclic GMP	1.1	63.3	5.1	57.6	calmodulin stimulates
II	cyclic AMP	...[b]	20.5	...[b]	65.5	no effect
	cyclic GMP	...[b]	21.5	...[b]	56.2	no effect
III	cyclic AMP	1.0	10.7	3.6	6.7	no effect
	cyclic GMP[c]	...	...	...	...	...

[a] Data were taken from ref 46. K_m and V_{max} values were determined by plotting reaction velocity vs. reaction velocity/substrate concentration, according to the method described by Hofstee.[47] [b] There is no high affinity site for hydrolysis of cyclic AMP or cyclic GMP by PDE II. [c] PDE III does not hydrolyze cyclic GMP to any appreciable degree.

notable exceptions, this insight was largely ignored by pharmacologists and medicinal chemists until it was demonstrated that these different molecular forms of phosphodiesterase can vary from one organ or cell type to another[32,33] and that the different forms can be selectively inhibited by various therapeutic agents.[33-36] Thus the potential now exists that agents can be developed that will reproduce the desirable effects of cyclic nucleotides, such as bronchodilation or increased myocardial contractility, and yet be free of the undesirable effects, e.g., increased heart rate or enhanced lipolysis.

This Perspective will examine the evidence for the existence of multiple molecular forms of phosphodiesterase within the cell, and will discuss the degree to which these different forms vary from cell type to cell type. In addition, the effects of reference phosphodiesterase inhibitors, such as the methylxanthines and papaverine, on the different molecular forms of phosphodiesterase as well as the inhibitory effects of several recently described selective phosphodiesterase inhibitors will also be discussed. Finally, the therapeutic potential for this new generation of selective phosphodiesterase inhibitors will be addressed.

Evidence for Multiple Molecular Forms of Phosphodiesterase

Credit for the initial report of multiple molecular forms of cyclic nucleotide phosphodiesterase (PDE) is generally given to Thompson and Appleman,[25] who in 1971 identified three forms of PDE in rat brain cortex. Since that time multiple molecular forms of PDE have been identified in a number of other tissues, using a variety of isolation methods. These methods include centrifugation,[37] gel electrophoresis,[38] isoelectric focusing,[39] and anion-exchange chromatography.[40]

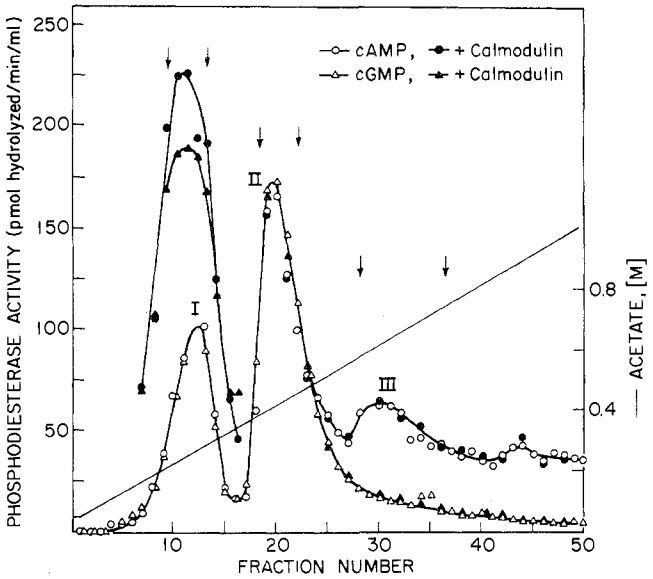


Figure 1. Separation of multiple molecular forms of phosphodiesterase using diethylaminoethyl (DEAE)-cellulose anion-exchange chromatography. Column fractions (~8 mL) were directly assayed for enzyme activity with use of either 1.0 μM ^{3}H-labeled cyclic AMP (○) or 1.0 μM ^{3}H-labeled cyclic GMP (△) as substrate. Filled symbols refer to enzyme activity in the presence of 0.1 U of calmodulin and 10 μM $CaCl_2$.

The initial excitement surrounding the identification of multiple forms of phosphodiesterase was tempered by reports that some forms of PDE were interconvertible in cell-free systems. In 1978, Epstein et al. concluded that a single form of phosphodiesterase isolated from rat uterus could be converted by trypsin treatment into two forms of PDE with different kinetic properties and substrate specificities.[41] Keravis and co-workers, however, were unable to duplicate Epstein's observation and suggested that the method used by Epstein to isolate phosphodiesterase was unable to resolve molecular forms of the enzyme prior to trypsin treatment.[42] The lack of uniformity with respect to isolation procedures described by Keravis has often made it difficult to compare the results obtained in different laboratories with regard to the number of phosphodiesterases present in various tissues. Thus, whereas in 1973 Amer and Mayol described the existence of two forms of PDE in human platelets,[37] a later study by Hidaka et al. showed that human platelets actually contained three forms of phosphodiesterase.[29] Similar discrepancies have also been reported for myocardial tissue[36,40,43] and for tracheal smooth muscle.[44,45] These

(28) Appleman, M. M.; Terasaki, W. L. *Adv. Cyclic Nucleotide Res.* **1975**, *5*, 153.
(29) Hidaka, H.; Asano, T. *Biochim. Biophys. Acta* **1976**, *429*, 485.
(30) Weiss, B.; Hait, W. N. *Ann. Rev. Pharmacol. Toxicol.* **1977**, *17*, 441.
(31) Bergstrand, H.; Lundquist, B.; Schurmann, A. *J. Biol. Chem.* **1978**, *253*, 1881.
(32) Wells, J. N.; Hardman, J. G. *Adv. Cyclic Nucleotide Res.* **1977**, *8*, 119.
(33) Hidaka, H.; Endo, T. *Adv. Cyclic Nucleotide Res.* **1984**, *16*, 245.
(34) Bergstrand, H.; Kristofferson, J.; Lundquist, B.; Schurmann, A. *Mol. Pharmacol.* **1977**, *13*, 38.
(35) Kariya, T.; Willie, L. J.; Dage, R. C. *J. Cardiovasc. Pharmacol.* **1982**, *4*, 509.
(36) Weishaar, R. E.; Quade, M.; Schenden, J. A.; Boyd, D. K.; Evans, D. B. *Pharacologist* **1983**, *25*, 551.
(37) Amer, M. S.; Mayol, R. F. *Biochim. Biophys. Acta* **1973**, *309*, 149.
(38) Weiss, B.; Fertel, R.; Figlin, R.; Uzunov, P. *Mol. Pharmacol.* **1974**, *10*, 615.
(39) Nemoz, G.; Prigent, A.-N.; Pico, M.; Pacheco, H. *Biochem. Pharmacol.* **1982**, *31*, 3353.
(40) Thompson, W. J.; Terasaki, W. L.; Epstein, P. M.; Strada, S. J. *Adv. Cyclic Nucleotide Res.* **1979**, *10*, 69.
(41) Epstein, P. M.; Pledger, W. J.; Gardner, E. A.; Stancel, G. M.; Thompson, W. J.; Strada, S. J. *Biochim. Biophys. Acta* **1978**, *527*, 442.
(42) Keravis, T. M.; Wells, J. N.; Hardman, J. G. *Biochim. Biophys. Acta* **1980**, *613*, 116.

Table II. Occurrence of Multiple Molecular Forms of Phosphodiesterase in a Number of Tissues and Cells

organ or cell type	no. of phospho-diester-ases	characteristics of phosphodiesterases	ref
human platelets	3	Low K_m, cyclic GMP specific, not stimulated by calmodulin High K_m, comparable affinity for cyclic AMP and cyclic GMP, not stimulated by calmodulin Low K_m, cyclic AMP specific, not stimulated by calmodulin	29, 33, 46, 49
rat ventricle	3	Low K_m, cyclic GMP selective, stimulated by calmodulin High K_m, comparable affinity for cyclic AMP and cyclic EMP, not stimulated by calmodulin Low K_m, cyclic AMP specific, not stimulated by calmodulin	28, 40, 50
guinea pig ventricle	3	Low K_m, comparable affinity for cyclic GMP and cyclic AMP, stimulated by calmodulin High K_m, comparable affinity for cyclic AMP and cyclic GMP, not stimulated by calmodulin Low K_m, cyclic AMP specific, not stimulated by calmodulin	46, 51
porcine coronary arteries	2	Low K_m, cyclic GMP selective, stimulated by calmodulin Low K_m, cyclic AMP specific, not stimulated by calmodulin	27
human aorta	3	High affinity, cyclic GMP specific, not stimulated by calmodulin Cyclic GMP selective, stimulated by calmodulin High affinity, cyclic AMP specific, not stimulated by calmodulin	33
human lung	3	Low K_m, cyclic GMP specific, stimulated by calmodulin High K_m, comparable affinity for cyclic GMP and cyclic AMP, not stimulated by calmodulin Low K_m, cyclic AMP specific, not stimulated by calmodulin	52, 53
rat liver	3	Low K_m, cyclic GMP selective, stimulated by calmodulin High K_m, comparable affinity for cyclic AMP and cyclic GMP, not stimulated by calmodulin, stimulated by low concentrations of cyclic GMP Low K_m, cyclic AMP selective, not stimulated by calmodulin	28
lymphocyte	1	Low K_m, cyclic AMP specific	54
monocytes	1	Low K_m, cyclic AMP specific	54

difficulties have been largely overcome by the adoption of diethylaminoethyl (DEAE)-cellulose anion-exchange chromatography for isolating the multiple forms of PDE. As described by Thompson and co-workers,[40] this procedure is reliable and straightforward, yielding reasonable quantities of phosphodiesterases that are quite stable.

An example of this method of separation is shown in Figure 1, in which the three molecular forms of PDE present in guinea pig left venticular tissue were discretely eluted from a DEAE column using a sodium acetate gradient. Cross-contamination can be eliminated by chromatography of pooled fractions of each peak.[40] In addition to the differences in substrate specificity that are apparent in Figure 1, the three molecular forms of cardiac PDE also vary according to their kinetic characteristics (K_m and V_{max}) and their ability to be stimulated by calmodulin. The kinetic characteristics of the three molecular forms of cardiac phosphodiesterase present in guinea pig ventricular muscle are summarized in Table I.

It is of interest to note that whereas in rat ventricular muscle type I phosphodiesterase is essentially a cyclic GMP selective enzyme,[40] in guinea pig ventricular muscle K_m and V_{max} values for cyclic GMP and for cyclic AMP hydrolysis by calmodulin-stimulated PDE (PDE I) are essentially the same. We have also observed that the PDE I in guinea pig atrial muscle likewise displays no clear preference for cyclic AMP or cyclic GMP.[48] In addition to differences in the substrate specificity of a particular form of phosphodiesterase, differences in the total number of phosphodiesterases present within the cell have also been described. Thus, whereas three molecular forms of PDE are present in cardiac muscle, in smooth muscle only two forms of phosphodiesterase have been identified.[27,46] The relevance of the different molecular forms of phosphodiesterase to the physiological control of cyclic AMP and cyclic GMP will be discussed in a later section of this Perspective.

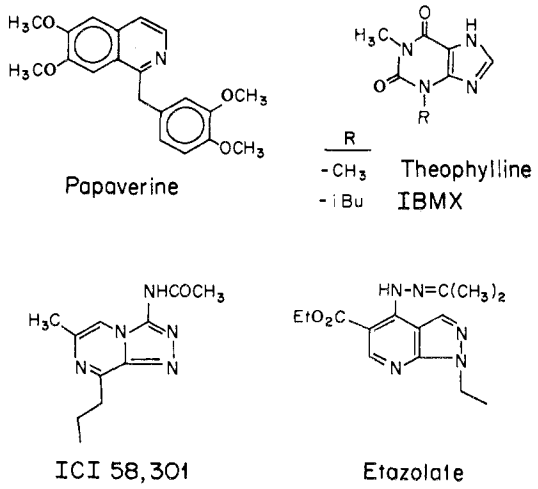
Since the primary focus of this Perspective is the concept of selective inhibitors of the different molecular forms of phosphodiesterase, a rigorous examination of all the different organs and cells in which multiple forms of phosphodiesterase have been identified will not be included. A compilation of information regarding the occurrence of multiple molecular forms of phosphodiesterase in a number of tissues and cells, as well as literature citations for obtaining additional information, is provided in Table II. As was previously mentioned, however, differences in the isolation procedure employed by various laboratories may make quantitative and qualitative comparisons of such information difficult.

Classes of Agents Characterized as Phosphodiesterase Inhibitors: Evolution from Nonselective to Selective Inhibition of Different Forms of Phosphodiesterases

Inhibition of phosphodiesterase activity has been reported for many different classes of compounds.[18] These include the prototypical nonselective inhibitors papaverine,[55] theophylline,[55] and 3-isobutyl-1-methylxanthine

(43) Tkachuk, V. A.; Lazarevich, V. G.; Severin, S. E. *Adv. Myocardiol.* **1982**, *3*, 541.
(44) Polson, J. B.; Krzanowski, J. J.; Szentivanyi, A. *Biochem. Pharmacol.* **1982**, *31*, 3403.
(45) Fredholm, B. B.; Brodin, K.; Strandberg, K. *Acta Pharmacol. Toxicol.* **1979**, *45*, 336.
(46) Quade, M. M.; Burrows, S. D.; Kobylarz, D. C.; Weishaar, R. E.; Evans, D. B. *Pharmacologist* **1984**, *26*, 106.
(47) Hofstee, B. H. J. *Science* **1975**, *116*, 329.
(48) Weishaar, R. E., unpublished observation.
(49) Yamamoto, T.; Yamamoto, S.; Osborne, J. C.; Manganiello, V. C.; Vaughan, M.: Hidaka, H. *J. Biol. Chem.* **1983**, *258*, 14173.
(50) Terasaki, W. L.; Appleman, M. M. *Metabolism* **1975**, *24*, 311.
(51) Weishaar, R. E.; Quade, M. M.; Schenden, J. A.; Boyd, D. K.; Evans, D. B., submitted for publication.
(52) Bergstrand, H.; Lundquist, B. *Biochemistry* **1976**, *15*, 1727.

Chart I. Structures of Nonselective Phosphodiesterase Inhibitors

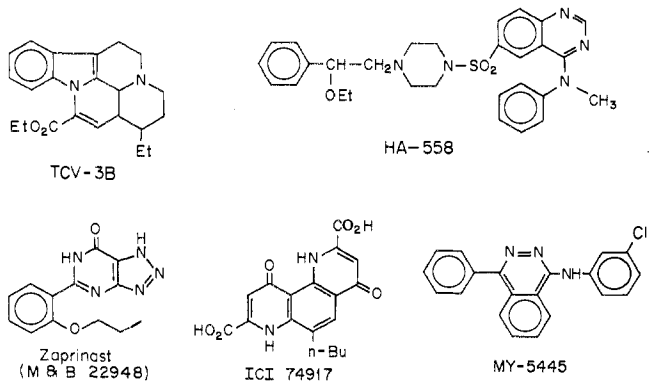


(IBMX),[55] as well as several purine isosteres, e.g., ICI 58,301,[56] and etazolate (SQ 20,009)[56,57] (Chart I), which are also generally considered to be nonselective inhibitors.

Among the first "selective" inhibitors of phosphodiesterase were certain 7-substituted and 8-substituted xanthines, which Wells and co-workers demonstrated to be relatively selective for calmodulin-stimulated phosphodiesterase in smooth muscle.[58,59] Other classes of agents were subsequently identified that generally exerted greater inhibitory effects on certain molecular forms of phosphodiesterase. For the purpose of this Perspective these latter agents which will be described below will be referred to as the "second generation" phosphodiesterase inhibitors.

PDE I Inhibitors. As previously illustrated in Figure 1, the activity of PDE I from guinea pig ventricular muscle can be stimulated several-fold by the Ca^{2+}–calmodulin complex. Such stimulation has previously been demonstrated in a number of other studies.[27,28,46,58,60,61] Several agents have been identified that inhibit the activity of this calmodulin-dependent phosphodiesterase. These include the phenothiazines chlorpromazine and trifluperazine, pimozide, calmidazolium (R 24,571), W-5, and W-7.[62-65] However, the observation that these agents inhibit calmodulin-stimulated phosphodiesterse activity indicates that they are acting as "calmodulin antagonists" and not as selective phosphodiesterase inhibitors.[62,64,65] Several studies have in fact shown that in addition to inhibiting calmodulin-stimulated phosphodiesterase activity these agents can also inhibit other calmodulin-stimulated enzymes, e.g., Ca^{2+}-ATPase and phosphorylase *b* kinase.[63,64] Thus, no effort has been made to include these agents in the present discussion of selective phosphodiesterase inhibitors.

Chart II. Selective PDE I Inhibitors



Recently, other agents were described that do exert a selective inhibitory effect on calmodulin-dependent phosphodiesterase activity. These include HA-558 and TCV-3B[33,65] and zaprinast (M & B 22,948) and ICI 74,197.[34] Unlike calmidazolium and W-7, Hidaka and co-workers have shown that HA-558 and TCV-3B exert comparable inhibitory effects on calmodulin-stimulated and basal phosphodiesterase activity.[33,65] On the basis of this difference, it has been proposed that these latter two agents selectively inhibit the hydrolytic site on the calmodulin-dependent phosphodiesterase rather than exerting any direct inhibitory effect on calmodulin or the calmodulin binding site on phosphodiesterase.[33,65] The structures of HA-558 and TCV-3B are included in Chart II.

In 1977, Bergstrand evaluated the inhibitory effects of a series of antiallergic agents on three partially purified forms of phosphodiesterase isolated from human lung.[34] Two of these compounds, zaprinast and ICI 74,197, were found to be selective inhibitors of calmodulin-stimulated phosphodiesterase.[34] The structures of these two agents are also shown in Chart II.

Several 1,4-dihydropyridine calcium channel blockers have recently been reported to be selective inhibitors of cardiac PDE I (e.g., IC_{50} of nifedipine for PDE I = 2 μM and for PDE II = 100 μM).[66] The therapeutic relevance of this selective inhibitory effect is unclear, since inhibition of PDE I is observed only at concentrations considerably greater than those required to block calcium entry via the slow channel.

In 1984, Hidaka and Endo reported that MY 5,445 (Chart II) potently inhibited a cyclic GMP specific, calmodulin-insensitive phosphodiesterase from human platelet (K_i = 0.5 μM) while exerting a lesser inhibitory effect on the cyclic GMP selective, calmodulin-sensitive phosphodiesterase from brain (K_i = 9.5 μM).[33] The authors have chosen to refer to the former enzyme as the FI PDE and the latter enzyme as the FII PDE, based on their elution rates from DEAE-cellulose. Although differences clearly exist between these two enzymes, for this Perspective both are included in this section since Quade et al. have recently demonstrated that the reference PDE I inhibitor zaprinast (M & B 22,948) inhibits both enzymes to comparable degrees (IC_{50} = 1.3 μM for calmodulin-insensitive cyclic GMP specific phosphodiesterase from human platelets and 3.4 μM for calmodulin-sensitive cyclic GMP selective phosphodiesterase from bovine coronary arteries).[46]

(53) Bergstrand, H.; Lundquist, B.; Schurmann, A. *J. Biol. Chem.* **1978**, *253*, 1881.
(54) Thompson, W. J.; Ross, C. P.; Pledger, W. J.; Strada, S. J.; Banner, R. L.; Hersh, E. M. *J. Biol. Chem.* **1976**, *251*, 4922.
(55) Wells, J. N.; Wu, Y. J.; Baird, C. E.; Hardman, J. A. *Mol. Pharmacol.* **1975**, *11*, 775.
(56) Davies, G. E. *J. Pharm. Pharmacol.* **1973**, *25*, 681.
(57) Weishaar, R. E., unpublished observations.
(58) Garst, J. E.; Kramer, G. L.; Wu, Y. J.; Wells, J. N. *J. Med. Chem.* **1976**, *19*, 499.
(59) Wells, J. N.; Garst, J. E.; Kramer, G. L. *J. Med. Chem.* **1981**, *24*, 954.
(60) Cheung, W. Y. *J. Biol. Chem.* **1971**, *246*, 2859.
(61) Kincaid, R. L.; Manganiello, V. C.; Odya, C. E.; Osborne, J. C.; Smith-Coleman, I. E.; Danello, M. A.; Vaughan, M. *J. Biol. Chem.* **1984**, *259*, 5158.
(62) Levin, R. M.; Weiss, R. *Mol. Pharmacol.* **1976**, *12*, 581.
(63) Hidaka, H.; Yamaki, T.; Naka, M.; Tanaka, T.; Hayashi, H.; Koboyashi, R. *Mol. Pharmacol.* **1979**, *17*, 66.
(64) Van Belle, H. *Cell Calcium* **1981**, *2*, 483.
(65) Hidaka, H.; Tanaka, T.; Itoh, H. *Trends Pharm. Sci.* **1984**, 237.

Table III. Effects of Various Selective and Nonselective Phosphodiesterase Inhibitors on Isolated Molecular Forms of Phosphodiesterase

compound	IC_{50}, μM; "IC_{25}, μM"; (K_i, μM); or [% inhibition at 10^{-5} M]			ref
	Ca^{2+}/calmodulin sensitive cyclic nucleotide phosphodiesterase (PDE I)	cyclic GMP sensitive cyclic nucleotide phosphodiesterase (PDE II)	low K_m, cyclic AMP phosphodiesterase (PDE III)	
theophylline	320[a]	210[a]	360[a]	46
isobutylmethylxanthine (IBMX)	3.4[b]	12[b]	6.9[b]	34
papaverine	9.0[b]	17[b]	6.6[b]	34
etrazolate (SQ 20,009)	(60)[c]		(12)[c]	71
ICI 58,301	(717)[c]		(217)[c]	71
zaprinast (M&B 22,948)	1.1[b]	170[b]	230[b]	34
ICI 74,917	16[b]	80[b]	280[b]	34
MY-5445	(9.5)[d]		(16)[e]	33
TCV-3B	(16)[d]		>500[e]	33
HA-558	(4.0)[d]		(270)[e]	33
dipyridamole	45[a]	4.0[a]	43[a]	68
sulmazole (vardax; AR-L 115)	>1000[a]	200[a]	500[a]	68
AR-L 57	265[a]	57[a]	390[a]	46
CI-914	>1000[a]	760[a]	6.1[a]	46
CI-930	>1000[a]	500[a]	0.9[a]	46
amipizone	[5%][a]	[0%][a]	[92%][a]	71
amrinone	>1000[a]	700[a]	35[a]	46
Y-590	[15%][a]	[6%][a]	[97%][a]	71
cilostamide (OPC 3689)	(22)[d]		(0.005)[e]	33
OPC 13,013	>1000[d]		0.19[e]	33
OPC 13,135	>1000[d]		0.073[e]	33
Ro 20-1724	250[b]	390[b]	9.1[b]	34
rolipram (ZK 62771)	"1400"[f]		"66"[f]	72
phthalazinol (EG 626)	(10)[d]		(1.4)[e]	33
fenoximone (MDL 17,043)	>1000[a]	520[a]	14[a]	46
piroximone (MDL 19,205)			8.6[g]	69

[a] Phosphodiesterases isolated from guinea pig ventricular muscle. [b] Phosphodiesterases isolated from human lung. [c] Phosphodiesterases isolated via differential centrifugation from sheep lung. The assumption is made that crude cGMP-PDE activity refers to the Ca^{2+}/calmodulin stimulated phosphodiesterase, whereas cAMP-PDE refers to the low K_m, cyclic AMP specific phosphodiesterase. [d] Phosphodiesterases isolated from human brain. [e] Phosphodiesterases isolated from human platelets. [f] A crude phosphodiesterase preparation was isolated from rabbit renal cortex. The assumption is made that crude cGMP-PDE activity refers to the Ca^{2+}/calmodulin stimulated phosphodiesterase, whereas cAMP-PDE refers to the low K_m, cyclic AMP specific phosphodiesterase. [g] Low K_m, cyclic AMP phosphodiesterase isolated from canine ventricular muscle.

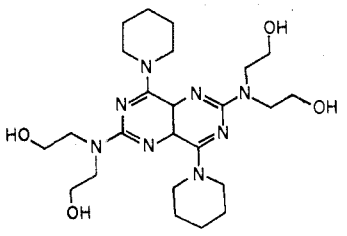
The pharmacology of the selective PDE I inhibitors will be discussed in greater detail in subsequent sections of this Perspective.

PDE II Inhibitors. At the present time, no agent has been identified that is a truly selective inhibitor of type II phosphodiesterase. This enzyme has also been referred to as the "cyclic GMP sensitive phosphodiesterase", since very low concentrations of cyclic GMP stimulate the hydrolysis of cyclic AMP by this enzyme.[28,32,67] Dipyridamole, AR-L 57, and sulmazole (AR-L 115BS) all exert a somewhat greater inhibitory effect on cardiac type II PDE than on type I PDE or type III PDE.[46,68]

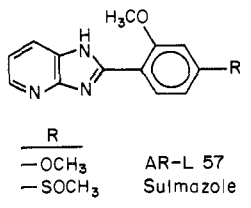
Contrary to its effect on cardiac muscle phosphodiesterases, dipyridamole inhibits PDE I and PDE II activity to a comparable degree in platelets.[46,68] The basis for this difference in inhibitory activity is not known at the present time, nor has it been established that inhibition of platelet PDE II (or platelet PDE I) activity represents the mechanism by which dipyridamole inhibits platelet aggregation, or that the cardiotonic activity of AR-L 57 and sulmazole is due to their inhibitory effects on cardiac PDE II activity.

The structures of these three agents are shown in Chart III.

Chart III. "Selective" PDE II Inhibitors



Dipyridamole

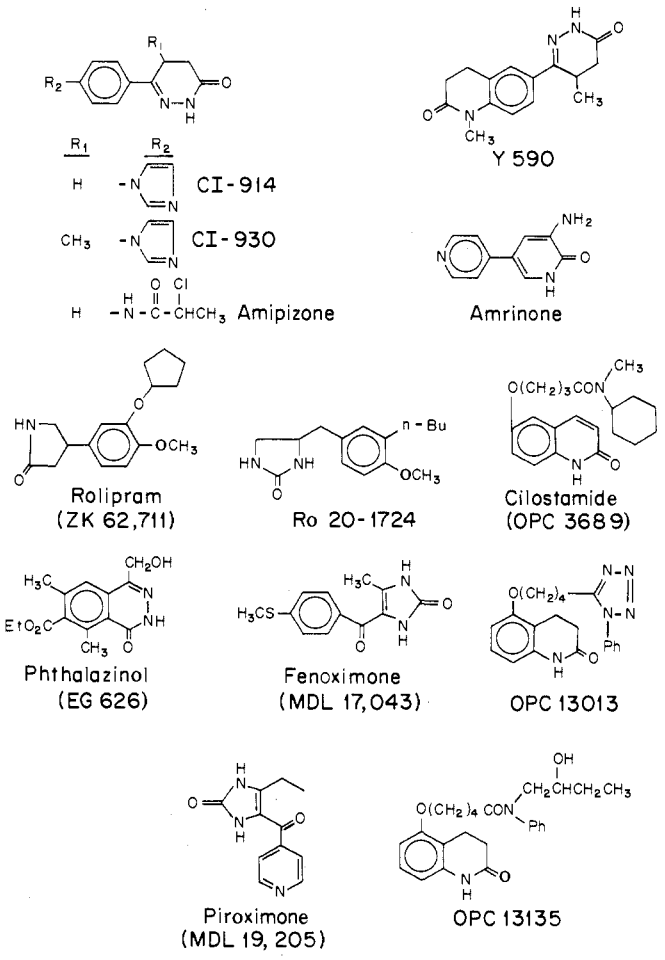


PDE III Inhibitors. Much interest has recently been focused on agents that selectively inhibit the activity of the low K_m, cyclic AMP specific phosphodiesterase (PDE III), particularly in cardiac muscle and in platelets, since selective PDE III inhibitors have been shown to posses cardiotonic activity[35,68] as well as to inhibit platelet aggregation.[33,69] The various agents that have thus far been shown to selectively inhibit PDE III are listed in Chart IV.

The inhibitory effects of the agents listed in Charts I–IV on the different molecular forms of phosphodiesterase are summarized in Table III. Since no study currently exists in which all of these agents were evaluated on phosphodiesterases from a single tissue or cell type, the values given in Table III are taken from a number of different literature sources. In addition, several previously unpublished results

(66) Norman, J. A.; Ansell, J.; Phillips, M. A. *Eur. J. Pharmacol.* **1983**, *93*, 107.
(67) Yamamoto, T.; Manganiello, V. C.; Vaughan, M. *J. Biol. Chem.* **1983**, *258*, 12526.
(68) Weishaar, R. E.; Burrows, S. D.; Kobylarz, D. C.; Quade, M. M.; Evans, D. B., submitted for publication.
(69) Mikashima, H.; Nakao, T.; Goto, K. *Thrombosis Res.* **1983**, *31*, 599.

Chart IV. Selective PDE III Inhibitors



from the authors' laboratory are also included in this table.

Pharmacologic Evaluation of Selective Phosphodiesterase Inhibitors

Although early studies with the selective "second generation" phosphodiesterase inhibitors focused primarily on evaluating the effects of these agents on partially purified or purified enzymes, later studies have attempted to prove that these agents in fact exert selective inhibitory effects on phosphodiesterase activity in isolated whole tissue. Thus, during the last 5 years the effects of zaprinast (M & B 22,948), rolipram, and other second generation phosphodiesterase inhibitors on a number of cyclic AMP and cyclic GMP dependent processes have been examined, including aggregation of platelets,[65] relaxation of vascular smooth muscle,[33,65,73-75] release of histamine from mast cells and basophils,[76] and cardiac muscle contractility.[77-79]

Several studies in particular have provided evidence that the second generation inhibitors such as zaprinast and CI-914, as opposed to the first generation inhibitors such as theophylline, caffeine, and papaverine, are indeed able to exert selective effects on cyclic nucleotide metabolism. In 1979 Kukovetz et al. examined the relaxant effects of nitroglycerin and sodium nitroprusside on circular strips of bovine coronary arteries in the presence and absence of zaprinast.[73] Previous studies have suggested that increases in tissue levels of cyclic GMP, which are believed to be due to a stimulant effect of both nitrolycerin and sodium nitroprusside on guanylate cyclase, are an important factor in mediating the vascular relaxant properties of these two agents.[80] Kukovetz et al. demonstrated that pretreament of the coronary arteries with the selective PDE I inhibitor zaprinast potentiated the relaxant effects of both nitroglycerin and sodium nitroprusside.[73] Zaprinast was also shown to potentiate the increases in tissue levels of cyclic GMP produced by these two agents. In addition, the relaxant effect of exogenously applied 8-bromo cyclic GMP was also potentiated by pretreatment with zaprinast.[73]

Lorenz and Wells have also shown that the ability of several substituted xanthines to potentiate the vascular relaxant effect of isoproterenol (which is mediated by cyclic AMP) or of sodium nitroprusside (which is mediated by cyclic GMP) is predictable from their ability to selectivity inhibit cyclic AMP- and cyclic GMP-phosphodiesterase activities, respectively.[75]

Antigen-induced histamine release from isolated mast cells and basophils has also been used as a means to demonstrate the selective inhibitory effects of "second generation" phosphodiesterase inhibitors. In 1981, Frossard and co-workers demonstrated that antigen-induced histamine release from human basophils could be inhibited by the selective PDE III inhibitor rolipram (ZK 62,711), whereas the selective PDE I inhibitor zaprinast (M & B 22,948) had no inhibitory effect on such release.[76] Conversely, zaprinast but not rolipram inhibited histamine release from rat mast cells.[76] These effects are presumably due to selective increases in cyclic AMP in the case of basophils and to selective increases in cyclic GMP in the case of mast cells, since the nonselective phosphodiesterase inhibitor theophylline inhibited histamine release from both cell types.[76] These studies support the premise that zaprinast, rolipram, and other "second generation" selective phosphodiesterase inhibitors are able to exert discrete effects on cyclic nucleotide metabolism, which were not previously possible with first generation phosphodiesterase inhibitors. More direct support for this hypothesis is provided by Hidaka and Endo, who demonstrated that MY 5,455, which is a selective inhibitor of the cyclic GMP specific phosphodiesterase in human platelets, increases cyclic GMP but not cyclic AMP in these cells[33] (Figure 2). In addition, recent studies in this laboratory have shown that the PDE III inhibitor CI-914 exerts a selective effect on cyclic nucleotide levels in cardiac muscle, increasing cyclic AMP levels in a concentration-dependent manner while having no effect on cyclic GMP levels.[79] The nonselective inhibitor theophylline, however, increases tissue levels of both cyclic AMP and cyclic GMP.[79] These results are illustrated in Figure 3. Bristol et al. have also shown a strong correlation between the inhibitory effects of CI-914, CI-930, amrinone, and fenoximone (MDL 17,043)

(70) Coulson, C. J.; Ford, R. E.; Marshall, S.; Walker, J. L.; Woodridge, K. R. H.; Bowden, K.; Coombs, T. J. *Nature (London)* **1977**, *265*, 545.
(71) Weishaar, R. E., unpublished observation.
(72) Fredholm, B. B.; Hedquist, P.; Vernet, L. *Biochem. Pharmacol.* **1978**, *27*, 2845.
(73) Kukovetz, W. R.; Holzmann, S.; Wurm, A.; Pöch, G. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1979**, *310*, 129.
(74) Holzmann, S. *J. Cardiovasc. Pharmacol.* **1983**, *5*, 364.
(75) Lorenz, K.; Wells, J. N. *Mol. Pharmacol.* **1983**, *23*, 424.
(76) Frossard, N.; Landry, Y.; Pauli, G.; Ruckstuhl, M. *Br. J. Pharmacol.* **1981**, *73*, 933.
(77) Roebel, L. E.; Dage, R. C.; Cheng, H. C.; Woodward, J. K. *J. Cardiovasc. Pharmacol.* **1982**, *4*, 721.
(78) Honerjäger, P.; Schäfer-Korting, M.; Reiter, M. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1981**, *318*, 112.
(79) Weishaar, R. E.; Quade, M. M.; Schenden, J. A.; Evans, D. B., submitted for publication.
(80) Katsuki, S.; Arnold, W.; Mittal, C.; Murad, R. *J. Cyclic Nucleotide Res.* **1977**, *3*, 23.

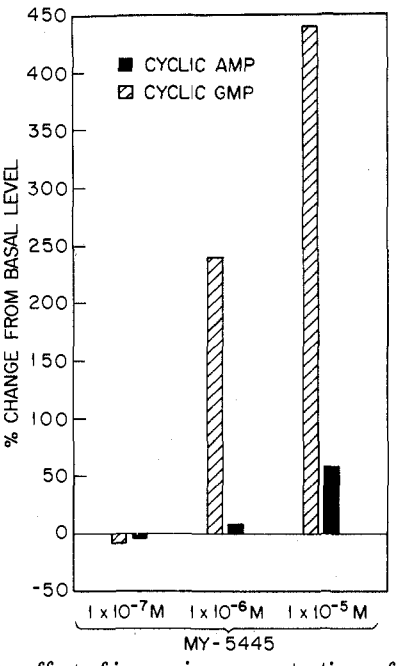


Figure 2. The effect of increasing concentrations of the selective cyclic GMP specific phosphodiesterase inhibitor MY-5445 on levels of cyclic AMP and cyclic GMP in isolated human platelets. The information contained in this figure was taken from data previously presented in a different format in ref 33.

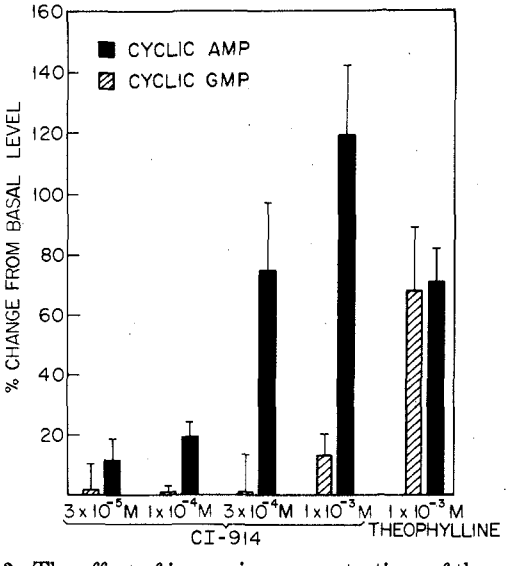


Figure 3. The effect of increasing concentrations of the selective cyclic AMP specific phosphodiesterase inhibitor CI-914, as well as the nonselective inhibitor theophylline, on levels of cyclic AMP and cyclic GMP in isolated guinea pig left atria.[79]

on cardiac PDE III actively and the in vivo cardiotonic effect of these agents.[81]

Therapeutic Utility of the "Second Generation" Phosphodiesterase Inhibitors

In 1975 Amer and McKinney reviewed what was then known regarding the cellular responses to cyclic AMP and cyclic GMP and outlined the potential therapeutic utility of agents that alter the intracellular levels of these two cyclic nucleotides.[82] According to the authors, such agents have potential clinical relevance in a variety of areas, including congestive heart failure, analgesia, glaucoma, hypertension, regulation of gastric acid secretion, cancer chemotherapy, and inflammation. Indeed, drugs that directly stimulate cyclic GMP synthesis such as the nitrates or stimulate cyclic AMP synthesis such as the β-receptor agonists have already proven effective agents for the treatment of certain conditions. However, agents that retard cyclic GMP or cyclic AMP hydrolysis by inhibiting phosphodiesterase activity, such as the nonselective "first generation" phosphodiesterase inhibitors theophylline and papaverine, have proven less effective therapeutic alternatives to the adenylate and guanylate cyclase stimulators. These latter agents generally lack potency, and their administration is often associated with a number of side effects, including tachycardia, tremor, increased rate of respiration, and, in the case of papaverine, gastrointestinal disturbances. Whether such side effects are associated with the fact that these agents increase both cyclic AMP and cyclic GMP have not been established.

Since the discovery of the "second generation" selective phosphodiesterase inhibitors such as zaprinast (M & B 22,948), rolipram, MY 5,445, and CI-914 is relatively recent, sufficient time has not elapsed to evaluate the efficacy of these agents in all the therapeutic areas for which Amer and McKinney previously suggested agents that alter cyclic nucleotide metabolism might prove beneficial. Nevertheless, recent developments have indicated that the "second generation" phosphodiesterase inhibitors may have considerable utility in at least three therapeutic areas. These are (i) as positive inotropic agents for the treatment of congestive heart failure, (ii) as mediator-release inhibitors for the treatment of asthma and related pulmonary disorders, and (iii) as platelet aggregation inhibitors for use in the treatment and/or prevention of ischemia.

Positive Inotropic Agents. The search for agents to replace digitalis for the treatment of congestive heart failure has led to the discovery of several non-catechol, non-glycoside cardiotonic agents that were originally considered to be acting via an entirely novel mechanism. The first such agent to be so characterized was amrinone.[83] Other such "novel" agents include carbazeran, fenoximone (MDL 17,043), piroximone (MDL 19,205), buquineran (UK 14,275), RMI 82,249, CI-914, CI-930, AR-L 57, and sulmazole (AR-L 115BS).[84] Recently, Weishaar et al. showed that many of these novel cardiotonic agents inhibit phosphodiesterase activity and also increase the level of cyclic AMP in isolated papillary muscles.[85] Subsequent studies have shown that four of these agents—amrinone, fenoximone, CI-914, and CI-930—are selective inhibitors of the low K_m, cyclic AMP specific phosphodiesterase (PDE III) present in cardiac muscle.[35,46,68,79] The ability of one such agent—CI-914—to selectively increase cyclic AMP levels in cardiac muscle has already been described.[79] These agents all exert considerably greater effects on in vivo cardiac contractility than do the first generation phosphodiesterase inhibitors theophylline and caffeine, and their administration is associated with a reduced incidence of side effects such as tachycardia.[86] The long-term utility of these agents in treating patients with con-

(81) Bristol, J. A.; Sircar, I.; Moos, W. H.; Evans, D. B.; Weishaar, R. E. *J. Med. Chem.* **1984**, *27*, 1099.
(82) Amer, M. S.; McKinney, G. R. *Ann. Rep. Med. Chem.* **1975**, *10*, 192.
(83) Alousi, A. A.; Farah, A. E.; Lesher, G. Y.; Opalka, C. J. *Circ. Res.* **1979**, *45*, 666.
(84) Bristol, J. A.; Evans, D. B. *Ann. Rep. Med. Chem.* **1981**, *16*, 83.
(85) Weishaar, R. E.; Quade, M.; Boyd, D.; Schenden, J.; Kaplan, H. R. *Drug Dev. Res.* **1983**, *3*, 517.
(86) Evans, D. B.; Burmeister, W. E.; Eldon, C. M.; McNish, R. W.; Potoczak, R. E.; Schenden, J. A.; Steffen, R. P.; Kaplan, H. R. *Pharmacologist* **1983**, *25*, 550.

gestive heart failure is currently under clinical investigation.

Agents for the Treatment of Pulmonary/Allergy Disorders. A variety of agents have been evaluated for the treatment of chronic obstructive pulmonary disorders such as chronic bronchitis, asthma, and emphysema. Of these, most act by increasing the level of cyclic AMP in bronchial smooth muscle, either by stimulating adenylate cyclase activity (β-receptor agonists) or by inhibiting the degradation of cyclic AMP (the "first generation" phosphodiesterase inhibitors). Use of the former is limited, however, by their direct effects on the heart and use of the latter by their lack of potency and also by the side effects which they produce.

Agents that alter intracelluar cyclic AMP levels also have proven useful for the prophylactic treatment of allergic reactions.[87] Modulation of mediator release by cyclic AMP was described by Lichtenstein and Margolis in 1968.[88] Subsequently, Orange et al. showed the inhibition of histamine and SRS-A release from human lung was related to the degree of elevation of tissue levels of cyclic AMP.[89] Although it was originally claimed that increases in cyclic GMP had the opposite effect, namely, enhancing mediator release,[90] subsequent studies by Coulson and co-workers showed a correlation between the ability of drugs to inhibit cyclic GMP hydrolysis and their ability to inhibit histamine release.[70] As mentioned previously, Frossard et al. have shown that histamine release from human basophils was inhibited by the selective PDE III inhibitor rolipram, while the selective PDE I inhibitor zaprinast effectively inhibited histamine release from rat mast cells.[76] Although the relevance of this observation to the prophylactic treatment of anaphylactic reactions in vivo has yet to be established, the ability of these agents to discretely alter cyclic nucleotide levels within the cell provides an excellent research tool for understanding the relationships between cyclic AMP and cyclic GMP and mediator release. In addition, the observation by Kukovetz et al. that zaprinast potentiates the smooth muscle relaxant effects of nitroglycerin and sodium nitroprusside may also imply that selective phosphodiesterase inhibitors could provide effective therapy for combined bronchospastic/allergic disorders.[73]

Inhibition of Platelet Aggregation

The relationship between increased levels of cyclic AMP and inhibition of platelet aggregation has been well documented,[91] and interference with cyclic AMP hydrolysis has been proposed as the mechanism of action of a number of reference platelet aggregation inhibitors, including dipyridamole.[91-94] Several recent studies have demonstrated the ability of "second generation" selective phosphodiesterase inhibitors to exert potent antiaggregatory effects. Hidaka and co-workers have shown that cilostamide (OPC 3,689), which is a selective inhibitor of platelet PDE III,[93] inhibits platelet aggregation induced by ADP, collagen, or arachidonic acid.[65] OPC 13,013, a derivative of cilostamide, has been shown to produce a concentration-dependent elevation in cyclic AMP levels in platelets.[33] Two other selective platelet PDE III inhibitors, Y-590 and amipizone,[72] have also been shown to be effective antiaggregation agents.[69,95]

Interference with the hydrolysis of cyclic AMP is a particularly attractive mechanism for inhibiting platelet aggregation since increases in intracellular cyclic AMP are presumed to impede calcium influx across the platelet cell membrane, an event that is essential to aggregation, whether such aggregation is stimulated by collagen, ADP, epinephrine, or thrombin.[91] Salzman and Weisenberger suggested that the antiaggregatory action of cyclic AMP is mediated by a cyclic AMP dependent protein kinase that phosphorylates a key membrane-bound protein associated with calcium influx.[96] When phosphorylated this latter protein can sequester calcium and thus prevent aggregation.

The involvement of cyclic GMP with platelet aggregation remains unclear. In 1973, Goldberg formulated his "Yin-and-Yang" hypothesis, which stated that the effects of cyclic GMP generally oppose those of cyclic AMP.[14] This hypothesis led to the proposal that increases in cyclic GMP may enhance platelet aggregation or otherwise counteract the antiaggregatory action of cyclic AMP.[97] Steiner et al. have in fact reported that cyclic GMP levels in platelets are increased by aggregatory agents such as ADP, epinephrine, thrombin, and collagen.[98] However, rather than promoting platelet aggregation, elevation of cyclic GMP may instead represent a physiological response to aggregation. Indeed, a recent report has shown that MY 5,445, which is a selective inhibitor of the cyclic GMP specific phosphodiesterase in platelets, is an effective inhibitor of ADP-, collagen-, and arachidonic acid induced platelet aggregation.[33,65] The observation that selective inhibitors of cyclic AMP phosphodiesterase as well as cyclic GMP phosphodiesterase both inhibit platelet aggregation is puzzling and indicates that further studies are required in order to establish the precise roles that cyclic AMP and cyclic GMP play in regulating platelet metabolism and platelet aggregation. Whether other selective inhibitors of the cyclic GMP specific phosphodiesterase in platelets such as M & B 22,948 are also effective inhibitors of platelet aggregation has not been reported.

While a number of questions remain to be answered regarding the role of cyclic nucleotides, particularly cyclic GMP, in platelet aggregation, the aforementioned studies clearly demonstrate the potential therapeutic utility of "second generation" selective phosphodiesterase inhibitors for the treatment of ischemia, vasospastic disorders, atherosclerosis, and other conditions in which a reduction in platelet aggregability is indicated.

Summary

With several notable exceptions, interest in the area of multiple molecular forms of phosphodiesterase remained relatively dormant during the decade following Thompson's discovery of more than one phosphodiesterase in brain in 1971.[25] Within the last several years, however, over 20 novel agents have been identified that exert se-

(87) Webb-Johnsor, D. C.; Andrews, J. L. *N. Engl. J. Med.* **1977**, *297*, 758.
(88) Lichtenstein, L. M.; Margolis, S. *Science* **1968**, *161*, 902.
(89) Orange, R. P.; Austen, W. G.; Austen, K. F. *J. Exp. Med.* **1971**, *134*, 136s.
(90) Kaliner, M.; Orange, R. P.; Austen, K. F. *J. Exp. Med.* **1972**, *136*, 556.
(91) Salzman, E. W. *N. Engl. J. Med.* **1972**, *286*, 358.
(92) Mills, D. C. B.; Smith, J. B. *Biochem. J.* **1971**, *121*, 185.
(93) Asano, T.; Ochiai, Y.; Hidaka, H. *Mol. Pharmacol.* **1977**, *13*, 400.
(94) Pichard, A.-L.; Hanoune, J.; Kaplan, J.-C. *Biochim. Biophys. Acta* **1972**, *279*, 217.
(95) Thyes, M.; Lehmann, H. D.; Gries, J.; König, H.; Kretzschmar, R.; Kunze, J.; Lebkücher, R.; Lenke, D. *J. Med. Chem.* **1983**, *26*, 800.
(96) Salzman, E. W.; Weisenberger, H. *Adv. Cyclic Nucleotide Res.* **1972**, *1*, 231.
(97) Chiang, T. M.; Dixit, S. N.; Kang, A. H. *J. Lab. Clin. Med.* **1976**, *88*, 215.
(98) Steiner, A. L.; Parker, C. W.; Kipnis, D. M. *J. Biol. Chem.* **1972**, *247*, 1106.

lective inhibitory effects on the various molecular forms of phosphodiesterase present within different cells. In addition, several studies have documented that such agents can produce discrete changes in cyclic AMP and cyclic GMP, an action that is not shared by "first generation" phosphodiesterase inhibitors such as theophylline.

The purpose of this Perspective is to provide some clarity to this rapidly evolving area of selective phosphodiesterase inhibitors. Thus, we have attempted to characterize the different forms of phosphodiesterase present in various tissues and cells according to their kinetic properties, substrate specificity, etc. and also to characterize those major classes of agents that have been shown to inhibit phosphodiesterase acitivity, whether selectively or nonselectively. In addition, we have described several therapeutic areas wherein selective phosphodiesterase inhibitors might prove efficacious, paying particular attention to those areas in which selective phosphodiesterase inhibitors have already been shown to exert beneficial effects, namely, stimulation of myocardial contractility, inhibition of mediator release, and inhibition of platelet aggregation. Although focusing on these three areas, it is obvious that the potential therapeutic utility of selective phosphodiesterase inhibitors could conceivably extend to several other areas in which modulation of cyclic nucleotides can have desirable effects, including cancer chemotherapy,[30] analgesia,[99] the treatment of depression,[100] Parkinson's disease,[101] and learning and memory disorders.[102] For example, the selective type III phosphodiesterase inhibitor rolipram has been shown to antagonize reserpine-induced hypothermia and also to potentiate yohimbine lethality, two tests that are indicative of antidepressant activity.[100] In addition, microinjection of the selective PDE III inhibitor Ro 20-1724 into the rat brain stem has been shown to produce analgesia.[100]

In conclusion, the observation that the different molecular forms of phosphodiesterase appear to be heterogeneously distributed in different tissues and cells and that the same form of phosphodiesterase may have somewhat different intracellular responsibilities implies that agents that exert a selective inhibitory effect on a single molecular form of phosphodiesterase may produce true tissue-selective responses, a characteristic not generally shared with currently available agents that alter cyclic nucleotide levels either via stimulation of adenylate cyclase (e.g., β-receptor agonists) or via nonselective inhibition of phosphodiesterase (e.g., first generation phosphodiesterase inhibitors). Given these latter observations, there is reason to hope that these new agents, the "second generation" selective phosphodiesterase inhibitors, by discretely influencing cyclic AMP or cyclic GMP levels in selected target organs or cells, may finally provide useful therapy for those clinical indications for which modulators of cyclic nucleotides have long been touted.

Acknowledgment. We acknowledge Dr. I. Sircar, D. Kobylarz, S. Burrows, and M. Quade for their expert assistance.

Registry No. Cyclic nucleotide phosphodiesterase, 9040-59-9.

(99) Levy, R. A.; Goldstein, B. D. *Pharm. Biochem. Behav.* **1981**, *15*, 501.
(100) Wachtel, H. *Neuropharmacology* **1983**, *22*, 267.
(101) Casacchia, M.; Meco, G.; Castellana, F.; Bedini, L.; Cusimano, C.; Agnoli, A. *Pharmacol. Res. Comm.* **1983**, *15*, 329.
(102) Randt, C. T.; Judge, M. E.; Bonnet, K. A.; Quartermain, D. *Pharmacol. Biochem. Behav.* **1982**, *17*, 677.